Asian Journal of Agriculture

| Asian J Agric | vol. 9 | no. 1 | June 2025
| E-ISSN: 2580-4537 |

Momordica charantia fruits photo by Salomé Bielsa

Published semiannually

PRINTED IN INDONESIA

E-ISSN: 2580-4537

Asian Journal of Agriculture

| Asian J Agric | vol. 9 | no. 1 | June 2025 |

ONLINE
http://smujo.id/aja

e-ISSN
2580-4537

PUBLISHER
Smujo International

ASSOCIATION
Society for Indonesian Biodiversity

INSTITUTION
Universitas Mulawarman, Samarinda, Indonesia

OFFICE ADDRESS
Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Balengkong No.1, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia. Tel./Fax.: +62-541-749159/738341, email: editors@smujo.id

PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Mulawarman
Samarinda, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Mulawarman Samarinda, Indonesia

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The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

Asian Journal of Agriculture

| Asian J Agric | vol. 9 | no. 1 | June 2025 | E-ISSN: 2580-4537 |

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 1-9 DOI: 10.13057/asianjagric/g090101

Comprehensive phytochemical analysis of *Momordica charantia* ethanol extract: Insights from Gas Chromatography-Mass Spectroscopy and in-silico ADMET studies

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Manuscript received: 4 August 2024. Revision accepted: 1 November 2024.

Abstract. *Mohanty SK, Nayak Y, Sahoo L. 2024. Comprehensive phytochemical analysis of* Momordica charantia *ethanol extract: Insights from Gas Chromatography-Mass Spectroscopy and in-silico ADMET studies. Asian J Agric 8: 1-9.* The *Momordica charantia* L. plant, also known as bitter gourd or bitter melon, is a crucial herbal remedy with a wide range of medicinal properties. It contains various essential phytocompounds, which contribute to its anti-inflammatory, antioxidant, and neuroprotective. The study investigates the phytochemical profile of *M. charantia* (bitter melon) ethanol extract using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, coupled with an in-silico ADMET assessment of the identified compounds. GC-MS analysis revealed a diverse range of phytoconstituents, including bioactive compounds such as Vitamin E, gentisic acid, cucurbitacin B dihydro, and various fatty acid esters, known for their potential therapeutic benefits. These compounds were further analysed using in-silico ADMET tools to predict their pharmacokinetic & Physiochemistry profiles. The results indicated that the majority of the identified compounds possess favourable ADMET properties, including good oral bioavailability and moderate toxicity levels, suggesting their potential as candidates for drug development. However, certain compounds, such as cucurbitacin, dihydro, and gentesic acid exhibited potential toxicity, necessitating careful consideration in therapeutic applications. The integration of GC-MS with in-silico ADMET analysis provides a data of the pharmacological potential and safety of *M. charantia* ethanol extract, supporting its traditional use and encouraging further exploration as a source of bioactive compounds for pharmaceutical development.

Keywords: Eco-toxicology, GC-MS analysis, in-silico ADMET, *Momordica charantia*

Abbreviations: ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity, BBB: Blood-Brain Barrier, GC-MS: Gas Chromatography-Mass Spectrometry, LogP: Logarithm of the partition coefficient (octanol-water), M.C: *Momordica charantia*, MS: Mass Spectrometry, MW: Molecular Weight, PDB: Protein Data Bank, TPSA: Topological Polar Surface Area

INTRODUCTION

Momordica charantia L., commonly known as bitter melon, is a tropical and subtropical vine belonging to the family Cucurbitaceae (Zhang et al. 2016). This plant is widely cultivated for its edible fruit, which is notably bitter and is used both in culinary and traditional medicinal practices across various cultures, particularly in Asia, Africa, and the Caribbean (Çiçek et al. 2022). The fruit, leaves, seeds, and roots of *M. charantia* have been extensively utilized in traditional medicine to treat a range of ailments, including diabetes, gastrointestinal disorders, and infections (Bortolotti et al. 2019). The ethno medicinal significance of *M. charantia* has spurred scientific interest, leading to numerous studies aimed at elucidating the bioactive compounds responsible for its therapeutic effects (Thakur et al. 2018).

The plant's bioactive components include a variety of phytochemicals, such as alkaloids, glycosides, flavonoids, triterpenes, and phenolic acids (Saeed et al. 2018). These compounds are known for their antioxidant, anti-inflammatory, antidiabetic, and anticancer properties, which contribute to the plant's medicinal efficacy (Bortolotti et al. 2019). Among these, phenolic compounds and flavonoids are particularly noteworthy due to their potent antioxidant activity, which plays a crucial role in neutralizing free radicals and mitigating oxidative stress-related diseases (Poolperm and Jiraungkoorskul 2017).

In recent years, the extraction and analysis of phytochemicals from medicinal plants have gained prominence, with ethanol emerging as a preferred solvent due to its ability to efficiently extract a broad spectrum of polar and non-polar compounds (Poolperm and Jiraungkoorskul 2017). Ethanol extracts of *M. charantia* have been the focus of several (Kim et al. 2018) studies aiming to identify and quantify the phytochemicals present, given the extract's potential for pharmaceutical and nutraceutical applications (Sorifa et al. 2017). The identification of these compounds is commonly performed using advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS), which allows for the separation, identification, and quantification of volatile and semi-volatile compounds in complex mixtures (Sun et al. 2021)

GC-MS is a powerful analytical tool that combines the separation capabilities of gas chromatography with the detection power of mass spectrometry (Saeed et al. 2018). This technique is particularly effective in the analysis of plant extracts due to its high sensitivity, specificity, and ability to analyze complex matrices (Barua et al. 2020). The application of GC-MS in the phytochemical analysis of *M. charantia* ethanol extract has revealed a diverse array of bioactive compounds, including various fatty acids, sterols, and essential oils. These compounds are known to possess significant biological activities, which contribute to the therapeutic potential of the plant (Stettin et al. 2020).

Over the past decade, research on *M. charantia* has expanded significantly, driven by the growing interest in natural products and the quest for novel therapeutic agents (Mohanty and Nayak 2024). Bitter gourd, also known as *M. charantia,* offers numerous health benefits and rich in essential nutrients like Vitamins C and A, it also provides powerful antioxidants that help combat oxidative stress and support immune function. Additionally, bitter gourd's anti-inflammatory and antimicrobial properties contribute to improved digestive health and detoxification (Hussain et al. 2018).

Studies demonstrated the rich phytochemical composition of *M. charantia* and its ethanol extract. For instance, recent research has highlighted the presence of key bioactive compounds such as charantin, momordicoside, and various cucurbitane-type triterpenoids, which are believed to be responsible for the plant's hypoglycemic and anticancer properties (Jia et al. 2017). Furthermore, the antioxidant potential of *M. charantia* has been extensively documented, with ethanol extracts showing significant free radical scavenging activity, which is attributed to the presence of phenolic compounds (Cortez-Navarrete et al. 2021).

Despite the extensive use of *M. charantia* (bitter melon) in traditional medicine for its diverse therapeutic properties, there remains a significant gap in the comprehensive phytochemical characterization of its ethanol extract. While previous studies have identified some bioactive compounds, a detailed analysis using advanced techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) has been limited. Moreover, the pharmacokinetic and toxicity profiles of these compounds, crucial for understanding their potential therapeutic applications, have not been thoroughly explored using in-silico Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) modeling. Addressing these gaps is critical to advancing the scientific understanding of *M. charantia* and optimizing its use in pharmaceutical applications.

The primary objective of this research is to perform a comprehensive phytochemical analysis of *M. charantia* ethanol extract using GC-MS to identify and quantify its bioactive constituents. Additionally, the study aims to predict the pharmacokinetic behavior and toxicity profiles of the identified compounds through in-silico ADMET analysis. This dual approach seeks to provide deeper insights into the therapeutic potential of *M. charantia*, facilitating its integration into modern medicinal practices and guiding future research on its pharmacological applications..

MATERIALS AND METHODS

Collection of plant materials

About 10 kg unripe fruits of bitter melon were purchased from the market in Soul Souk Organic Farm (www.soulsoak.com) in the month of October 2023, verified by Department of Botany, Centurion University of Technology and Management Bhubaneswar, Khordha, District Odisha, India.

Chemical and reagents

All the chemicals and Reagent were purchased from Sigma Aldrich and Heridity Bio-Academy, Bhubaneswar, Odisha.

Preparation of plant extract

Ten kg of bitter melon fruit was washed, and then cut into small slices, and then oven-dried at 50°C for a day. A dried sample was pulverized to fine powder by an electronic blender SR no-LAMK1WD184195, which was kept at 4°C until usage. The pulverized powder (850 g) was soaked in 90% Ethanol (1 L) and then extracted using a Soxhlet extractor for 3 days until extraction was completed. The Wattman No. 1 filter paper was used to filter the exract and then the filtrate was evaporated by the rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) at 190-220 rpm and 40-50°C for 24 hours under reduced pressure to get the amorphous solid mass. The extract yield was 12%. This crude extract was used for the phytochemical investigation of secondary metabolites, and GS-MS tests were carried out in triplicate (Nirupama et al. 2018).

Phytochemical screening

The fruit extract was subjected to preliminary phytochemical screening by following methods as described by Tupe et al. (2013).

Alkaloids

To screen the alkaloid: 1.0 mL extract was mixed with 5ml diluted hydrochloric, filtered on a steam bath and then 1.0 mL Mayer's reagent (CAS no- 56602-33-6) was added to 1.0 mL of filtrate into a separate tube. The formation of a cloudy, faint yellow colour confirmed the presence of alkaloids

Steroids

The screening for the presence of steroids was carried out by using M. Liebermann-Burchard's test .in this test 0.5 mL of extract was dissolved in 2.0 mL of acetic anhydride (CAS no-285977-80-2) 99% purity and cooled in ice, then 1.0 mL conc. H_2SO_4 was added resulting in the formation of a blue green ring that indicates the presence of steroids.

Tannins

A volume of 1.0 mL of the plant extract was mixed with an equal volume of Ferric chloride (CAS no-7705-08-0) 97% purity, resulting in to the formation of a reddish-brown precipitate indicating the presence of tannins.

Flavonoids

Screening of flavonoids was carried out by the Ferric chloride test in the ethanolic fruit extract of *M. charantia*. 0.2 mL of the extract was added to 10% $FeCl_3$ (CAS no-7705-08-0) and was shaken vigourously. A woody brownish precipitate formed indicating the presence of flavonoids..

Saponins

A frothing test was carried out to determine the presence of saponin in the fruit extract of *M. charantia*. In the test, 0.2 mL of the extract was mixed with 5.0 mL of distilled water and then shaken for 20 min. Formation and persistence of foams was observed indicating the presence of saponins.

Cardiac-glycoside

The cardiac-glycoside screening was done using Legal's method. In this method 1.0 mL of the extract was dissolved in 5.0 mL pyridine to which 2 drops of 2% Sodium Nitroprusside (CAS no-13755-38-5) and 2 drops of 20% NaOH was added. Appearance of deep red colour appeared, gradually fading to brown, indicating the presence of cardenolide.

Carbohydrate tests

Extract (1.0 mL) was added to 2.0 mL Fehling's solution and boiled for 5 min. A red precipitate was observed indicating the presence of reducing sugars; 1.0 mL extract was added to 2.0 mL Bradford's reagent and boiled for 1 min resulting in to the formation of red precipitate indicating the presence of reducing monosaccharides; 1.0 mL extract was added to 1.0 mL Molisch's reagent and 1.0 mL conc. H_2SO_4 was carefully added. A reddish ring was observed indicating the presence of carbohydrates.

Anthraquinones

The Borntrager's test screened the anthraquinone. 2.0 g extract in 10 mL ethanol, steamed for 5 min and filtered, 2.0 mL filtrate was added to 2.0 mL chloroform, shaken thoroughly. After 5 mins chloroform layer was taken off and to it 5.0 mL of distilled water was added and then shaken with 5.0 mL of dilute ammonia solution. Expected red colour did not appear in ammonia upper phase indicating the absence of anthraquinones.

GC-MS analysis method

The ethanol extract was subjected to GC-MS analysis by SHIMADZU QP-2010 Gas-Gas-Chromatography-Mass Spectroscopy. A fused silica column packed with Elite-5 ms, 5% Diphnvl, 95% Dimethyl polysiloxane (CAS no-63148-68-9), 30 mm × 0.25 mm × 0.25 μm separated the components. Separation of components was carried out with helium as the carrier gas at a constant flow of 1 mL/minutes. The sample extract of 2 μL is injected into the instrument. It was detected by the turbo gold mass detector with the aid of the Turbo mass 5.2 software. The oven was maintained at a temperature of 70°C with 2 min holding. The temperature of the injector was 280°C. The line inlet was at 260°C, while the source temperature was at 200°C. Mass spectra were collected at 70 eV, scan interval of 0 S, and fragment from 45-450 Da. The Mass Spectroscopy (MS) was scanned in 36 min. The interpretation on mass spectrum GCMS is based on the database of National Institute standard and technology, NIST, with more than 62,000 patterns.

Ligand preparation and filtration

Bioactive phytocompounds from *M. charantia* were collected from GC-MS analysis of ethanolic extract. The 3D structures of these chemicals were retrieved from the PubChem database. Using Discovery Studio 4.0's "prepare ligand" protocol, the ligands were cleaned, their 3D coordinates computed, and conformations were generated. These compounds were then filtered based on their molecular characteristics using Lipinski's "rule of five," which assesses bioavailability. This rule states that compounds should have a molecular mass of less than 500 daltons, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a log P (octanol-water partition coefficient) not greater than 5 (Lipinski 2004).

Drug-likeness property

Drug-likeness is a qualitative seed in drug design to assess a compound's potential as a drug based on its molecular properties affecting Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) (Chan et al. 2010). These properties are critical, as they account for nearly 60% of drug failures during clinical phases (Zhang et al. 2023). Therefore, predicting ADMET characteristics analysis by “Swiss-ADME” software (Daina et al. 2017) is essential for discovering new treatments. The compounds were analyzed for their ADMET properties to ensure they are quickly absorbed, delivered to their intended site of action, do not transform into harmful metabolites, and are efficiently excreted from the body. This analysis helps in identifying drug-like candidates suitable for further development.

RESULTS AND DISCUSSION

GC-MS profiling

Phytochemical screening-based test results of the Ethanolic fruit extract of *M. charantia* are presented in Table 1 and Figure 2. The results show the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlorotannins, cardiac glycosides, and carbohydrates, while it does not show the presence of anthraquinones. Table 2 shows the various compounds present in the plant, as identified by the GC-MS analysis. The major compound present in the Ethanolic fruit extract of *M. charantia* as identified by GCMS was gentisic acid with RT 16.544 and 8.406% relative peak area. The structure of gentisic acid was given in Figure 1, molecular formula as $C_7H_6O_4$, molar mass as 150.12 g/mol and biologically active as antioxidant (Table 3). Also, results from the fruit extract of *M. charantia* identified other compounds such as Vitamin E, 1- Pentadecyne, Cucurbitacin B Dihydro, Cis-9-hexadecenal, Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14- methyl-, methyl ester, β-sit.

In silico Swiss ADME analysis

The integration of ADMET profiling with phytochemical analysis provides a robust framework for evaluating the therapeutic potential of bioactive compounds. While Gas Chromatography-Mass Spectroscopy (GC-MS) identifies the chemical constituents of *M. charantia*, ADMET analysis offers crucial insights into the pharmacokinetics and safety of these compounds, bridging the gap between in vitro findings and potential clinical applications.

ADMET profiling allows for a comprehensive understanding of how compounds are absorbed in the body, distributed through tissues, metabolized by liver enzymes, excreted, and whether they possess any toxic effects. In silico ADMET studies are highly significant in this context, as they provide predictive data on the bioavailability and safety of compounds prior to in vivo experimentation. These predictions are critical for the rational design of future pharmacological studies and drug development processes. For instance, compounds that exhibit poor absorption or high toxicity can be flagged early, saving time and resources in the drug discovery pipeline.

In this particular study, ADMET analysis offers invaluable insights into how the bioactive phytoconstituents of *M. charantia* behave within biological systems. By simulating interactions with human physiological pathways, this approach reveals whether these compounds can effectively reach therapeutic concentrations in the body, whether they are likely to accumulate in specific tissues, and how they are metabolized into active or inactive forms. The toxicity assessment aspect of ADMET also helps in identifying safe dosage ranges, which is crucial for avoiding adverse effects in future preclinical and clinical trials.

The integration of ADMET with phytochemical analysis not only underscores the therapeutic relevance of the compounds but also ensures that only those with favorable pharmacokinetic and toxicity profiles are pursued further. This approach maximizes the potential for safe, efficacious use of *M. charantia* in treating various ailments.

Table 1. The phytochemical screening of *Momordica charantia*

Phytochemicals	Result
Alkaloids	+
Steroids	+
Tannins	+
Flavonoids	+
Saponins	+
Cardiac- glycosides	+
Carbohydrates	+
Phlobatinins	+
Anthraquinone	-

Table 3. Properties of gentisic acid

Properties	Given as
Molecular formula Molar mass	$C_7H_6O_4$
Biological property	150.12 g/mol Antioxidan

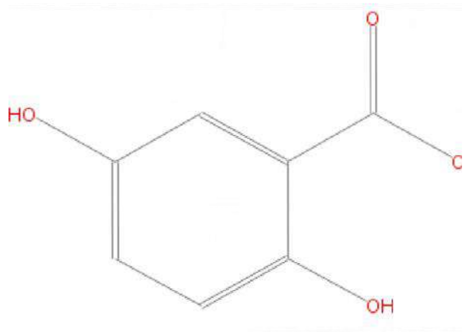

Figure 1. Structure of gentisic acid

Table 2. The list of chemical compounds from *Momordica charantia* fruit ethanolic extract

Components	R.T*. (minutes)	% Relative area peak
Vitamin E	14.698	0.331
Gentisic acid	16.544	8.406
1- pentadecyne	17.034	4.976
Cucurbitacin B Dihydro	18.498	1.674
Cis-9-hexadecenal	20.295	5.082
Hexadecanoic acid, methyl ester	20.683	ND
Pentadecanoic acid14-methyl-, methyl ester	21.713	ND
β-sitoserol	21.838	0.618
Stigmasterol	24.811	2.653
Oleic acid	25.156	ND
Stigmastan-3-ol	25.965	0.928
Ethyl-4,5-dimethyl-Phenol	26.565	ND
Linoleic acid	27.355	ND

Table 4. Prediction of the physiochemistry of a few chemicals found in *M. charantia*

Compound	Formula	Molecular weight	Fraction Csp3	Rotational Bond(RB)	Hydrogen Bond Acceptor (HBA)	Hydrogen Bond Donor (HBD)	Metabolic Ratio(MR)	Total Polar Surface Area (TPSA)	Estimated solubility (ESOL)	Lipophilicity Conseco Log p
Vitamin E	$C_6H_8O_2$	117.13 g/mol	0.50	0	2	1	30.14	37.30 Å²	Soluble	1.38
Gentisic acid	$C_7H_6O_4$	275.33 g/mol	1.00	9	0	0	59.80	0.00 Å²	Moderate soluble	5.82
1- pentadecyne	$C_{15}H_{28}$	201.39 g/mol	1.00	11	0	0	69.41	0.00 Å²	Moderate soluble	4.23
Cucurbitacin	$C_{32}H_{46}O_8$	250.49 g/mol	1.00	15	0	0	88.64	0.00 Å²	Poorly soluble	4.23
DihydroCis-9-hexadecenal	$C_{32}H_{65}NO_3$	132.42 g/mol	0.45	4	4	1	99.73	45.59 Å²	Moderate soluble	4.36
Hexadecanoic acid,	$C_{19}H_{38}O_2$	186.45 g/mol	0.50	5	1	1	94.67	20.23 Å²	Soluble	3.89
Pentadecanoic acid14-methyl-	$C_{15}H_{30}O_2$	196.65 g/mol	0.71	5	1	1	129.37	20.23 Å²	Moderate soluble	3.00

Note: -shows Fraction Csp3≤0.25, permissible range: & lt; 500 g/mol Acceptable ranges for HBA and HBD are ≤10 and ≤5, respectively, for hydrogen bond acceptors and donors. ESOL stands for water solubility, Log p for high lipophilicity (recommended range: ≤5), Molar Refractivity (MR): 40 to 130 is the acceptable range. Topological polar surface area or TPSA

Table 5. Prediction of the pharmacokinetics of pharmacophores obtained from *M. charantia*

Compound	Gastrointestinal Absorption (GIA)	Blood-Brain Barrier Permeability (BBB P)	P-glycoprotein Substrate (P-gpS)	Cytochrome (CYP1A2) Inhibitor	Cytochrome (CYP2C19) Inhibitor	Cytochrome (CYP2C9) Inhibitor	Cytochrome (CYP2D6) Inhibitor	Cytochrome (CYP3A4) Inhibitor	Log Kp
Vitamin E	High	Yes	No	No	No	No	No	No	-6.76 cm/s
Gentisic acid	Low	No	No	Yes	No	No	No	No	-6.01 cm/s
1- pentadecyne	Low	No	No	Yes	No	No	No	No	-2.40 cm/s
Cucurbitacin B Dihydro	Low	No	No	Yes	No	No	No	No	-1.20 cm/s
Cis-9-hexadecenal	High	Yes	No	No	No	No	Yes	No	-4.23 cm/s
Hexadecanoic acid, methyl ester	High	Yes	No	Yes	No	Yes	No	No	-3.01 cm/s
Pentadecanoic acid14-methyl-, methyl ester	Low	No	No	No	No	Yes	No	No	-1.45 cm/s

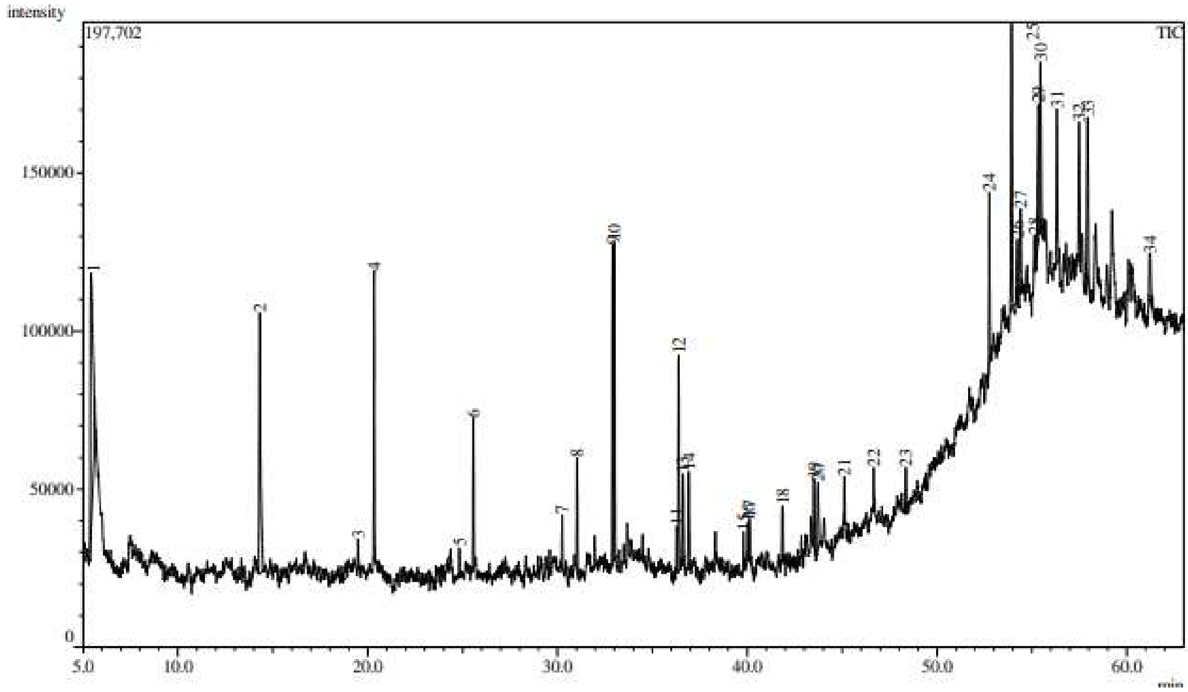

Figure 2. The GC-MS graph of ethanolic extract of *Momordica charantia* fruit

Physicochemical properties

Developing a potential drug candidate requires careful consideration of ethical and physicochemical parameters, which are crucial for Absorption, Distribution, Metabolism, and Excretion (ADME). These parameters also impact other stages in the drug development process. It is essential to assess how a drug will interact with living organisms, identifying desirable and undesirable molecular characteristics.

The molecular weights of all compounds were found to be under 500 g/mol, as shown in Table 4. Each compound had no more than ten rotatable bonds, and their molar refractivity ranged from 40 to 130, also detailed in Table 4. Topological Polar Surface Area (TPSA) is another critical factor related to drug bioavailability; compounds that are highly orally bioavailable and passively absorbed typically have a TPSA of less than 75 Å² (Johari et al. 2019). According to Table 4, all selected compounds are polar, with Vitamin E having the lowest TPSA value. The TPSA values ranged from 29.54 Å² to 71.69 Å², indicating potential for good oral bioavailability.

All seven compounds in Table 4 showed good to moderate water solubility, supporting effective oral absorption, with Log S/Log P values ranging 5.82. While most compounds were very soluble, gentisic acid were soluble, and the remaining compounds were moderately soluble.

This analysis indicates that the selected phyto-compounds from *M. charantia* exhibit favourable physicochemical properties and ADME profiles, supporting their potential as effective drug candidates for Anti-diabetic, anti-inflammatory like properties is showing.

Pharmacokinetics properties

Pharmacokinetics involves the study of drug disposition within the body, including parameters such as volume of distribution, elimination half-life, clearance, and bioavailability. Bioavailability is the proportion of a drug that reaches the bloodstream after administration, expressed as a percentage. It also informs the frequency and duration of drug administration. Cytochrome P450 (CYP) superfamily enzymes play a significant role in drug metabolism, except for those that can induce transporters. The permeability of a compound across cell membranes is another crucial factor, which can be indicated by its partition coefficient (Kp). A higher Kp value (cm/s) signifies lower membrane permeability.

Discussion

ADMET profiles and physicochemical properties of these compounds underscore the importance of understanding solubility, bioavailability, and toxicity in the development of effective therapeutic agents. Vitamin E, a lipid-soluble antioxidant, exhibits good oral bioavailability due to its hydrophobic nature, although its high molecular weight may limit aqueous solubility and influence its absorption. Gentisic acid, on the other hand, is a small polar molecule with moderate aqueous solubility, likely leading to good absorption, though its polarity may restrict its distribution across lipid membranes. This compound’s structural similarity to salicylic acid suggests potential anti-inflammatory and antioxidant activities.

1-Pentadecyne, a long-chain alkyne, is highly hydrophobic, which may allow for good absorption through lipid membranes, but its poor water solubility could limit overall bioavailability. The compound’s potential antimicrobial properties warrant further investigation, though its medicinal chemistry remains underexplored. Cucurbitacin B dihydro, a highly hydrophobic triterpenoid, presents challenges in oral administration due to poor solubility but holds promise in anti-cancer therapy. However, its potent biological activity raises concerns about toxicity, emphasizing the need for careful dosing strategies.

Cis-9-hexadecenal, an unsaturated aldehyde, shows moderate absorption and distribution, particularly in lipid-rich tissues, with its reactive aldehyde group influencing metabolism and excretion pathways. Although primarily studied in the context of flavoring and pheromone research, its therapeutic applications are not well established. Hexadecanoic acid methyl ester and pentadecanoic acid 14-methyl, methyl ester, both highly hydrophobic esters, are expected to be we ll absorbed but face challenges in bioavailability due to poor solubility.

This is based on the presumption that within the fruit of *M. charantia* lies a diverse group of biologically active chemical constituents; hence, a detail phytochemical screening and GC-MS analysis is done. The present investigation of phytochemical screening of ethanolic extracts of MC fruits showed the presence of alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, phlorotannins, carbohydrates, and terpenoids. GCMS analysis confirmed the presence of Vitamin E, gentisic acid, Stigma sterol, β-sitosterol, and cucurbitacin B, which are important medicinal compounds. All those compounds are secondary metabolites and among the important constituents of medicinal plants. It has been reported that secondary metabolites of plants exert a wide range of biochemical activities on physiological systems. Elsewhere, it was reported that the alkaloids, saponins, glycosides and phenolic constituents are present in *M. charantia*.

Apart from Vitamin E, gentisic acid has been also known as an antioxidant agent acting as free radicals terminators. gentisic acid is, hence, a pharmaceutical important compound under physiological functions. Cucurbitacin B, which exerts ant proliferative activity against breast cancer, glioblastoma multiform, and myeloid leukemia cells, was also found. According to Subha et al. (2021), Saponins are natural surfactants. Saponins are steroid glycosides with an amphipathic characteristic, a foaming feature, and strong biological activity. They also possess fungicidal and bactericidal activities. Flavonoids are polyphenol compounds, widely distributed in plants and acting as secondary metabolites, presenting antioxidant activity. This offers protection against the damage in blood vessels, reducing the risk of cardiovascular diseases, cancer prevention, and the improvement of the immune system. The analysis also revealed the possible anticancer properties of *M. charantia*, due to the presence of cucurbitane-type triterpenoids.

In this study, gentisic acid was identified as a significant phytocomponents of *M. charantia* ethanol extract through comprehensive GC-MS analysis. Gentisic acid, a known phenolic compound, is recognized for its potent antioxidant and anti-inflammatory properties. The presence of this compound within *M. charantia* adds to the plant's pharmacological profile, offering insights into its traditional use in managing various ailments, particularly those related to oxidative stress and inflammation. Absorption, distribution, metabolism, excretion, and other stages of the drug development process are significantly influenced by the physicochemical characteristics and ethical considerations of created prospective therapeutic candidates. It is crucial to assess the ways in which medicine will interact with biological systems and to consider the aspects of these disciplines that a molecule should have and those that it should not have. As seen in Table 4, the molecular weight of every molecule showed a value that satisfied the necessary ≤500 g/mol criteria. Each chemical under investigation has less than 10 rotatable bonds. Additionally, Table 4 illustrates that the compounds' molar refractivity values range from 40 to 130. TPSA clarified yet another crucial element pertaining to the drug's bioavailability. TPSA of less than 140 Å2 is exhibited by highly oral accessible and passively absorbed compounds (Johari et al. 2019). As can be seen in Table 4, every chemical that was chosen is polar; Vitamin E has the lowest TPSA value, with values ranging from 0.00 Å2 to 45.59 Å2, Whereas gentisic acid shows high TPSA value, ranging from 0.00 Å2 to 59.80 Å2 All seven of the compounds in Table 4, with Log p values ranging from 1.38 to 5.82, have good to moderate water solubility and may be useful for oral adsorption. While gentisic acid, 1-Pentadecyne, DihydroCis-9-hexadecenal, and Pentadecanoic acid14-methyl- are moderately soluble, cucurbitacin is poorly soluble. Vitamin E and hexadecanoic acid are soluble.

The identification of gentisic acid, along with other bioactive compounds, is supported by recent research findings that have highlighted its strong antioxidant, anti-inflammatory, and neuroprotective activities. Comparing the current findings with recent reports reveals its distinct advantages over other phytochemicals in terms of both efficacy and safety.

A study by Paredes-López et al. (2018) confirmed the potent antioxidant properties of gentisic acid, which can effectively neutralize free radicals and protect against oxidative stress-related damage. When compared to other well-known antioxidants such as quercetin and Vitamin C, gentisic acid demonstrated comparable or superior activity in reducing oxidative stress markers. Its dual function as both an antioxidant and an anti-inflammatory agent makes it particularly valuable in addressing chronic diseases linked to inflammation and oxidative damage, such as neurodegenerative disorders and cardiovascular diseases (Sarkar et al. 2020).

In comparison with other phytochemicals identified in this study, such as quercetin and quinine, gentisic acid presents a unique profile. Quercetin, a flavonoid, is widely recognized for its antioxidant and anti-inflammatory activities but often suffers from poor bioavailability, limiting its clinical effectiveness (Aherne and O'Brien 2002). In contrast, in-silico ADMET analysis for gentisic acid revealed favorable pharmacokinetic properties, including high gastrointestinal absorption (GIA) and good blood-brain barrier permeability (BBB P), making it a more viable candidate for oral administration and neuroprotective applications. Moreover, gentisic acid's solubility and low molecular weight provide better metabolic clearance, reducing potential toxicity risks, as highlighted in a recent study by Deepa et al. (2021). When compared to quinine, gentisic acid offers a broader therapeutic scope. While quinine has well-established antimalarial properties, its use is limited due to concerns about side effects such as cardiac arrhythmias and drug interactions (Pukrittayakamee et al. 2018). Gentisic acid, on the other hand, exhibits a much safer toxicity profile with fewer adverse effects, even at higher doses, as demonstrated in recent toxicity screenings (Pradhan et al. 2022). Additionally, its ability to act as a free radical scavenger enhances its potential in treating a wide range of conditions, from inflammation to oxidative stress-induced cell damage.

In summary, gentisic acid stands out as a promising phytochemical with superior ADMET characteristics, antioxidant efficacy, and safety profile when compared to other bioactive compounds in *M. charantia*. The results from this study align with and expand upon recent research, underscoring gentisic acid's potential for therapeutic applications in chronic diseases.

In conclusion, the study investigates the physicochemical properties of *M. charantia* fruit, focusing on its solubility, bioavailability, and toxicity. It reveals that Vitamin E, gentisic acid, Stigma sterol, β-sitosterol, and cucurbitacin B are important medicinal compounds. The phytochemical screening and GC-MS analysis of the ethanolic fruit extract of *M. charantia* revealed various compounds. Gentisic acid, an antioxidant, has physiological functions, while Cucurbitacin B has ant proliferative activity against breast cancer, glioblastoma multiform, and myeloid leukemia cells. Saponins are natural surfactants with strong biological activity. The comprehensive phytochemical analysis of *M. charantia* ethanol extract using Gas Chromatography-Mass Spectroscopy (GC-MS) and subsequent in-silico ADMET studies highlighted gentisic acid as a significant phytocomponents. The identification of gentisic acid within the extract underscores its potential therapeutic value, given its well-documented anti-inflammatory, antioxidant, and analgesic properties. The in-silico ADMET profiling further corroborated the suitability of gentisic acid for drug development, demonstrating favourable ADMET characteristics. These findings suggest that gentisic acid contributes substantially to the pharmacological effects of *M. charantia* and warrants further investigation for its role in therapeutic applications, particularly in the context of chronic inflammatory and oxidative stress-related diseases.

ACKNOWLEDGEMENTS

We express our sincere gratitude to all who contributed to this review. Special thanks to our team for their insightful feedback, which significantly improved this manuscript. We acknowledge the foundational work of the researchers we cited, whose studies were invaluable to our analysis. We also appreciate the Institution (Centurion University of Technology and Management, Bhuabneswar, Odisha, India) for supporting us.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 10-22 DOI: 10.13057/asianjagric/g090102

Optimizing raised bed dimensions for enhanced maize yield, water footprint reduction, and improved soil moisture dynamics under furrow irrigation

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Manuscript received: 24 August 2024. Revision accepted: 2 November 2024.

Abstract. *Islam MT, Bhuyan MY, Das N, Jahan N, Rahman MM, Jewel MAH, Adham AKM. 2024. Optimizing raised bed dimensions for enhanced maize yield, water footprint reduction, and improved soil moisture dynamics under furrow irrigation. Asian J Agric 8: 10-22.* Effective water management is crucial for sustainable agriculture, especially in regions facing water scarcity. This study examines the impact of different Raised Bed (RB) dimensions on maize (*Zea mays* L.) yield, Water Footprint (WF), and soil water content under furrow irrigation, with a focus on water conservation. The experiment, conducted at Bangladesh Agricultural University, Bangladesh, utilized a randomized complete block design featuring four irrigation treatments: the Conventional Method (CM) and three RB configurations with widths of 25 cm, 65 cm, and 110 cm. The RB65 treatment significantly improved maize yield, achieving 13.1±1.4 t/ha compared to 9.89±1.11 t/ha with CM. Additionally, RB65 peaked in water productivity, reducing irrigation water use by 37.72%, highlighting its potential for water conservation. Soil moisture retention was consistently higher across all RB treatments, with RB65 showing the greatest retention at depths up to 80 cm. It also recorded the lowest green WF (88.6±10.07 l/kg) and blue WF (12.63±1.43 l/kg), resulting in the lowest total WF (101.22±11.50 l/kg). These findings suggest that RB systems, particularly the 65 cm configuration, optimize water usage and enhance maize productivity, making it a viable strategy for resource management in water-limited areas. The study recommends adopting the RB65 configuration to maximize water efficiency and crop yields, contributing to food security and sustainable agricultural practices. However, these results are based on specific geographical and climatic conditions, limiting their generalizability to other regions or crops. Future research should explore long-term studies across diverse agro-ecological zones and examine various crops to validate the broader utility of the RB65 configuration.

Keywords: Maize yield, raised bed irrigation, soil water content, sustainable agriculture, water footprint, water management

INTRODUCTION

Water is undeniably crucial for the agricultural industry, serving as the foundation of food production. Agriculture, as the largest user of global water resources, accounts for approximately 70% of freshwater withdrawals, primarily for irrigation (Jeong and Zhang 2020). This heavy reliance places agriculture at the heart of global water scarcity challenges. While essential for food production, conventional agricultural practices, particularly inefficient irrigation methods, contribute significantly to water depletion, exacerbating the very scarcity upon which agriculture depends (Roushan et al. 2023). Among surface irrigation technologies, furrow irrigation is the most prevalent due to its simplicity and cost-effectiveness. It is widely used in water-stressed regions, such as arid and semi-arid areas, where water management is critical (Akbar et al. 2016; Mekonnen et al. 2020). Studies in Egypt and South Asia demonstrate that furrow irrigation can reduce water use while maintaining or improving crop yields for staple crops like maize and wheat (Sarker et al. 2020; Ismail et al. 2021; Yigezu et al. 2021). However, despite its popularity, furrow irrigation is associated with inefficiencies such as deep percolation and runoff, leading to significant water loss (Setu et al. 2023). Thus, optimizing these systems is vital for sustainable water management.

The adoption of improved water-saving agricultural practices, such as enhanced furrow irrigation and Raised Bed (RB) systems, can have profound socioeconomic impacts. These methods not only conserve water but also increase crop yields, thereby promoting food security and offering economic benefits to smallholder farmers, especially in developing countries like Bangladesh, where water scarcity limits agricultural potential (Sarker et al. 2020). Additionally, water-efficient practices reduce production costs, increase labor efficiency, and enhance resilience to climate variability, ultimately improving the livelihoods of rural communities (Yigezu et al. 2021). By boosting irrigation efficiency, these technologies significantly enhance water productivity and enable better resource management across agricultural systems (Islam et al. 2022; Rahman et al. 2022). Notably, 97.8% of farmers' fields are irrigated using surface methods, underscoring the widespread dependence on traditional systems, despite their water loss issues (Setu et al. 2023). Therefore, more efficient practices like improved furrow irrigation are critical to addressing these inefficiencies and supporting sustainable agriculture.

Maize (*Zea mays* L.), one of the most widely cultivated crops, is crucial for global food security (Das et al. 2018; Tizhe et al. 2023; Diri and Kedoneojo 2024). However, climate-related shocks have caused a 3.8% decline in maize yields in recent decades, with projections of a 24% decrease by the end of the 21st century (Markos et al. 2023). This is because of poor soil nutrients, water scarcity, and soil nitrogen deficiency (Islam et al. 2017). In developing countries like Bangladesh, water scarcity hinders agricultural potential (Makate et al. 2019). Traditional flooding irrigation is inefficient, leading to significant water loss and reducing aeration and nitrogen absorption (Majeed et al. 2015; Rahman et al. 2022).

Water Footprints (WFs) are a crucial indicator for expressing water resources needed for goods and services, categorized into green, blue, and grey WFs (Khan and Ali 2024). Use of WF in managing limited water resources among competing sectors is emerging as a highly promising approach. In particular, agricultural water management stands to benefit significantly from this method, as WF variability is strongly influenced by factors such as climate, soil types, and water management strategies (Feng et al. 2021). Recent studies focus on the WF of specific crops, including maize, across global, national, and regional scales (Chapagain and Hoekstra 2011; Elbeltagi et al. 2020; Li et al. 2020; Al-Gaadi et al. 2022). These studies highlight the importance of understanding the spatiotemporal variability of WFs and their implications for crop production.

Maize requires sufficient water, especially during early growth, flowering, and seed-filling stages, as water scarcity at these stages can severely impact production (Muslimah et al. 2023). Waterlogging during flowering can reduce grain output by about half (Kaur and Kaur 2022). Thus, improving water management is vital to boost maize yields (Muslimah et al. 2023). Planting on RBs is an effective water-saving technique that mitigates the impact of flooding during irrigation and heavy rainfall (AbdelRahman and Arafat 2020). RB planting enhances water absorption efficiency and evenly distributes irrigated water, reducing water usage by up to 50% and increasing yields by 20-25% compared to traditional irrigation systems (Verhulst et al. 2011; Rashwan et al. 2024). However, the effectiveness of RBs depends on factors like bed size, implementation method, sowing technique, and seed rates (Akbar et al. 2016; Yigezu et al. 2021). Improper sizing of RB installation can lower yields (Yigezu et al. 2021).

Furrow-irrigated RB cultivation systems are crucial for sustainable agriculture, providing efficient water use and increased yields (Shah et al. 2024). The bed size (i.e., width) of an RB can have significant effects on soil wetness, thereby, influencing crop growth and yield (Pan et al. 2019; Duan et al. 2021). While the benefits of RB systems under varied agricultural conditions are well-documented, the specific effects of different RB dimensions on maize cultivation remain poorly understood. Although existing research highlights the water-saving benefits of RB farming, there is a significant lack of studies on how varying RB widths affect crop yield and WFs.

This study aims to investigate effects of different furrow-bed configurations on enhance maize yield, water productivity, and soil water content. It also seeks to provide a comprehensive understanding of the WFs associated with maize cultivation, crucial for developing sustainable agricultural practices and improving resource management in regions with limited water availability. The research will contribute valuable insights to optimize irrigation practices, ultimately supporting food security and sustainable water use in agriculture.

MATERIALS AND METHODS

Experimental site with soil and climatic conditions

The experiment was conducted at the Bangladesh Agricultural University (BAU) farm in the Mymensingh District, Bangladesh, following the procedures outlined in Figure 1. The study site is situated in agro-ecological zone 9, at coordinates 24.75° N latitude and 90.50° E longitude, with an elevation of 18 meters above mean sea level (Figure 2). A comprehensive analysis of the soil's physical, chemical, and biological properties was conducted by the Agri-Varsity Humboldt Soil Testing Laboratory. According to the Bangladesh Agricultural Research Council (BARC 2005), the soil texture class of the experimental field is silt loam, underlain by sandy loam, characteristic of the Old Brahmaputra Floodplain. Key soil characteristics measured include bulk density (1.31 g/cm³), field capacity (31.33%), electrical conductivity (250-345 µS/cm), and wilting point (16.05%).

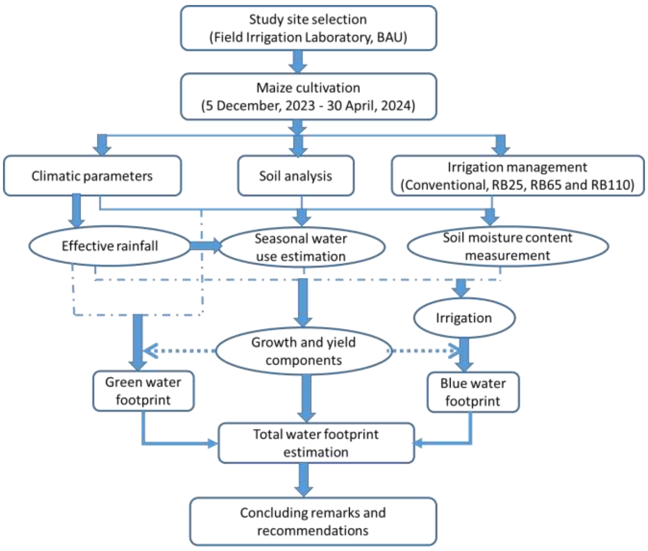

Figure 1. Flow diagram outlining the methodologies used in this study. RB denotes the raised bed-based furrow irrigation technique, implemented with varying bed widths of 25 cm, 65 cm, and 110 cm

Table 1. Weather data for the experimental site throughout the maize growing period (from 5 December, 2023 to 30 April, 2024)

Parameters	Months				
	December	January	February	March	April
Rainfall (mm)	26.5	0	17.3	72	0.2
Mean maximum air temperature (°C)	24.1	20.1	23.7	27.2	33.5
Mean minimum air temperature (°C)	18	13.8	17.7	21.1	26.1
Monthly average relative humidity (%)	84.2	86.5	76.4	76.3	76.5
Mean evaporation (mm)	2.15	1.6	2.63	3.5	4.79
Mean wind speed (km/h)	0.37	1.71	2.6	4.6	7.63
Mean sun shine (hours)	5.56	3	5.93	6.93	7.19

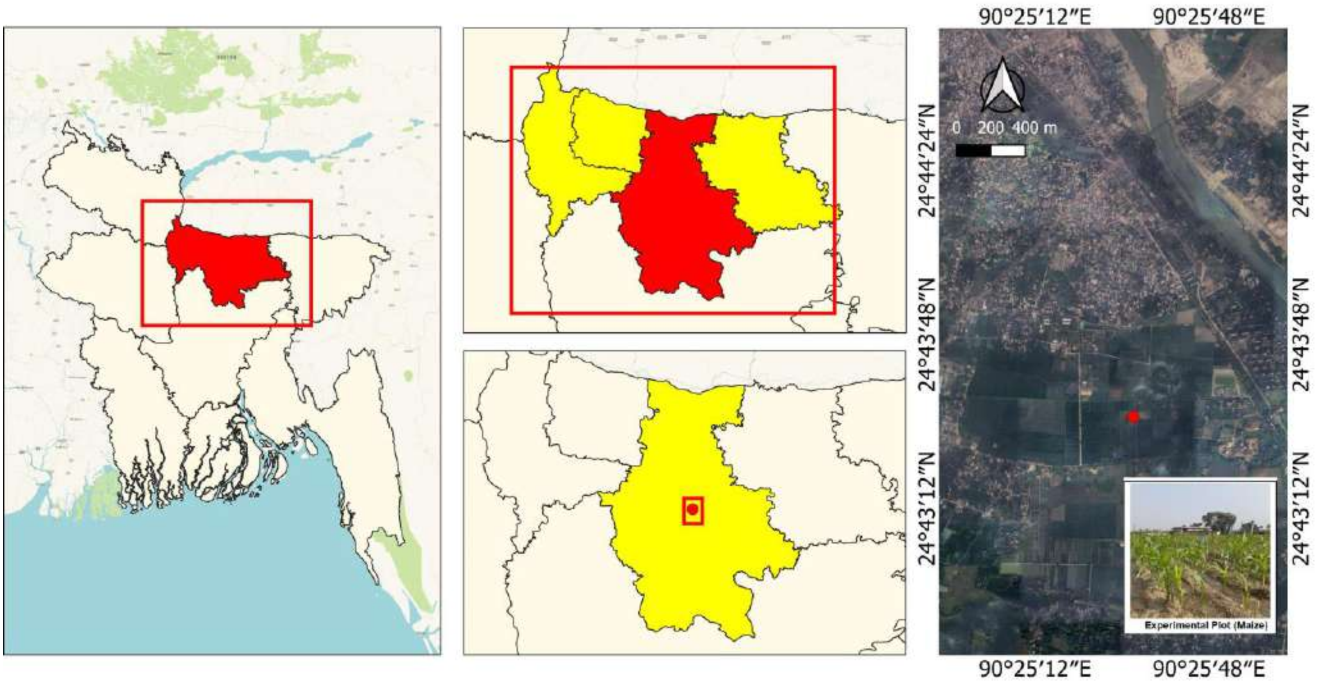

Figure 2. Geographical location of the experimental site at the Field Irrigation Laboratory of Bangladesh Agricultural University, Mymensingh, on the map of Bangladesh

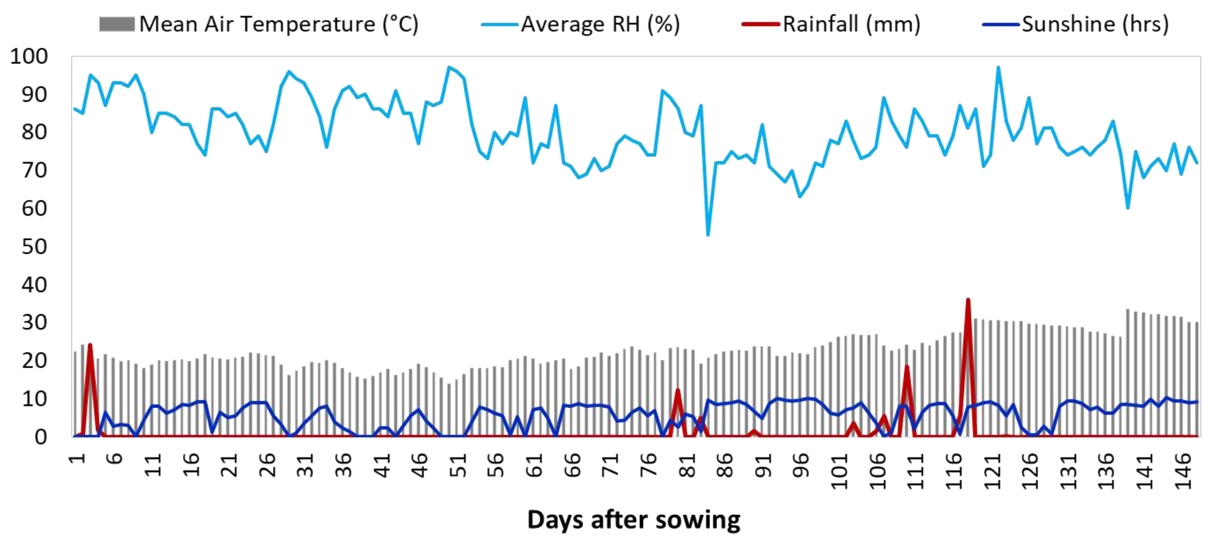

Figure 3. Weather data recorded at the experimental site during the days following maize sowing

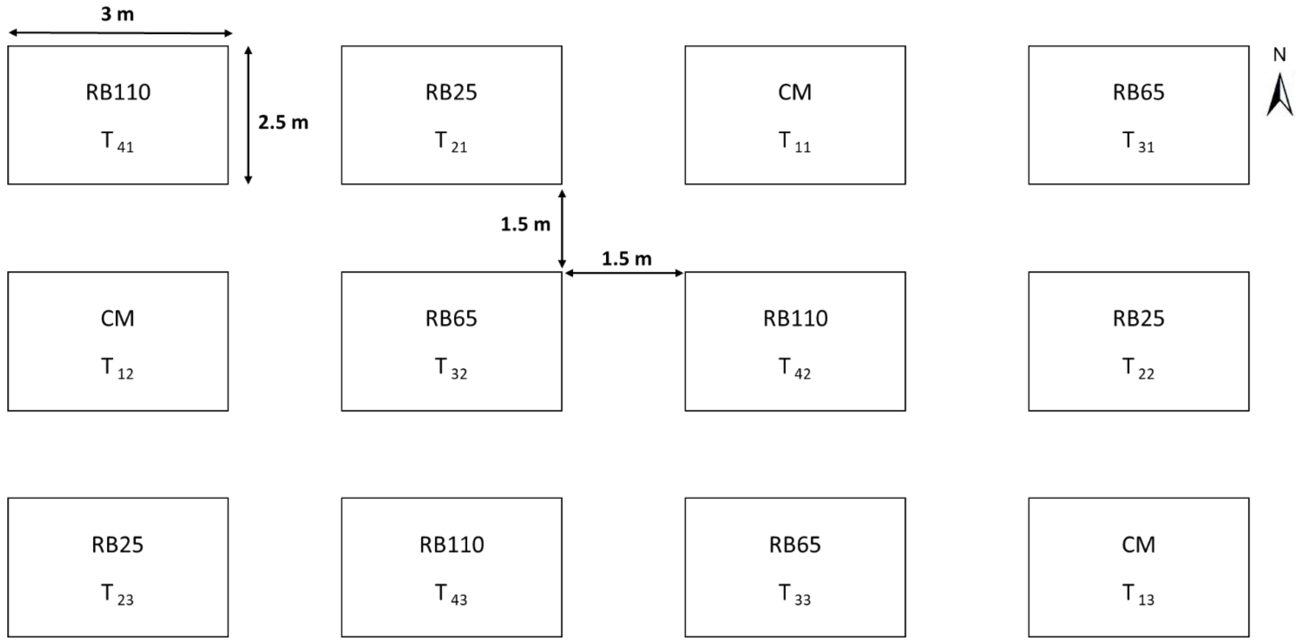

Figure 4. Layout of the field experiment, illustrating the different irrigation treatments: CM represents the Conventional Method, while RB110, RB65, and RB25 denote Raised Bed (RB)-based irrigation methods with bed widths of 110 cm, 65 cm, and 25 cm, respectively

Climatic variables, including temperature, sunshine hours, wind speed, relative humidity, rainfall, and evaporation, were gathered from the Bangladesh Agricultural University (BAU) weather station, operated by the Bangladesh Meteorological Department (Table 1). Additionally, a plot was created to visualize meteorological data, such as sunshine, rainfall, relative humidity, and mean air temperature, illustrating the weather conditions during the maize growing season (Figure 3).

Experimental setup and irrigation treatments

A randomized complete block design was employed for the experimental layout, comprising 12 plots, each measuring 3 meters by 2.5 meters. Four different irrigation treatments were tested, with each treatment replicated three times. The treatments included a Conventional Method (CM) as a control and three irrigation strategies tailored to different bed widths (25 cm, 65 cm, and 110 cm). In all treatments, plant spacing within each plot was standardized to approximately 20 cm between plants to ensure uniformity. Each plot consisted of six rows, with 13 plants per row, spread across a 3-meter-wide area with 25-cm furrows. The row-to-row distance was consistently maintained at 50 cm across all treatments. For the RB-based irrigation conservation method with a 25-cm bed size (RB25), planting was conducted over six beds, with one row per bed. For the 65-cm bed size (RB65), planting was arranged over three beds, with two rows per bed, while for the 110-cm bed size (RB110), planting took place over two beds, with three rows per bed. Figure 4 illustrates the field configuration used for this experiment. The specific treatments with various irrigation techniques and bed sizes are as follows:

T_{11}, T_{12} and T_{13} = Conventional Method (CM), i.e., flooding irrigation

T_{21}, T_{22} and T_{23} = RB-based conserving irrigation with 25 cm bed size (RB25)

T_{31}, T_{32} and T_{33} = RB-based conserving irrigation with 65 cm bed size (RB65) and

T_{41}, T_{42} and T_{43} = RB-based conserving irrigation with 110 cm bed size (RB110)

Field works and data recording

The field was meticulously prepared before the experiment, involving plowing, harrowing, and planking to ensure optimal soil conditions. It was cleared of weeds and crop residues. Fertilizer application followed standard guidelines (AIS 2019) for hybrid maize production in Bangladesh, using 550 kg/ha of urea, 250 kg/ha of triple super phosphate, 200 kg/ha of muriate of potash, and 250 kg/ha of gypsum. Maize seeds (*Taj* variety) were sown at a rate of 25 kg/ha. Uniform intercultural practices were applied to all plots, except for irrigation treatments. Irrigation management was based on soil moisture content and key growth stages. Moisture levels were monitored using a soil moisture profiler (Delta-T Device Co., UK), and irrigation water was applied into furrows in RB plots until water level reached the bed level. Rainfall data were collected from the nearby BAU weather station. Weed control was conducted twice, and the crop was harvested at full maturity.

Determination of leaf area and harvest indices of maize

Plant growth dynamics are widely expressed by Leaf Area Index (LAI), which measures the one-sided leaf area per unit ground area of a crop field. During the growing season, leaf area measurements were taken three times: the first measurement was taken 34 Days After Sowing (DAS), followed by subsequent measurements at 40 and 69 DAS, respectively. A LI-3100 Leaf Area Meter was used to measure leaf area of collected samples. The LAI was computed using the following equation (Islam et al. 2022; Munmun et al. 2024).

$$\mathrm{LAI} = \frac{\text{Leaf area of the sample (m}^2)}{\text{Area of land covered by the sample (m}^2)}$$

Crop maturity was determined by observing the browning of the plant's husks and leaves. To establish initial plant density, the number of plants within a square meter of each plot was counted. Plant height was measured from the ground to the highest spike, and the number of ears per plant was recorded. At maturity, a 1 m x 1 m area in the central section of each plot was designated for harvesting, with the rest of the plot also harvested separately. The yield from the marked area was collected, labeled, and sun-dried to gather grain. Yield was estimated at ~12% of grain moisture content. For calculating harvest indices, the perimeter and length of each ear were measured, and the number of rows and grains per ear was counted. Ears were picked, dried, and shelled plot by plot. A sample of one thousand clean, dried grains was weighed to calculate yield, which was extrapolated to estimate the total plot yield in kilograms per hectare (kg/ha). Straw yield was determined by drying and weighing straw from the 1 m² area, with results also converted to kg/ha. The biological yield, recorded for each plot in kg/ha, was the sum of the grain and straw yields. The harvest index (HI) was calculated using the following formula (Hossain et al. 2019; Islam et al. 2022; Munmun et al. 2024):

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Estimation of water productivity and water footprints

The total crop-water use during the growing season was calculated by summing the irrigation water applied and the rainfall received. The seasonal Effective Rainfall (ER) was calculated according to the methodologies detailed by Islam et al. (2015). Crop-water use was mathematically represented as described by Michael (1978):

WU = IR + ER
Where:
WU: Seasonal crop-water use (cm)
IR: Total irrigation water applied (cm)
ER: Seasonal effective rainfall (cm)

Crop-water productivity, also known as Field Water Use Efficiency (FWUE), is calculated using the ratio of crop yield to the total amount of water utilized during the crop's growing cycle (Hossain et al. 2019; Islam et al. 2022; Munmun et al. 2024).

$$\mathrm{FWUE} = \frac{\mathrm{Y}}{\mathrm{WU}}$$

Where:

FWUE: Field-water use efficiency or crop-water-productivity (kg/ha/cm)
WU: Seasonal crop-water use in the crop field (cm)
Y: Grain yield (kg/ha)

The concept of WF, as proposed by Hoekstra and Hung (2003), is an indicator of water appropriation, indicating the volume of water used by a crop (Rodriguez et al. 2015). When discussing WF for crop production, the terms GWF and BWF refer to the consumption of precipitation and irrigation water, respectively (Islam et al. 2024). These metrics enable the assessment of their respective contributions to the Total Water Footprint (TWF), which is the sum of GWF and BWF, expressed in liters per kilogram of maize.

$$\mathrm{GWF} = \frac{\mathrm{GWU}}{\mathrm{Yield}}$$

$$\mathrm{BWF} = \frac{\mathrm{BWU}}{\mathrm{Yield}}$$

$$\mathrm{TWF} = \mathrm{GWF} + \mathrm{BWF}$$

Measurement of soil moisture content

Soil moisture content readings were obtained from the selected experimental plots three times a day. Due to restricted access to some plots, measurements were taken from 4 of the 12 plots, with one plot representing each technique under investigation. A HH2 moisture meter (Delta-T Device Co., UK) was used to record soil moisture content at predetermined depths of 10 cm, 20 cm, 30 cm, 40 cm, and 80 cm.

Statistical analyses

Using the methodology of Gomez and Gomez (1984), an Analysis of Variance (ANOVA) was conducted to evaluate the effects of various treatments on the growth, yield-contributing variables, and FWUE of irrigated maize. The standard error method was employed to assess the significance of differences between treatment means.

RESULTS AND DISCUSSION

Growth and yield-contributing attributes of irrigated maize

The study analyzed various growth and yield-contributing attributes of irrigated maize under different RB dimensions and the CM. Key metrics such as plant height, ear length, ear perimeter, productive kernel number per ear, kernel weight per ear, and 1000-kernel weight were measured (Table 2). The CM treatment resulted in the tallest plants with an average height of 224.167±38.81 cm, while the shortest plants were observed in the RB110 treatment, averaging 212.81±31.80 cm. Ear length and ear perimeter are critical indicators of maize yield potential. The RB65 treatment outperformed others, achieving the highest ear length (17.385±0.64 cm) and ear perimeter (12.11±0.38 cm). Significant differences in ear length were noted between the RB65 and CM treatments, with the former showing superior results. However, no significant differences in ear perimeter were detected among the treatments. Regarding productive kernel number per ear and kernel weight per ear, the RB65 treatment again showed superior performance with 278.98±31.74 kernels per ear and a kernel weight of 91.22±7.85 g. Additionally, the highest 1000-kernel weight was observed under the RB65 treatment (319.33±13.6 g), followed by the RB110 treatment (305.67±2.08 g). Moreover, the analysis of LAI for irrigated maize, as depicted in Figure 5, reveals significant variability across different RB treatments throughout the growing season. The RB110 treatment consistently demonstrated the highest LAI, reaching its peak at 69 DAS. Conversely, the RB25 treatment consistently produced the lowest LAI values across all growth stages. The LAI values for RB65 and RB110 treatments were relatively close, with RB65 only slightly lower.

Table 2. Growth and yield-contributing characteristics of irrigated maize

Treatment	Plant height (cm)	Ear length (cm)	Ear perimeter (cm)	Productive kernel number per ear	Kernel weight per ear (g)	1000 kernel weight (g)
CM	224.167±38.81	16.92±0.75[ab]	12.06±0.52	263.80±46.86	80.66±7.33	312.67±11.02
RB25	216.53±23.86	16.65 ±1.29[ab]	11.82 ±0.31	262.38±29.23	79.5±10.8	302±23.58
RB65	219.08±29.96	17.385±0.64[a]	12.11±0.38	278.98±31.74	91.22±7.85	319.33±13.6
RB110	212.81±31.80	15.6±0.46[b]	11.81±0.28	240.07±25.18	72.04±17.8	305.67±2.08
Significance	NS	*	NS	NS	NS	NS
CV (%)	14.44	24.2	16.47	39.17	15	5.16
$LSD_{0.05}$	0.48	0.22	0.85	0.47	0.32	0.30

Note: Different letters, a and b, in results indicate a significant difference at a level of 0.05; asterisk symbol '*' indicates that the difference among the data was statistically significant; CV, Coefficient of Variation; LSD, Least Significant Difference; NS, Non-Significant; CM, Conventional Method, and RB110, RB65, and RB25 denote Raised Bed (RB)-based irrigation methods with bed widths of 110 cm, 65 cm, and 25 cm, respectively

Table 3. Effect of various irrigation practices on grain yield, harvest index, and water footprints of cultivated maize

Treatment	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)	Harvest index (%)	GWF (l/kg)	BWF (l/kg)	TWF (l/kg)
CM	9.89±1.11	21.01±2.16a	30.9±3.27	32.01±0.01b	117.24 ±13.35	92.45±10.52a	209.7±23.87a
RB25	11.96±1.7	17.545±0.47ab	28.51±2.15	38.31±0.03ab	105.807±15.83	82.7±12.37a	188.51±28.20ab
RB65	13.1±1.4	18.54±2.24ab	31.63±2.4	41.46±0.05a	88.6±10.07	12.63±1.43b	101.22±11.50c
RB110	10.35±3.77	16.06±2.17b	26.4±5.9	38.30±0.06ab	112.119±50.61	12.758±5.75b	124.87±56.37bc
Significance	NS	*	NS	*	NS	*	*
CV (%)	20.84	13.62	13.1	13.16	25.01	78.58	33.4
LSD 0.05	0.37	0.06	0.36	0.12	0.47	<0.001	0.016

Note: Different letters, a, b and c, in results indicate a significant difference at a level of 0.05; asterisk symbol '*' indicates that the difference among the data was statistically significant. CV: Coefficient of Variation, LSD: Least Significant Difference, NS: Non-Significant, GWF: Green Water Footprint, BWF: Blue Water Footprint, TWF: Total Water Footprint, CM: Conventional Method, and RB110, RB65, and RB25 denote Raised Bed (RB)-based irrigation methods with bed widths of 110 cm, 65 cm, and 25 cm, respectively

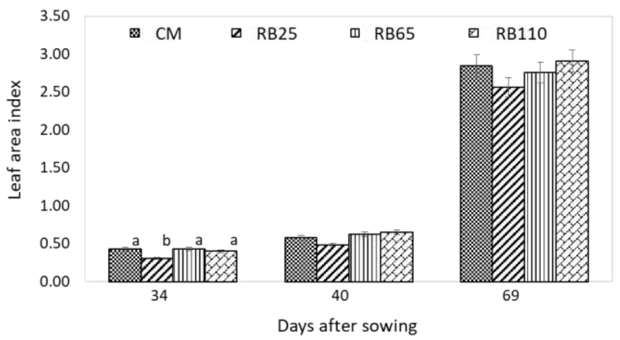

Figure 5. Comparison of leaf area index values for irrigated maize across different days after sowing using various irrigation techniques; error bars represent the standard deviation of the mean, and different letters (a and b) indicate a significant difference at the 0.05 significance level

Yield and Harvest Index (HI)

The study examined the impact of different irrigation methods on maize grain yield and HI, with detailed findings presented in Table 3. The RB65 method demonstrated the highest effectiveness, achieving a grain yield of 13.1±1.4 t/ha, significantly surpassing the yield of 9.89±1.11 t/ha observed with the CM. Additionally, the RB65 treatment resulted in the highest HI of 41.46±0.05%, indicating a more efficient conversion of biomass into grain yield compared to the significantly lower HI of 32.01±0.01% recorded for the CM method. The RB65 treatment also achieved the highest total biological yield at 31.63±2.4 t/ha, further underscoring its effectiveness. In contrast, the RB110 treatment, which utilized a 110 cm bed width, recorded the lowest straw yield (16.06±2.17 t/ha) and biological yield (26.4±5.9 t/ha). The CM treatment, however, showed an increased straw yield of 21.01±2.16 t/ha.

Water footprints of cultivated maize

The WF analysis presented in Table 3 reveals significant variations across different irrigation treatments for maize cultivation, offering valuable insights into WUE. The CM exhibited the highest TWF of 209.7±23.87 l/kg, indicating the least efficient water use. In contrast, the RB treatments showed markedly improved WUE, with RB65 demonstrating the lowest TWF of 101.22±11.50 l/kg, representing a reduction of over 50% compared to CM. The BWF results are particularly noteworthy, with CM showing the highest value of 92.45±10.52 l/kg, while RB65 and RB110 demonstrated significantly lower BWFs of 12.63±1.43 l/kg and 12.758±5.75 l/kg, respectively. Regarding the GWF, the study observed values ranging from 88.6±10.07 l/kg for RB65 to 117.24±13.35 l/kg for CM. The RB25 treatment recorded a GWF of 105.807±15.83 l/kg, while RB110 showed a GWF of 112.119±50.61 l/kg.

Water productivity with irrigation water-saving

The data presented in Figure 6 reveals significant variations in water productivity and irrigation water savings across different bed widths, emphasizing the advantages of RB systems. Notably, the RB65 technique demonstrated the highest water productivity, attributable to its optimal 65 cm bed width. This configuration improved water distribution while reducing losses through deep percolation and runoff. Regarding irrigation water savings, the RB110 system emerged as the most efficient, achieving a 37.72% reduction in water usage compared to the CM. Similarly, the RB65 system saved 36.12% of irrigation water. Conversely, the RB25 system achieved only a 0.386% water savings.

Relationships between maize productivity parameters under conventional and RB65 treatments

The RB65 technique has so far shown superior performance in maize productivity parameters compared to other RB dimensions. To explore this further, a correlation analysis was conducted (Table 4) to gain insights into the relationships between maize growth parameters under both CM and RB65 treatments. This analysis aimed to identify the key growth parameters affecting maize productivity in these treatments. In the CM treatment, plant height, ear length, and the number of productive kernels per ear showed strong positive correlations with grain yield and kernel weight per ear, highlighting their importance in determining maize productivity. Notably, plant height had a highly significant correlation with both grain yield (r=0.999, p<0.01) and kernel weight per ear (r=0.999, p<0.05), indicating that taller plants tend to yield more grain and heavier kernels. Additionally, the number of productive kernels per ear strongly correlated with grain yield (r=0.999, p<0.05) and kernel weight per ear (r=0.997, p<0.05), emphasizing kernel productivity as a crucial factor in maize yield under conventional irrigation. Under the RB65 treatment, correlation dynamics shifted due to the optimized water distribution and soil moisture. Although plant height still positively correlated with grain yield and kernel weight per ear, the strength of these correlations decreased (r=0.679 for grain yield and r=0.923 for kernel weight). Interestingly, ear length and the number of productive kernels per ear became more influential in this treatment. Ear length showed a very high correlation with grain yield (r=0.997) and straw yield (r=0.991). Moreover, the number of productive kernels per ear had even stronger correlations with grain yield (r=0.999) and straw yield (r=0.999). These results suggest that, under RB65, ear length and kernel number are more significant in driving overall productivity than plant height.

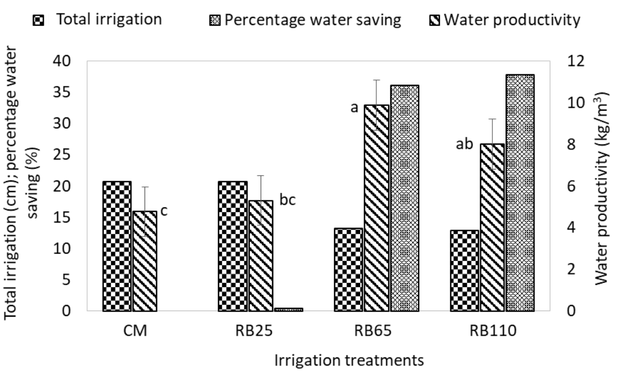

Figure 6. Water productivity and percentage of water savings for different irrigation techniques in maize cultivation; error bars represent the standard deviation of the mean, and different letters (a, b, and c) indicate significant differences at a 0.05 significance level

Table 4. Pearson correlation matrix (r) of maize parameters measured under (a) CM and (b) RB65 treatments

(a) CM treatment									
	PH	**EL**	**EP**	**PKNE**	**KWE**	**1000 KW**	**GY**	**SY**	**HI**
PH	1.000								
EL	0.940	1.000							
EP	0.731	0.455	1.000						
PKNE	0.999*	0.924	0.762	1.000					
KWE	0.999*	0.949	0.713	0.997*	1.000				
1000 KW	0.950	0.999*	0.481	0.935	0.958	1.000			
GY	0.999**	0.936	0.738	0.999*	0.999*	0.946	1.000		
SY	0.999*	0.956	0.696	0.995	0.999*	0.965	0.998*	1.000	
HI	0.858	0.632	0.978	0.881	0.844	0.655	0.864	0.831	1.000

Table 4. Continue

(b) RB65 treatment									
	PH	**EL**	**EP**	**PKNE**	**KWE**	**1000 KW**	**GY**	**SY**	**HI**
PH	1.000								
EL	0.617	1.000							
EP	0.922	0.874	1.000						
PKNE	0.697	0.994	0.921	1.000					
KWE	0.923	0.872	0.999**	0.919	1.000				
1000 KW	0.720	0.990	0.933	0.999*	0.931	1.000			
GY	0.679	0.997	0.910	0.999*	0.909	0.998*	1.000		
SY	0.719	0.991	0.932	0.999*	0.931	0.999**	0.998*	1.000	
HI	0.462	0.983	0.770	0.958	0.768	0.948	0.965	0.949	1.000

Note: *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), PH: Plant Height, EL: Ear Length, EP: Ear Perimeter, PKNE: Productive Kernel Number per Ear, KWE: Kernel Weight per Ear, 1000 KW: 1000 Kernel Weight, GY: Grain Yield, SY: Straw Yield, and HI: Harvest Index

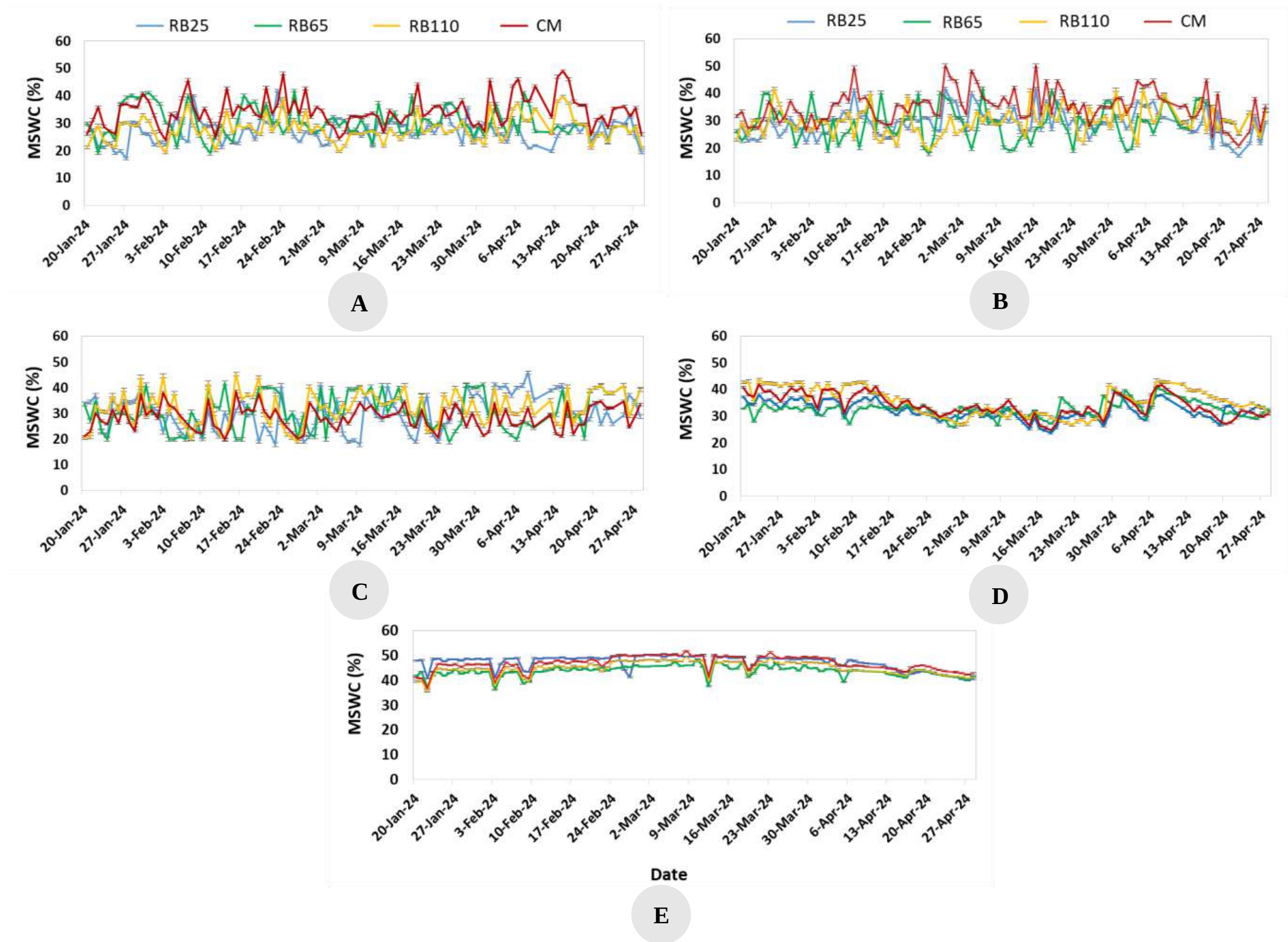

Figure 7. Comparison of mean soil water content (MSWC) across various irrigation treatments at different soil profile depths of A. 10 cm, B. 20 cm, C. 30 cm, D. 40 cm, and E. 80 cm during maize cultivation; error bars indicate the standard deviation of the mean

Effects of treatments on soil moisture content in irrigated maize field

The analysis of soil moisture dynamics across different RB treatments reveals significant variations in moisture retention at various soil depths (Figure 7), which are crucial for supporting healthy maize growth. At a depth of 10 cm (Figure 7.A), the RB65 treatment-maintained soil moisture levels that were sufficiently close to those of the CM treatment. Among these, the RB110 treatment retained the most moisture, followed by RB65 and RB25. At a 20 cm depth (Figure 7.B), the trend of higher soil moisture content in RB treatments persisted, with RB110 again showing the highest levels. As the depth increased to 30 cm (Figure 7.C), RB65 and RB110 demonstrated comparable soil moisture content, both significantly higher than RB25 and CM. Moreover, at 40 cm depth (Figure 7.D), RB110 continued to outperform other treatments in moisture retention, followed by RB65 and RB25. Finally, at 80 cm depth (Figure 7.E), RB110 maintained its advantage, retaining the highest soil moisture content among all treatments. The variation in soil moisture content across treatments can be partly attributed to the high rainfall observed during the initial and later stages of the crop growth period, coupled with uneven distribution throughout the crop cycle.

Discussion

Growth and yield-contributing attributes

The findings presented in Table 2 indicate that the CM treatment resulted in the tallest plants, suggesting that traditional methods may still have advantages in terms of overall plant height. However, the RB65 treatment's superior performance in ear length and perimeter highlights the potential benefits of RB systems in improving yield-contributing attributes, likely due to enhanced water management and nutrient availability. This aligns with findings by He et al. (2015), who noted that RB systems tend to improve ear length and perimeter. The minimal variation in plant height across treatments suggests that irrigation methods may have a limited effect on this particular growth parameter, as also observed by Brar (2013). Moreover, the RB65 treatment's superior kernel number per ear and kernel weight per ear are consistent with the results reported by Tanveer et al. (2014), who also found that RB planting systems enhance kernel numbers. The absence of significant differences in kernel weight across treatments, as noted by He et al. (2015), suggests a complex interaction between irrigation methods and yield attributes. The high 1000-kernel weight observed under the RB65 treatment corroborates the findings of Mehta et al. (2011), who reported similar improvements in 1000-kernel weight under RB planting.

The LAI analysis (Figure 5) reveals that the RB110 treatment's optimal leaf area expansion is likely due to its ability to retain and evenly distribute water, especially during critical growth phases. This finding is consistent with previous studies by Pan et al. (2019) and Duan et al. (2021). The consistently low LAI values in the RB25 treatment may be attributed to limitations in water and nutrient distribution, a notion supported by Kashif et al. (2018). The similarity in LAI between RB65 and RB110 treatments suggests that beyond a certain bed width, the benefits in LAI may plateau, as noted by Yigezu et al. (2021). Moreover, the observed increase in LAI during the final development stage across all treatments may reflect maize's physiological response to increased water demand during flowering and grain filling. This phenomenon is particularly evident in the RB110 treatment, where the bed's capacity to retain and distribute water contributes to a late-stage increase in LAI. These findings underscore the importance of bed width in influencing not only LAI but also other growth parameters, thereby contributing to higher yield potential under optimal irrigation management, as highlighted by Islam et al. (2022) and Munmun et al. (2024).

Yield and Harvest Index (HI)

The results clearly demonstrate that the RB65 method is significantly more effective in improving maize grain yield and HI compared to the CM. The RB65 treatment achieved a grain yield of 13.1±1.4 t/ha, surpassing the yield observed under CM. This outcome aligns with studies by Kaur and Kumar (2018) and Kaur and Kaur (2022), which also reported increased productivity using optimized RB methods. Additionally, the higher HI of 41.46±0.05% in RB65, compared to 32.01±0.01% in CM, indicates more efficient biomass conversion into grain yield. This finding is consistent with Mehta et al. (2011) and Tanveer et al. (2014), who noted substantial improvements in grain yield and HI with optimized bed dimensions. The RB65 treatment also demonstrated the highest total biological yield (31.63±2.4 t/ha), reinforcing its superiority in maximizing maize productivity.

In contrast, the RB110 treatment's lower straw and biological yields suggest that excessively wide beds may not be as effective for maize cultivation. Wider beds, while efficient at reducing water losses through runoff and evaporation, may disrupt the balance between plant population density and resource availability, such as water and nutrients. The RB110 treatment had fewer plants per unit area compared to RB65, leading to reduced biomass and grain yield. This supports the findings of Yigezu et al. (2021), who observed decreased crop productivity with overly wide beds due to inadequate plant populations and uneven water distribution. Furthermore, the increased straw yield in the CM treatment (21.01±2.16 t/ha) may be attributed to greater plant height under frequent irrigation, but the trade-off between grain and straw yield highlights the inefficiency of conventional methods. Overall, the RB65 treatment emerges as a more sustainable and productive alternative to CM, optimizing both water use and crop productivity (Pan et al. 2019; Duan et al. 2021).

Water footprints

The results presented in Table 3 clearly indicate that the RB65 treatment offers the most efficient water use among the irrigation methods studied. The substantial decrease in TWF for RB65, which reduced water use by over 50% compared to the CM method, aligns with previous studies that reported significant water savings through RB systems (Mekonnen et al. 2020; Chandra et al. 2023). This efficiency in water use is critical, particularly in regions facing water scarcity. The dramatic reduction in BWF for the RB treatments, especially the RB65 configuration, highlights the method's ability to optimize irrigation water use. This finding is consistent with research by Akbar et al. (2016) and Ismail et al. (2021), who reported similar reductions in water use with RB systems. The lower BWF observed in RB treatments suggests a more efficient use of irrigation water, crucial for sustainable agricultural practices. The variation in GWF across treatments, despite the assumption of constant green water use, underscores the importance of yield differences in water footprint calculations. This finding highlights the need to consider both water use and crop productivity when assessing the overall efficiency of irrigation systems, as emphasized by

Li et al. (2020) and Feng et al. (2021). The RB65 treatment's superior performance in both BWF and GWF suggests it offers an optimal balance between water conservation and yield enhancement, aligning with the findings of He et al. (2015), Yadav et al. (2018), and Munmun et al. (2024).

However, it is important to interpret these results within the study's limitations. The assumption of constant green water use across treatments may oversimplify the complex soil-plant-atmosphere interactions inherent in different irrigation regimes. Future studies should consider employing lysimeter experiments to provide more accurate measurements of crop evapotranspiration, runoff, seepage, and percolation losses, as suggested by Rodriguez et al. (2015), Mekonnen et al. (2020), Bhatt et al. (2021). The variability in WF results across treatments emphasizes the need for site-specific optimization of irrigation strategies. As noted by Chapagain and Hoekstra (2011) and Elbeltagi et al. (2020), factors such as local climate, soil characteristics, and crop varieties can significantly influence water footprints. While the RB65 treatment showed promising results in this study, its performance may vary in different agro-ecological contexts.

Water productivity with irrigation water-saving

The results clearly highlight the superior water productivity of the RB65 technique, which is likely due to its optimal bed width of 65 cm (Figure 6). This configuration enhances water distribution and reduces losses through deep percolation and runoff, aligning with findings by Akbar et al. (2016), who observed similar improvements in soil properties and water efficiency under comparable conditions. The inefficiency of traditional flood irrigation methods, as demonstrated by the CM's lowest water productivity, underscores the need for adopting more efficient irrigation practices, as highlighted by Islam et al. (2022). Moreover, the RB110 system's efficiency in achieving a 37.72% reduction in water usage further underscores the critical role of bed width in water conservation (Figure 6). The broader beds in the RB110 treatment required less irrigation due to fewer furrows, a finding that aligns with Yigezu et al. (2021), who emphasized the effectiveness of wider beds in reducing water consumption by minimizing uncropped areas. Similarly, the RB65 system's 36.12% water savings demonstrate its balance between water use efficiency and crop yield. On the other hand, the RB25 system's minimal water savings of 0.386% highlight the limitations of narrower beds, which require more frequent irrigation due to faster drying, as supported by Pan et al. (2019).

These findings underscore the effectiveness of wider RBs, particularly those with bed widths between 65-110 cm, in optimizing water productivity and conserving irrigation water. This approach represents a valuable strategy for sustainable agriculture in water-scarce regions. The results are consistent with studies by Ismail et al. (2021) and Shah et al. (2024), who reported similar benefits of RB techniques in enhancing water productivity and crop yields. Furthermore, Kaur (2011) and Asif et al. (2022) observed improved grain yield and water productivity in maize under RB systems, attributing these outcomes to enhanced root development, nutrient uptake, and water availability. In conclusion, this study contributes to the growing body of evidence that RB systems, particularly those with wider bed dimensions, are highly effective in improving water productivity and conserving irrigation water.

Relationships between maize productivity parameters

The correlation patterns in Table 4 indicate that, in the CM treatment, plant height directly influences grain yield and kernel weight. This aligns with Tanveer et al. (2014) and Kaur and Kumar (2018), who identified plant height as a key determinant of maize productivity under traditional irrigation. The strong positive correlation between plant height and grain yield in this study supports this relationship, suggesting that taller plants are generally more productive in the CM setup. In contrast, the RB65 treatment shows a different pattern where ear length and the number of kernels per ear have a greater impact on yield than plant height. The weaker correlation between plant height, grain yield, and kernel weight in RB65 indicates a shift in productivity dynamics. Instead, the correlation coefficients for ear length and kernel number per ear highlight their enhanced role in yield under the RB65 system. This observation aligns with Yigezu et al. (2021), who found that bed dimensions, particularly in RB systems, positively affect yield-contributing traits like ear length and kernel number. The results underscore RB65’s optimized water distribution, which favors traits that directly boost yield. Overall, the analysis shows the efficacy of RB65 in altering maize productivity: while plant height is crucial in conventional methods, RB65 prioritizes ear length and kernel productivity for higher yield. This supports Mehta et al. (2011), emphasizing that bed dimensions significantly influence crop productivity. The shift in productivity dynamics suggests that RB65 can optimize water and nutrient distribution, offering a promising strategy for maize cultivation, especially in water-scarce regions. Further research on different RB dimensions could refine sustainable agricultural practices across various conditions. The evidence here suggests that adopting the RB65 method can enhance maize yield and water use efficiency, providing a significant advantage over traditional irrigation methods.

Soil moisture dynamics

The results in Figure 7 highlight the superior performance of RB65 in retaining soil moisture at different depths, likely due to its optimized bed width, which promotes water infiltration and minimizes evaporation. Wider beds, such as RB65, enhance the surface area for water percolation, leading to greater moisture retention within the soil profile. This aligns with the findings of Verhulst et al. (2011), who observed that optimized raised beds reduce surface runoff and improve infiltration, thus increasing moisture retention. Research has shown that RB systems, especially those with intermediate to wider bed widths like RB65 and RB110, are effective in maintaining higher soil moisture levels at deeper profiles. Yadav et al.

(2018) and AbdelRahman and Arafat (2020) similarly reported that wider beds reduce runoff and enhance water infiltration, improving soil moisture dynamics. Kaur et al. (2024) also found that wider bed configurations tend to retain more moisture compared to narrower ones, particularly at deeper soil levels. The increased moisture retention observed in deeper layers, especially at 40 cm and 80 cm, highlights the importance of improved infiltration and reduced evaporation, a characteristic that wider beds like RB110 offer. Studies by He et al. (2015) and Munmun et al. (2024) further support this, demonstrating that wider beds are more effective in retaining soil moisture, which is crucial for sustaining crops during periods of water scarcity. The RB110 system's ability to retain moisture in deeper soil layers is especially important for supporting maize root systems during drought conditions, as also noted by Duan et al. (2021), who emphasized the role of wider beds in enhancing crop resilience under water-limited conditions.

However, the additional moisture retained at deeper layers in RB110 may not always be advantageous. Maize typically has a rooting depth of around 60 cm, and thus the excess water retained at depths beyond this may not be fully utilized by the plant, especially during critical growth stages such as flowering and grain filling. As a result, while RB110 excels in moisture retention, this does not necessarily translate into better water use efficiency or higher crop yields. Verhulst et al. (2011) and He et al. (2015) emphasized that moisture distribution within the root zone is more critical for crop productivity than the total amount of water retained in the soil.

In contrast, the RB65 configuration, with its narrower bed width, offers a more balanced distribution of moisture, keeping water accessible within the root zone. This supports better crop growth, particularly during the grain-filling stages. The RB65 system's ability to retain soil moisture at optimal levels across various depths ensures better water distribution and supports higher grain yields and water use efficiency. Moreover, the reduced efficiency observed in the narrower RB25 and excessively wide RB110 treatments suggests that extreme variations in bed width do not provide the balance necessary for effective irrigation management. Selecting the appropriate bed width, such as the RB65 configuration, emerges as a promising technique for achieving sustainable maize cultivation, particularly in regions facing water scarcity. The RB65 system not only optimizes water retention but also maximizes yield and WUE, presenting a valuable strategy for sustainable agriculture.

Overall, this study highlights the critical role of RB dimensions in optimizing maize yield, WF, and soil moisture content under furrow irrigation. Among the tested configurations, the RB65 setup emerged as the most effective, significantly boosting yield and water productivity while reducing irrigation water use by 37.72%. It also maintained optimal soil moisture at various depths, ensuring efficient water distribution and retention—key advantages for regions facing water scarcity. Although the RB110 treatment demonstrated superior water retention, it underperformed in yield due to lower plant density and inefficient water utilization. In contrast, RB65 achieved a balance between moisture retention and crop yield by maintaining higher plant density and improving water distribution within the maize root zone. These findings emphasize the limitations of overly wide RB configurations like RB110 and the importance of carefully selecting bed widths to optimize water efficiency and crop productivity. The RB65 technique presents a promising solution for enhancing agricultural efficiency and resilience in water-scarce areas. For future studies, more precise WF estimations should incorporate crop evapotranspiration measurements and lysimeter experiments, and long-term research on different RB dimensions across various crops and soil conditions will be essential for refining sustainable agricultural practices. This research provides valuable insights for policymakers and farmers aiming to implement water-saving irrigation techniques to support agricultural sustainability in water-limited environments**.**

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the Bangladesh Agricultural University Research System (BAURES) for the project titled "Effect of different raised bed sizes under furrow irrigation method on yield and water productivity of maize" (Project No. 2023/1939/BAU).

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 23-30 DOI: 10.13057/asianjagric/g090103

Vulnerability determinant of rice farmers to climate change in Ebonyi State, Nigeria

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Manuscript received: 26 July 2024. Revision accepted: 3 November 2024.

Abstract. *Emmanuel OE, Nnenna OM, Nkiruka B-CG, Henry AK, Ebuka OJ, Ogbuji EC, Thankgod EK, Chinenyenwa T-AA, Ugochukwu NE, Jerry UE, Chibueze NF. 2024. Vulnerability determinant of rice farmers to climate change in Ebonyi State, Nigeria. Asian J Agric 8: 23-30.* Climate change has become a reoccurring nightmare regarding agricultural production with negative impacts. The study researched the vulnerability determinants of rice farmers to climate change in Ebonyi State, Nigeria. Rice farmers numbering 80 were picked using the purposive sampling method. A structured questionnaire was used for data collection, and mean, frequency counts, percentage, and probit regression models were employed in data analysis. Results show that the rice farmers were in their productive age, male, married, experienced, and relatively educated. Late onset of rainfall (98.8%), increased temperature (97.5%), prolonged drought (95.0%), severe windstorm (95.0%), and high evaporation rate (92.5%) were the most perceived climate change occurrences in the state. The use of improved varieties of seeds (100%), effective use of pesticides (96.3%), efficient application of fertilizers (95.0%), and diversification of crops (90.0%) were the primary adaptation measures to climate change. Age, education, access to capital, farming experience, access to climate change information, gender, and extension contacts were major determinant factors in the climate change vulnerability of rice farmers. High cost of fertilizer (100%), lack of timely access to inputs (97.5%), inadequate capital (97.5%), poor extension service (96.3%), and inadequate lands (90%) constrained rice production in the state. Farmers should practice more climate-smart agriculture to overcome the adverse effects of climate change on rice production.

Keywords: Climate change, determinants, probit model, rice farmers, vulnerability

INTRODUCTION

In Nigeria and other nations worldwide, rice (*Oryza sativa* L.) is a crucial crop that contributes significantly to food security, employment development, and income generation (FAO 2023). It is the most cereal crop consumed globally across all continents (Africa, Asia, Europe, America, etc.) (FAO 2022). Sub-Saharan Africa, Bangladesh, Cambodia, Myanmar, Laos, and Vietnam consume more than 50% of their calories from rice. In contrast, Thailand, the Philippines, Malaysia, India, Nepal, and Sri Lanka consume 20-44% of calories from rice. Nigeria, Africa's leading rice producer, consumes over 20% of its calories from rice intake (FAO 2022). As a staple food, rice cultivation is significant in African countries, including Nigeria. Rice cultivation employs unemployed youths, is a source of household income, food intake, and energy, and contributes to about 15% of agriculture's gross domestic product in Nigeria (NBS 2023). It equally serves as a source of raw materials for manufacturing industries in Nigeria. Rice production is predicted to rise in Nigeria due to the recent government restrictions on cereal imports, including rice (Bichi et al. 2023). The rules were imposed in good faith to encourage and boost local rice production in the country to meet the growing demand for grain foods. However, the above objective of the Nigerian government is still far-fetched owing to several factors and climate change issues that are prevalent in the country with its attendant consequences (FAOSTAT 2024). Nigeria is particularly vulnerable to climate change due to its excessive reliance on rain-fed agriculture, which distorts crop yield and causes farmer poverty (Onyeneke 2021). Extreme weather events and climate change negatively impact rice production, causing poor yields and harvest. Delays in food production and delivery caused by a rise in the frequency and intensity of extreme weather events drive food prices (Ho et al. 2022). Interestingly, food insecurity is rising in Nigeria due to crop growers' vulnerability and susceptibility to climate change (FAO 2022). Nigerian rice farmers are currently dealing with several challenges, including vulnerability to climate change, which affects crop farming operations and agricultural productivity. The worrisome development has decreased rice productivity and yield, resulting in low income, starvation, hunger, and poverty (Van-Oort and Zwart 2018). Extreme weather events like droughts, floods, temperature swings, windstorms, rainfall, evaporation, relative humidity, etc., threaten rice cultivation and result in significant output losses. High temperature reduces rice plant yields while simultaneously promoting the growth of pests, weeds, and insects that prey on rice crops (Onyeneke et al. 2021). Rising temperature distorts the root and development of rice, causing poor yields. For instance, a temperature of 25 to 28°C is ideal for rice seedling growth; any higher temperature would result in losses for rice growers. Variations in precipitation trends raise the risk of short-term crop failure and long-term production drops (Arifah et al. 2022). High precipitation lowers rice plants' capacity for photosynthetic activity, leading to low yields.

Consequently, high rains cause flooding of rice fields, leading to rice seedling erosion and soil nutrient depletion. As the soil gets hotter, evapotranspiration decreases, resulting in low moisture levels that impede rice growth and development (Saud et al. 2022). A high evaporation rate produces rice of lesser quality and quantity, negatively impacting yield and market value. During a drought, the rice field becomes dry, which damages the planted seedlings and results in yield losses. Planting rice seedlings in an environment with high relative humidity hinders their germination and growth. Elevated relative humidity leads to a rise in water loss and yellowing of rice plants, which impacts photosynthetic functions (Tang et al. 2023). In recent times, rice farmers in Ebonyi State have suffered from adverse effects of climate change and have become more vulnerable to the changing climate with attendant evidence of poor yield and low economic returns, which have devastated the farmers and plunged them into abject penury (Osuji et al. 2023). Before now, the Ebonyi State was reputed as the rice hub of the nation and in Sub-Saharan Africa, but surprisingly, its status regarding rice production is fast declining due to rice farmers' vulnerability to climate change and extreme weather conditions (Onyeneke et al. 2021).

Previous studies (Ojo and Baiyegunhi 2020; Sharma et al. 2022; Habib-Ur-Rahman et al. 2022; Chen et al. 2023a; Joseph et al. 2023; Dar et al. 2024) have examined the impacts of climate change on cereal crops such as rice using both primary and secondary data and these studies focused mainly on direct and indirect impacts of climate change on rice crops. However, interestingly, it has yet to explore the vulnerability determinants of rice farmers to climate change in Nigeria, thereby making this study the first to consider and explore this aspect of study in Nigeria's context. The study considered the socio-economic factors and their relationship with farmers' vulnerability to climate issues. The study also contributed to the body of literature by adding new scientific knowledge to the existing literature, making the study of greater significance. The study set out to achieve the following objectives: the socio-economic characteristics of rice farmers, the perceived climate change occurrences encountered by rice farmers, the adaptation mechanisms of rice farmers to climate change, determinants of the vulnerability of rice farmers to climate change, and factors impeding rice production of farmers in the State.

MATERIALS AND METHODS

Study area

The research was done in Ebonyi State, Nigeria (Figure 1). The state has its location in South-East region with an estimated population of 3,242,500 persons (NPC 2023). The state practice agriculture on large scale and is reputed as the rice hub of the nation. The state is made up of 13 Local Government Areas (LGAs) (Abakaliki, Izzi, Ezza-North, Afikpo-South, Ohaukwu, Ebonyi, Oniocha, Ishielu, Ezza-South, Ikwo, Afikpo-North, Ohaozara and Ivo) with total land area of 5,533 km^2 and Latitude: 6°10' 40.7028" and Longitude: 7°57' 33.4296". The state experiences two seasonal vegetation; the rainy and dry seasons which influence agricultural activities in the state. Rainfall is around 1200 mm, while temperature ranges between 27°C to 28°C.

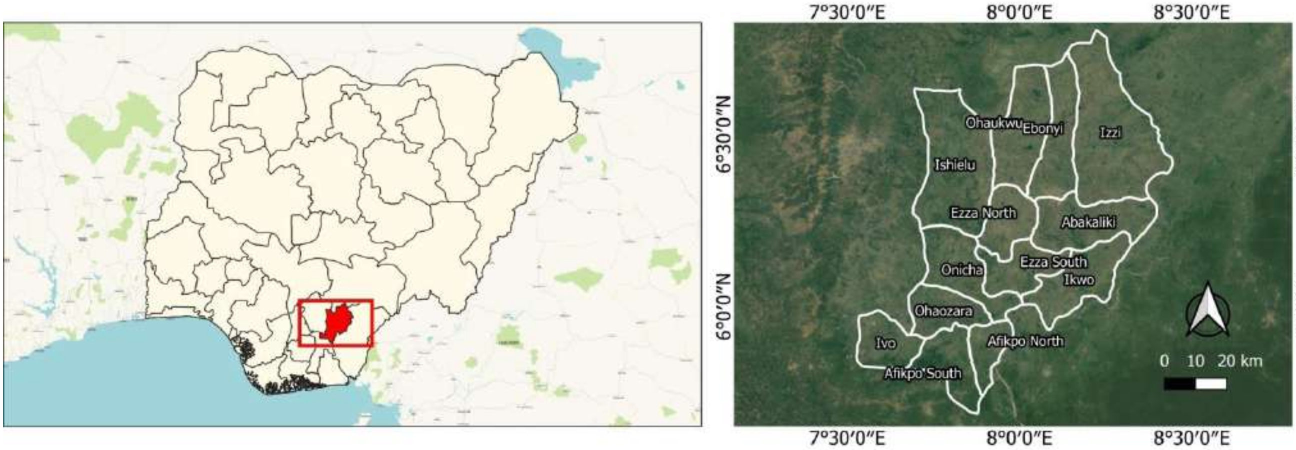

Figure 1. Map of Ebonyi State, Nigeria showing the study locations

Sampling techniques

A purposive sampling technique was used for this study. In the first instance, four Local Government Areas (LGAs) (Ikwo, Izzi, Ezza-North, and Ezza-South) were picked from the 13 LGAs in the state. These were selected based on their predominant rice cultivation, which distinguishes them from other LGAs (ESMANR 2023). The second stage involved another purposive selection of six communities out of the four LGAs, resulting in 24 communities. The six communities were noted for extensive rice cultivation in the state (ESMANR 2023). From the total registered rice farmers in the 24 communities, four independent rice farmers were selected to sum up 96 farmers. The four rice farmers were selected based on their extensive scale rice cultivation, primarily for commercial purposes.

Method of data collection

Primary data were collected from the 96 rice farmers using structured questionnaire designed for the study and out of the selected 96 rice farmers, only 80 rice farmers provided useful information for data analysis. Sixteen (16) questionnaires were dropped due to inconsistent and incomplete information required for the study.

Method of data analysis and model specification

Data were analyzed using descriptive statistics (mean, frequency count, and percentage) and the probit regression model. The probit regression model is a scientific analytical tool used to model dichotomous dependent variables with multiple input variables (Ismael and Duleba 2023). It has an advantage over other analytical tools in that it produces precise results with good statistical degrees that are not spurious or erroneous, hence its suitability for the study. The probit model was used to model the vulnerability determinants of rice farmers to climate change. Note that the vulnerability of the rice farmers to climate change was measured using the climate vulnerability criterion (Kim and Kwon 2022), which is expressed as climate sensitivity plus exposure minus adaptation. In this study, the farmers' susceptibility to climate risks with adaptation equals one and otherwise equals zero (0). Hence, the probit model accommodates dichotomous dependent variables (1 and 0) (Ismael and Duleba 2023).The probit model is explicitly expressed follows;

$$P_i = Pr(Y = 1/X) = Pr(I_i \leq I_i) = Pr(Z_i \leq B/X_i) = F(BX_i)$$

$$F(I_i) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{BXi} e - z^{2/2}\, dz$$

Where:

Pi = Pr = {Probability of ith farmers' climate sensitivity plus exposure minus adaptation= 1 and otherwise = 0}

F = Standard normal cumulative distributed function

B = Coefficients

X_i= represents the independent variables such as;

X_1 = Age (years)

X_2 = Education (No. of years spent in school)

X_3 = Household size (No. of persons)

X_4 = Farm size (Ha)

X_5 = Farming experience (No. of years)

X_6 = Participation in climate change workshops (No. of times participated)

X_7 = Access to climate change information {Accessed = 1, otherwise = 0}

X_8 = Extension contacts (No. of visits)

X_9 = Farm income (Naira)

X_{10} = Adaptation to climate change {Adapted = 1, otherwise = 0}

RESULTS AND DISCUSSION

Socio-economic characteristics of rice farmers

Table 1 presents the socio-economic characteristics of rice farmers. The majority of the farmers were within the age of 41-50 years with a mean age of 48 years. This implies that the rice farmers were young and in their most productive age which could enhance rice production in the area. Increase in age is associated with increase in farm production experiences which aid farm operations and other related activities (De-Vos et al. 2023). The majority of the rice farmers were male (86.3%), in comparison to the female (13.8%). This implies that rice production was dominated by the male farmers. This supports the fact that men are breadwinners and tend to be involved in crop production to fend for their families (Chen et al. 2023b). The majority of the farmers were married (51.3%), the singles (26.3%) and widowed (22.5%). This implies that the married farmers dominated rice production. The table shows that 32.5% of the rice farmers had primary education, 11.3% tertiary education, 15.0% had non-formal education. Thus the majority of the farmers 41.3% had secondary education. This implies that the rice farmers were relatively educated to understand rice cultivation and production principles (Arifah et al. 2023). The majority of the rice farmers, 63.8% had household sizes between 5-8 persons with a mean household size of 7 persons. This implies that the household size regarding the rice farmers was relatively large and could support their farming strength and production activities. The majority of the rice farmers, 73.7% were involved with farming and other non-farming activities while about 26.3% of them were fully engaged in farming activities only. This shows that the rice farmers combined rice cultivation with other production activities to raise farm income and livelihood sustenance (Kobayashi et al. 2023). The majority of the farmers, 47.5% had farm sizes within 1.1-2.0 hectares with a mean hectare of 1.8. This implies the prevalence of small farm holdings in rural communities. This is partly due to land tenure systems and land scarcity which limit large scale agricultural production. The table shows that about 20% of the rice farmers had extension contacts between 1-2 times, 10% had extension contacts between 3-4 times while the majority of the rice farmers, 70% had no extension contacts. The mean extension contact was approximately 2 contacts, which implies that the rice farmers had relatively low contacts with extension agents, which shows that the rice farmers did not benefit much from the extension agents (Chen et al. 2023a). The majority of the farmers, 87.5% do not belong to cooperative society while 12.5% belongs to cooperative societies. This implies that a lower percentage of the rice farmers benefited from cooperative societies. The mean participation in workshop/training was 1.3 times per cropping year. This means that the rice farmers had a low participation in workshop/training regarding rice cultivation and production principles. The majority of the rice farmers, 67.5% had farming experience ranging between 11-20 years. The mean farming experience of the rice farmers was 18 years, proving that the rice farmers had experience in rice production. Farming experience helps farmers to improve on their production capacity and overcome inherent farm problems and challenges besetting farmers (Lansing et al. 2023). Table 1 shows that the rice farmers obtained their capital from banks (10%), friends/relatives, (41.3%), while a higher percentage (48.7%) accessed their capital from personal savings. This shows that majority of the rice farmers started their rice cultivation from their own personal savings which is common in rural societies. The table shows that the land for rice farming was acquired through pledge (7.5%), purchase (13.8%), through gifts (18.8%), through lease/rent (23.8%) and majority (36.3%) via inheritance. This implies that the land used for rice farming was obtained via inheritance as this is common in rural societies (Yin et al. 2021). The majority of the farmers (37.5%) made use of family labour, while (31.2%) made use of hired labourers. This implies that the labour used in rice cultivation came majorly from family labour than hired labour. This could be due to the cheap source of family labour.

Perceived climate change occurrence encountered by rice farmers

Table 2 presents the perceived climate change occurrences encountered by rice farmers. The table shows that 95% of the rice farmers acceded to prolonged drought. This implies the long absence of water or delayed rainfall, negatively affecting crop production. Prolonged drought causes stunted growth in rice farming, affecting yield (Chen et al. 2023b). The result shows that 90% indicated increased rainfall intensity. This implies overbearing rainfall, which could lead to the erosion of newly planted rice seedlings and the washing away of soil nutrients and vegetative soil supports, causing total crop failure and poor performance (Sharma et al. 2022). About 97.5% of the rice farmers identified increased temperature; this implies that the rice farmers experienced increasing temperature, and this could lead to dryness of the soil moisture contents, which limits rice production and increases disease and pest infestations, which attacks rice plants, leading to poor yield and low harvest (Joseph et al. 2023). High temperatures oppose rice cultivation by destroying soil microorganisms, which support soil efficacy and soil-fauna microbial activities. About 95.0% assented to severe windstorms; severe windstorm implies fierce, destructive, and unfavorable wind patterns that cause severe havoc on rice plants (Dar et al. 2024). Excessive wind destroys rice planted in the field, leading to poor growth and yield performance. In addition, severe windstorms uproot newly planted and already growing rice seedlings, causing total crop damage and failure. High relative humidity was identified by 80% of the rice farmers. High relative humidity promotes the growth and emergence of rice pests and diseases that feed on rice plants, causing crop failure and low yield (Ojo and Baiyegunhi 2020). It affects the photosynthetic processes of rice plants, leading to stunted growth and poor root and shoot development. About 98.8% of the rice farmers indicated a late onset of rainfall; this implies a delay in rainfall, which leads to a prolonged drought season, thereby negatively affecting rice production. Late onset of rainfall distorts farming activities and the calendar, affecting the yearly planting of crops—delayed rains cause land dryness, which does not support rice cultivation (Habib-Ur-Rahman et al. 2022). Early cessation of rainfall was identified by 87.5% of the rice farmers. This implies the sudden and unexpected cessation of rainfall which severely affects rice production. Early rain cessation is inimical to rice cultivation as it distorts rice growth and overall development causing poor yield and harvest. It makes already planted rice seedlings to lose support of soil moisture and causes yellowing of rice leaves (Bichi et al. 2023). High evaporation rate was indicated by 92.5% of the farmers. High evaporation rate implies the quick loss of water to the atmosphere thereby depleting soil-moisture contents and soil-water needed for overall growth rice crop growth and development. The sudden losses of soil-water causes rice field dryness mostly in the vegetative soil-layers which debase rice yield. About 81.3% of the rice farmers' experienced high atmospheric pressure, high atmospheric pressure prolongs rice crop germination time, delays the growth rate of young rice plants and impede root and shoot development in rice plants (Saud et al. 2022). It also affects soil microbial organisms responsible for soil build-up sand other soil-activities needed for optimal rice growth. It equally distorts the photosynthetic activities in rice plants leading to low yield and economic losses.

Adaptation mechanism of rice farmers to climate change

Table 3 presents the adaptation mechanisms of rice farmers to climate change. The table shows 88.8% of rice farmers adopted the irrigation methods. This implies the artificial or alternate water provisions during drought periods. Our agricultural system is dependent on rain fed agriculture making rice farmers vulnerable to climate change (Ali et al. (2021). Adoption of irrigation method substitute for rain absence by providing rice farmers with the needed water required for rice production throughout the cropping season and this ensures maximum rice yield. Use of improved seed varieties was adopted by all the rice farmers (100%). This implies the use of proven rice seedlings that is resistant to rice pests and diseases and adverse climatic and weather conditions (Amadu et al. 2020). These improved rice seedlings ensures improved rice yield, performance and optimal harvest. Use of developed farm machineries was adopted by 12.5% of rice farmers. This implies the deployment of modern farm machines such as tractors, planters, harrowers, ridgers, etc. in rice cultivation. These developed implements facilitate rice cultivation in the face of climate change and adverse weather conditions (Arifah et al. 2022).Its usage enhances rice yield and improved land productivity while displacing the use of crude implements which retards yield. Effective use of pesticides on farm was adopted by 96.3% of the rice farmers. Efficient and effective use of pesticides assists in controlling rice pests and diseases caused by high temperatures and frequent rainfalls. Most indigenous rice varieties are vulnerable to pest infestations and disease and thus the use of pesticides which should be properly applied based on specifications (Adekunmi et al. 2022). Efficient application of fertilizers on farm was adopted by 95.0% of rice farmers. Due to negative impacts of climate change on rice field, farmers' experiences low yields and poor harvest leading to economic losses and time invested in cultivation. As a result the use of inorganic fertilizers seems appropriate and efficient in remedying these ugly impacts of climate change on rice fields. The use of fertilizers improves rice production and enhances better performance both in land yield and productivity (Yakubu et al. 2021). The table further reveals that 96.3% of the rice farmers adopted the use of crop rotation. Crop rotation refers to a proven and verified land management system used to mitigate the negative impacts of changing climate and ensures sustainable crop yield with other crops inclusive. It helps to break pest and disease cycles caused by adverse weather condition and reinforces nutrient supply required for optimal rice yields. About 88.8% of rice farmers adopted changing of cultivation date. The change of cultivation dates is necessary considering climate change negative effects on rice production (Gbemavo et al. 2022). Changing of cultivation date are effective strategies in dealing with continued variations of precipitation and temperature. Change of dates allows farmers to suit their cropping in respect to variations in climate change per time. Adoption of plant spacing was adopted by 81.3% of rice farmers. Crop spacing assist in the development of rice roots, shoots, and overall crop growth. It helps in effective distribution of light, water, and nutrients required in rice plants (Akanbi et al. 2022). Crowding of rice plants in the face of climate change leads to overall crop failure and input-resource sabotage. The use of improved soil conservation methods was adopted by 80% of rice farmers. This connotes that the rice farmers practiced improved soil conservation methods such as mulching, erosion control, use of drainage systems, etc. in mitigating climate change and improving rice production (Khan et al. 2022a). Improved soil conservation methods helps to maintain soil fertility, biodiversity and aid in soil developmental processes and nutrient capacity of soils leading to improved yields. Diversification was adopted by 90% of the rice farmers. Diversification is a multi-level processes in which crop farmers engage in other income yielding occupation to guard against the negative effects of climate change on agriculture and crop production. Diversification allows crop farmers to explore other areas and engage productively should there be any climate eventualities (Ocheni et al. 2021).

Table 1. Socio-economic characteristics of rice farmers

Variable	Frequency	Percentage
Age		
20-30	15	18.8
31-40	22	27.5
41-50	35	43.8
51 and above	08	10.0
Mean	48	
Sex		
Male	69	86.3
Female	11	13.8
Marital status		
Single	21	26.3
Married	41	51.3
Widowed	18	22.5
Level of education		
Primary	26	32.5
Secondary	33	41.3
Tertiary	09	11.3
Non formal	12	15.0
Household size		
1-4	22	27.5
5-8	51	63.8
9-12	07	8.8
Mean	07	
Occupation		
Farming only	21	26.3
Farming and others	59	73.7
Farm Size		
0.1-1.0	35	43.8
1.1-2.0	38	47.5
2.1-3.0	07	8.7
Mean	1.8	
Extension contact		
1-2	16	20.0
3-4	08	10.0
None	56	70.0
Mean	1.6	
Cooperative membership		
Yes	10	12.5
No	70	87.5
Participation in workshop/training		
1-2	13	16.3
3-4	06	7.5
None	61	76.2
Mean	1.3	
Farming Experience		
1-10	18	22.5
11-20	54	67.5
21-30	08	10.0
Mean	18	
Source of Capital		
Banks	08	10.0
Friends/relatives	33	41.3
personal savings	39	48.7
Source of land		
Inheritance	29	36.3
Lease/rent	19	23.8
Gift	15	18.8
Purchase	11	13.8
Pledge	06	7.5
Source of labour used		
Family	30	37.5
Hired	25	31.2
Both	25	31.2

Table 2. Perceived climate change occurrence encountered by rice farmers

Perceived climate change occurrence	*Frequency	Percentage (%)
Prolonged drought	76	95.0
Increased intensity of rainfall	72	90.0
Frequent floods	67	83.8
Increased temperature	78	97.5
Severe windstorm	76	95.0
High relative humidity	64	80.0
Late onset of rainfall	79	98.8
Early cessation of rainfall	70	87.5
High evaporation rate	74	92.5
High atmospheric pressure	65	81.3

Note: *Multiple Responses

Table 3. Adaptation mechanism of rice farmers to climate change

Adaptation mechanism of rice farmers	*Frequency	Percentage
Adoption of irrigation methods	71	88.8
Use of improved varieties of seeds	80	100.0
Use of developed farm machineries	10	12.5
Effective use of pesticides on farm	77	96.3
Efficient application of fertilizers on farm	76	95.0
Adoption of crop rotation system	77	96.3
Changing of cultivation date	71	88.8
Adoption of plant spacing	65	81.3
Adoption of improved soil conservation methods	64	80.0
Diversification of crops	72	90.0

Note: *Multiple Responses

Table 4. Determinants of vulnerability of rice farmers to climate change

Variable	Coefficient	t-value	S.E
Constant	0.762	1.901**	0.400
Age	-6.436	-3.951***	1.629
Education	9.540	3.351***	2.847
Household size	-16.853	-0.702ns	24.007
Access to capital	-0.967	-2.821**	0.342
Farming experience	-3.601	-4.843***	0.743
Farm size	0.841	-0.788ns	1.067
Access to climate change information	-10.421	-3.902***	2.671
Participation in climate change workshops	0.562	-1.001ns	0.561
Gender	-0.887	-1.990**	0.446
Extension contacts	-13.742	-2.002**	6.864
R^2	0.881		
F-value	42.099		
N	80		

Note: ***, ** indicates significant at 1 and 5% levels

Table 5. Identified factors impeding rice production of farmers

Identified factors	*Frequency	Percentage
Lack of timely access to inputs	78	97.5
Inadequate lands	72	90.0
High cost of fertilizers	80	100.0
Increased water flooding	57	71.3
Incidence of pests and diseases	71	90.0
Inadequate capital	78	97.5
High cost of labor	69	86.3
Poor extension service	77	96.3
Inaccessibility of climate change	66	82.5
information	70	87.5
Poor storage facilities	80	100.0
Crude processing equipment		

Note: *Multiple responses

Determinants of vulnerability of rice farmers to climate change

Table 4 presents the determinants of vulnerability of rice farmers to climate change. The R^2 value of 0.881 explained about 88.1% of the total variations in the vulnerability of rice farmers to climate change. The F value of 42.099 was significant and further indicates the goodness of fit of the model used. Age coefficient was negative at 1% and significant; this implies that aging farmers tend to be more vulnerable to climate change compared to younger farmers. This could also mean the inability of aging farmers to access vital information on climate change ahead of time to mitigate its negative effects on rice production. Typically age of a farmer is very important in agricultural production and climate change mitigation (Khan et al. 2022b). Education coefficient was significant at 1% and was statistically positive; indicating that the higher the educational level of the rice farmers, the less vulnerable they will be to climate change issues. Education is known to empower farmers with the requisite knowledge and understanding of variations in climate and weather conditions. It exposes farmers in accessing vital information regarding climate change and its adverse effects (Osuji et al. 2023). Access to capital was significant at 5% and was negative, indicating that access to capital determines the vulnerability of rice farmers to climate change. Inability of the rice farmers to access capital limits the purchase of planting materials such as improved and resistant seed varieties, chemicals for pests and disease control, fertilizers, etc. which is essentially used in mitigating climate change adverse impacts on crop production. In addition, this further implies that failure of the rice farmers to access capital makes them more vulnerable to climate change (Onyeneke et al. 2021). Farming experience was significant at 1% and statistically negative. This implies that inexperienced farmers suffers most from the negative consequences of climate change and are more vulnerable to climate change adverse effects. More so, while experienced farmers are better-off with changes in climate, inexperienced ones are at a loss and are faced with severe climate change impacts which affects their crop yield and outputs (FAO 2022). Access to climate change information was significant at 1% and statistically negative; indicating that access to climate change information could lead to the vulnerability of the rice farmers to climate change. Inability of the rice farmers to access climate change information makes them more vulnerable to climate change negative effects. Failure to access climate change information exposes farmers to the dangers associated with climate change which manifests in crop failures and poor yields (Ho et al. 2022). Gender was significant and statistically negative at 5%, this implies that the female farmers are more vulnerable to climate change than the male farmers. This could be due to their feminine nature and inability to observe climate change variations and undue weather conditions per cropping season. This also could imply inability of the female farmers to take proactive measures to mitigate negative effects of climate change upon observations and access to information (Van-Oort and Zwart 2018). Extension contacts was significant and statistically negative at 5%, indicating that rice farmers with no extension contacts becomes more vulnerable to climate change when compared with farmers with good exposure to extension contacts. Furthermore, poor extension contacts limits diffusion and accessibility of climate change information and thus exposes the farmers to vulnerability of climate change. Extension service is a vital tool in overcoming climate change incidence and occurrence regarding crop production (Arifah et al. 2022).

Identified factors impeding rice production of farmers

Table 5 presents the factors impeding rice production of farmers. The table shows that 97.5% of rice farmers perceived lack of timely access to inputs to be a limiting factor in the production of rice. This implies that inability of the farmers to access farming inputs on time constrained rice production (Chen et al. 2023a). Inadequate lands were identified by 90% of the rice farmers. This shows that the rice farmers cultivated on small portions of lands. This could be due to land tenure systems and land scarcity which limit large scale rice production (Sharma et al. 2022). High cost of fertilizer was indicated by 100% of the rice farmers. This implies that high cost of fertilizer contributed to low rice production. Most of the rural farmers are resource poor and may not be able to afford the high cost of fertilizers, and this could be a serious challenge for the farmers. Increased water flooding was identified by 71.3% of rice farmers. This implies that the issue of climate change contributing to water flooding of rice field can be devastating leading to total crop failure (Joseph et al. 2023). About 90% of the rice farmers indicated incidence of pest and diseases to be a limiting factor in rice production. This shows the prevalence of pests and disease attacks on rice plants which reduce rice yields. Inadequate capital was identified by 97.5% of the rice farmers. Inadequate capital is a major challenge facing rural farmers, inability of the farmers to access capital from banks and other micro-finance institutions limits large scale production (Dar et al. 2024). High cost of labor was identified by 86.3% of the rice farmers. This connotes that inability of the rice farmers to afford high labour fares could be a huge constraints to rice production. This stems from rural-urban migration of youths to cities in search of greener pastures. Poor extension service was identified by 96.3% of rice farmers. This means that lack of access to extension services impeded rice production. Poor extension services limit the dissemination of farm information, innovation and technical know-how regarding crop production (Ojo and Baiyegunhi 2020). Inaccessibility of climate change information was indicated by 82.5% of rice farmers. This implies that inability of the rice farmers to access climate change information constrained rice production. Issues of climate change relating to high temperature, irregular rainfall, high evaporation rate, relative humidity, etc. are remarkable impediments to rice production (Habib-Ur-Rahman et al. 2022). Poor storage facility was indicated by 87.5% of the rice farmers. Issue of storage facilities limits large scale cultivation of rice, as harvested rice is prone to grain losses and spoilage and further resulting in economic losses. Crude processing equipment was identified by 100% of the rice farmers. This means that the farmers encountered so many losses during the processing of rice resulting from the use of crude processing equipments. This further connotes that value addition in rice processing is jeopardized via the use of crude processing tools.

In conclusion, the study's findings show that the rice farmers were more male, married, experienced, and productive age, with a household size of 7 persons. Notable perceived climate change occurrences were prolonged drought, increased intensity of rainfall, frequent floods, increased temperature, and severe windstorms. To mitigate climate change, the farmers employed some adaptation measures such as adopting irrigation methods, improving varieties of seeds, developing farm machinery, effective use of pesticides, efficient application of fertilizers, and adopting a crop rotation system. Age, education, access to capital, farming experience, access to climate change information, gender, and extension contacts appeared to be significant determinants of climate change vulnerability for rice farmers. Lack of timely access to inputs, inadequate lands, high cost of fertilizers, increased water flooding, pests and diseases, and inadequate capital-constrained rice production of farmers. Farmers should practice more climate-smart agriculture to overcome the adverse effects of climate change on rice production. Farmers should seek early climate change information to mitigate the adverse effects of climate change on rice production.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 31-39

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090104

Antimicrobial activity and identification of secondary metabolites in summer oyster mushroom (*Pleurotus ostreatus*) extracts

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Manuscript received: 27 August 2024. Revision accepted: 23 November 2024

Abstract. *Devi NB, Kalpana CA. 2025. Antimicrobial activity and identification of secondary metabolites in summer oyster mushroom (*Pleurotus ostreatus*) extracts. Asian J Agric 9: 31-39.* Consumption of oyster mushrooms is increasing due to their unique flavor, meaty taste, and extensive medicinal potential. The main aim of this study is to analyze and compare antimicrobial assays and identify secondary metabolites present after different drying techniques of oyster mushrooms. Oyster mushrooms were dried three different ways: Sun-Dried (SD), Cabinet-Dried (CD), and Freeze-Dried (FD). The dried samples were powdered and macerated using ethanol as a solvent extract and ultrasound treated. An antimicrobial assay was performed for the extracts using two Gram-positive and two Gram-negative bacteria. Gentamycin was used as a positive control and ethanol as a negative control. Further phytochemical screening was performed on the extracts to identify secondary metabolites, and other organic and inorganic compounds were identified by Fourier Transform Infrared Spectroscopy (FTIR) analysis. The study revealed that the mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *Staphylococcus aureus* were 15.6 mm, 17.2 mm and 13 mm respectively while against *Bacillus cereus* were 0 mm, 0 mm and 14 mm respectively. And the mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *Escherichia* were 12.6 mm, 7.8 mm and 15.4 mm respectively whereas against *Pseudomonas aeruginosa* were 1 mm, 0 mm and 11.4 mm respectively. The study also found that all three samples contained good amounts of alkaloids and carbohydrates, flavonoids, sterols, anthraquinones, and cardiac glycosides. All the samples showed bands in the IR regions of 400-4000 cm^{-1}. The study concluded that the FD oyster mushroom preserved more secondary metabolites that had potential role in antimicrobial activity. The present work extends the finding of previous work by adopting ultrasonication as an extraction process, to compare the antimicrobial activity and identification of secondary metabolites of different drying method for summer-grown oyster mushroom cultivated in Coimbatore District of Tamil Nadu, India.

Keywords: *Dhingri*, FTIR, lyophilization, phytochemical, ultrasonication

Abbreviations: CD: Cabinet-Dried, FD: Freeze-Dried, FTIR: Fourier Transform Infrared Spectroscopy, SD: Sun-Dried, UAE: Ultrasonication-Assisted Extraction

INTRODUCTION

Oyster mushrooms (*Pleurotus ostreatus)*, also well known as *dhingri* in India, belong to the family Basidiomycetes and are the second most produced edible fungus due to their rich nutritional properties and easy cultivation (El-Razek et al. 2020). The best growing seasons for mushrooms are March/April (summer mushrooms) in hilly areas and September/October (winter mushrooms) in lower regions. The *P. ostreatus* is one of the most commercially cultivated *Pleurotu*s species. Growing *Pleurotu*s species is easy and inexpensive; it can be grown yearly in under-maintained climate conditions. In India, oyster mushrooms are mostly cultivated and produced in eastern regions like Odisha and West Bengal, most of the northeastern states, southern regions, such as Karnataka and Andhra Pradesh, and western regions, such as Maharashtra and Madhya Pradesh in Central India (National Horticulture Board of India) grey oyster mushrooms and white oyster mushrooms are suited to cultivation in Tamil Nadu (TNAU Agritech Portal).

Mushrooms have a long history of consumption and cultivation around the world because of their unique umami flavor and meaty taste, as well as their medicinal properties (Zhou et al. 2024). The *P. ostreatus* is a rich source of major nutrients, including protein, carbohydrates, dietary fiber, minerals, and vitamins, especially Vitamin D (Corrêa et al. 2016) and it is low in fat, calories, and cholesterol (Fakoya et al. 2020). The Vitamin D content is naturally undetectable in indoor-cultivated fresh mushrooms unless irradiated with natural or artificial ultraviolet rays (Hu et al. 2020). Mushrooms are the only vegan source of Vitamin D2 since the conversion of ergosterol to ergocalciferol occurs by natural or artificial ultraviolet rays, which produce adequate Vitamin D in mushrooms (Cardwell et al. 2018). One study on the application of UV-B irradiation and air-drying club together with oyster mushroom powder for the production of Vitamin D (ergocalciferol) revealed that it could be the best strategy to deliver ergocalciferol in foods (Pedrali et al. 2020). According to the study findings from NHANES III, a lower risk of total mortality rate is associated with the consumption of mushrooms in US adults (Ba et al. 2021).

Oyster mushrooms are also rich sources of bioactive compounds that have antioxidant, antimicrobial, antitumor, and anti-inflammatory effects. A previous study showed that aqueous extracts of the fruiting body of FD oyster mushrooms had the strongest inhibition of the growth of most fungi, and an alcohol-based extract had less inhibition of antimicrobial activity against most tested microorganisms (Younis et al. 2015). Another study by El-Razek et al. (2020) showed that aqueous and ethanol extracts of oyster mushrooms had significant antibacterial properties against *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus.*

Oyster mushrooms contain both primary and secondary metabolites. Primary metabolites are essential for the growth and development of an organism. In contrast secondary metabolites are chemical compounds essential for biological activity that are produced by the organisms for protection against environmental shocks or predatory threats (Herawati et al. 2021). Secondary metabolites, also known as phytochemicals, include alkaloids, flavonoids, sterols, terpenoids, anthraquinones, and glycosides and are more concentrated in the fruiting parts of mushrooms (Al Qutaibi and Kagne 2024). In a previous study by the same authors (Nongmaithem and Chinnapan 2023) on the effect of the screening of phytochemical constituents, some oyster mushrooms were FD, and others were exposed to sunlight for 30 minutes followed by FD, then extracted into a powder in different solvents (aqueous, methanol, and ethanol). The study observed that more secondary metabolites were found in FD ethanol extracts, while protein was found in higher quantities in the second sample. Still, there was no difference in the secondary metabolites present in the methanol extracts in the samples.

Since mushrooms contain 80-90% moisture, they are highly perishable (Schill et al. 2021); therefore, it is necessary to reduce their water content by adopting drying methods that extend their shelf life (Van Hung et al. 2020). Sun Drying (SD), Cabinet Drying (CD) and Freeze Drying (FD) were adopted in the present study. SD is low cost and traditional method of drying. SD preserved organoleptic characteristics and physio-chemical properties of oyster mushroom (Maray et al. 2018). Cabinet Drying (CD) is a simple and easy method, has been promoted as appropriate technologies at lower temperature because of low investment. Freeze Drying (FD) is advanced, costly and excellent method of drying which retains the color, texture and nutrients of foods (Shams et al. 2022). No previous studies have highlighted specifically summer harvested oyster mushrooms. The present work extends the finding of previous work by adopting ultrasonication as extraction process and the comparison of different drying methods of *P. ostreatus* in terms of the identification of secondary metabolites, antibacterial activity from Coimbatore District, Tamil Nadu, India.

The main goal of this study was to evaluate the antibacterial activity against two Gram-positive and two Gram-negative bacteria, identify bioactive compounds, and screen primary and secondary metabolites (phytochemicals) from oyster mushroom powder extracts produced via different drying methods.

MATERIALS AND METHODS

Sample procurement and identification

Fresh oyster mushrooms (*P. ostreatus*) were purchased from a farm in Coimbatore, Tamil Nadu, India. They were grown in a mushroom hut (maintained temperature of 18-26°C due to the hot climate in Tamil Nadu) and harvested between April and May (summer mushroom). Sample identification was carried out at the Centre of Advanced Studies in Botany, University of Madras, Tamil Nadu.

Sample preparation

Oyster mushrooms were cleaned and washed thoroughly, and their fruiting parts were separated from their stalks. They were cut into pieces (1×1 cm^2) and divided into three portions, then dried by the following methods.

Sun drying

Sliced oyster mushrooms were Sun-Dried (SD) at 30-35°C, gills up toward the sunlight, for 2-3 days from 11 am to 3 pm when UV was extreme (10±1) in Coimbatore District, Tamil Nadu. SD is the traditional and most convenient method of drying for the removal of moisture content from the mushrooms.

Cabinet drying

Thinly sliced oyster mushrooms were arranged on a tray in a single layer. A cabinet dryer was preheated for 15 minutes to obtain a constant temperature of 50-55°C, sample was kept in the dryer until the moisture was lost (around 10 hours).

Freeze drying

Sliced mushrooms were kept in a lyophilizer at 45±5°C with vacuum pressure of about 0.062 mbar for 36 h until their moisture was lost. Freeze-Dried (FD) is among the best methods for removing moisture without changing the color or texture of food products and with minimal volume change (Bhatta et al. 2020).

Drying extends the shelf life of oyster mushrooms. The dried oyster mushrooms were powdered finely in a grinding machine, sieved through a 0.5 mm pore-size sieve, and kept in an airtight container <2°C for further analysis. The samples were labeled Sun-Dried (SD), Cabinet-Dried (CD), and Freeze-Dried (FD) oyster mushroom powder.

Extract preparation from powdered oyster mushrooms

Ultrasonication-Assisted Extraction (UAE) was adopted for solvent extraction. UAE is a non-conventional method of solvent extraction from mushroom powder using an ultrasound device. The UAE method of extraction is reported to yield high ergosterol and metabolites from mushrooms in less time than the Soxhlet extraction method (Papoutsis et al. 2020). The protocol followed for the extraction of phytochemicals and metabolites was that of Patil et al. (2018) with some modifications to the solvent used and time.

Five grams of SD, CD, and FD oyster mushroom powder were added separately to three 250 mL glass beakers and mixed with 100 mL of ethanol (HPLC grade). Ethanol is Generally Recognized as Safe (GRAS) which was adopted as a solvent extract other than methanol and water because of non-toxic and easy to evaporate. Nutraceutical and food supplements of mushroom extracts, either aqueous or alcohol-based could be dried or solution, and are available as powder or tablets form (Radzki et al. 2023). All three samples were covered with aluminum foil to prevent evaporation during extraction and were ultrasonicated (Labman digital ultrasonicator cleaner, 100W power rating, 40±3 Khz ultrasonication frequency) for 30 minutes at 50±5°C. After ultrasonication, the extracts were centrifuged at 3500 rpm for 15 minutes. The supernatant was separated and filtered through 11µm Whatman filter paper. The extracts were stored at -4°C until further analysis.

Antimicrobial assay

An antibacterial assay was performed following the Kirby-Bauer method in accordance with the standards set by the Advanced Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India. In brief, 4-5 mL of broth medium was incubated at 35°C for 2-6 hours to maximize turbidity. The culture was diluted in sterile saline to 1-2 x 10^8 CFU/mL for *S. aureus* (ATCC 6538), *B. cereus* (ATCC10987), *E. coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC27853). The medium was punctured to make a 6 mm diameter well and the extracts were filled in different concentrations, such as 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL. The Petri plates were inverted for complete diffusion, and the inhibition zones were examined by measuring the diameter (in mm) formed around the well after 24 hours' of incubation at 37°C. The zones were measured using a standard (Hi-Media) scale (Witasari et al. 2022). *Gentamycin*, well established antibiotic was randomly selected (easily available) as a positive control and ethanol as a negative control because ethanol was used as a solvent for extraction of bioactive compounds from oyster mushrooms. The two Gram-positive bacteria such as *S. aureus* and *B. cereus*, while two Gram-negative bacteria namely *E. coli*, and *P. aeruginosa* were studied against positive control "Gentamycin" since there was less reported of the specific bacteria in different drying of oyster mushroom extracts.

Phytochemical screening

Identification of the phytochemical constituents in naturally available food facilitates the finding of potent pharmacological and therapeutic activity. Conventional ways of performing phytochemical screening tests are more economical, easily affordable, and still good choices for qualitative phytochemical constituents. Phytochemical constituents were screened as described in Shaikh and Patil (2020) as follows:

Identification of alkaloids in mushroom extracts by Mayer's test and Dragendorff's test. A color change of creamy color precipitate and reddish brown precipitate respectively denoted the presence of alkaloids.

Identification of flavonoids by adding concentrated H_2SO_4 test. A change in color to reddish orange color indicated the presence of flavonoids in the extract. Identification of sterols in the mushroom extracts by Libermann's test, the presence of a reddish brown ring indicated that the extract contained sterols. Anthraquinones was identified by Borntrager's test, reddish orange color indicated the presence of anthraquinones. Identification of protein by 2% Ninhydrin reagent, showing a color of purple indicated the presence of protein in the extracts. Identification of carbohydrates by Molisch's test and Fehling's test), showing violet ring and red precipitate indicated the presence of carbohydrates. Identification of cardiac glycosides by Keller-Killani test, forming of brown ring indicated the presence of cardiac glycosides in the mushroom extracts.

Identification of functional groups by FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) of the extracts was performed to identify the presence of functional groups. FTIR analysis is the most powerful and fastest tool to detect the functional groups present in a substance (Mane and Khilare 2021). FTIR (SHIMADZU) was performed for the liquid extracts in the region between 400-4000 cm^{-1}.

Statistical analysis

Zone of inhibition against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa* in different concentrations of SD, CD, and FD oyster mushroom extracts were calculated and compared within the group by one sample t-test at 95% confidence interval of the difference. A significant 2-tailed was analysed and *p*-value of <0.01 and <0.05 are at 99% and 95% significant respectively. It was performed in IBM SPSS Statistics version 21.

RESULTS AND DISCUSSION

Antimicrobial susceptibility of oyster mushroom extracts dried with different methods

SD oyster mushroom powder extract, CD oyster mushroom extract, and FD oyster mushroom powder extract were subjected to an antimicrobial activity test (Kirby-Bauer method). Many studies have reported that *Pleurotus* spp. has medicinal properties, including antimicrobial, antioxidant, anti-viral, anti-diabetic, and anti-inflammatory properties (Masri et al. 2017). A study on antimicrobial activities in methanol, petroleum ether, and acetone extracts of agro-waste-produced oyster mushrooms against five Gram-positive and five Gram-negative bacteria revealed that methanol extract had a broader spectrum of inhibition against the bacteria, followed by acetone extract and petroleum ether (El-Razek et al. 2020). In the current study, ethanol extracts of oyster mushrooms dried with different methods were analyzed for antimicrobial assays.

The pictorial zone of inhibition against the Gram-positive and Gram-negative bacteria by different concentrations of extracts of oyster mushroom dried by different methods (10µL, 20µL, 30µL, 40µL, and 50µL) (Figure 1). The zone of inhibition against Gram-positive and Gram-negative bacteria by the positive control (Gentamycin) and negative control (ethanol) (Figure 1.A). The pictorial zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) by the SD oyster mushroom powder extract (Figure 1.B). The zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria by the CD oyster mushroom powder extract (Figure 1.C). The pictorial zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) by the FD oyster mushroom powder extract (Figure 1.D).

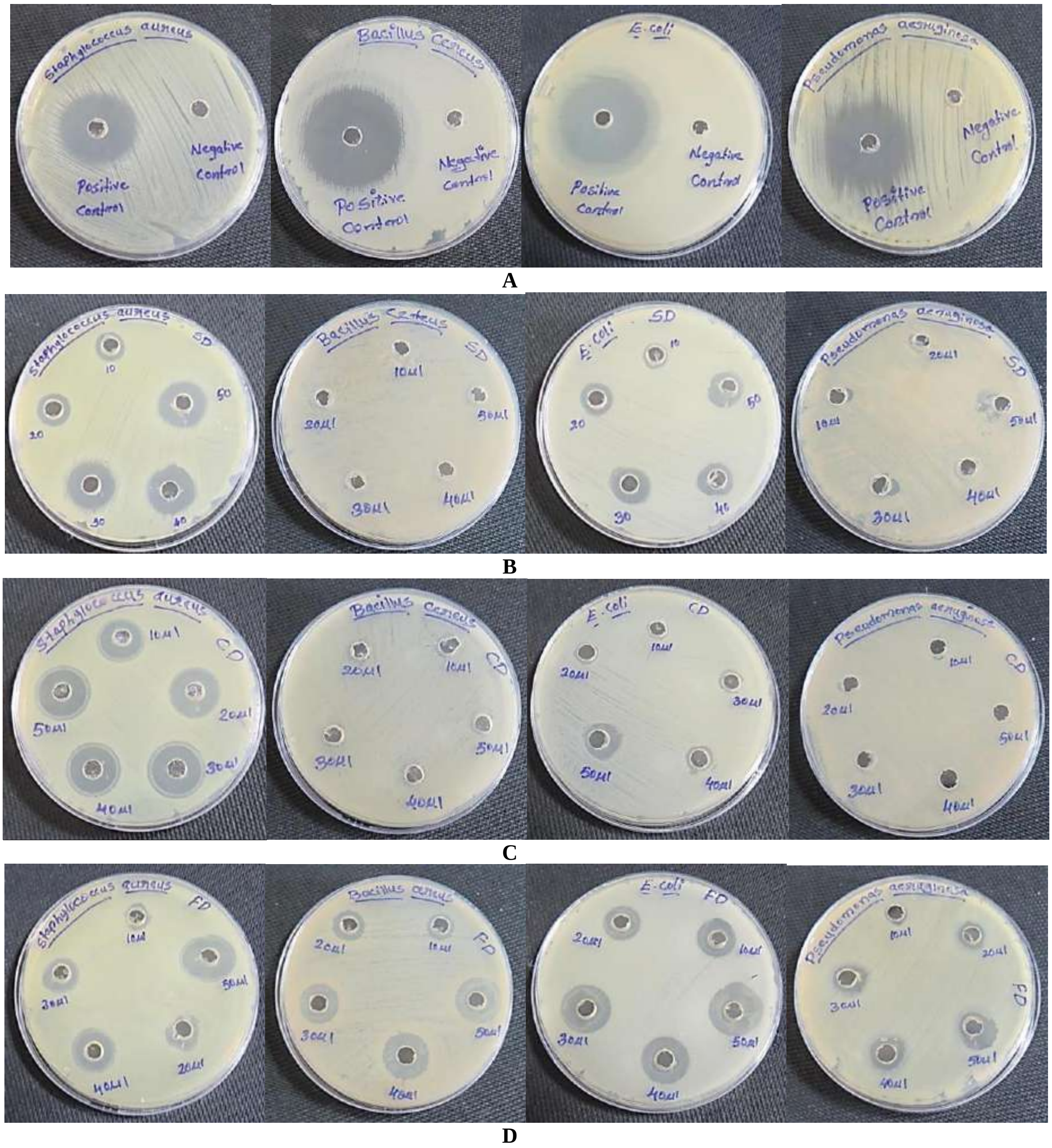

Figure 1. Zone of inhibition against Gram-positive and Gram-negative bacteria by: A. Positive control (Gentamycin) and negative control (ethanol), B. SD oyster mushroom powder extract, C. CD oyster mushroom powder extract, D. FD oyster mushroom powder extract

Table 1. Diameter of inhibition against two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) in different concentrations of SD, CD, and FD oyster mushroom extracts

Concentration (µL)	Zone of Inhibition (mm)											
	Gram-positive						Gram-negative					
	S. aureus (mm)			*B. cereus* (mm)			*E. coli* (mm)			*P. aeruginosa* (mm)		
	SD	CD	FD	SD	CD	FD	SD	CD	FD	SD	CD	FD
10	11	15	9	0	0	9	9	0	14	1	0	8
20	12	18	10	0	0	12	11	8	13	0	0	9
30	18	18	13	0	0	14	15	8	16	4	0	12
40	18	17	14	0	0	17	14	9	16	0	0	13
50	19	18	19	0	0	18	14	14	18	0	0	15
Mean zone of inhibition (mm)	15.6	17.2	13	0	0	14	12.6	7.8	15.4	1	0	11.4
p-value	0.001**	0.00**	0.002**	NS	NS	0.001**	0.000**	0.025*	0.000**	0.266	NS	0.001**
Standard (*Gentamycin*)	28 mm			35 mm			31 mm			29 mm		
Negative control (ethanol)	0			0			0			0		

Note: SD: Sun-Dried oyster mushroom extract, CD: Cabinet-Dried oyster mushroom extract, FD: Freeze-Dried oyster mushroom extract. ** significant level at 99.9%, * significant level at 95%, NS: Not Significant

The zone of inhibition by SD, CD, and FD oyster mushroom extracts in different concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) against two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) compared with positive control (standard Gentamycin). The higher the concentration, the greater the zone of inhibition observed, and the zone of inhibition is denoted in mm. The zone of inhibition by standard Gentamycin was 28 mm and 35 mm for the two Gram-positive bacteria (*S. aureus* and *B. cereus*), respectively, and 31 mm and 29 mm for the two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), respectively. The negative control, ethanol, did not show a zone of inhibition of either the Gram-positive or Gram-negative bacteria. The mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *S. aureus* were 15.6 mm, 17.2 mm and 13 mm respectively while against *B. cereus* were 0 mm, 0 mm and 14 mm respectively. The average zone of inhibition by SD, CD and FD oyster mushroom extracts against *E. coli* were 12.6 mm, 7.8 mm and 15.4 mm respectively whereas against *P. aeruginosa* were 1 mm, 0 mm and 11.4 mm respectively (Table 1).

The CD oyster mushroom extract had a greater ability to inhibit the growth of the Gram-positive bacteria *S. aureus* (p=0.000) followed by SD (p=0.001) and FD (p=0.002) oyster mushroom extracts, there was no significantly difference of drying methods against *S. aureus* while the FD oyster mushroom extract had more ability to inhibit the Gram-positive bacteria *B. cereus* (p=0.001)so, there was significantly affect by drying method to inhibit this bacteria, whereas the other two extracts had no zone of inhibition. The FD oyster mushroom extract had a higher inhibition zone, followed by the SD and CD oyster mushroom extracts for the Gram-negative bacteria *E. coli,* a non-significant difference was observed within the samples. The FD oyster mushroom extract had more ability to inhibit *P. aeruginosa* (p=0.001) than the SD or CD oyster mushroom extracts, as either no inhibition zone or much less inhibition was found for both. So, a significantly difference was observed. A study has shown the significant impact of aqueous and ethanol extracts of freeze-dried oyster mushrooms in antibacterial properties against *S. typhimurium*, *E. coli*, and *S. aureus* (El-Razek et al. 2020). In the present study, thermal degradation of bioactive compounds was observed in SD and CD oyster mushroom extracts compared to FD. SD and CD oyster mushroom extracts did not show antimicrobial properties to inhibit against *B. cereus* and *P. aeruginosa.* In contrast FD oyster mushroom extract showed antimicrobial properties to inhibit both Gram-positive and Gram-negative, showed statistically significant. Therefore, the study demonstrated that different drying methods might affect the bioactive compounds that are responsible for antimicrobial properties since FD oyster mushroom extracts preserved bioactive compounds followed by SD and CD (Table 1).

Phytochemical screening

Phytochemical screening was performed to observe the secondary metabolites present in extracts of oyster mushrooms dried using different methods. Studies have shown that macro-fungi like oyster mushrooms have been used in the pharmaceutical industry due to the presence of secondary metabolites (Al Qutaibi and Kagne 2024). One study by Rahimah et al. (2019) found that 70% ethanol of oyster mushroom extract contained a total phenolic compound (6.67 µg/1 mg) equivalent to 1 mg gallic acid. This same study also confirmed that aqueous was a better solvent than ethanol or acetone. Another study (Senthilkumar et al. 2015) demonstrated that major phytochemical constituents from edible wild *P. ostreatus* collected from the Dharmapuri District, Tamil Nadu, were found to be higher in ethanol extracts than other solvents, such as ethyl acetate, chloroform, and hexane. Ethanol is the most common solvent used for extraction other than water and methanol due to its dissolvability of polar and non-polar solvents; special techniques to be adopted for drying by using water for extraction, and the use of methanol for extraction are limited due to its toxic effect (Rahimah et al. 2019). Ethanol is also recognized as Generally Recognized as Safe GRAS by the US Food and

Drug Administration (FDA 2024). In the present study, ethanol was used as a solvent extraction to screen for 16 phytochemical constituents, and their presence in the extracts of oyster mushroom powders dried by different methods was compared.

Out of 16 phytochemical constituents screened, alkaloids and carbohydrates were present in high amounts in all three samples, shown in yellow as oyster mushroom extract was high in alkaloids, which has the potential role of antibacterial properties. Flavonoids, sterols, anthraquinones, and cardiac glycosides were found appreciably in all three extracts (shown in light green). Anthocyanin was found in the FD oyster mushroom extract in appreciable amounts but not detected in the SD or CD oyster mushroom extracts. Protein was found in appreciable quantities in the SD and CD oyster mushroom extract and high amounts in the FD oyster mushroom extract. Thus, FD retained more anthocyanin and protein content in the mushrooms than did other methods of drying. Terpenoids, phenolic compounds, quinones, tannins, saponins, lignin, coumarins, and volatile oils were not detected (shown in green) in any sample. Such metabolites might be lost during drying or processing. The potential effect of secondary metabolite content in fungi is closely related to its growing conditions, the extraction process, and the solvent used for solvent extraction (Herawati et al. 2021) (Table 2).

Identification of functional groups by FTIR analysis

Mushrooms naturally contain high amounts of polysaccharides, high protein, and low fat. The nutritional quality of mushrooms is closely related to their growing conditions. *Pleurotus* spp. cultivated on paddy straw was found to be abundant in protein and carbohydrates, high in fiber, and low in fat, so it is an excellent choice for a high-protein, low-calorie diet (Aswathy et al. 2024). The functional groups of the SD, CD, and FD oyster mushroom extracts were identified by FTIR analysis in the region of 400-4000 cm^{-1} (Figure 2). According to a previous study (Wickramasinghe et al. 2023): The important broadband lies in the region of 3300 cm^{-1} because the O–H and C–H group stretching predicts the availability of moisture. The band around 2900-2880 cm^{-1} represents the stretching of CH_2 and CH_3 (fatty acids in the cell walls). The band at 1740 cm^{-1} might be the result of the carbonyl stretching of alkyl esters, and the band between 1650-1560 cm^{-1} was determined to be amides of protein. The band between 1500-750 cm^{-1} might be polysaccharide C–O stretching, which is linked with protein, lipids, and polysaccharides. The region around 850-750 cm^{-1} has been identified as a polysaccharide anomeric configuration, the β- glycosides might be linked to the band at 890 cm^{-1}, and α- glycosides might be linked to the band between 860-810 cm^{-1}.

The major peaks for all three samples. The consolidated graph was prepared in Origin software 2024b (learning version). All three samples (SD, CD, and FD) show three specific bands in the region 400-4000 cm^{-1}. The peaks were found to be similar, but there were slight quantitative differences within the bands. SD, CD, and FD were found at the bands 3341.31 cm^{-1}, 3319.76 cm^{-1}, and 3338.62 cm^{-1}, respectively, which depict the availability of moisture. The bands at 2888.62 cm^{-1}, 2891.31 cm^{-1}, and 2888.23 cm^{-1} for SD, CD, and FD, respectively, show the stretching of CH_2 and CH_3, which showed the presence of sterols in phytochemical screening (Table 2). The bands at 1641.01 cm^{-1} (SD) and 1632.93 cm^{-1} (FD) represent the presence of proteins (Table 2), while there was no specific band for the CD extract. Thermal treatment might denatured proteins present in CD oyster mushroom extract. The bands at 1382.33 cm^{-1}, 1385.02 cm^{-1}, and 1387.22 cm^{-1} for the SD, CD, and FD extracts, respectively, show that the presence of the C–O group belongs only to polysaccharides (Akcay and Yalcin 2021) which denoted highly presence of carbohydrates in all the three mushroom extracts (Table 2). The sharp peaks at 1050.89, 1050.89, and 1053.59, respectively, might represent the presence of alkaloids, protein, lipids, and polysaccharide vibration (Table 2). The bands in the regions 878.44 cm^{-1}, 875.74 cm^{-1}, and 886.52 cm^{-1} respectively represented the presence of β- glycosides which showed the presence of flavonoids, cardiac glycosides in the SD, CD, and FD oyster mushroom extracts (Figure 2). There was a significant difference in protein content and no other bioactive compounds present in the three samples.

Table 2. Phytochemical constituents observed in sun-dried, cabinet-dried, and freeze-dried oyster mushroom extracts

Phytochemical constituents	Sun-dried oyster mushroom	Cabinet-dried oyster mushroom	Freeze-dried oyster mushroom
Alkaloids	High amount	High amount	High amount
Anthocyanin	Absent	Absent	Appreciable amount
Anthraquinone	Appreciable amount	Appreciable amount	Appreciable amount
Carbohydrates	High amount	High amount	High amount
Cardiac glycosides	Appreciable amount	Appreciable amount	Appreciable amount
Coumarins	Absent	Absent	Absent
Flavonoids	Appreciable amount	Appreciable amount	Appreciable amount
Lignin	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Absent
Protein	Appreciable amount	Appreciable amount	High amount
Quinones	Absent	Absent	Absent
Saponins	Absent	Absent	Absent
Sterols	Appreciable amount	Appreciable amount	Appreciable amount
Tannin	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Volatile oils	Absent	Absent	Absent

Note: ■: Absent, ■: Appreciable amount, ■: High amount

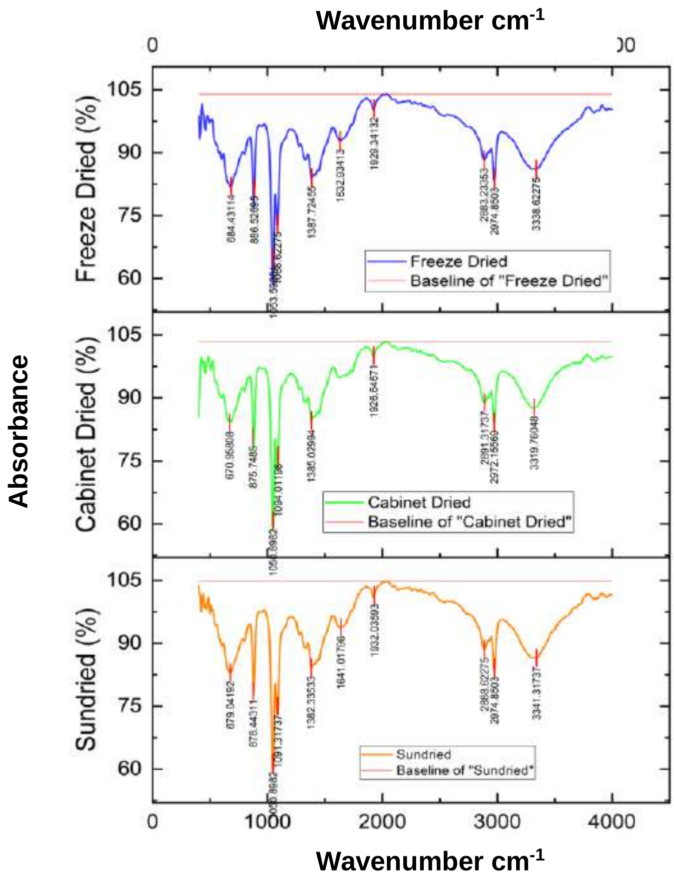

Figure 2. FTIR spectra of sun-dried, cabinet-dried, and freeze-dried oyster mushroom extracts region between 400-4000 cm.^{1}

Discussion

Oyster mushrooms are well known for having nutraceuticals and pharmaceutical properties in mankind. They are highly perishable and have a short shelf life of 3-4 days at room temperature because of low ethylene production and high rate of respiration. Spoilage, browning, and off-flavor might happen if the mushrooms are not stored properly. Drying is the best way to extend the shelf life of mushrooms by removing moisture from them. There are different drying methods to reduce moisture content–traditional drying (sun drying) and advanced drying (cabinet drying and freeze drying) were adopted in the present experiment to evaluate whether the different drying methods impact the antimicrobial activity, content of primary and secondary metabolites as well as bioactive compounds. However, the practice of Sun Drying (SD) is affected because of environmental and weather conditions, and the products may be contaminated by pests, insects, dirt, etc. But it is still being adopted because of its convenience and low cost among other drying methods. Cabinet Drying (CD) is drying in a hot dryer with a high temperature within a few hours; it does not depend on weather conditions while Freeze Drying (FD) is an advanced technology that dries at low temperature, it retains nutrients more than other drying methods. Dried and powdered mushrooms can be stored for a year in well air-sealed conditions; this can be incorporated with food products and may be an alternate source of meat for the vegan population (Van Hung et al. 2020).

Ethanol is GRAS which is commonly used as a solvent extract because of non-toxic and easy to evaporate. UAE is a non-conventional method of solvent extraction which was adopted in this experiment because of the less time consumption as well as yielding more by this extraction process (Papoutsis et al. 2020). Characterization of antimicrobial activity, identification of secondary metabolites and other bioactive compounds of different drying of oyster mushrooms for various reasons. Primarily, ethanol extract among the three extracts (SD, CD and FD mushroom extracts) had maximum inhibition against Gram-positive and Gram-negative bacteria. Our findings revealed (shown in Table 1) that FD mushroom extract had maximum inhibition ability against both the Gram-positive bacteria and Gram-negative bacteria followed by SD and CD oyster mushroom extracts. Secondarily, to observe secondary metabolites from the three different extracts. Our finding observed that (Table 2) FD retained protein more than SD and CD; drying techniques did not affect the presence of secondary metabolites. Finally, to identify and compare whether drying techniques influenced functional groups present in all three extracts. The findings showed that (Figure 2) there was no effect in functional groups by drying.

The present study shows that SD, CD, and FD oyster mushroom extracts had average zones of inhibition against *S. aureus* at 15.6 mm, 17.2 mm, and 13 mm respectively, and the average zone of inhibition against *B. cereus* at 0 mm, 0 mm, and 14 mm respectively. SD, CD, and FD oyster mushroom extracts had an average zone of inhibition against *E. coli* at 12.6 mm, 7.8 mm, and 15.4 mm respectively whereas against *P. aeruginosa* at 1 mm, 0 mm, and 11.4 mm respectively. It revealed that the SD and CD oyster mushroom extracts have the potential role of inhibiting bacterial growth against one Gram-positive and one Gram-negative bacteria. In contrast, FD oyster mushroom extract has the highest ability to inhibit both Gram-positive and Gram-negative bacteria. Gram-positive bacteria (*B. cereus*) strain and Gram-negative bacteria (*P. aeruginosa*) were more resistant to SD and CD extracts. Ahmed et al. (2022) compared the antibacterial activity of three solvent extracts (methanol, petroleum ether, and acetone) of oven-dried *P. ostreatus* against Gram-positive and Gram-negative bacteria. Methanol extract showed the average zone of inhibition at 18 mm, 13 mm, 9 mm, and 16 mm against *S. aureus, B. cereus, E. coli,* and *P. aeruginosa* respectively. Petroleum ether extract showed the average zone of inhibition at 18 mm against *S. aureus* whereas it showed negative results for the other three bacteria. Acetone extract showed the average zone of inhibition at 20 mm, 20 mm, and 15 mm against *S. aureus, B. cereus,* and *E. coli* respectively, and a negative result for *P. aeruginosa.* The methanol extract had more antibacterial activity followed by acetone extract and petroleum ether extract was least and Gram-negative bacteria strains were more resistant than Gram-positive bacteria strains.

The phytochemical screening of SD, CD, and FD oyster mushroom ethanol extracts revealed the presence of high amounts of alkaloids, and carbohydrates in all three extracts. It showed a high amount of protein content in FD oyster mushroom extract whereas there was an appreciable amount of protein in SD and FD extracts. All three extracts presented appreciable amounts of flavonoids, sterols, anthraquinones, and cardiac glycosides. Anthocyanin was present in FD oyster mushroom extract whereas it was absent in SD and CD oyster mushroom extracts. A study was reported by Effiong et al. (2024) on the phytochemical constituent of five different solvent extracts (ethanol, methanol, aqueous, chloroform, and n-Hexane) of hot oven-dried *P. ostreatus.* All the extracts were qualitatively present of alkaloids, flavonoids, carbohydrates, tannins, anthocyanins and betacyanins, saponins, phenols, coumarins, quinones, terpenoids, and cardiac glycosides. In contrast, phlorotannins and betacyanins were absent in chloroform and n-hexane extracts. Either glycosides or acids were completely absent in all five extracts. Fakoya et al. (2020) demonstrated the presence of alkaloids, saponins, phenols, tannins, protein, carbohydrates and in ethanol and aqueous extract sun-dried *P. ostreatus* extract while triterpenoids and glycosides were found only in ethanol extract.

From the FTIR analysis, the present study demonstrated that all the major peaks of the three extracts (SD, CD and FD oyster mushroom) fell between the regions 400-4000 cm^{-1}. The stretching bands fell in the specific regions which have significantly related to the presence of sterols, alkaloids, proteins, lipids, carbohydrates, polysaccharide vibration, β- glycosides, flavonoids, and cardiac glycosides in all three samples. The bands at 1641.01 cm^{-1} (SD) and 1632.93 cm^{-1} (FD) represent the presence of proteins while there was no specific band for the CD extract, it might be denatured of protein during drying (Figure 2). FTIR analysis of four different oven-dried mushrooms (*Agaricus bisporous*, *P. ostreatus*, *Calocybe* species, and *Ganoderma lucidum*) powder showed specific regions with little difference in quantitative. All the bands are shown in IR-spectra correlated with the oyster mushroom components of protein, fat, carbohydrates, moisture, and polysaccharides including glucans (Wickramasinghe et al. 2023).

In conclusion, oyster mushrooms (*P. ostreatus*) have multiple benefits, as shown through the presence of antimicrobial properties, and they are rich in primary and secondary metabolites like alkaloids, carbohydrates, flavonoids, sterols, anthraquinones, and cardiac glycosides which have a potential role to play in inhibiting Gram-positive and Gram-negative bacteria, also found as the presence of bioactive compounds in the extracts from FTIR analysis. Research on different varieties of oyster mushroom using different techniques of extraction with other solvents can be implemented for future aspects to forge on pharmaceutical and food industries. This study also recommends fortifying traditional food products with oyster mushroom powder to improve their nutritional quality, and it will be therefore necessary to analyze its bioavailability.

ACKNOWLEDGEMENTS

Financial support from the National Fellowship for Scheduled Caste (NFSC) by Ministry of Social Justice and Empowerment, Government of India is deeply acknowledged.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 40-51

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090105

Effects of different application doses of black soldier fly frass *Hermetia illucens* (Diptera: Stratiomydae) on soybean plant performances and arthropod abundance

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Manuscript received: 20 September 2024. Revision accepted: 26 November 2024.

Abstract. *Yudistira DH, Sandi YU, Wirabumi BA, Damayanti A, Wikandari P, Sato S. 2025. Effects of different application doses of black soldier fly frass* Hermetia illucens *(Diptera: Stratiomydae) on soybean plant performances and arthropod abundance. Asian J Agric 9: 40-51.* Food waste poses a global challenge, contributing approximately 3.49 billion tons of CO_2 to the atmosphere annually. Recycling this waste into valuable soil nutrients is a viable solution. This study focuses on black soldier fly *Hermetia illucens* L. (Diptera: Stratiomydae) larvae, which convert organic waste into protein-rich biomass and frass, a potential soil amendment. However, the effects of black soldier fly frass on crop performance and biodiversity remain underexplored. We investigated the impact of three different frass doses (0, 2.5, and 5 tons/ha) on the growth of the Tsuruoka soybean variety (*Glycine max* L.), *dadachamame*. Results showed no significant differences in plant height (p=0.07), but higher doses increased leaf count (p<0.01) and SPAD values significantly (p<0.001), with 2.5 tons/ha improving leaf count by 26% and SPAD values by 16%. Yield analysis revealed that 2.5 tons/ha increased the number of filled pods by 29% (p<0.05) and reduced empty pods by 52% (p<0.001), thereby enhancing pod quality. Arthropod analysis indicated that 5 tons/ha significantly increased overall arthropod abundance (p<0.001), particularly predators (p<0.001) and herbivores (p<0.001), while 2.5 tons/ha balanced predator abundance, thereby controlling herbivores and supporting biodiversity. These findings suggest that an application dose of 2.5 tons/ha is optimal for improving edamame yield and quality while maintaining a balanced arthropod ecosystem, demonstrating black soldier fly frass as a nutrient-rich, eco-friendly input that supports sustainable agriculture.

Keywords: Biodiversity, food waste recycling, organic farming, sustainable agriculture, trophic interaction

Abbreviations: BSF: Black Soldier Fly, WAT: Weeks after treatment

INTRODUCTION

Approximately one-third of the world's food production is wasted annually, accounting for roughly 40% of all waste (FAO 2011). Food waste is estimated to cost the global economy up to $936 billion and release around 3.49 billion tons of CO_2 into the environment (FAO 2011). In some countries, food waste has become a major problem. For example, food waste has long been an issue in Japan, where 17 million tons of food were wasted in 2017 alone, mostly from the retail, food, and wholesale industries (Oishi 2019).

Recycling food waste offers a cost-effective and environmentally friendly alternative by repurposing various nutrients, which aligns with the Sustainable Development Goals (SDGs) related to responsible consumption and production (SDG 12), climate action (SDG 13), and life on land (SDG 15). According to Noor et al. (2014), composting is an effective method for managing food waste, significantly reducing the volume of waste requiring landfill disposal. It also offers numerous benefits, including enhanced mineralization of plant nutrients, suppression of plant pests and diseases, and improved soil structure and stability (Martínez-Blanco et al. 2013; Ayilara et al. 2020). Advanced technologies have facilitated faster composting, such as through the use of microorganisms to decompose complex organic materials (Guanzon and Holmer 1993).

Recently, employing insects for organic material decomposition has emerged as a global trend. *Hermetia illucens* L. (Diptera: Stratiomydae), commonly known as the black soldier fly (BSF), is recognized for its beneficial role in reducing organic waste by converting it into biomass. The treatment of black soldier fly larvae (BSFL) is being explored as a waste reduction strategy in various countries (Fischer et al. 2021; Basri et al. 2022) due to the simplicity, speed, and minimal space requirements for BSFL rearing (Rummel et al. 2021). Beyond waste reduction, BSFL offers additional value by bioconverting low-value food waste into valuable fertilizer and protein-rich larvae (Basri et al. 2022). The residual material after harvesting the larvae, termed “frass”, consists of leftover BSFL feed, exuviae, feces, and microorganisms (Schmitt and de Vries 2020).

Organic farming was developed to reduce the impact of chemical use in conventional agriculture. Organic farming focuses on a comprehensive strategy that integrates high-quality output with environmentally friendly methods, thereby contributing to biodiversity, resource conservation, and animal welfare (Dinis et al. 2015). Organic yields are generally lower than conventional yields, but the main goal is sustainability (Seufert et al. 2012; Muller et al. 2017). Although yields may be lower, organic farming compensates for this by promoting higher biodiversity (Gong et al. 2022). Organic farming increases species diversity by approximately 30%, especially in areas with intensive land use (Tuck et al. 2014). This is due to organic farming avoids synthetic chemicals, making it safer for many species (Bengtsson et al. 2005). Organic fertilizer plays a key role in organic farming by enhancing microbial activity and improving soil physical properties and fertility (Chatzistathis et al. 2021). Among the various options, black soldier fly frass stands out as a promising component for organic fertilizers.

Black soldier fly frass (BSF frass) enhances soil quality, fertility, and nutrient content (Klammsteiner et al. 2020; Beesigamukama et al. 2021; Gärttling and Schulz 2022; Gebremikael et al. 2022; Beesigamukama et al. 2022). It is recommended to increase soil organic matter as well as nitrogen, phosphorus, and potassium levels (Anyega et al. 2021; Menino et al. 2021). According to Gärttling and Schulz (2022), BSF frass is notably rich in dry matter nutrients, containing nitrogen, ammonium nitrogen, phosphate, and potassium totaling over 1% of its dry weight. Several studies have shown that BSF frass positively affects plant growth and yield (Agustiyani et al. 2021; Anyega et al. 2021; Borkent and Hodge 2021; Fischer et al. 2021; Menino et al. 2021). Many studies have already investigated the effects of BSF frass on plant performance and soil nutrients. However, there is still limited information on the optimal application doses and its impact on organic farming agroecosystems, particularly concerning the structure of arthropod communities.

Soybean (*Glycine max* L.) is an important crop, especially in Japan, where the immature seeds, known as edamame, are a popular product (Koshika et al. 2022). Tsuruoka, a city in Japan, is renowned for producing a local variety of edamame called ‘*dadachamame*.’ The high-quality cultivar *Shirayama-dadachamame* is particularly well-known for its umami and sweet flavor (Kamimura et al. 2016). This study aims to investigate the optimal application dose of BSF frass and its impact on agroecosystems, specifically focusing on the structure of arthropod communities. While previous research has highlighted the positive effects of BSF frass on soil quality, fertility, and plant growth, there remains a gap in our understanding of the appropriate amount of BSF frass to use in edamame crops and its potential effects on the broader ecosystem, particularly arthropod populations.

In this study, however, we did not include a chemical fertilizer as a positive control. Although chemical fertilizers are widely used in conventional farming and serve as a benchmark for evaluating the effectiveness of alternative fertilizers, this study focuses exclusively on organic frass applications. This choice aligns with our objective to prioritize organic methods; however, it limits the study’s ability to make broad claims about the efficacy of frass as a complete substitute for chemical fertilizers. By addressing this gap, we aim to provide valuable insights into the sustainable utilization of BSF frass in agriculture while minimizing any unintended ecological consequences.

MATERIALS AND METHODS

Land preparation and transplanting

The research was conducted on an experimental farm at the Faculty of Agriculture, Yamagata University, Tsuruoka, Japan, from May to June 2022. We utilized a local soybean variety, *dadachamame* cv. ‘Shirayama’. Seedlings were raised in a greenhouse using seed trays filled with commercial seedling soil Kumaya planter soil (Kumaya, Japan). The experimental field, measuring 13.0 × 15.0 m, was divided into 6.0 × 1.0 m plots (Figure 1). Soil preparation involved cultivation with a hand tractor two weeks before planting. Seedlings, approximately 14 days old, were transplanted into individual holes spaced 50 cm apart in both directions. The total number of plants in each plot was 15. The field maintenance was free from chemical input, including pesticides and chemical fertilizers, to represent organic field farming.

Frass preparation

Black soldier flies were obtained from the Yamadai Mizuabu Laboratory, a specialized research facility for black soldier fly studies within the Faculty of Agriculture at Yamagata University, Japan. The larvae were fed food waste collected from Yamagata University Co-op Vert and Tsuruoka Municipal Shonai Hospital, primarily composed of noodles, vegetables, bread, and rice, with a smaller portion of meat. This food waste was processed and homogenized before being provided to the larvae (Table 1). Over a 14-day period, approximately 7 kg of food waste and vegetables in a 5:2 ratio was given to the larvae in a 14-liter container. At the end of this feeding period, frass and prepupae were manually harvested using a 1 mm sieve. The frass contained insect skins, which are rich in chitin. The C/N ratio, total nitrogen (TN), and total carbon (TC) of the frass were analyzed using a total nitrogen analyzer (Sumigraph NC-220 F, Sumika Chemical Analysis Service, Ltd.). Total phosphorus (P) was measured by means of the vanadomolybdophosphoric acid colorimetric method (Kuo 1996) using UV-visible spectrometry, and total potassium (K) content was assessed through atomic absorption spectrometry (Mizuno and Minami 1980) (Table 2).

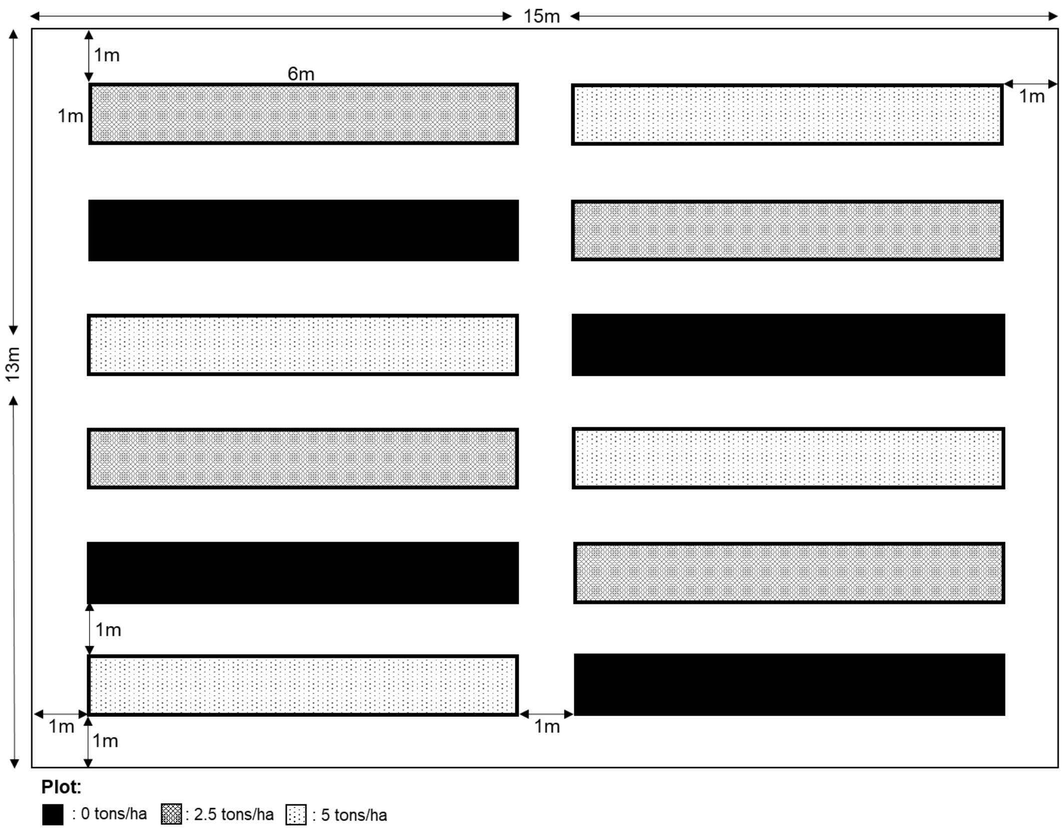

Figure 1. The experimental field plot design

Table 1. List of the BSF larvae feed and nutrient content

Product name	Source	Nutrient content (g)			
		Carbohydrate	Protein	Fat	Salt
Mini Tempura bowl (ミニ丼天丼)	University	75.2	9.4	7.8	2.4
Teriyaki chicken soba bowl (照秋チキンのそばる丼)	University	79	17.6	9.6	4.7
Fried rice and fried chicken (ミニチャハン＆唐揚げ)	University	63.4	11.9	13.6	1
Ramen (ラーメン)	University	45.8	15.4	8.7	5.4
Beef rib bowl (牛カルビ丼)	University	76.4	8.5	5.3	2.3
Chocolate marble snack (チョコマーブル)	University	41.3	5.2	10.5	0.8
Fried fish bento (のり弁当Ｂ（白身魚フライ）)	University	89.8	21.6	30	4
Sandwich（ゆでたまごとハムサンド)	University	28.1	10.5	12.7	2.2
Rice ball (おにぎり)	University	32.8	2.9	0.9	0.8
Carrot skin	Hospital	9.58	0.93	0.24	-
Raddish skin	Hospital	8.36	1.17	0.13	-
Cabbage	Hospital	5.58	1.44	0.12	-
Eggplant skin	Hospital	5.7	0.19	0.19	-

Note: The nutrient content of the product was provided by the company and is included on the product labels. The nutrient content of vegetable waste provided is the amount per 100 g of waste

Table 2. The chemical properties of BSF frass

Chemical properties	Concentration (%)
Total N	4.16±0.15
Total P	8.70±0.67
Total K	4.80±0.27
Total C	30.88±3.67

Frass application

The experiment involved applying BSF frass across different plots one week before transplanting the edamame plants. Three application dose treatments were applied: (i) 0 tons/ha, (ii) 2.5 tons/ha, and (iii) 5 tons/ha. Each treatment was replicated six times.

Plant performance

The characteristics of 20 edamame plants in each treatment were measured, including plant height (cm), number of leaves, and leaf color. The leaf color was measured on the shade of green it was, determined by the SPAD (soil plant analysis development) meter (SPAD-502, Konica). Other parameters were observed after the harvest time. The harvested edamame plants were assessed for their yield, total number of pods, number of empty pods, and the dry weight of the seeds. Drying was conducted in a natural convection oven (DS-64, Yamato) at a temperature of 70°C for three days.

Arthropod collection and identification

Soil arthropods were collected using pitfall traps made from 545 mL plastic cups (CP92-545, Daiso Industries Co., LTD). Each cup was filled with 200 mL of water and 1 mL of detergent to instantly kill the trapped arthropods. The traps were buried approximately 20 cm below the soil surface and placed in the middle of the experimental plots. Each plot contained three traps arranged in a straight line, spaced 15 cm apart. The traps were set for 24 hours and then collected and inspected. Pitfall traps were deployed during the 2nd, 3rd, and 4th weeks after transplanting (WAT). In this experiment, we used only macro-arthropods due to their measurable impacts on pest dynamics.

Foliar arthropods were collected using yellow sticky traps (Holiver, Arista Life Sciences). Each trap measured 10 × 10 cm and was mounted on poles 50 cm above the soil surface. These traps were also left for 24 hours before being collected and inspected. Yellow traps were set on July 12, 2022 and August 3, 2022. In each plot, two traps were placed between plants, with a distance of 50 cm between traps. Each treatment includes a total of 12 traps.

Collected arthropods were identified to the genus level in the laboratory at Yamagata University using a stereo microscope (SMZ800, Nikon). Arthropods were classified to morpho-genus level according to literature such as Higley and Boethel (1994) and Borror et al. (1996). The data on arthropods were then categorized according to their ecological functions.

Data analysis

All statistical analyses were performed using R version 4.2.1. Before conducting the analyses, the normality of the data was tested using the Shapiro-Wilk test (p>0.05), confirming that the data followed a normal distribution. Plant performance parameters and yield were analyzed using a one-way analysis of variance (ANOVA), followed by a post-hoc analysis using the Tukey test at a significance level of p<0.05. Abundances of soil and foliar arthropods were analyzed using a likelihood ratio (LR) test in the Generalized Linear Model (GLM) and implemented using Poisson error. After this, the deviance from the GLM was analyzed using the Chi-square test.

The community structure analyzed by the non-metric multidimensional scaling (NMDS) using the “vegan” package was performed to generate the visualization of assemblage composition on a matrix of Bray-Curtis dissimilarities of abundance data (square root transformed). To test the null hypothesis of species composition after the treatments, we applied analysis of similarities (ANOSIM) on the matrix of Bray-Curtis with 999 permutations. The Shannon diversity (H’) index were determined using the following formula (Ludwig and Reynold 1984):

$$H' = -\Sigma[(ni/N) \text{ In } (ni/N)]$$

The Richness (R) index was determined using the formula:

$$R = (S-1) / \text{In } N$$

The Evenness (E) index was determined using the formula:

$$E = H'/\text{In } S$$

The Simpson dominance (C) index was determined using the formula:

$$C = \Sigma \ (ni/N)$$

Where:
Ni : Total individuals of species i
N : Total individuals found
S : Number of species found.

RESULTS AND DISCUSSION

Effect of different application doses of BSF frass on edamame plants

Plant performances

The study evaluated the impact of three different application doses (0, 2.5, and 5 tons/ha) on plant performance, by measuring plant height, number of leaves, and SPAD values (Table 3). No significant differences were found in plant height (df=2, χ^2=5.39, p=0.07), with a height average of 63.79±1.04 cm, 70.28±2.72 cm, and 71.18±3.10 cm for the application doses of 0, 2.5, and 5 tons/ha, respectively. However, plant height showed increases of 10 and 12% for the application doses of 2.5 and 5 tons/ha. The number of leaves was significantly affected (df=2, χ^2=6.87, p<0.01), with increases of 26% and 31% for the application doses of 2.5 and 5 tons/ha. SPAD values, which indicate chlorophyll content, significantly increased by 16 and 20% for the same treatments (df=2, χ^2=113.9, p<0.001).

Regarding the yield components, no significant differences were found in total pod count (df=2, χ^2=1.68, p=0.43) and pod dry weight (df=2, χ^2=1.18, p=0.55). However, the average number of empty pods showed significant differences across treatments (df=2, χ^2=16.63, p<0.001). The application dose of 2.5 tons/ha significantly increased the number of filled pods by 29% compared to the control (df=2, χ^2=5.88, p<0.05) and reduced the number of empty pods by 52%, indicating improvement of pod quality (Table 2).

The results indicate that although higher application doses did not impact overall yield or seed dry weight, they improved specific yield components, particularly with the application dose of 2.5 tons/ha. This application dose notably enhanced leaf number, chlorophyll content, and number of seeds, and reduced the number of empty pods. An application dose of 2.5 tons/ha is recommended to optimize edamame plant performance and pod quality.

Effects of different application doses of BSF frass on arthropods

Soil arthropod abundance

The study assessed the impacts of three different application doses of BSF frass on the abundance of soil arthropods in an edamame field, identifying 17 species across the treatments (Table 4). The results indicated a significant increase in the total number of arthropods with the highest dose (Table 4, df=2, χ^2=17.93, p<0.001). To be specific, the application of 5 tons/ha resulted in a substantial 313% higher number of arthropods per plot of about 19.40±8.50 individuals/plot compared to the control (0 tons/ha), which had the lowest number of arthropods of about 4.70±1.50 individuals/plot. Additionally, there was a 246% increase in the number of arthropods in the application of 2.5 tons/ha, which resulted in about 5.60±1.60 individuals/plot.

Table 3. Plant performances at three different application doses of BSF frass

Plant performances	Application doses		
	0 tons/ha	2.5 tons/ha	5 tons/ha
Plant growth characteristics			
Height (cm)	63.79±1.04^a	70.28±2.72^a	71.18±3.10^a
Number of leaves	20.26±1.8^a	25.58±2.08^b	26.59±1.57^b
SPAD	38.10±0.69^a	44.14±0.31^b	45.75±0.53^b
Yield attributes			
Yield (g/plant)	89.41±7.78^a	92.96±6.90^a	75.58±11.23^a
Number of filled pods	58.43±4.28^a	64.46±2.87^b	69.66±9.29^b
Number of empty pods	28.83±2.26^b	13.90±1.96^a	20.75±3.35^a
Seed weight (g)	86.60±06.53^a	111.83±04.51^a	77.400±16.17^a

Note: Average ± standard error. The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

Table 4. The average number of individuals (± standard error) of soil arthropod species at three different application doses of BSF frass

Order	Species	Role	Application doses of BSF frass			Notation
			0 tons/ha	2.5 tons/ha	5 tons/ha	
Coleoptera (adult)	*Scarites terricola* Bonelli, 1813	PR	0.39±0.13	0.67±0.15	1.17±0.37	*
	Harpalus variipes Bates, 1883	PR	0.39±0.09	0.22±0.09	0.22±0.12	N.S.
	Tachys laetificus Bates, 1873	PR	0.00±0.00	0.72±0.30	1.56±0.42	***
	Bembidion spp.	PR	0.00±0.00	0.00±0.00	0.61±0.34	***
	Ectinoides insignitus Lewis, 1894	HV	0.00±0.00	0.11±0.11	0.00±0.00	N.S.
	Cardiophorus sp.	N	0.03±0.02	0.03±0.02	0.00±0.00	N.S.
	Harpalus sp.	PR	0.06±0.03	0.00±0.00	0.08±0.04	N.S.
	Holotrichia kiotonensis Brenske, 1894	HV	0.03±0.02	0.00±0.00	0.03±0.02	N.S.
	Anomala flavipennis Burmeister, 1844	HV	0.06±0.03	0.00±0.00	0.00±0.00	N.S.
	Popillia japonica Newman, 1838	HV	0.36±0.16	0.19±0.08	0.22±0.09	N.S.
Orthoptera	*Gryllus bimaculatus* De Geer, 1773	HV	0.22±0.09	0.17±0.07	0.50±0.23	N.S.
	Gryllotalpa orientalis Burmeister, 1838	HV	0.06±0.03	0.03±0.02	0.61±0.24	***
Isopoda	*Armadillidium vulgare* (Latreille, 1804)	N	0.00±0.00	0.08±0.04	0.00±0.00	*
	Armadillidium sp.	N	0.00±0.00	0.00±0.00	0.06±0.03	N.S.
Lithobiomorpha	*Lithobius* sp. (1)	PR	0.03±0.02	0.03±0.02	0.22±0.12	N.S.
	*Lithobius*sp. (2)	PR	0.22±0.07	0.14±0.08	0.19±0.06	N.S.
Trichoptera	Unknown	N	0.00±0.00	0.11±0.05	0.00±0.00	N.S.
Lepidoptera (larva)	*Spodoptera* sp.	HV	0.03±0.02	0.00±0.00	0.00±0.00	N.S.
	Spodoptera litura	HV	0.25±0.10	0.08±0.08	0.47±0.32	N.S.
Hymenoptera	*Tetramorium tsushimae* Emery, 1925	PR	0.03±0.02	0.11±0.05	5.39±1.97	***
Arachnida	*Pardosa astrigera* L.Koch, 1878	PR	0.78±0.14	0.69±0.15	1.00±0.21	N.S.
	Pardosa agraria Tanaka, 1985	PR	0.08±0.04	0.11±0.05	0.06±0.03	N.S.
	Pardosa spp.	PR	0.00±0.00	0.06±0.05	0.00±0.00	N.S.
Average			4.70±1.50^b	5.60±1.60^b	19.40±8.50^a	

Note: PR: Predator, N: Neutral, HV: Herbivore. Average ± standard error. Stars indicate statistical significance based on GLM analysis with Poisson distribution (*** p<0.001, ** p<0.01, * p<0.05, N.S. p<0.05). The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

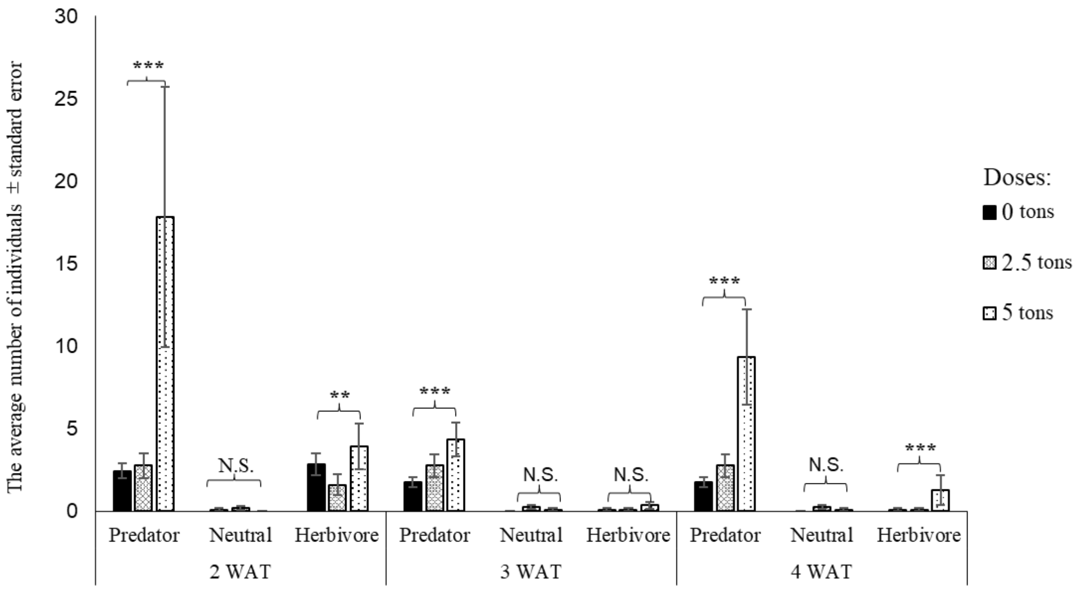

Figure 2. The average number of individuals of soil arthropod species according to their trophic groups at three different application doses of BSF frass at 2, 3, and 4 weeks after treatment (WAT). Significant differences were determined using multiple comparison tests (Tukey's test) (* p<0.05, ** p<0.01, *** p<0.001, N.S. p<0.05)

Predators were the dominant trophic group across all doses, with the 5 tons/ha treatment showing a significant 124% increase in predator abundance compared to the control (Figure 2, df=2, χ^2=18.12, p<0.001). The adult ground beetle was the dominant predator group, comprising seven species. Among these, the *Tetramorium tsushimae* ant was notably abundant in the highest dose treatment, with a statistically significant increase compared to other doses (Table 3, df=2, χ^2=63.65, p<0.05). Herbivores also showed increased abundance with the 5 tons/ha dose (Figure 2, df=2, χ^2=9.29, p<0.001), with *Gryllotalpa orientalis* as the most abundant herbivore, the number of which was significantly higher in the 5 tons/ha plots than that of the control (Table 3, df=2, χ^2=18.73, p<0.05). Detritivores, however, showed no significant difference across treatments (Figure 2, df=2, χ^2=4.21, p=0.12).

As seen in Figure 2, predator abundance at 5 tons/ha was approximately 433% higher than that of the control and 282% higher than that of the 2.5 tons/ha dose. Herbivores also reached peak abundance with the 5 tons/ha treatment, showing an 83% increase compared to the control and a 215% increase compared to the 2.5 tons/ha dose. In contrast, neutral arthropods did not show significant differences across doses (p<0.05) and displayed low average counts, suggesting negligible practical impact of BSF frass on neutral groups. Over time, no significant differences in trophic group abundances were found between weeks after application (predator: df=1, χ^2=1.35, p=0.244; detritivore: df=1, χ^2=3.92, p=0.4; herbivore: df=1, χ^2=0.0001, p=0.99), though specific weeks did show notable patterns. At 2 weeks after treatment (WAT), both predator and herbivore abundances were highest with the 5 tons/ha dose (predator: df=2, χ^2=217.92, p<0.001; herbivore: df=2, χ^2=12.43, p<0.001). At 3 WAT, predator abundance peaked again in the 5 tons/ha dose (df=2, χ^2=13.82, p<0.001). At 4 WAT, predator and herbivore numbers were significantly higher with the 5 tons/ha dose (predator: df=2, χ^2=83.14, p<0.001; herbivore: df=2, χ^2=22.62, p<0.001).

Considering the results above, the 5 tons/ha dose is recommended to increase predator and herbivore abundance, supporting overall arthropod diversity, while the 2.5 tons/ha dose has a minimal effect on neutral arthropods due to their low abundance across treatments.

Foliar arthropod abundance

A total of 2,634 foliar arthropods were collected across three different application doses of BSF frass (0, 2.5, and 5 tons/ha). The results indicated a significant effect of frass on the abundance of foliar arthropods (Table 5, df=2, χ^2=100.87, p<0.001). To be specific, the control treatment (0 tons/ha) had the highest number of arthropods at 105.00±17.80 individuals/plot. The application doses of 2.5 tons/ha resulted in a significantly lower number of arthropods at 52.00±4.50 individuals/plot, a 50.5% decrease compared to the control. The application doses of 5 tons/ha had a total number of arthropods at 62.30±17.20 individuals/plot, representing a 40.7% decrease compared to the control. These results suggest that although the control plots had the highest abundance of arthropods, the application doses of BSF frass at both 2.5 and 5 tons/ha reduced arthropod numbers significantly.

Table 5. The average number of individuals (± standard error) of foliar arthropod species at three different application doses of BSF frass

Order	Species	Role	Application doses of BSF frass			Notation
			0 tons/ha	**2.5 tons/ha**	**5 tons/ha**	
Diptera (adult)	*Musca domestica* Linnaeus, 1758	N	4.50±0.81	0.67±0.14	1.42±0.42	***
	Sarcophaga peregrina (Robineau-Desvoidy, 1830)	N	0.00±0.00	0.25±0.25	4.42±0.16	***
	Bradysia sp. (Fungus gnat)	N	3.33±1.32	3.58±1.09	9.83±2.90	***
	Culiseta nipponica LaCasse & Yamaguti, 1950	N	8.00±2.32	0.83±0.30	2.25±0.79	***
	Dolichopus plumipes (Scopoli, 1763)	N	2.00±0.39	0.75±0.30	0.67±0.31	**
	Drosophila sp.	N	0.17±0.11	0.00±0.00	0.33±0.33	N.S.
	Cricotopus sp.	N	0.00±0.00	0.17±0.11	0.00±0.00	N.S.
	Agromyza wistariae Sasakawa, 1961	HV	0.00±0.00	7.33±0.92	0.42±0.42	***
	Cerodontha denticornis (Panzer, 1806)	HV	0.00±0.00	1.67±0.99	0.25±0.25	***
	Gnoriste mikado Okada, 1939	N	0.00±0.00	0.83±0.21	0.00±0.00	***
	Dysmachus sp.	PR	0.00±0.00	0.17±0.11	0.00±0.00	N.S.
	Eupeodes bucculatus Rondani, 1857	PO	0.17±0.11	0.83±0.51	0.00±0.00	**
Coleoptera (adult)	*Aulacophora* sp.	HV	0.08±0.08	0.33±0.22	0.33±0.14	N.S.
	Altica cyanea (Weber, 1801)	HV	0.00±0.00	0.00±0.00	0.17±0.11	N.S.
	Psylliodes sp.	HV	0.00±0.00	0.33±0.14	0.17±0.17	*
	Phyllotreta cruciferae (Goeze, 1777)	HV	0.00±0.00	0.33±0.22	0.00±0.99	N.S.
	Chaetocnema sp.	HV	0.33±0.22	0.00±0.00	0.17±0.11	N.S.
	Tachys sp.	PR	0.00±0.00	0.00±0.00	0.17±0.11	N.S.
	Propylea japonica (Thunberg, 1781)	PR	0.33±0.14	0.00±0.00	0.17±0.11	N.S.
	Coccinella septempunctata Linnaeus, 1758	PR	0.17±0.11	0.08±0.08	0.50±0.23	N.S.
	Ectinoides insignitus (Lewis, 1894)	PR	0.17±0.11	0.08±0.08	0.17±0.11	N.S.
Hymenoptera (adult)	*Pemphredon* spp.	PR	0.25±0.18	0.42±0.19	0.42±0.19	N.S.
	Xiphozele compressiventris Cameron, 1906	PR	0.00±0.00	0.17±0.11	0.00±0.00	***
	Cotesia plutellae (Kurdjumov, 1912)	PR	6.00±1.11	0.17±0.11	0.83±0.32	***
	Synopeas sp.	PR	0.50±0.23	12.08±1.53	3.67±1.79	***
	Pachyneuron formosum Walker, 1833	PR	0.00±0.00	0.00±0.00	0.17±0.17	N.S.
	Brachyponera chinensis (Emery, 1895)	PR	1.67±0.45	0.25±0.13	0.50±0.23	**
Hemiptera	*Acyrthosiphon pisum* (Harris, 1776)	HV	8.17±1.55	8.67±0.47	5.50±1.60	**
	Macrosteles quadrilineatus (Forbes, 1885)	HV	13.00±1.87	3.17±0.75	11.83±3.65	***
	Geocoris sp.	HV	0.33±0.14	0.25±0.13	0.83±0.44	N.S.
Lepidoptera (adult)	*Lycaena* sp.	HV	0.00±0.00	0.17±0.11	0.42±0.15	*
	Colias philodice Godart, 1819	HV	0.00±0.00	1.00±0.30	0.17±0.11	***
	Unknown	HV	0.08±0.08	0.17±0.11	0.00±0.00	N.S.
	Spodoptera sp.	HV	0.17±0.11	0.17±0.11	0.17±0.17	N.S.
Orthoptera	*Conocephalus longipennis* (Haan, 1843)	HV	0.75±0.43	0.08±0.08	0.83±0.37	**
	Gryllus bimaculatus De Geer, 1773	HV	0.67±0.43	0.08±0.08	0.67±0.36	*
	Chorthippus latipennis (Bolívar, 1898)	HV	0.00±0.00	0.08±0.08	0.00±0.00	N.S.
Thysanoptera	*Thrips* sp.	HV	54.33±14.60	6.83±1.22	14.92±9.55	***
Average			105±17.8^{a}	52±4.5^{b}	62.3±17.2^{b}	

Note: PR: Predator, HV: Herbivore, N: Neutral, PO: Pollinator. Average ± standard error. Stars indicate statistical significance based on GLM analysis with Poisson distribution (*** p<0.001, ** p<0.01, * p<0.05, N.S. p<0.05). The same letters after means within the same column indicate there is no significant difference according to the Tukey test results, with a significance level of 5%

The absence of BSF frass led to the highest overall arthropod abundance with herbivores particularly dominant. Thrips were significantly more abundant without BSF frass, constituting about 86% of their total population (Table 5, df=2, χ^{2}=588.47, p<0.001). The application doses of 2.5 tons/ha supported the highest abundance of predatory arthropods, which represented about 58% of the predator total, notably *Synopeas* sp. (Table 5, df=2, F=19.11, p<0.05). Neutral insects were most abundant in the application doses of 5 tons/ha, making up 38% of the total neutral population, but this difference was not statistically significant (df=2, χ^2=67.41, p=0.174). The Fungus gnat showed a significant increase at 5 tons/ha, comprising about 58% of their total (Table 5, df=2, χ^2=554.16, p<0.001). According to Figure 3, predators were significantly more abundant at 2.5 tons/ha, representing approximately 53% of the predator total (df=2, χ^2=29.48, p<0.001), and this dose also supported a diverse range of other beneficial insects.

Figure 3 showed there were no statistically significant differences in the abundance of all trophic groups between months (predator df=1, χ^2=1.24, p=0.26; neutral df=1, χ^2=3.03, p=0.08; herbivore df=1, χ^2=1.60, p =0.20) in all treatments (i.e., plots with 0, 2.5 and 5 tons/ha of BSF frass). However, predators were abundant in July (df=1, χ2=8.03, p<0.001) only, and herbivores were abundant in July and August (Figure 3: July df=1, χ^2=109.95, p<0.001; August df=1, χ^2=102.07, p<0.001). Given these findings, the application dose of 2.5 tons/ha of BSF frass is recommended as it optimally balances the promotion of beneficial predators while controlling the overall abundance of herbivores and neutral insects.

Soil arthropod community structure

The study analyzed the diversity of soil arthropods in edamame fields treated with different doses of BSF frass using various diversity indices. In Table 6, the diversity index (H') indicated a slightly higher diversity index at 2.5 tons/ha compared to 0 tons/ha, and lower diversity at 5 tons/ha, suggesting that moderate dose frass application promotes higher species diversity. The Richness (R') index was highest at 2.5 tons/ha, indicating the presence of more species in this treatment. The Evenness (E') index showed that species were more evenly distributed at lower doses, implying a balanced community structure. Simpson dominance (C') index was higher at 5 tons/ha, suggesting that a few species dominated the community at this higher dose. Given the Bray-Curtis similarity coefficient, NMDS (Figure 4) was conducted on the community structure of soil arthropods. NMDS analysis showed a good fit (stress=0.08) and an overlap between treatments, indicating similar community structures across different frass applications. This was supported by ANOSIM results (R=0.03, *p*=0.0014), indicating statistically significant differences in arthropod communities among treatments, with an even distribution of high and low ranks within and between groups. Given these findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields, as it optimally enhances species diversity and maintains a balanced arthropod community without the dominance issues observed at higher doses.

Table 6. Diversity, Richness, Evenness, and Simpson Dominance indices of soil and foliar arthropods at different application doses of BSF frass

Indices	Application doses		
	0 tons/ha	2.5 tons/ha	5 tons/ha
Soil arthropods			
H'	2.26	2.34	1.97
R'	5.41	5.64	5.41
E'	0.81	0.82	0.71
C'	0.13	0.13	0.22
Foliar arthropods			
H'	1.76	2.43	2.39
R'	7.01	8.94	8.52
E'	0.56	0.70	0.70
C'	0.30	0.13	0.13

Note: H': Diversity indices, R': Richness indices, E': Evenness indices, and C': Simpson dominance

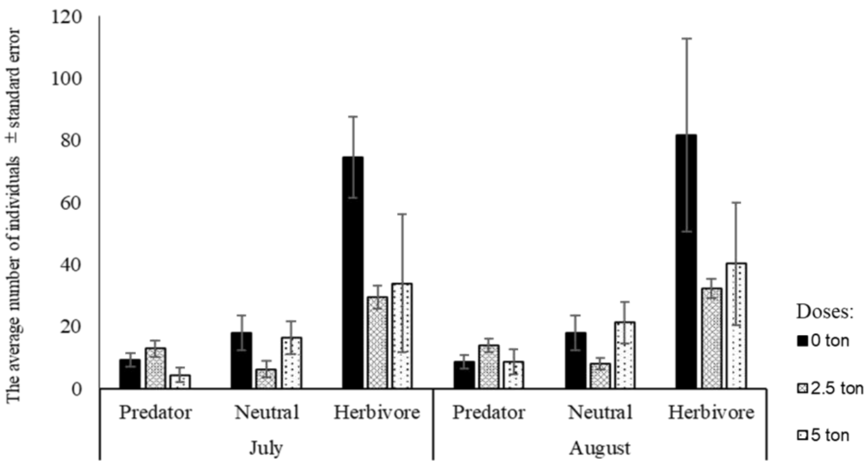

Figure 3. The average number of individuals of foliar arthropod species according to trophic groups at three different application doses of BSF frass at 2, 3, and 4 weeks after treatment (WAT). Significant differences were determined using multiple comparison tests (Tukey's test) (* *p*<0.05, ** *p*<0.01, *** *p*<0.001, N.S. *p*<0.05)

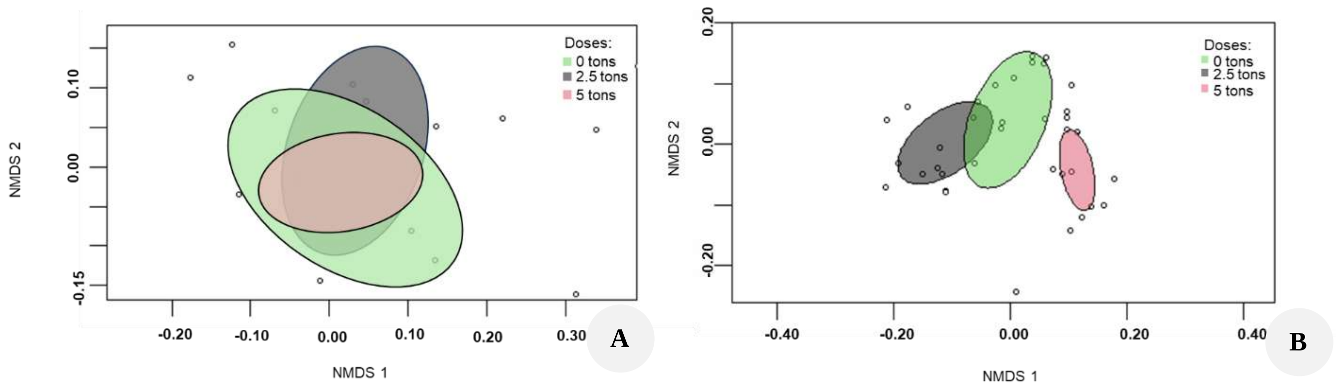

Figure 4. The non-metric multidimensional scaling (NMDS) ordination of A. Soil arthropods and B. Foliar arthropods community structure at three different application doses of BSF frass

Foliar arthropod community structure

The study analyzed the diversity of foliar arthropods in edamame fields treated with different doses of BSF frass using various diversity indices. In Table 6, the Shannon diversity (H') indices were higher at both 2.5 tons/ha and 5 tons/ha compared to the control (0 tons/ha), suggesting that frass application enhances species diversity. The Richness (R') index was highest at 2.5 tons/ha, indicating a greater number of species in this treatment. The Evenness (E') index showed that species were more evenly distributed at 2.5 tons/ha and 5 tons/ha, suggesting a balanced community structure at these doses. The Simpson dominance (C') index was highest at 0 tons/ha, indicating that a few species dominated the community without frass. The NMDS (Figure 4) analysis showed a good fit (stress=0.11) and an overlap existed between plots treated with 5 tons/ha and those with 0 tons/ha. However, this overlap was supported by ANOSIM results (R=0.59, p<0.001), indicating a significant difference in arthropod communities among treatments, with substantial differentiation between groups. Given these findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields, as it optimally enhances species diversity and maintains a balanced arthropod community without the dominance issues observed at higher or zero doses.

Discussion

This study demonstrated the impact of different application doses of BSF frass on the performance and yield of edamame plants and on the abundance of soil and foliar arthropods. The results revealed varied effects, providing insights into optimal dose recommendations for maximizing plant growth, yield, and insect diversity. Plant performance data indicated that an application dose of 2.5 tons/ha was optimal. These findings are consistent with a previous study on lettuce, which revealed that higher concentrations of BSF frass led to stunted plant growth (Chiam et al. 2021). A previous study by Beesigamukama et al. (2021) also indicated that 2.5 tons/ha is sufficient for plant performance and yield. Similarly, Kawasaki et al. (2020) noted that a high dose of BSF frass could cause damage. However, these findings differ from other studies, which observed increased plant height with high applications of BSF frass on mustard, kale, swiss chard, and maize (Abiya et al. 2022; Tanga et al. 2022). This suggests that each plant has different requirements for BSF frass dose, with edamame needing only 2.5 tons/ha.

The edamame yield study further illustrated the specific effects of different application doses of BSF frass. It increased plant nitrogen (N) uptake by up to 23% and had higher nitrogen recovery efficiencies than those of other organic fertilizers (Beesigamukama et al. 2020). The quality of the frass also plays a crucial role in influencing soil nitrogen availability and subsequent plant growth (Kagata and Ohgushi 2012). The enhanced nitrogen levels in the soil are particularly beneficial for soybean pod filling, given soybeans' high nitrogen requirement (Serafin-Andrzejewska et al. 2024). These findings highlight the efficacy of using 2.5 tons/ha of BSF frass to optimize edamame yield through improved nitrogen management and pod filling, thereby enhancing overall plant productivity.

Furthermore, the study explored the community structure and abundance of soil and foliar arthropods in response to different application doses of BSF frass. Given the study's findings, an application dose of 2.5 tons/ha of BSF frass is recommended for edamame fields. This dose optimally enhances soil and foliar arthropod diversity, maintains a balanced arthropod community, and supports the highest abundance of beneficial predatory arthropods while controlling herbivore populations. The mechanism behind these changes likely involves multiple factors, one of which is the nutrient enrichment in the soil resulting from treatment with BSF frass (Hasibuan et al. 2022; Shahbuddin et al. 2023). Increased plant biomass offers more foliage for herbivores to feed on, supporting larger herbivore populations (Bonser and Reader 1995; Katz 2016). Predators, benefiting from the larger prey base, also increase in number, contributing to a more balanced and dynamic ecosystem (Snyder and Evans 2006).

The results showed that higher doses of organic fertilizer can increase the population of soil arthropods (Lin et al. 2013; Wang et al. 2015). In this research, BSF frass increased organic matter and nutrients in the soil, which can create a more favorable environment for soil arthropods. This is also consistent with studies that found that the high content of organic matter enhances arthropod abundance (Gunadi et al. 2002; Kautz et al. 2006; Salah et al. 2018; Viketoft et al. 2021). BSF frass contains nitrogen, phosphorus, potassium, micronutrients, and beneficial microbes that enhance soil health (Watson et al. 2021; Boudabbous et al. 2023). Research by Peng et al. (2023) found that insect frass improves soil structure by forming aggregates, enhancing aeration and root penetration. This improved structure also retains moisture longer, benefiting plants and soil organisms (Beesigamukama et al. 2021). The microbes in BSF frass increase microbial activity, which is crucial for nutrient cycling (Houben et al. 2020; Poveda 2021; Gebremikael et al. 2022), and can work synergistically with plant growth-promoting rhizobacteria (PGPR) (Fuertes-Mendizábal et al. 2023). Additionally, nutrients such as calcium and magnesium in BSF frass play key roles in plant physiology and soil pH regulation, promoting a balanced soil environment (Gärttling and Schulz 2022; Lopes et al. 2022). The results showed that ants dominate in the highest doses. Ants thrive in soil with high nutrient content (Farji-Brener and Werenkraut 2017; Vaidya and Vandermeer 2021), which is supported by Vaidya and Vandermeer (2021), who found that ants prefer plants with higher fertilizer levels. These findings highlight the positive impact of high organic matter and nutrient content on predator populations, particularly ground beetles and ants, in agricultural fields. The organic material might change the environmental condition of the soil. In the soil that was treated with organic fertilizer, the number of microorganisms was higher by 7% compared to the soil without any application of fertilization (Bebber and Richards 2022). The soil microorganisms are highly likely to become the source of food for microfauna such as detritivores and indirectly increase the predator presence. In other words, organic fertilization enhances the complexity of ecological relationships between microbes and microfauna, impacting food web dynamics and predator-prey interactions (Suleiman et al. 2019). This combination supports soil-dwelling arthropods, contributing to pest control and nutrient cycling, which enhances overall soil health and plant productivity (Kuťáková et al. 2018).

The application of high doses of BSF frass decreased the number of herbivores. This condition is in contrast with the application of other types of fertilizer that result in herbivores having the highest abundance in the higher doses (Chen et al. 2008; Trisnawati et al. 2015; Rashid et al. 2017). The nutrient enrichment of the soil from fertilizer enhances plant growth, providing more food and habitat resources for arthropods (Hasibuan et al. 2022; Shahbuddin et al. 2023). However, BSF frass potentially decreases the herbivore population by increasing the plant defense mechanism (Lopes et al. 2022). Plants grown with organic fertilizer often have higher concentrations of plant defense chemical compounds than they do if grown with synthetic fertilizers (Staley et al. 2011). In addition to enhancing plant resilience to herbivores, organic fertilizers can support biocontrol activities. Through bottom-up impacts, herbivore abundance is decreased and parasitism rates are increased due to better soil nutrient content and plant metabolism (Gu et al. 2022).

In conclusion, the study provides valuable insights into the multifaceted effects of different application doses of BSF frass on edamame plant performance, yield, and arthropod communities. Given these findings, an application dose of 2.5 tons/ha of BSF frass is optimal for edamame fields, resulting in enhanced plant performance, yield, and arthropod diversity. The findings align with previous studies indicating that moderate dose frass applications improve plant growth and soil health, driven by nutrient enrichment and improved soil structure. However, further research is warranted to elucidate the long-term effects of BSF frass on plant-soil-arthropod interactions and its broader implications for sustainable agriculture.

ACKNOWLEDGEMENTS

This research was funded by Yamagata University, Japan. We would like to express our sincere gratitude to all members of the Yamadai Mizuabu Laboratory, Yamagata University, Japan for their invaluable support throughout this research. We also extend our appreciation to the reviewers for their constructive comments and insightful advice, which greatly contributed to the improvement of this manuscript.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 52-59 DOI: 10.13057/asianjagric/g090106

Effect of selenium and zinc fortification on the physiochemical and sensory properties of Iraqi soft cheese

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Manuscript received: 18 September 2024. Revision accepted: 2 December 2024.

Abstract. *Al-Garory NH, Al-Musa RS, Al-Hashimi AG. 2025. Effect of selenium and zinc fortification on the physiochemical and sensory properties of Iraqi soft cheese. Asian J Agric 9: 52-59.* Food industries, including the dairy industry, have developed to improve the health condition as they are the foods that provide the body with important nutritional components such as minerals, whose levels decrease after industrialization processes. This study aimed to evaluate the impact of fortifying Iraqi soft cheese with zinc and selenium. The study focused on the physicochemical properties, rheological behavior and sensory characteristics of the cheese along with peroxide value as an indicator of oxidative stability. Zinc and selenium elements were added at a concentration of 200 mg/L to milk for cheese making. The result showed that an increase in peroxide value with increasing storage time and a decrease in fat oxidation for zinc and selenium were noted in treatments compared to the control treatments made from buffalo milk (T1) and cow's milk (T2). This may be due to the presence of selenium and zinc, which reduce fat oxidation, and lower pH values after storage. No effect of selenium and zinc fortification was observed on the acidity values of cheese, while the chemical composition values varied as selenium fortification did not affect the percentage of fat and protein. Microbial tests also showed an increase in bacteria number after 10 days of storage compared to the samples after manufacturing, as well as increase in total bacterial number after fortification. The hardness and consistency of cheese increased during storage and after addition of minerals compared to control samples. Cheese flexibility decreased after storage and fortification. The sensory indicators were almost similar in control and fortified samples.

Keywords: Buffalo milk, cow milk, functional foods, Iraqi soft cheese, microbial counts, peroxide value, rheological properties

INTRODUCTION

In recent years, new methods and practices have been developed to improve the nutritional value and therapeutic benefits of food as well as maintain quality and safety standards in accordance with globally accepted laws (Terry and Diamond 2018). This has led to increased interest in functional foods, including fortified foods, especially with increased consumer awareness to maintain health and get rid of diseases as they do not cause side effects like synthetic drugs (Reuben et al. 2023). Functional food science arose from the link between food science and medical science, as researchers studied food components and their health effects by studying changes in health behavior and the balance of health indicators in the body (Lubna and Ahmad 2023). Such studies led to determining the health effects and safe doses of functional foods, which is basically a global marketing term for food if it is proven to have a beneficial effect on body functions in health and disease (Fawi and Salman 2023). The dairy industry is one of the most functional industries in the world due to the use of probiotics in its manufacture (Uzunsoy 2024). One of these foods is cheese, which is made from coagulating milk caseins and separating it from the whey using microbial starters that modify the appearance, texture, odor, nutrient composition, improve quality and extend shelf life (Reuben et al. 2023). Essential trace elements play an important role as cofactors for some enzymes involved in cellular metabolism and growth and most of them are involved in protein, carbohydrate, lipid, and energy metabolism (Fawi and Salman 2023). They are essential for human growth and development, muscle and nerve function, normal cellular functioning, and the synthesis of certain hormones and connective tissues (Reuben et al. 2023). The role of trace elements in bioprocessing may provide the key to understanding the etiology of certain diseases such as cancer (Çelebi et al. 2023). In the last decade, studies have focused intensively on determining the levels of trace elements in cancer patients to understand the nature of the relationships between cancer and trace elements (Naumova et al. 2020). The expected role of trace elements will enable us to understand the causes of cancer, provide a rapid diagnostic method and establish effective treatment methods (Al-Fartusie and Mohssan 2017). Recently, the use of cheeses fortified with minerals has increased due to their important benefits, Milk contains small amounts of zinc and selenium, estimated at less than 1 mg per cup and among these minerals is selenium, which has a role in the balance of the human body and the work of the immune system, as well as being an antioxidant and a cofactor for many enzymes (Batool et al. 2018). It also affects the redox balance and metabolism for hormones, including thyroid hormone and protecting the body from infections (Uzunsoy 2024). Its deficiency can lead to cardiovascular diseases, neuromuscular diseases, cancer, infertility, diabetes, depression, immune diseases, and increased intake causes poisoning, liver disease, deterioration of blood composition, bone tissue deformation, neurological disorders, and type 2 diabetes (Terry and Diamond 2018). Zinc and selenium are an essential element in food and are the second most abundant elements after iron (Çelebi et al. 2023). It is used as an antioxidant and anti-inflammatory, as well as essential for liver function and metabolism, and its lack of dietary intake or increased elimination through excretion or abnormal metabolism causes liver disease (Lubna and Ahmad 2023). Zinc is essential for the catalytic activity of about 100 enzymes and has a role in immune function, protein synthesis, wound healing, DNA synthesis and cell division (Maret and Sandstead 2015). Human lack a specific storage system for zinc, so its intake is necessary to maintain its viability, its deficiency leads to growth retardation, loss of appetite and impotence in men (Mostafa 2022). The aim of this study was to evaluate the effect of fortifying Iraqi soft cheese with zinc and selenium of these products, or the bacterial strains used, and to optimize the daily Se and Zn requirements of the consumer. The result of this study may be beneficial to people, especially those with zinc and selenium deficiency, or vegetarians who do not consume meat, as well as its nutritional value and its therapeutic and health benefits.

MATERIALS AND METHODS

Experimental design

The present study was conducted in the Department of Food Science, Faculty of Agriculture, University of Basrah, Iraq. The cow's milk was obtained from the Agricultural Research Station, and Iraqi buffalo milk from a breeder in the Garma area in Basra. The fungal microbial rennet *Rhizomucor pusillus* used was produced by the Japanese company Meito Sangyo Co, Ltd, within the validity period and according to the manufacturer's recommendations. The selenium and zinc were purchased from the British company BDH.

Cheese manufacturing

Soft cheese was made according to the method of Fasale et al. (2017) after pasteurizing the milk at 65°C for 15 minutes and cooling to 45°C. Zinc and selenium elements were added at a concentration of 200 µg/L. The rennet was used according to the manufacturer's instructions. Samples made from buffalo milk (T1) and cow's milk (T2) were considered as control treatments, while buffalo milk cheese treatments with added zinc and selenium were T3 and T4, and cow's milk cheese treatments were (T5) and (T6). Three replicates were made for each concentration which were left for 30 minutes at 45°C. The curd was cut into cubes, filtered and stored at 4°C. The peroxide value, physicochemical, microbial, rheological, and sensory properties of soft cheese were calculated over three storage periods of 0, 3, 7 and 10 days.

Cheese analysis

Peroxide value estimation

The peroxide value was estimated based on (AOAC 2008). 0.5 g of Buffalo and cow cheese oil were dissolved in 10 mL of chloroform, after that 15 mL of acetic acid, and saturated 1 mL of potassium iodide were added. Each of these solutions was gently stirred and incubated in the dark for 10 minutes. After incubation, 20 mL of distilled water and 0.3 mL of (1%) starch was added to the solution and mixed. The solutions were then titrated with thiosulphate 0.01 N till the color was transparent.

Physiochemical tests

pH was estimated using a Sartorius pH meter (Germany) and acidity by titration with 0.1 N NaOH. The percentage of protein was evaluated by Kjeldahl's method to estimate total nitrogen and multiplied by the protein factor 6.38. The percentage of fat was determined by Kerber's method, while moisture and ash were estimated by burning method (AOAC 2008).

Microbial test

Plate count agar technique was used to determine the total viable microbial count in the samples (Ali et al. 2020). Serial dilutions of the samples were prepared up to 10^5 and 10^6. From each dilution, 1 mL aliquots were plated on Plate count agar medium and incubated in an incubator at 32±1°C for 48 hours. Petri dishes were placed upside-down to prevent condensation from dripping onto the agar surface. After incubation, plates were placed on a colony counter to enumerate the bacterial colonies. Total bacterial counts in soft cheese were estimated using nutrient agar medium (Oxoid) and *Escherichia coli* using MaConkey agar medium (Holland) according to the manufacturer's recommendations.

Rheological tests

Tissue properties, such as elasticity, cohesion, and stiffness were estimated using a Testometric Texture Analyzer M350-10CT (AOAC 2008).

Sensory tests

Sensory evaluation of soft cheese parameters was performed by 10 specialized evaluators from the Department of Food Science, College of Agriculture, Basra University. The test, included flavor, texture, and appearance qualities (El-Desoki and Hamed 2022).

Data analysis

The data was statistically analyzed using CRD, ANOVA and LSD tests to calculate significant differences between the mean coefficients at a probability level of 0.05 using Genstat software 12.1 (Schmuller 2017).

RESULTS AND DISCUSSION

Peroxide value

The peroxide value is an indicator of fat oxidation and product spoilage. The peroxide values for both control cheese (T1 and T2) and fortified cheese (T3, T4, T5 and T6) are presented in Figure 1. The results of statistical analysis showed that there were significant differences ($P<0.05$) in the peroxide value of the buffalo milk cheese samples T3 and T4 compared to the cow's milk cheese samples. This may be due to higher fat percentage in buffalo milk compared to cow milk, the peroxide value of buffalo milk cheese increased compared to cow milk cheese. The results also showed that there were significant differences ($P<0.05$) in the peroxide value with increasing storage periods. The peroxide value increased as the storage periods progressed, at storage time 0 it was 0.32, 0.25, 0.32, 0.30, 0.25 and 0.22 $MeqO_2$/kg respectively, which increased to 0.88, 0.66, 0.58, 0.54, 0.51 and 0.52 $MeqO_2$/kg at the storage period of 10 days. The antioxidant activity of cheese is due to the degradation of proteins by proteolytic enzymes and the production of bioactive peptides with the ability to inhibit lipid peroxidation due to their content of the amino acids histidine, methionine, cystine and tryptophan which possess antioxidant activity by capturing free radicals, inhibiting peroxides and binding to metal ions (Uzunsoy 2024). A decrease in lipid oxidation was observed for zinc and selenium treatments (T3, T4, T5 and T6) compared to control treatments T1 and T2. The presence of selenium and zinc reduces lipid oxidation, free radical formation, and metallic taste production (Batool et al. 2018).

pH and acidity

Figures 2.A and 2.B exhibit the pH values and percentage of acidity of control cheese treatments (T1 and T2) and buffalo and cow milk cheese fortified with zinc and selenium (T3, T4, T5 and T6) during storage periods of 0, 3, 7 and 10 days. The results showed that significant differences were observed at a probability level of $P<0.05$ for pH but there was no difference for acidity values in all treatments. The pH values of (T1) and (T2) immediately after manufacturing were 6.23 and 6.25, and for (T3, T4, T5 and T6) were 6.21, 6.22, 6.4 and 6.25, respectively. The post-storage values decreased in all treatments, reaching 5.61, 5.64, 5.61, 5.63, 5.63 and 5.64% after the 10^{th} day of storage at 4°C (in refrigerator). This can be attributed to the continued activity of starter bacteria during storage to produce lactic acid, reducing pH and raising acidity.

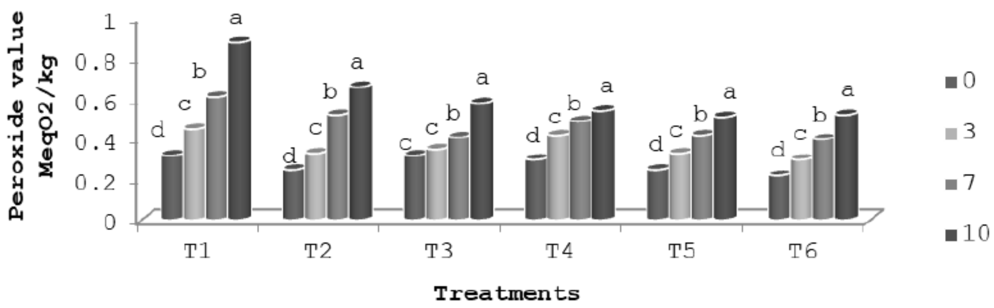


Figure 1. Peroxide value ($MeqO_2$/kg) of soft cheeses made from buffalo and cow milk fortified with zinc and selenium during different storage periods. *Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P<0.05$)

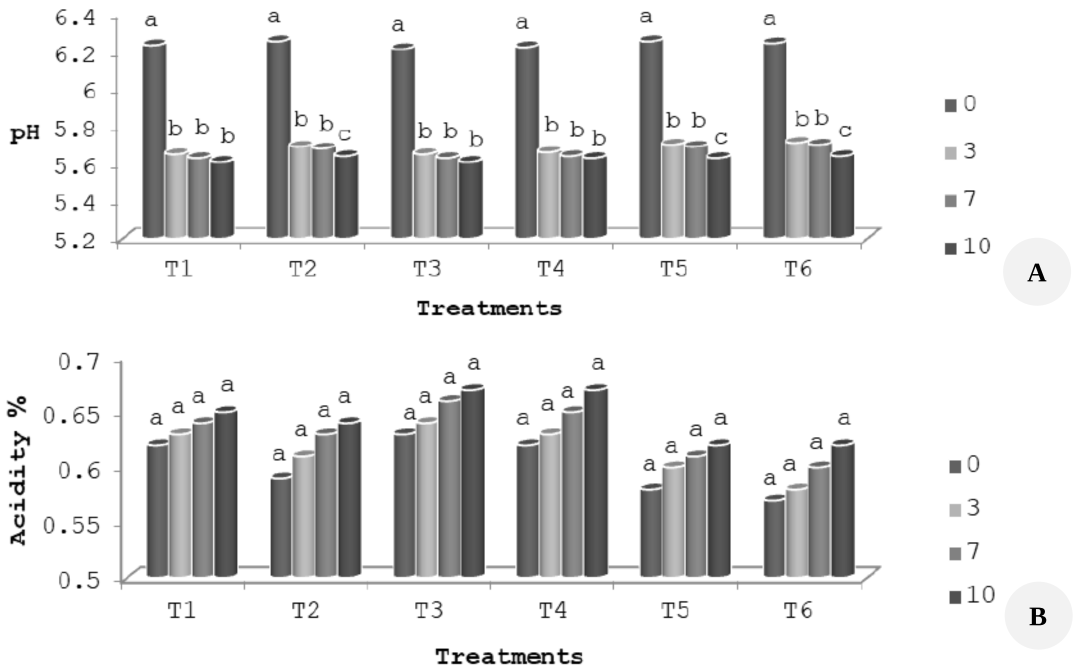


Figure 2. A. pH and B. Acidity of soft cheeses made from buffalo and cow milk fortified with zinc and selenium during different storage periods. *Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P<0.05$)

Table 1. Chemical composition of soft cheese made from buffalo and cow's milk fortified with zinc and selenium during different storage periods

Treatments	Storage periods/days	Ash %	Moisture %	Fat %	Protein %
T1	0	3.68[a]	59.70[a]	15.68[a]	20.98[a]
	3	3.81[a]	59.52[a]	15.69[a]	21.12[a]
	7	3.83[a]	59.32[a]	15.75[a]	21.16[a]
	10	3.83[a]	59.09[a]	15.80[a]	21.20[a]
T2	0	3.61[a]	62.40[a]	14.40[a]	19.61[a]
	3	3.74[a]	62.30[a]	14.44[a]	19.67[a]
	7	3.75[a]	62.22[a]	14.48[a]	19.73[a]
	10	3.77[a]	62.12[a]	14.56[a]	20.77[a]
T3	0	3.78[a]	59.57[a]	15.68[a]	20.97[a]
	3	3.80[a]	59.46[a]	15.69[a]	21.15[a]
	7	3.83[a]	59.22[a]	15.73[a]	21.17[a]
	10	3.85[a]	59.05[a]	15.81[a]	21.21[a]
T4	0	3.75[a]	59.57[a]	15.65[a]	20.95[a]
	3	3.78[a]	59.45[a]	15.67[a]	21.13[a]
	7	3.80[a]	59.20[a]	15.70[a]	21.19[a]
	10	3.84[a]	59.10[a]	15.83[a]	21.22[a]
T5	0	3.70[a]	62.30[a]	14.40[a]	19.60[a]
	3	3.73[a]	62.28[a]	14.43[a]	19.66[a]
	7	3.75[a]	62.18[a]	14.45[a]	19.72[a]
	10	3.78[a]	61.07[a]	14.53[a]	20.75[a]
T6	0	3.69[a]	62.30[a]	14.35[a]	19.60[a]
	3	3.70[a]	62.26[a]	14.40[a]	19.67[a]
	7	3.74[a]	62.14[a]	14.43[a]	19.75[a]
	10	3.79[a]	61.02[a]	14.54[a]	20.76[a]

Note: *Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability (P<0.05)

Similar results were found by El-Desoki and Hamed (2022) who observed a decrease in pH of selenium-fortified cheese after 60 days, which amounted to 4.43 compared to the values on the first day of manufacture of 6.71. This can be explained by continuous activity of starter bacteria during the storage process without the effect of selenium and zinc on bacterial activity. No effect of selenium and zinc fortification was observed on the acidity values calculated on the basis of lactic acidity of the cheese, as the percentage of acidity immediately after processing for (T1) and (T2) (0.59 and 0.62%). For fortified cheese (T3, T4, T5 and T6), pH was 0.57, 0.58, 0.62 and 0.63%, respectively. The results also show that pH for all treatments increased with storage, so the values after 10 days were 0.65, 0.64, 0.67, 0.67, 0.62 and 0.62%, respectively. These results are consistent with El-Desoki and Hamed (2022) who found that acidity at the end of storage period after 60 days increased to 1.38% compared to 0.21% immediately after manufacturing.

Chemical composition

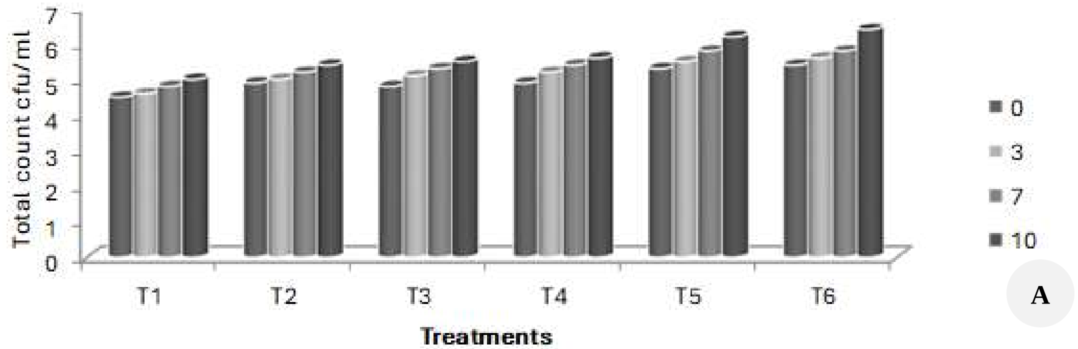
Table 1 shows the percentages of protein, fat, moisture and ash content of (T1, T2, T3, T4, T5 and T6) during a storage of 0, 3, 7 and 10 days. The results of statistical analysis indicate that there were no significant differences in the values of protein, fat, moisture and ash at a probability level of P<0.05. Protein content increased with increasing storage time in all treatments (T1, T2, T3, T4, T5 and T6) 21.20, 20.77, 21.21, 21.22, 20.75 and 20.76 after 10 days of storage. The increase in protein content as the storage period increases is due to the decrease in moisture content and the increase in solids content, including protein (Batool et al. 2018). The percentage of fat increased with storage periods, and varied between the control and the zinc and selenium-fortified treatments. Zinc fortification had no significant effect on it. The fat percentage at day 0 was 15.85, 14.40, 15.68, 15.68, 14.40 and 14.35% for (T1, T2, T3, T4, T5 and T6), which increased after 10 days to 15.85, 14.56, 15.81, 15.83, 14.53 and 14.54%. According to Batool et al. (2018), selenium fortification did not influence the fat and protein content of cheese. The reduction in fat percentage observed during cheese production was primarily due to the fermentation process. Furthermore, decrease in fat percentage during storage was attributed to the progressive degradation of fat components over time. A decrease in the percentage of moisture was observed for all samples as the storage time progressed. The moisture in day 0 decreased from 59.70, 62.40, 59.57, 59,57, 62.30, 62.30% to 59.09, 62.12, 59.05, 59.10, 61.07 and 62.02% after 10 days. The ash content increased during storage for all treatments and the values after 10 days were 3,83, 3.77, 3.85, 3.84, 3.78, and 3.75%. The values also increased after zinc and selenium fortification where the ash content in T1 and T2 reached 3.68 and 3.61%. The values of treatments (T3, T4, T5 and T6) also increased after fortification to 3.78, 3.75, 3.70 and

3.69%, respectively. The reason for the high percentage of total solids, including proteins and fats, is due to evaporation and low moisture content in storage and consolidation, which increases the percentage of solids (Al-Shaikh and Doosh 2018). About 32% of the zinc in cow's milk is bound to casein and about 63% is bound to colloidal calcium phosphate, so casein has a greater ability to bind to zinc. It is about 32% of the zinc in cow's milk is bound to casein and about 63% is bound to colloidal calcium phosphate, so casein has a higher ability to bind zinc, while lactoferrin has a low ability to bind to zinc. This minimizes zinc loss in whey, enhances its retention in the cheese, and consequently increases the ash content in the final product (Ianni et al. 2020; Sorice et al. 2024)

Microbial test

Total bacterial count

The logarithm of total bacterial counts and coliform counts (cfu/gm) for treatments (T1, T2, T3, T4, T5 and T6) during storage for 0, 3, 7 and 10 days are presented in Figures 3.A and 3.B. Statistical analysis revealed that there were no significant differences at a probability level of $P<0.05$ between all treatments. Higher total number of bacteria was observed with increasing zinc and selenium fortification, and higher number was observed upon storage periods. The number in cheese treatments made from cow's milk was higher than that of buffalo milk treatments, as total number (T1, T2, T3, T4, T5 and T6 at 0 time were 4.5, 4.9, 4.8, 4.9, 5.3 and 5.4 cfu/gm, respectively. These number increased after 10 days to 5.0, 5.4, 5.5, 5.6, 6.2 and 6.4. Low number of coliform bacteria in (T1) and (T2) increased after storage and fortification, where after 10 days reached 2.0, 2.5, 2.3, 2.4, 2.6, and 2.9 cfu/gm in comparison to 1.2, 1.5, 1.4, 1.5, 1.7 and 1.9 cfu/gm at 0 day. Inadequate manufacturing or pasteurization processes add additional bacterial populations to the naturally occurring lactic acid bacteria in dairy products. Selenium fortification does not reduce these populations, which are caused by contamination after cheese manufacturing and increase during storage, while a decrease in coliform bacteria populations can be observed due to the presence of lactic acid bacteria that work to eliminate spoilage pathogens (Naumova et al. 2020). Aquilanti et al. (2012) found that zinc fortification of cheese reduces the growth of different types of bacteria, and mentioned that 25 ppm is capable of inhibiting *Lactobacillus arabinosus*, and positive ions Mn, Ca, Mg and Sr counteract the inhibition of zinc by removing cations from proteins when they bind to zinc. The toxicity of zinc to bacteria was observed to decrease with decreasing pH due to the interaction of Gram-positive bacterial cell components such as lactic acid bacteria with zinc. El-Desoki and Hamed (2022) mentioned that fortification of cheese with selenium increases the number of *Lactobacilli* and *Streptococcus* bacteria, as the total number of bacteria immediately after manufacture in control samples and samples fortified with different percentages of selenium 0.5, 0.8 and 1.0 ppm (Na_2SeO_3) reached 5.5, 6.2, 6.5 and 7.1 cfu/gm, which increased to 8.2, 8.6, 9.0 and 9.3 cfu/gm, respectively after 60 days storage at a refrigerator.



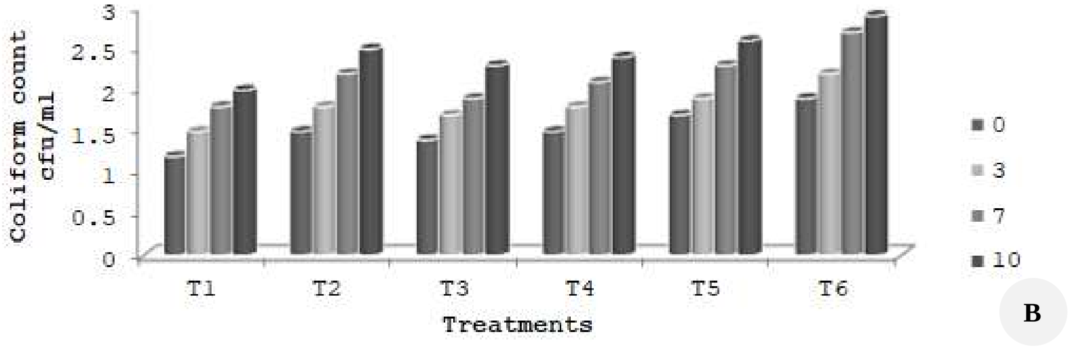


Figure 3. Microbial tests of soft cheeses made from buffalo and cow's milk fortified with zinc and selenium during different storage periods. A. Total count and B. Coliform counts

Rheological tests

The rheological test is an important test to determine the characteristics of food products and is one of the main factors, along with flavor and appearance, that determines the sensory acceptance of that food by the consumer (Dos Santos Rocha et al. 2022). Table 2 shows the rheological tests of soft cheese made from buffalo and cow's milk and fortified with zinc and selenium during different storage periods. Statistical analysis indicates that there were significant differences in the values of rigidity and flexibility and no differences in the values of cohesion at a probability level of $P<0.05$. Higher hardness and firmness were observed in the cheese during storage and mineralization compared to the control sample, while lower elasticity values were observed during storage and fortification. At 0 day hardness value of samples (T1, T2, T3, T4, T5 and T6) were 38.3, 35.2, 47.6, 38.0, 64.8 and 53.2 g, which increased after 10 days to 45.3, 40.9, 57.7, 68.6, 68.3 and 59.9 g. Cohesion values at the beginning of storage were 0.45, 0.43, 0.50, 0.59, 0.64 and 0.63%, increased to 0.65, 0.55, 0.63, 0.70, 0.75 and 0.71% after 10 days storage. The reason for the increase in hardness upon storage is due to the decrease in moisture upon evaporation, and the higher hardness in the mineralized samples compared to the control samples is due to the higher proportion of solids, and this is directly proportional to the cohesiveness of the samples (Liu et al. 2015). The results showed that after 10 days elasticity decreased to 2.0, 3.5, 1.2, 3.0, 3.2 and 3.0 mm. Loss of elasticity after storage is due to increase in proteolysis or reduction in moisture content, which reduces its elasticity (Picciotti et al. 2022; Azorín et al. 2024).

Sensory tests

Table 3 exhibits the sensory tests of soft cheese made from buffalo and cow's milk fortified with zinc and selenium during different storage periods. The results showed that there were significant differences in the values of sensory characteristics at a probability level of $P<0.05$. Lower quality indicators for flavor, appearance, and texture were observed for (T1) and (T2) compared to (T3, T4, T5 and T6) samples, where the flavor values were 47.9, 47.5, 47.5, 47.3, 47.2 and 47.4 immediately after manufacturing. Similarly, appearance values were 8.5, 8.4, 8.2, 8.4, 8.2 and 8.3. All values increased after storage at 4°C, and the control samples were slightly higher for all treatments. The sensory quality indicators of cheese play an important role for the consumer compared to its chemical composition, which decreases after storage and expiration, resulting in discoloration and flavor changes due to high acidity (Naumova et al. 2020). The texture is directly proportional to the amount of added minerals, which leads to an increase in hardness after fortification with metals such as zinc (Sorice et al. 2024).

Table 2. Rheological tests of soft cheeses made from buffalo and cow's milk fortified with zinc and selenium during different storage periods.

Treatments	Storage periods/days	Elasticity/mm	Cohesion %	Hardness/gm
T1	0	4.0^{a}	0.45^{a}	38.3^{d}
	3	3.7^{b}	0.49^{a}	40.1^{c}
	7	3.2^{b}	0.55^{a}	43.9^{b}
	10	2.0^{c}	0.65^{a}	45.3^{a}
T2	0	4.6^{a}	0.43^{a}	35.2^{d}
	3	4.3^{a}	0.45^{a}	37.5^{c}
	7	3.8^{b}	0.48^{a}	39.3^{b}
	10	3.5^{b}	0.55^{a}	40.9^{a}
T3	0	3.5^{a}	0.50^{a}	47.6^{d}
	3	3.1^{a}	0.54^{a}	49.0^{c}
	7	2.9^{b}	0.60^{a}	53.4^{b}
	10	1.2^{c}	0.63^{a}	57.7^{a}
T4	0	4.0^{a}	0.59^{a}	38.0^{d}
	3	3.9^{b}	0.60^{a}	41.4^{c}
	7	3.7^{b}	0.67^{a}	45.1^{b}
	10	3.0^{b}	0.70^{a}	68.6^{a}
T5	0	4.1^{a}	0.64^{a}	64.8^{b}
	3	3.9^{b}	0.69^{a}	66.5^{a}
	7	3.7^{b}	0.72^{a}	67.2^{a}
	10	3.2^{b}	0.75^{a}	68.3^{a}
T6	0	4.2^{a}	0.63^{a}	53.2^{c}
	3	3.9^{b}	0.66^{a}	55.0^{b}
	7	3.3^{b}	0.68^{a}	56.2^{b}
	10	3.0^{b}	0.71^{a}	59.9^{a}

Note: *Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P<0.05$)

Table 3. Sensory tests of soft cheeses made from buffalo and cow's milk fortified with zinc and selenium during different storage periods

Treatments	Storage periods/days	Appearance (10)	Texture (40)	Flavor (50)	Total (100)
T1	0	8.5[a]	35.2[a]	47.9[a]	91.6
	3	8.0[a]	34.5[a]	46.7[a]	89.2
	7	7.6[b]	32.8[b]	44.5[b]	84.9
	10	6.4[c]	30.9[c]	42.6[c]	79.9
T2	0	8.4[a]	34.8[a]	47.5[a]	90.7
	3	8.0[a]	34.0[a]	46.3[a]	88.3
	7	7.3[b]	32.1[b]	44.2[b]	83.6
	10	6.2[c]	30.5[c]	41.7[c]	78.4
T3	0	8.2[a]	36.7[a]	47.5[a]	90.4
	3	7.6[b]	35.5[b]	46.2[a]	87.3
	7	7.0[b]	33.8[c]	43.6[b]	82.4
	10	6.1[c]	31.0[d]	41.7[c]	77.8
T4	0	8.4[a]	36.5[a]	47.3[a]	90.2
	3	8.2[a]	35.3[b]	46.3[a]	87.8
	7	7.5[b]	33.9[c]	43.5[b]	81.9
	10	6.5[c]	31.0[d]	41.4[c]	77.9
T5	0	8.2[a]	35.7[a]	47.2[a]	90.1
	3	7.8[a]	35.3[a]	46.5[a]	88.6
	7	7.0[b]	32.0[b]	44.0[b]	83
	10	6.0[c]	30.2[c]	41.5[c]	77.7
T6	0	8.3[a]	35.5[a]	47.4[a]	90.2
	3	7.5[a]	35.2[a]	46.4[a]	88.1
	7	7.3[a]	32.0[b]	44.2[b]	83.5
	10	6.5[b]	30.5[c]	41.4[c]	78.4

Note: *Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($P<0.05$)

In conclusion, the results of fortification of Iraqi soft cheese resulted in a decrease in the peroxide value after the addition of zinc and selenium compared to the control samples manufactured from buffalo and cow milk and a reduction in pH by a small ratio. The variation of chemical composition values and high bacterial growth were observed after the fortification of these metals. Rheological test results showed that hardness and cohesion values increased, leading to reduced elasticity of the cheese. There was no significant variation in sensory quality indicators for flavor, texture, and appearance. The result enables the fortification of dairy products with zinc and selenium elements to offset the shortage of these minerals in food, especially for vegetarians who do not consume meat but consume dairy products.

ACKNOWLEDGEMENTS

We would like to gratitude to the Department of Food Science, College of Agriculture, University of Basrah, Iraq for supporting this research work.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 60-67 DOI: 10.13057/asianjagric/g090107

Potentiality of *Eichhornia crassipes* as partial bovine feed: A step towards managing its invasiveness

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Manuscript received: 21 June 2024. Revision accepted: 5 December 2024.

Abstract. *Guchhait S, Paria DS, Shrestha R, Pradhan P, Roy A. 2025. Potentiality of Eichhornia crassipes as partial bovine feed: A step towards managing its invasiveness. Asian J Agric 9: 60-67.* Menace of invasive species is rampant in tropical and subtropical areas and is a threat to the integrity of regional aquatic ecosystems. Bio-utilization has been suggested as one of the means of controlling the populations of invasive species. However, invasive plants such as Water Hyacinth (WH) (*Eichhornia crassipes*) are also known to accumulate heavy metals. From the literature survey, the young leaves of WH collected from freshwater ponds were found to have the lowest amounts of heavy metals in comparison to the roots as well as other fodder plants for bovines. This study aims to determine the effects of substituting common traditional bovine feed used in Eastern India with young leaves of the invasive plant WH collected from freshwater ponds on milk production, cow health, and its economic aspect. Eight cows aged between 3.7 to 6.7 years were considered for the study. In the control (0%) collection, they were fed with partial replacement feed composed of 5%, 10%, 15%, 20%, and 25% of total cow feed with young WH leaves. During the investigation, we assessed feed replacement-dependent changes in milk production and cow health to determine the EC_{50}, the optimal replacement level, and the efficacy of partial feed replacement with WH. From the study, it was known that an increase in WH% in the partially replaced feed had a positive effect on milk production while it had an adverse effect on health (stomach upset). Further, replacement feed with the range of 12.19-14.89% WH (maximum 15%) was identified to be optimum in terms of both milk production, cow health, and monetary contribution from milk output. So, the idea may be considered a step towards controlling the population of WH.

Keywords: Aquatic invasive plant, bio-utilization, cow health, milk production, tolindex, TPR-FPR

INTRODUCTION

Eichhornia crassipes (Mart.) Solms, commonly known as Water Hyacinth (WH), is native to the Amazon basin (Eckert et al. 2016). Originally valued for its ornamental beauty, WH was introduced to many tropical and subtropical regions (Bakrim et al. 2022). However, its high proliferation rate and competitiveness have rated it among the top 10 most invasive weeds (Narayanan et al. 2017; Yan and Guo 2017), and it is included in the IUCN's list of the 100 most dangerous invasive species (Bakrim et al. 2022).

Introduced to India during the British period, WH has become a common nuisance in the country's freshwater bodies. The plant has broad, thick, glossy, ovate leaves, long, spongy stalks, and blackish-purple roots, sometimes rising to one meter above water (Heuzé et al. 2015). Its seeds remain viable for up to 28 years (Sullivan and Wood 2012), and its runners can double its population in just two weeks (Gettys et al. 2014; Punitha et al. 2015; Rezania et al. 2015). This plant causes navigation interference, obstruction to water flow, evapotranspiration, and poses risks to hydroelectric and irrigation systems (Gettys et al. 2014; Dersseh et al. 2019). It adversely affects water quality, reducing dissolved oxygen, nutrient level, and pH (Gopal 1987; Rezania et al. 2015), leading to drastic changes in freshwater ecosystems and fish mortality and impacting fisheries (Datta et al. 2021). Infested areas face significant environmental challenges and socio-economic loss (Rezania et al. 2015; Basaula et al. 2021).

Controlling WH is a global challenge. Common methods include chemical control (non-ecofriendly herbicides), physical control (non-cost-effective mowers and dredgers), and biological control (specific insects and fungi), with each method having their own merits and constraints (Greenfield et al. 2006). Reducing nutrient runoff from catchment areas can also help control WH (Pullen et al. 2014; Dersseh et al. 2019). While these methods aim to destroy WH in the populations, utilization offers another solution. Water hyacinth can be converted into enriched vermicompost (Sridevi et al. 2016) and used as animal feed (Tham 2015). However, eradicating WH is difficult, and most efforts focus on minimizing economic costs and ecological damage (Villamagna and Murphy 2010). Studies suggest that current WH management practices have not been fully successful in long-term control (Rezania et al. 2015).

Water hyacinth is known to accumulate heavy metals, but young leaves collected from freshwater ponds are reported to have negligible heavy metal content (As 2.58 ppm, Pb 2.976 ppm, Zn 31.15 ppm, Cu 9.08 ppm, Cr 2.19

ppm) (Chaudhuri et al. 2008), and at least 18% crude protein making them suitable as fodder (Nyman et al. 2015; Indulekha et al. 2019). Young leaves have been utilized as a partial replacement for para-grass (*Brachiaria mutica* (Forssk.) Stapf) in ruminant diets (Tham 2012; Wimalarathne 2019). Tham (2015) used various forms of WH (fresh, wilted, pelleted, boiled, chopped, ground) as partial replacement in diets for ruminants, pigs, rabbits, fish, ducks, and geese with consistently positive results, reassuring the effectiveness of this method. WH also has high cellulose (36.5±0.9) and hemicellulose (22±0.5) levels, serving as energy sources for ruminants (Oyeoka et al. 2021). Therefore, to avoid bloat, studies recommend not exceeding 30% WH replacement for growing cattle (Greenfield et al. 2006) and 50% for sheep (Ilo et al. 2020).

The author's group has studied the plant diversity of freshwater lakes, estuaries, as well as coasts, along with their economic contributions (Manna et al. 2019; Roy et al. 2016; 2022). The present work aims to identify the potential of young WH leaves as a partial feed replacement for domestic lactating cows, calculate the optimum level of WH partial replacement, and assess its role in milk production and animal health. Additionally, the study discusses strategies for bio-utilization and WH population control in freshwater ponds of West Bengal, India, which involves replacing traditional feed with the WH partial replacement methods.

MATERIALS AND METHODS

Study region and selection of cows

The study was conducted in the fall of 2020 in the tropical coastal villages of Kotbarh (21.966556°N, 87.526306°E; rural area), Sarada (21.798556°N, 87.760306°E; rural area) and Kanthi (21.791333°N, 87.763944°E; semi-rural area), located in the East Medinipur District of West Bengal, India. These areas primarily derive their income from agriculture and animal husbandry. The study selected eight cows of a common indigenous breed, each in mid-lactation, and the cows were aged between 3.7 and 6.7 years, each accompanied by one calf aged between 7 and 9 months.

Preparation of fodder

In the control set, the cows were fed the traditional fodder used in the plains of West Bengal, India. This diet included paddy straw, starchy decoction derived from boiling rice, vegetable wastage, rice polish, and green grasses. For the experimental set, we introduced a unique feeding method. Fresh, young, unfurling leaves of the WH with minimally developed swollen petioles (Burke et al. 2014) were collected from the local wetlands situated 500 m to 1.5 km from nearby households. The collected leaves were manually chopped into small pieces (Mani 2018) and were boiled thoroughly for 20-25 minutes in unleaded earthen pots, with edible salt (NaCl) added according to the cow's requirements. After boiling, the broth was discarded (Masifwa et al. 2001), and freshwater was added. The diet was then carefully adjusted, ensuring that 5% to 25% of the total feed was replaced with boiled young leaves of WH (Table 1). Additionally, 3 to 5 L of starchy decoction derived from boiling rice (Bhaat'’er Fan; Bhaat=Boiled Rice, Fan=sieved off starchy decoction in Bengali) were collected from households and added to the mixture. No commercial cow feed was used for either the control or experimental set.

Table 1. The feed chart for control (0% WH) and experiment (5-25% WH) sets for eight cows, each receiving 5 L of water daily

Set of cows	% and corresponding weight (g) of WH replacement in the feed	Rice polish (g)	Paddy straw (g)	Vegetable waste (g)	Total fodder (g)
Cow 1 and 2	0% (Control)	350	5,500	1,750	7,600
	5%; 380 g	332.5	5,225	1,662.5	
	10%; 760 g	315	4,950	1,575	
	15%; 1140 g	297.5	4,675	1,487.5	
	20%; 1520 g	280	4,400	1,400	
	25%; 1900 g	262.5	4,125	1,312.5	
Cow 3 and 8	0% (Control)	500	5,000	1,750	7,250
	5%; 362.5 g	475	4,750	1,662.5	
	10%; 725 g	450	4,500	1,575	
	15%; 1087.5 g	425	4,250	1,487.5	
	20%; 1450 g	400	4,000	1,400	
	25%; 1812.5 g	375	3,750	1,312.5	
Cow 4 and 5	0% (Control)	667	6,800	1,750	9,217
	5%; 460.85 g	633.65	6,460	1,662.5	
	10%; 921.7 g	600.3	6,120	1,575	
	15%; 1382.55 g	566.95	5,780	1,487.5	
	20%; 1843.4 g	533.6	5,440	1,400	
	25%; 2304.25 g	500.25	5,100	1,312.5	
Cow 6 and 7	0% (Control)	250	7,000	1,750	9,000
	5%; 450 g	237.5	6,650	1,662.5	
	10%; 900 g	225	6,300	1,575	
	15%; 1350 g	212.5	5,950	1,487.5	
	20%; 1800 g	200	5,600	1,400	
	25%; 2250 g	187.5	5,250	1,312.5	

Control readings for milk production and cow health were recorded for three consecutive days. Standard milking techniques were consistently applied throughout the study, with cows milked twice daily at fixed times each morning and evening to maintain uniformity in data collection. From the 4th day to the 10th day, 5% of each cow's total feed was replaced with boiled young leaves of WH, and milk production readings were taken from both morning and evening milkings on the 8th to 10th days. On the 11th day, the feed replacement percentage was increased to 10%, with production readings again recorded during both morning and evening sessions on the 15th to 17th days. This incremental replacement continued, reaching up to 25% by the 22nd day. Final milk production readings were collected from the morning and evening milkings on the 36th to 38th day. The detailed feed chart is presented in Table 1. Standard weighing machines were used to measure both traditional feeds and young leaves of WH, while standard measuring cylinders were employed for precise milk production measurements.

Statistical analysis

Results were analyzed using Anova: Two-Factor without replication in MS Excel, and percentage-wise results for WH were graphically plotted using a box plot in OriginPro 2018. The analysis focused on three main aspects: (i) Determining the optimum WH percentage by calculating the True Positive Rate (TPR) and False Positive Rate (FPR) for both productivity and health effects and deriving the trend line intersection; (ii) Calculating the optimum WH percentage using tolerance index, with calculations performed in R (ver 4.0.3) using tolindex package (Ver.0.1.0) (Pradhan and Guchhait 2019); and (iii) Calculating EC_{50}, which represents the concentration at which the replacement feed exerts half of its maximal 'complementary' effect, and identifying the optimal percentage by finding the crossover point where the complementation trend line intersects the health trend line.

The literature review suggested that incorporating WH into animal feed could increase milk production (production factor) (Sayanthan et al. 2024). However, our research has revealed the potential for feed replacement-dependent negative health impacts due to changes in traditional feed diets (health factor) (Abdelhamid and Gabr 1991; Tham 2012; Wimalarathne 2019; Oyeoka et al. 2021). These findings have significant implications for the animal nutrition and veterinary science field. The Receiver Operating Characteristic (ROC) analysis, a popular tool for clinical dose identification, was employed to predict efficacy from a given drug concentration and to identify optimal cut-off for a test, combining the highest true positive rate with the lowest false positive rate (Clements et al. 2018). To determine the appropriate cut-off for both production and health factors, proportions of Sensitivity/ True Positive Rate (TPR) and Specificity/ False Positive Rate (FPR) were plotted in an efficiency plot following Clements et al. (2018). TPR and FPR for WH concentrations ranging from 5% to 25% were calculated in MS Excel with the following formula:

TPR (Production Factor) 5~25 / = SUM(INC 5~25 : INC 25)/SUM(INC 5 : INC 25)

FPR (Production Factor) 5~25/ = SUM(DEC5~25 : DEC 25)/SUM(DEC 5 : DEC25)

TPR (Health Factor) 5~25/ = SUM(unhlty5~25 : unhlty25)/SUM(unhlty5 : unhlty25)

FPR(Health factor)5~25/ = SUM(hlty5~25 : hlty25)/SUM(hlty5 : hlty25)

Where:

Inc: Number of cases with an increase in milk production

dec: Number of cases with a decrease in milk production

unhlty: Number of cases with unhealthy cows

htly: Number of cases with healthy cows

The tolerance index for individual replacement feed concentrations ranging from 5% to 25% was calculated to determine the ideal feed tolerance (Clements et al. 2018). These calculations were performed in the R Program, identifying the highest TAvg as the ideal % of feed replacement. For this purpose, individual WH% replacement sets were assessed using two tolerance indices (T1 and T2) with the following formulas:

$T_1 = ((C_{step} \times (1/D_{step}+H_{step})) / ((I_{step}+N_{step}) \times 1000)) \times I_{step})$

$T_2 = ((C_{direct} \times (1/D_{direct}+H_{direct})) / ((I_{direct}+N_{direct}) \times 1000)) \times I_{direct})$

Where:

C_{step}: Stepwise sum of increase or decrease in milk produced by eight cows e.g., cumulative WH5% milk output of all cows (increase and decrease) compared to cumulative control output and henceforth

C_{direct}: Comparison of the sum of increase or decrease in milk produced by eight cows in various treatments (5% to 25%) directly with control output (0%)

D_{step}: Proportion of the decrease in milk production at a particular concentration evident from C_{step}

D_{direct}: Proportion of decrease in milk production at a particular concentration evident from C_{direct}

H: Proportion of cows without stomach upset at a particular concentration

I_{step}: Proportion of the increase in milk production at a particular concentration evident from C_{step}

I_{direct}: Proportion of the increase in milk production at a particular concentration evident from C_{direct}

N_{step}: Proportion of neutral/ no change in milk production at a particular concentration evident from C_{step}

N_{direct}: Proportion of neutral/ no change in milk production at a particular concentration evident from C_{direct}.

The EC_{50} was calculated from the plot of the concentration-wise ratio of Effect (E)/Concentration (C) % (or % response) on the y-axis plotted against concentration in the x-axis, using a polynomial trend line of order 2. The EC_{50} was determined as the value of x when y = 50. For calculating higher ECs, such as EC_{75}, Hill Slope or slope factor (H) was calculated using the formula: (Highest E/C – Lowest E/C)/(Highest Effect-Lowest Effect), which measures steepness (higher H) or shallowness (lower H) of a curve, and standard feed replacement-response curve having H value of 1. The higher targeted EC, such as EC_{75} (75% response), was then calculated from the formula (75/(100-75))^1/value of H*value of EC_{50}.

For economic analysis, the rate of July 2020 for unskilled labor (Rs 257 for 8 hours, 30 minutes of work including 30 minutes of rest, but without additional food cost) was derived from the official website of the Labour Department, Government of West Bengal (WBLC 2020). During 2019–2020, the rate of milk/liter in rural West Bengal fluctuated between Indian Rupee (INR) 40-42; for the current analysis, the higher rate was considered. Polynomial equations of the trend line were solved for y=50 (EC_{50}) using the equation-solving platform of WolframAlpha (WolframAlpha 2020). No cost of fuel costs was additionally incorporated as farmers utilized plant debris from around the farmyard and waste rice straw to fuel the fodder preparation process.

RESULTS AND DISCUSSION

The study demonstrated that gradually replacing traditional feed with WH led to a significant increase in milk production (F 73.16>F critical 2.29, P≤0.05). Post hoc testing using the Holm-Bonferroni correction further clarified these findings. Comparisons showed significant increases in milk production at 5%, 10%, and 15% WH feed replacement levels (corrected P-values of 0.0083, 0.0042, and 0.0032, respectively). In comparison, no significant changes were observed at 20% and 25% replacement levels (corrected P-values of 0.1674 and 0.8587, respectively). However, when feed replacement reached 20%, one out of eight cows showed signs of stomach upset, indicated by irregular excreta and decreased milk productivity, which necessitated a return to the control feed level for that cow. At 25% replacement, six out of eight cows experienced stomach upset, and both the mean and median milk output values showed a noticeable drop post-15% WH replacement (Figure 1). These findings align with Tham (2012), who also observed signs of indigestion in cattle after 25% feed replacement. Due to these adverse effects, feed replacement with a higher concentration of *E. crassipes* was halted, and cows were returned to their normal (control) feed regime. It's important to note that milk output responses varied among individual cows, with ANOVA indicating a significant difference in feed utilization capacities (F=4.86>F critical 2.49, P≤0.05). This highlights the need for personalized feeding strategies to ensure the well-being of each cow.

The optimum feed replacement level with TPR-FPR analysis

The True Positive Rate (TPR) and False Positive Rate (FPR) for WH % feed replacement, along with their gradient proportions, are presented in Tables 2 and 3. The alternative hypothesis posited that increasing WH% would positively affect productivity but negatively affect health. For productivity, true positives were recorded up to 15% WH. Beyond 15% WH (20-25%), false negative results were observed. Conversely, false positive results for productivity were noted within the 5-15% WH concentration, while true negative cases were observed in the 20-25% WH range.

For health, true positives were recorded in the 20-25% WH range, while false negative cases were noted within the 5-15% WH range. Similarly, false positive results for productivity were found within 5-15% WH concentration, and true negative cases were noted in 20-25% WH range. The accuracy of the TPR-FPR analysis for WH productivity was 0.813, compared to 0.4 for the same analysis for health. Based upon the x-axis values at the intersection of individual TPR and FPR trend lines for productivity and health (Figures 2 and 3), the ideal WH% for replacement feed was determined to be between 12.192-14.765% (Table 4).

The optimum feed replacement level with tolindex

The tolindex, which incorporates productivity, health factors, and neutral values (where effects do not change between treatment percentages), was calculated for each WH replacement percentage. In Table 5, for immediate treatment comparison (T1), WH 10% showed the highest value of 4.60, whereas the control-based comparisons (T2) showed the highest value of 11.95 for WH 15%. On average, WH 15% had the highest Tavg, followed by WH 10%. However, despite WH 15% achieving the highest T2 value, its T1 value (3.60) was lower than the T1 (3.75), suggesting WH 10% as a more consistent option with high T1 (4.60) and T2 (8.35) values. Notably, the Tavg values for WH 20% and 25% were negative, indicating a synergistic decline in health and milk production. The tolindex was developed as an R package and hosted on GitHub (Pradhan and Guchhait 2019).

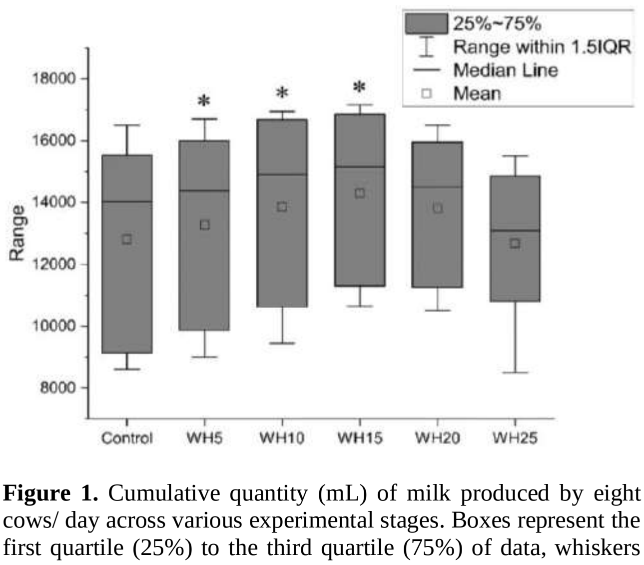

Figure 1. Cumulative quantity (mL) of milk produced by eight cows/ day across various experimental stages. Boxes represent the first quartile (25%) to the third quartile (75%) of data, whiskers indicate the range within the 1.5 × Interquartile Range (IQR), a bar within each box represents the median, and a small square represents the mean. An asterisk (*) above the bars for WH5, WH10, and WH15 denotes statistically significant increases in milk production compared to the control (Holm-Bonferroni adjusted p < 0.05)

Table 2. TPR-FPR analysis of productivity related to WH partial replacement

WH%	Decrease	Increase	TPR	FPR	TPR proportion	FPR proportion
5		16	1	1	0.389	0.218
10		16	0.746	1	0.290	0.218
15		16	0.492	1	0.191	0.218
20	7	9	0.238	1	0.0926	0.218
25	10	6	0.095	0.588	0.0370	0.128

Note: TPR: True Positive Rate, FPR: False Positive Rate, Decrease: Decrease in milk production, Increase: Increase in milk production. Accuracy of TPR-FPR analysis: 0.813

Table 3. TPR-FPR analysis of cow health related to WH partial replacement

WH%	Healthy	Unhealthy	TPR	FPR	TPR proportion	FPR proportion
5	8		1	1	0.206	0.384
10	8		1	0.758	0.206	0.290
15	8		1	0.515	0.206	0.198
20	7	1	1	0.273	0.206	0.105
25	2	6	0.857	0.061	0.176	0.023

Note: TPR: True Positive Rate, FPR: False Positive Rate, Healthy: Number of healthy cows, Unhealthy: Number of unhealthy cows. Accuracy of TPR-FPR analysis: 0.4

Table 4. Trendline equations of TPR-FPR for productivity and health and value of x-axis at the intersection of individual TPR and FPR trendlines of productivity and health

Proportion	Trendline equation	R^2	Intersection
FPR (productivity)	$y = -0.0005x^2 + 0.0118x + 0.1641$	0.8571	12.192
TPR (productivity)	$y = 0.0002x^2 - 0.0254x + 0.5136$	0.9974	
FPR (Health)	$y = 7E\text{-}05x^2 - 0.0201x + 0.4837$	0.9998	14.765
TPR (Health)	$y = -0.0002x^2 + 0.0039x + 0.1882$	0.8571	

Note: Trendline equation: Quadratic equation of order 2

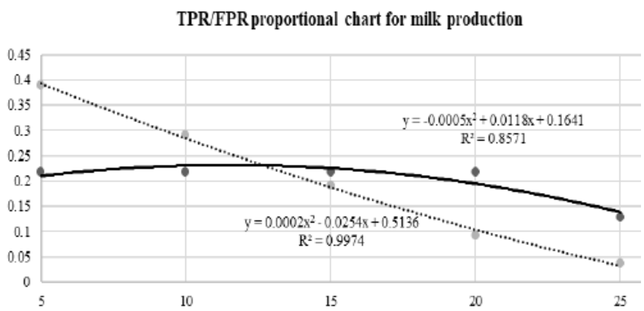

Figure 2. TPR/FPR proportional chart for milk production

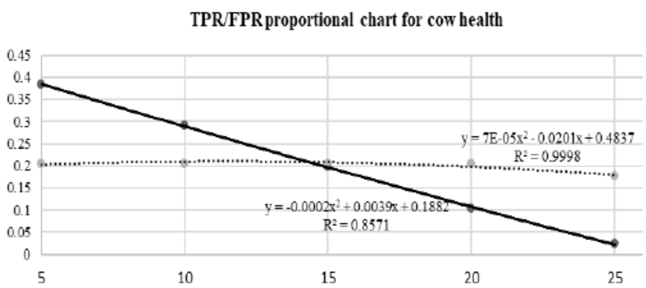

Figure 3. TPR/FPR proportional chart for cow health

Calculation of EC_{50}

EC_{50} values were calculated for both productivity across seven cows (with WH administration ranging from 0-25%) and for cow number 5 (with WH administration ranging from 0-20%), as well as for health. The EC_{50} values for productivity ranged from 5.28 to 6.57, while for health, the EC_{50} value was 5.19 (Table 6). This indicated that a 5% WH replacement was nearly equivalent to 50% of the effective concentration for the cows. Additionally, the Hill Slope (H) value for the health factor was 0.253, suggesting less accuracy in predicting higher EC values (e.g., EC_{75}, EC_{90}). In contrast, the H value for productivity was close to 1, indicating the productivity curve was closer to the standard curve, which allowed for a more accurate prediction of higher EC values based on productivity.

Economic analysis

In rural southern parts of West Bengal, it was found that 73.63 minutes were invested daily per cow for tasks ranging from cleaning the cows and cowshed to preparing feed and milking. Collecting young WH leaves was not a dedicated task due to the abundance of waterbodies and available WH vegetation around the villages. This activity was typically carried out by locals on the way home from other prioritized tasks, taking between 4.69 to 21.16 minutes and resulting in the collection of 0.36 to 2.3 kg of young WH leaves (Tables 1 and 7). The WH leaves collection times were added to the cow rearing and milking 'control' time, however calculations were also made excluding WH harvesting time. Including the WH collection time led to a gradual increase in investment costs, while excluding collection time showed a decline in costs supplemented by WH input, which refers to the use of WH leaves as a cost-effective feed supplement.

WH at 15% had the highest average monetary value (INR) of milk per day, yielding a higher return and profit compared to the control when calculated without a time factor. This finding underscores the potential for profit at WH 15%, which should be a source of optimism for farmers and policymakers. However, when factoring in the collection time, the control showed the highest profit margin (INR 27.65), with WH 10% close behind (INR 27.14). It was also observed that investing additional time beyond WH 15% did not yield adequate returns in milk production and resultant monetary benefits. Therefore, if WH harvesting time was not considered, collectors could still achieve profit at WH 15% (Table 7).

Table 5. Optimal feed replacement levels with Tolindex values for milk productivity and bovine health: Comparative analysis across WH percentages

	5%	10%	15%	20%	25%	Trend line equation	Max X
T1	3.75	4.60	3.60	-8.00	-5.09	$y = 0.0007x^4 - 0.0441x^3 + 0.8641x^2 - 5.6424x + 15.431$	14.89
T2	3.75	8.35	11.95	7.79	-0.38	$y = 0.0023x^4 - 0.1245x^3 + 2.2884x^2 - 16.593x + 43.664$	12.99
T_{Avg}	3.75	6.48	7.78	-0.11	-2.73	$y = 0.0015x^4 - 0.0843x^3 + 1.5763x^2 - 11.118x + 29.548$	13.79

Note: T1: Compared to immediate set, T2: Compared to control, Avg: Average of T1 and T2, R2: 1 for all three equations, Trend line equation: Quadratic equation of order 4, Max X: Value of X axis on the peak of trend line

Table 6. Trend line equations derived from WH% concentration vs average effect/concentration plot. EC_{50} was derived from the x value of the trend line when y=50

	Trend line equation	R^2	EC_{50}	Hill Slope (*H*)
Cow 5	$y = -0.1405x^2 + 6.9411x + 17.286$	0.9999	5.28	0.987
Cows 1,2,3, 4,6,7,8	$y = -0.0908x^2 + 5.5587x + 17.408$	0.9992	6.57	1.27
Health	$y = -20.395x^2+120.84x-27.373$	0.9938	5.19	0.253

Note: Trend line equation: Quadratic equation of order 2. Values of Cow 5 and the other 7 cows were taken separately

Table 7. Role of WH and time investment for its collection in daily profit from the sale of milk

WH%	Milk production (mL/day)	Value (INR 42/L)	Return above control (INR)	Time investment (INR)	With time factor			Without time factor		
					Feed investment (INR)	Investment compared with control (INR)	Profit (INR)	Feed Investment (INR)	Investment compared with control (INR)	Profit (INR)
0	2,134.38	89.64	-	73.63	61.99	-	27.65	22.57	-	67.07
5	2,212.50	92.93	+3.28	84.18	66.68	+4.69	26.24	21.61	-0.96	71.32
10	2,308.33	96.95	+7.31	91.81	69.81	+7.82	27.14	20.65	-1.92	76.30
15	2,383.33	100.10	+10.46	99.94	73.20	+11.21	26.90	19.69	-2.88	80.41
20	2,235.71	93.90	+4.26	110.50	77.88	+15.89	16.02	18.72	-3.85	75.18
25	2,225.00	93.45	+3.81	119.44	83.15	+21.16	10.30	19.20	-3.37	74.25

Note: Milk production: Average quantity of milk (mL)/day, Value: Average monetary value (INR) of milk/day, Return: Increase in milk production above control in monetary terms, Time investment: Average time invested (minutes) per cow, Feed investment: Investment on rice polish, straw, salt

Environmental considerations

Unmanaged WH populations in water bodies are known to outcompete native aquatic species, reduce oxygen levels for aquatic wildlife, and create ideal habitats for water-borne vectors like mosquitoes (Lahon et al. 2023). Daily harvesting of WH cover would control the spread of WH colonies and allow sunlight to reach native submerged plants. These plants, in turn, would help restore the oxygen-depleted by fast-growing WH colonies, thus supporting aquatic wildlife survival.

The study showed that an optimal 15% level of partial replacement feed would result in each cow utilizing 1.24 kg of WH per day, while a 10% level would amount to 0.83 kg per day (Table 1). The bovine density in the East Medinipur District is reported to be 220/km^2 (NDDB 2017), suggesting that within a 1 km^2 area, 182.6 kg to 277.76 kg of WH could be utilized daily. This could be a significant step towards controlling WH populations.

Lahon et al. (2023) reported that WH biomass output is highest during the summer season (43-51%; May-August), drops to 3-11% during September-December, and is 16-31% during January-February. Therefore, harvesting efforts to eradicate WH should be intensified during September-February when the plants are already stressed due to low biomass output. This strategy could potentially lead to significant economic benefits. However, maximum harvesting from an economic perspective may be achieved during May-August.

The use of WH as animal feed is prevalent in Southeast Asian countries, both for ruminants and non-ruminants, in both raw and boiled forms (Masifwa et al. 2001; Tham 2015). However, few studies have tested bovine feed tolerance to WH replacement (Tham 2015; Ilo et al. 2020). The current study suggests an optimal WH replacement level in a feed to be 12.19-14.89% (maximum 15%), which is within the previously suggested maximum of 30% for cattle (Ilo et al. 2020). The milk output from the optimal WH replacement of 15% was found to be 2.3833 L; however, the lack of comparative data from other studies underscores the need for further research in this area.

Regarding heavy metal content, WH leaves collected from freshwater ponds have been reported to contain 2.976 ppm of Pb, which is below the level found in paragrass (5.84 ppm) and much below the alfalfa haylage (271 ppm). In the Indian context, WH has the lowest Zn content (31.15 ppm) compared to hybrid napier (43.426 ppm). However, only in the case of As and Cu do sorghum (0.424 ppm and 8.685 ppm, respectively) have lower values than WH (2.58 ppm and 9.08 ppm) (Jafari 2010; Nyman et al. 2015). Compared to bovine feeds discussed in the study of Jafari (2010), leaves of WH from fresher ponds are reported to have much lower amounts of heavy metal content (Nyman et al. 2015). However, it is recommended to avoid WH leaves collected from polluted areas, sewage canals (Nyman et al. 2015), as well as Arsenic and Fluoride affected areas (Chaudhuri et al. 2008).

In conclusion, the experiment proved to be an efficient means of partially replacing traditional feed for dairy cattle in eastern India while managing the population of WH. Analyses such as TPR-FPR for calculating the optimum WH% and the development and use of the tolindex R package yielded comparable results, with ideal WH levels ranging from 12.19-14.77% (TPR-FPR) and 12.99-14.89% (tolindex). While a 5.19-6.57% WH replacement initiated a 50% response in milk production (EC_{50}), the optimal effect was observed within 12.19-14.89% WH, with a maximum effect at 15%. At this level, milk production and associated monetary returns were maximized, resulting in an 8.15-11.67% increase in milk yield and additional returns of INR 7.31-10.46 over control values when WH was used as a partial replacement feed replacement within the 10-15% range. While heavy metal accumulation in WH is a known concern, studies have reported low heavy metal content in young WH leaves. Therefore, to minimize this risk, the study recommends selecting pollution-free freshwater sites, collecting only young leaves, and boiling them before use. Future research could extend this study by evaluating the protein, fat, and heavy metal composition of milk derived from WH-fed cows to assess both nutritional benefits and safety better. This additional data would contribute valuable insights to support the broader application of WH as a viable partial feed replacement.

ACKNOWLEDGMENTS

The authors are thankful to the Chairman, Member Secretary, Senior Research Officer, and other officials of the West Bengal Biodiversity Board, India, for providing necessary help and facilities as and when required. Our sincere gratitude also goes to the local people of Purba Medinipur District, India, because, without their involvement, the present work would not have been possible.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 69-83

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090108

Evaluation of land potential for organic farming development and implications for achieving Sustainable Development Goals (SDGs) in Sleman District, Yogyakarta, Indonesia

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Manuscript received: 28 September 2024. Revision accepted: 6 December 2024.

Abstract. *Romadhona S, Soedarmo SPK, Mussadun. 2025. Evaluation of land potential for organic farming development and implications for achieving Sustainable Development Goals (SDGs) in Sleman District, Yogyakarta, Indonesia. Asian J Agric 9: 69-83.* The land potential is a critical factor in managing and utilizing land for agriculture. Land with high potential can support quality crop growth, increase production yields, and contribute to food security. Identifying and mapping land potential helps farmers and policymakers make informed decisions to optimize land use, increase production efficiency, and minimize negative environmental impacts. This research highlights the importance of land potential analysis in land use optimization for the agricultural sector in Sleman District, which has a strategic role in the regional economy and community welfare. Using quantitative descriptive analysis and scoring techniques, this research identifies high-potential land for agriculture capable of producing quality crops and high productivity. An overlay approach of multiple maps was used to generate comprehensive new information on land potential, providing essential insights for more efficient and sustainable agricultural decision-making and planning. The novelty of this research lies in integrating scoring and map overlay methods in the analysis of land potential, which has yet to be widely applied in the local context, thus making a new contribution to the literature on agricultural land management in the region. The results of this study identified land potential in Sleman based on the Land Potential Index (IPL) classification. The highest land potential class (IPL) covers 45,741 hectares or 79.77% of the total land area and is spread across all sub-districts. The very high IPL class covers 7,239 hectares or 12.62%, spread across Moyudan, Minggir, Seyegan, Godean, Gamping, Mlati, Depok, Berbah, Prambanan, Kalasan, Ngemplak, Ngaglik, Candi, and Pakem. The medium IPL class covers 3,772 hectares (6.57%), while the low class is only 583 hectares (1.01%). This data shows that most areas have high land potential, essential for planning sustainable agricultural development.

Keywords: Agricultural development, geographic information system, index potential land, organic farming, sustainable development goals

INTRODUCTION

Indonesia is an agricultural country with the agricultural sector as one of its leading sectors. The sustainability of the agricultural sector, especially food crop farming, is under serious threat, namely the shrinking of agricultural land in Indonesia due to the massive conversion of productive agricultural land to non-agricultural use (Romadhona et al. 2023). The increasing need for land, the scarcity of fertile and potential agricultural land, and the competition for land use between the agricultural and non-agricultural sectors, require appropriate technology in an effort to optimize land use in a sustainable manner (Horrillo et al. 2020). To be able to utilize land resources in a directed and efficient manner, it is necessary to have complete data and information regarding the condition of climate, soil and other physical environmental characteristics, as well as the growing requirements for the plants being cultivated, especially plants that have good market opportunities and economic significance (Tscharntke et al. 2021). Sustainable development has become a global concern realized through the Sustainable Development Goals (SDGs), an international agenda that includes 17 key goals to achieve a balance between social, economic and environmental needs by 2030 (MacPherson et al. 2020). The SDGs have an important role in driving the transformation of development practices, especially in the agricultural sector which plays a central role in supporting food security and reducing global poverty. In the local context, the implementation of the SDGs in the agriculture sector is a challenge, especially in terms of efficient and sustainable land resource management. Various studies emphasize the importance of implementing environmentally friendly agricultural practices (Dwiartama et al. 2024).

Sleman District, located in the Special Region of Yogyakarta, is one of the regions that has great potential in the agricultural sector. Its strategic geographical location, with fertile soil and favourable climate, makes Sleman a leading region in agriculture. Sleman is famous for its quality agricultural products, such as salak pondoh, organic rice, and various vegetables and fruits (Widiati et al. 2017). Agricultural sector in Sleman District is a strategic sector and plays an important role in the regional economy and community survival, especially in its contribution to the Regional Revenue and Expenditure Budget, providing employment opportunities and providing food in Sleman District (Devi et al. 2021). Therefore, the concept of spatial planning in Sleman District refers to the model of growth centers which emphasizes services for the agricultural product processing industry (Romadhona et al. 2020). A use of land in a suitable location will be very profitable, not only in the agricultural sector, but also in industry, trade, education and so on. Agricultural areas located in areas with high potential fertility will produce larger harvests compared to agricultural areas with low soil fertility.

The research area faces significant challenges due to rapid urbanization and land-use changes from 2013 to 2023. These changes have led to an increase in agricultural land but also added pressure on the sustainability of agricultural practices. Rapid urbanization can result in land fragmentation, conversion of agricultural land to non-agricultural uses, and increased pollution and environmental degradation (Widayani et al. 2022). These conditions make the spatial assessment of land potential crucial. Proper spatial analysis can help identify the most suitable areas for the development of organic and horticultural agriculture, thereby optimizing sustainable land use and reducing negative environmental impacts.

The development of organic agriculture in Sleman District has gained significant momentum with the issuance of Sleman Regent Regulation (PERBUP) No. 62 of 2023 concerning the Development of Area-Based Organic Agriculture. This regulation provides a strong legal foundation and strategic direction for the sustainable and competitive development of organic agriculture in Sleman. Organic agriculture, which prioritizes environmentally friendly and sustainable practices, is considered a solution to various problems faced by the conventional agricultural sector, such as soil degradation, declining water quality, and loss of biodiversity. On the other hand, organic agriculture also promises added economic value for farmers through products that have higher market value and wider market access, both domestically and internationally (Triyono et al. 2023). Mapping recommendations for organic agricultural land in Sleman District is an effort to implement sustainable agriculture and improve the quality of regional products through organic farming. The mapping of organic agricultural land recommendations needs to consider the potential and suitability of the land (Wacano et al. 2012). Evaluating land suitability potential is a crucial step to identify environmental limits in sustainable land use planning. This relates to the assessment of land performance for specific uses, namely crop production.

Another important focus is the absence of specific maps indicating locations with the potential for organic farming development in Sleman District. Without detailed and accurate maps, it is difficult for stakeholders to effectively plan and direct land development. Comprehensive mapping is needed to identify areas with soil and climate conditions suitable for organic farming and to determine sustainable land development strategies (El Baroudy 2016). With such maps, local governments, research institutions, and farmers can collaborate more efficiently in developing more planned and focused organic farming. Therefore, this research aims to fill this gap by mapping potential and providing land development directions in Sleman to support the enhancement of more sustainable and profitable organic farming implementation.

MATERIALS AND METHODS

Study area

Sleman District, Yogyakarta, Indonesia (Figure 1) has an area of 57,482 Ha or 574.82 km^2 covering 17 sub-districts, 86 villages, and 1,212 hamlets. Most of the area is fertile agricultural land with technical irrigation in the west and south, and the land varies between paddy fields, tegal, yards, and forests. The topography is divided into four elevation classes: <100 m (10.79%), 100-499 m (75.32%), 500-999 m (11.38%), and >1000 m (2.60%). The land potential index is used to assess the feasibility of agricultural development, taking into account altitude and soil fertility for various commodities, especially in sub-districts with altitude <100 m such as Moyudan, Minggir, Godean, Prambanan, Gamping, and Berbah, to >1000 m in sub-districts such as Turi, Pakem, and Cangkringan.

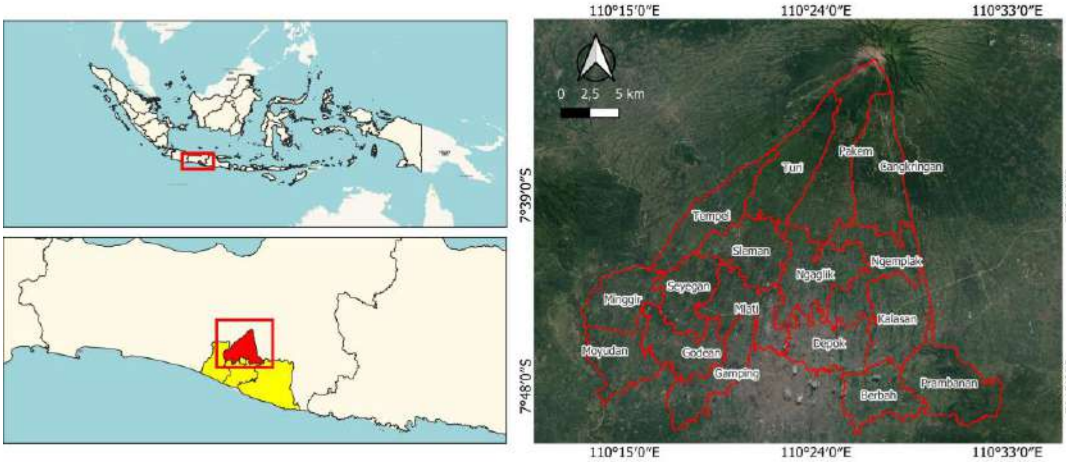

Figure 1. Map of research location in Sleman District, Yogyakarta, Indonesia

Procedures

In Figure 2, the flowchart for determining the Land Potential Index (IPL) for agriculture is a crucial process involving the thorough collection and analysis of data to support sustainable agriculture. This process begins with the collection of mosaic data, which includes spatial and non-spatial data such as topography, soil types, climate, and current land use. This data is then intricately overlaid using geographic information systems (GIS) to produce a land potential map. The next step is the assessment and weighting of each parameter based on its influence on agricultural productivity. The result of this analysis is a land potential index map that identifies the most suitable areas for the development of organic and non-organic agriculture. This analysis is essential as it provides accurate and detailed information to decision-makers to optimize land use, minimize environmental impact, and enhance agricultural production efficiency, all of which contribute to sustainable farming practices.

Determining the land potential index will be more efficient if presented spatially (spatial variability). The boundaries for each potential land area can be known with certainty, as the spatial pattern and the most important thing is the absolute position. Therefore, we need the most efficient method to process and analyze spatial and attribute data containing other information for making a Land Potential Index Map (Karimi et al. 2018). The land potential index is carried out by dividing classes, namely an evaluation carried out by grouping land into several categories based on five parameters: slope, soil type, lithology, rainfall, and disaster vulnerability. Land potential classification is the grouping of land into special units according to its ability to be used optimally and its treatment so that it can be used continuously.

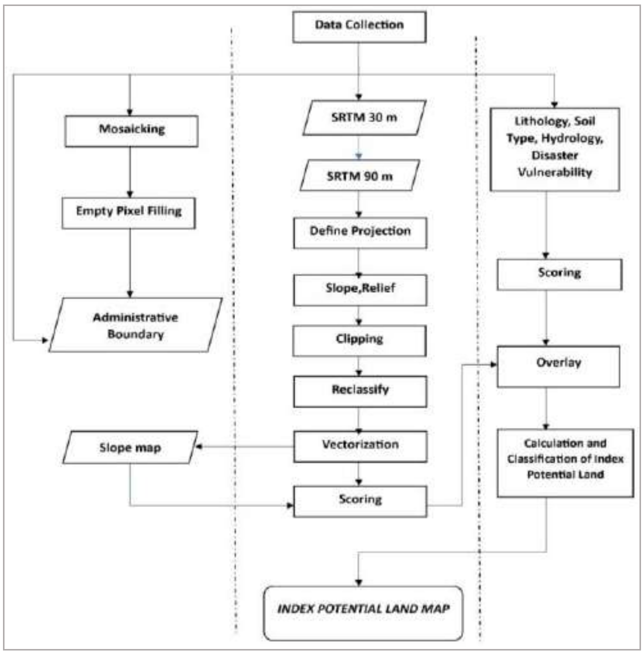

Figure 2. Research stages for land potential index assessment

Therefore, this classification system aims to group land that can be cultivated according to its potential and obstacles to sustainable production. The system is based on inhibiting factors and other potential hazards still acceptable in land classification (Castoldi and Bechini 2010).

Land potential index is carried out using a tiered quantitative method for each supporting variable except the erosion vulnerability parameter because it is a limiting variable. Erosion vulnerability is used as a multiplier in the summation of all supporting variable scores. Land potential is expressed by a numerical value called the Land Potential Index (IPL). The amount of land potential index is determined by evaluating 5 rational formula calculation factors, following the rational formula calculation formula, which describes the relationship between various environmental variables that influence each other in determining the level of sustainable agriculture (Marco et al. 2021; Devianti et al. 2022).

The data analysis technique used in this research is quantitative descriptive analysis, namely by scoring. This research also uses the overlay technique of several maps to generate new information, which is then analyzed. Each parameter map consisting of a slope map, lithology map, soil texture map, groundwater potential map, and landslide hazard map was assessed based on the reference table of land potential index parameter assessment and overlaid (Chiaka et al. 2024) . The Land Potential Index (IPL) states the relative potential of land for public use. The higher the IPL value, the higher the land's capability when used for land processing activities to provide optimal results. Land potential can be classified using a formula.

IPL formula = $(R + L + T + H) \times B$

Where:
IPL: Land Potential Index
R: Value of slope factor
L: Value of lithology factor
T: Value Soil type factor
H: Value of hydrological factors
B: Value of disaster vulnerability or barrier

Table 1 explains that the Land Class Characteristics Information provides a comprehensive overview of the Index of Land Potential (IPL), which is an important metric for determining the relative potential of land for public use. The HDI value is directly correlated with the ability of the land to deliver optimal crop yields when used for various land management activities. Higher IPL values indicate higher land capability, which indicates its suitability for agricultural and other land-intensive activities. Classification of land potential is done systematically using a specialized formula that takes into account factors such as soil quality, topography, climatic conditions and water availability. This classification helps in identifying the most suitable areas for development, ensuring sustainable land management practices.

Table 1. Characteristics of land class information

Class	Obstacle	Land characteristics
Land class with very high capabilities	Land in this class has obstacles that limit its use, suitable for all kinds of agricultural uses (Hartati et al. 2018; Firmansyah et al. 2022)	This class is characterized by flat soil, little danger of erosion, deep solum, generally well-drained, easy to cultivate, can hold water well and is responsive to fertilization (Abdelrahman et al. 2016)
Land class with high land potential	Land in this class has several obstacles that narrow crop choices or require land preservation measures (Islam et al. 2018)	This class is characterized by rolling slopes, moderate erosion hazard, moderately shallow soil depth, and moderate drainage.
Land class with medium capabilities	Suitable for all agricultural businesses where the threat of damage is greater than on high land classes (Hartati et al. 2018)	This class is characterized by slightly sloping slopes, or very sensitive to erosion hazards, poor drainage, very slow soil permeability, shallow solum, low water holding capacity, low soil fertility and is not easily repaired.
Land class with low capability	This land can be planted, but the choice of crops that can be planted is very limited. Can be used for various types of agricultural uses with the threat and danger of greater damage to the land.	This class is characterized by steep slopes, great erosion sensitivity, low water holding capacity, and high salinity (Hossen et al. 2021)
Land class with very low capabilities	This land is usually not cultivated but is used as a protected area.	This land is characterized by a high risk of erosion, and frequently experiences flooding, rocky soil, and soil in swampy areas that are difficult for drainage.

Data analysis

Method used in this research is a field survey method with purposive sampling technique applied to each Index Potential Land (IPL) class. Purposive sampling was chosen because it allows researchers to deliberately select sample locations that are considered representative based on certain criteria, such as soil type, topography, and land use relevant to the research. In addition to the field survey, in-depth interviews with key informants were also conducted to support the final results. The key informants in this study were the head of the Agriculture Office and local farmers. These interviews aimed to obtain in-depth information on farming conditions and practices that could affect the potential of the land. For data analysis, tiered quantitative and qualitative methods were used with the support of Geographic Information System (GIS) technology (Montgomery et al. 2016). Tiered quantitative analysis was conducted by scoring each parameter that contributes to the determination of Land Management Index (LMI), such as soil fertility, water availability and other environmental factors however, for the analysis of agricultural land potential, the Index of Land Potential (IPL) is more often the first choice as its evaluation includes more attributes, resulting in a more complete and holistic assessment of land suitability for agriculture. Each parameter is given a weight based on its level of influence on agricultural land productivity (Kumar 2019). IPL is a method to identify the potential of land, which is very useful for sustainable management based on the capability and potential of the land. The determination of IPL uses parameters such as slope, hydrology, soil type, lithology, and disaster vulnerability, where each parameter has unique characteristics that contribute to the assessment of IPL. This research aims to provide a comprehensive understanding of land potential to support agricultural sustainability in Sleman District.

Land use generally depends on the capabilities of the land and on the location of the land (Wondimu and Ayansa 2022). For agricultural activities, land use depends on land capacity classes which are characterized by differences in properties that become obstacles to its use such as soil texture, slope of the land surface, ability to hold water and the level of erosion that has occurred. The Land Potential Index (IPL) is an approach that uses the assessment of several parameters according to their influence on the potential of a land. The parameters used in determining the land potential index are slope, relief, lithology, soil solum depth, soil texture, hydrology and disaster vulnerability (Li 2023). The land potential index is processed using the Geographic Information System (GIS) spatial method so that the location and area can be known in map form. Sleman based on relief or topography variables, slope, lithology, depth of soil solum, soil texture, hydrology and vulnerability to disasters (erosion) (Herzberg et al. 2019).

The score assessment for soil texture parameters and soil solum depth is mentioned in Tables 2 and 3. Table 2 presents the classification of soil texture, which includes different types of textures such as clay, sand, and silt. This classification is important for determining the ability of land to support organic farming, taking into account factors such as water retention ability, soil aeration, and nutrient availability. Soil texture can be used to determine the classification of soil texture distribution and suitability soil texture is based on land cover, soil texture is the level of fineness of the soil that is seen of the particles contained in a soil (Santos-Francés et al. 2022). Soil texture is one of the most common soil properties applied and is usually called the soil grain size. Soil texture is related closely related to the movement of water and dissolved substances, air, the movement of heat, weight land volume, specific surface area, ease soil compacts (compressibility) (Maleki et al. 2022).

In Table 3, soil depth is categorized based on the ability of the soil to support plant root systems and water retention. The categories include very shallow, shallow, medium, deep and very deep soils. Latosol soil type gets a texture score of 2 and a depth score of 5. The Andosol soil type gets a texture score of 5 and a depth score of 4. The Mediterranean soil type gets a texture score of 5 and a depth score of 5. The Regosol soil type gets a texture score of 1 and a depth score of 1. Soil type The one with the best score for assessing the land potential index is Mediteran. The type and texture of soil can chemically influence the availability of nutrients in the soil and physically the depth of the soil solum can influence soil permeability, soil structure and soil water availability. The assessment of slope, relief and erosion vulnerability parameter scores is mentioned in Tables 4 and 5.

The chapter on the land potential class index method in Table 4 related to slope and relief provides a detailed analysis of how slope and relief characteristics affect land potential. This method uses topographic criteria-based classification to determine the land potential index, where slope and relief are the main factors (Pimenta et al. 2021). Land slope is a certain height difference in the existing relief on a landform. Determination of the average land slope for each mapping groups can be done by creating relationships between points. The length of one line indicates the same slope. The slope of the land indicates the character of the area which must be considered in land use guidelines. Relief is the perpendicular (vertical) difference between the high and low parts of the earth's surface. Relief influences the potential of land based on the ease of access to the land for use or utilization (Maulida and Munir 2022).

The land potential class index in Table 5 shows the varying levels of disaster vulnerability for various land types in Sleman District. Based on the processed data, land with high potential tends to have a lower risk of disaster vulnerability compared to land with medium and low potential. Areas with low to high slopes and flat to mountainous relief, respectively, get a slope score and relief score of 5 to 1. Areas without disaster vulnerability get a score of 1, light vulnerability gets a score of 0.8, moderate vulnerability gets a score of 0.7, severe vulnerability gets a score of 0.6, and very severe vulnerability gets a score of 0.5. Erosion is the main barrier to sustainable land use. Identification of erosion on forest land is needed to determine the type and level of erosion and the percentage of the eroded area on a map unit to plan practical soil conservation efforts. The erosion hazard level can be predicted based on field conditions by paying attention to sheet erosion, rill erosion, and gully erosion. Another approach to predicting the level of erosion hazard, which is relatively more straightforward, is to pay attention to the soil surface lost (on average) per year compared to non-eroded soil, characterized by the presence of the A horizon. A dark color usually characterizes the A horizon because it relatively contains material higher organic (Mandal et al. 2022).

In analyzing methods to determine the potential of organic farming land, it is necessary to conduct an in-depth evaluation of various environmental, economic, and social factors that affect land suitability. Using multi-criteria approaches such as the Analytic Hierarchy Process (AHP) or Geographic Information System (GIS) helps integrate spatial and non-spatial data to evaluate land suitability based on parameters such as soil quality, water availability, topography, and market accessibility. In addition, this analysis should also consider sustainable agricultural practices and their impact on biodiversity and climate change mitigation. This holistic approach ensures that organic farming is productive and profitable and contributes to the SDGs' overall achievement.

Table 2. Soil texture score assessment

Code	Texture	Type of soil	Honor
T1	Rough	Regosol, litosol, organosol	1
T2	Rather rough	Podsolic, andosol	4
T3	Currently	Brown alluvial, andosol, mediterranean	5
T4	Somewhat subtle	Gley humus, renzina, podsol	3
T5	Fine	Grumusol, latosol, alluvial gray	2

Table 3. Soil solum depth score assessment

Code	Depth (cm)	Type of soil	Honor
S1	Very deep >100	Alluvial, latosol, mediteran, podzolic, grumusol	5
S2	Within 75-100	Andosol, podsol	4
S3	Medium 50-75	Renzina, planosol	3
S4	Shallow 30-50	Gley humus, hydromorph	2
S5	Very shallow <30	Regosol, litosol	1

Source: (Nurda et al. 2020)

Table 4. Slope and relief score assessment

Class	Slope (%)	Honor	Relief	Honor
I	0-5	5	Flat-sloping	5
II	5-15	4	Wavy	4
III	15-25	3	Low hilly	3
IV	25-45	2	Hilly	2
V	>45	1	Mountainous	1

Source: (Nurda et al. 2020)

Table 5. Disaster Vulnerability Factors (Erosion)

Code	Disaster vulnerability level	Honor
E1	Very heavy	0.5
E2	Heavy	0.6
E3	Currently	0.7
E4	Light	0.8
E5	Without	1.0

Source: (Nurda et al. 2020)

RESULTS AND DISCUSSION

Land with high and very high potential values indicates the ability of the land to be utilized productively based on its soil type, groundwater availability, parent rock and slope conditions. The nutrient-rich soil and abundant groundwater make this land ideal for productive agriculture. The priority use of this land is for agricultural development, although some land is used for settlements due to few limiting factors This is due to several limiting factors, such as low land slope, good soil texture, and accessibility to infrastructure. These conditions make the land suitable not only for agriculture but also for housing development. The impact of this dual land use must be managed wisely through comprehensive spatial planning, taking into account the balance between the need for productive agricultural land and housing development in Sleman District. Land with a medium-class index of land potential (IPL) is usually utilized for limited cultivation, forest areas, tourist parks, or settlements. In contrast, land with a low IPL has limited potential due to disaster risk and poor relief conditions, making it more suitable for protected areas. This analysis uses the parameters of soil type, aquifer productivity, slope and rock type. However, for more accurate results, it is necessary to consider additional data such as soil texture, availability of irrigation channels, and other morphology. The implication is that land use must consider its potential and limitations to be optimal and sustainable.

The lithological parameter in Table 6 indicates that pyroclastic rocks achieve the highest lithology score of 8, followed by massive igneous and coarse-grained clastic sedimentary rocks with a score of 5, and fine-grained clastic sedimentary rocks with the lowest score of 2. The lithology factor represents the parent soil material that contains minerals that influence their availability nutrients in the soil (Peano et al. 2014). The content of nutrients that come from rocks, such as P and K, in the soil really depends on the rocks that make up the soil. The forms of P and K are basically contained in a form that is not available or is slowly available, but with certain treatments it can dissolve the P and K so that they can be available to plants. However, through specific treatments such as soil tillage and fertilizer application, phosphorus (P) and potassium (K) can be mobilized and become more available. Newly formed pyroclastic deposits from the volcanic activity of Mount Merapi contain high levels of potassium (K) but low levels of phosphorus (P). Over time, following volcanic disturbances, the nitrogen (N) content in the soil tends to increase (Utami et al. 2018).

Relief and slope

Based on the data in Table 7 and Figure 3, most of Sleman District has undulating relief conditions with an area of 30,063 hectares or around 52.43% of the total district area. This condition is spread evenly across all sub-districts in Sleman. In addition, flat to gentle relief conditions are also quite significant, covering an area of 19,457 hectares or 33.93% of the total area. This shows that almost half of Sleman District has varied topography, from flat to undulating, which can affect various aspects, including land use and agricultural practices. Next, there are low hilly conditions with an area of 4,714 Ha with a percentage of 8.22% in several areas, namely Prambanan, Berbah and Gamping, next in sequence you will find areas with hilly conditions with an area of 2,147 Ha with a percentage of 3.74%, conditions that will rarely found in the Sleman area with mountainous conditions, namely an area of 955 Ha, with a percentage of 1.66%, mostly in the Cangkringan and Pakem areas. Relief is a factor that is closely related to climate, especially temperature. The higher the relief, the lower the temperature on the land, thus affecting the soil formation process. This means that the relief affects the physical properties of the soil in an area (Nurda et al. 2020).

The slope gradient significantly affects the processes of weathering and soil development, washing and transport of soil (El Behairy et al. 2022). Based on the data listed in Table 8, most of the Sleman District area is in the 5-25% slope class, which is spread across all sub-districts with a land area of 30,063 hectares or around 52.43% of the total area. In addition, areas with a slope class of 0-5% cover a land area of 19,457 hectares, which represents 33.93% of the total area of Sleman District. These two slope classes show the dominance of land with low to moderate slope levels, which are generally more suitable for various types of agricultural and residential land uses. These slope classes play an important role in spatial planning and regional development, especially in supporting agricultural activities and infrastructure development with this class spread across all sub-district areas, then in class 15-25% with an area of 4,714 Ha with a percentage of 8.22% in this class in the Prambanan, Berbah and Gamping areas, in the slope class 25-45 area 2,147 with a percentage of 3.74% this cass is spread across several areas of Cangkringan, Pakem, Turi, Prambanan and Godean.

Table 6. Lithological parameter assessment

Code	Rock type	Honor
Lb	Massive igneous rocks	5
Lp	Pyroclastic rocks	8
Lk	Coarse grained clastic sediment	5
Lh	Fine grained clastic sediment	2
Lg	Calcareous and metamorphic sediments	3
Ll	Limestone	5
La	Alluvium/coluvium	10

Source: Nurda et al. 2020

Table 7. Slope class in Sleman District, Yogyakarta, Indonesia

Relief			
Class	Area (ha)	Percentage	Region
Mountainous	955	1.66%	Cangkringan, Pakem
Hilly	2147	3.74%	Cangkringan, Pakem, Turi, Prambanan, Godean
Low hilly	4714	8.22%	Prambanan, Berbah, Gamping
Wavy	30063	52.43%	Spread across all district areas
Flat-sloping	19457	33.93%	Spread across all district areas

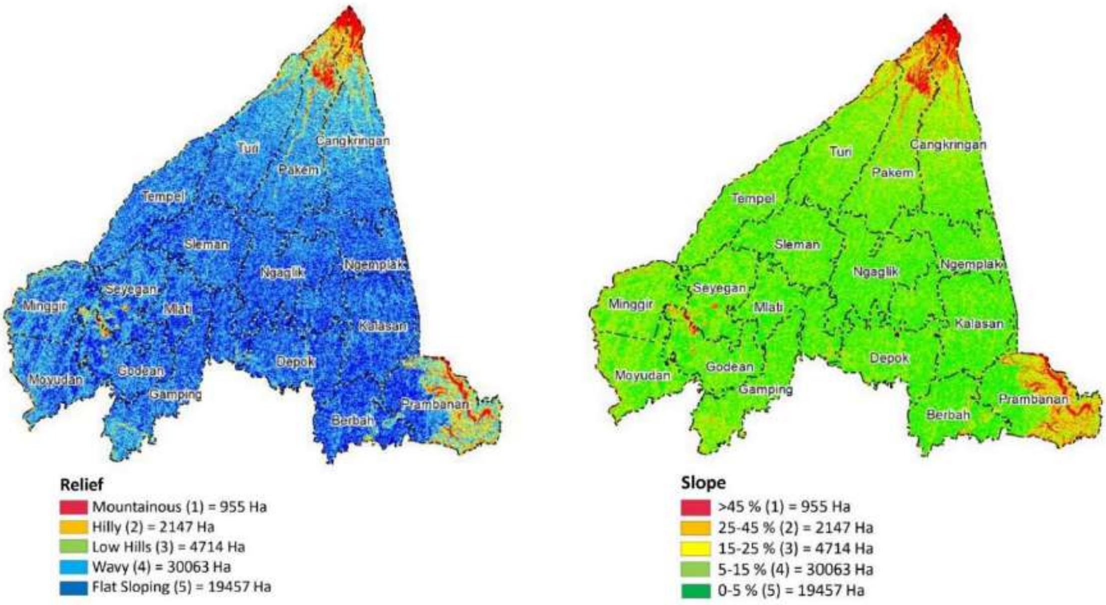

Figure 3. Relief map and slope map of Sleman District, Yogyakarta, Indonesia

Table 8. Slope class in Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
>45 %	955	1.66%	Cangkringan, Pakem
25-45 %	2147	3.74%	Cangkringan, Pakem, Turi, Prambanan, Godean
15-25 %	4714	8.22%	Prambanan, Berbah, Gamping
5-25 %	30063	52.43%	Spread across all district areas
0-5 %	19457	33.93%	Spread across all district areas

Connection between land slope and other parameters is quite dominant. Usually in different topography, which means the slope is different, the soil development is also different. Differences in soil development also mean there are differences in characteristics. Soil development is also influenced by the direction of the slope, because differences in slope will affect the speed of rock weathering into soil. Thus, the slope of the slope usually contains the consequences of differences in soil texture, drainage conditions, plant types and soil depth (Hossen et al. 2021).

Lithology and soil depth

Knowledge of lithology is very important in agriculture because it affects the physical and chemical properties of soil. Table 9 of the research results related to lithology in the research area shows that most of the lithological conditions in the Sleman District area, namely 98.88% or 56,694 Ha, are pyroclastic rocks. According to research conducted by (Astari et al. 2022) also explained that pyroclastic rocks are the result of volcanic activity, especially from the eruption of Mount Merapi which is located in the northern region and is part of Sleman District. The mountain is located on the boundary line between Yogyakarta and Central Java. These rocks consist of volcanic fragments such as volcanic ash, which are deposited during eruptions (Faozan et al. 2022). Pyroclastic sedimentary rocks are sedimentary rocks composed of volcanic material, the result of eruptions that have undergone transportation and deposition. The eruption of Mount Merapi that occurred had a negative impact on the community, but on the other hand there were great benefits in it, namely re-fertilizing the existing land. Soil becomes fertile again due to the nutrients contained in volcanic ash. This process is known as soil recovery, which helps improve the fertility of agricultural land. Shortly after the pyroclastic flow occurred, the process of soil formation began through the weathering of the elements and minerals contained therein. In the agricultural sector, apart from always providing new materials in the soil formation process, Pyroclastic material also provides a suitable growing place for plants by providing plant nutrients in the minerals it contains. Figure 4 map of the distribution of lithological research results in Sleman District shows massive igneous rocks, with 1.05% or 603 Ha, mostly spread in the Gamping area, then fine-grained clastic deposits with a percentage of 0.06% or 39 Ha, which are scattered in the Godean District area. The depth of the soil greatly determines plant growth. Shallow soil will be limited in its ability to provide water and other nutrients. Besides that, the depth of the soil really determines whether the land can be cultivated or not. In shallow soil, soil management will actually turn the subsoil upwards, which has consequences disruption of plant growth (Rachman et al. 2020). In the classification of land capability and suitability, the soil depth factor is very taken into account and determines. The depth of this soil can vary in various locations, and affects the productivity and quality of agricultural land. Regarding the depth of the soil in the Sleman District area, it is divided into 2 classes, namely very shallow and very deep. The effective depth factor of the soil will greatly influence the development of plant roots, if the depth is relatively thin it will inhibit root development.

Table 10 shows that the soil depth condition in Sleman District is very deep with a land area of 55,779 Ha or 97.28% of the area with very deep class soil depth characteristics spread throughout the sub-district area, then the soil depth condition is very shallow with a land area of 1557 Ha or 2.71% of the area with very shallow in Figure 4 can be explained related to the distribution of the soil depth distribution map covering Pakem and Tempel sub-districts. Deeper soil layers can also contain more nutrients due to the process of accumulating organic matter and minerals over thousands of years. Plants planted in deep soil solums are usually more fertile and productive because they have better access to nutritional resources.

Soil texture and hydrogeology

The soil texture most sensitive to erosion is very fine sand and dust. Therefore, the higher the dust content in the soil will maintain soil quality, Table 11 shows the condition of soil texture in the Sleman District area mostly with a land area of 34.686 Ha or 60.49% of the area can be explained in Figure 5 related to the distribution of soil texture in this condition, which is scattered in Tempel, Turi, Sleman, Ngaglik, Ngemplak, Kalasan, Mlati and Seyegan sub-districts, then with a medium soil texture class with an area of 18,121 Ha or 31.60% of conditions with medium texture spread in the Minggir, Moyudan, Godean, Gamping, Depok, Berbah and Prambanan sub-district, next in sequence namely the moderate class fine with a land area of 2,972 Ha or 5.18% spread across the Prambanan, Gamping and Berbah Sub-districts, next is the coarse soil texture class with an area of 1,557 Ha or 2, influence of soil texture on agricultural land is related to the soil's ability to store water, provide nutrients, support plant roots, and influence aeration and drainage (Mishra et al. 2015).

Hydrogeology affects the availability of groundwater in an area. If the land is located in an area with abundant groundwater, agriculture or activities that require water will be easier to carry out. On the other hand, if the land is located in an area with limited access to groundwater, then land use must be regulated. Table 12 shows that most of the hydrogeological conditions in Sleman District fall into the High Productivity class with a fairly wide distribution in this class with a total area of 46,119 Ha or 80.43% which can be seen in Figure 5 hydrogeology distribution map spread across all sub-district areas, then the Medium Productivity class with a total distribution area of 6444 Ha or 11.23%. Next in sequence is the medium-small productivity class with a total land area of 1390 Ha or with a percentage of 2.42%, then the rare groundwater class with a total area of 3382 Ha or 5.89%.

Table 9. Lithology class of Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
Fine Grained Clastic Sediment	39	0.06%	Godean
Massive Igneous Rocks	603	1.05%	Gamping
Pyroclastic Rocks	56694	98.88%	Spread across all sub-district areas

Table 10. Soil depth classes in Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
Very shallow	1557	2.71%	Pakem, Tempel
Very deep	55779	97.28%	Spread across all sub-district areas

Table 11. Soil texture classes of Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
Rough	1557	2.71%	Pakem, Cangkringan
Fine	34686	60.49%	Tempel, Turi, Sleman, Ngaglik, Ngemplak, Kalasan, Mlati, Sayegan
It's a bit subtle	2972	5.18%	Prambanan, Gamping, Berbah
Currently	18121	31.60%	Moyudan, Godean, Gamping. Depok, Berbah, Prambanan

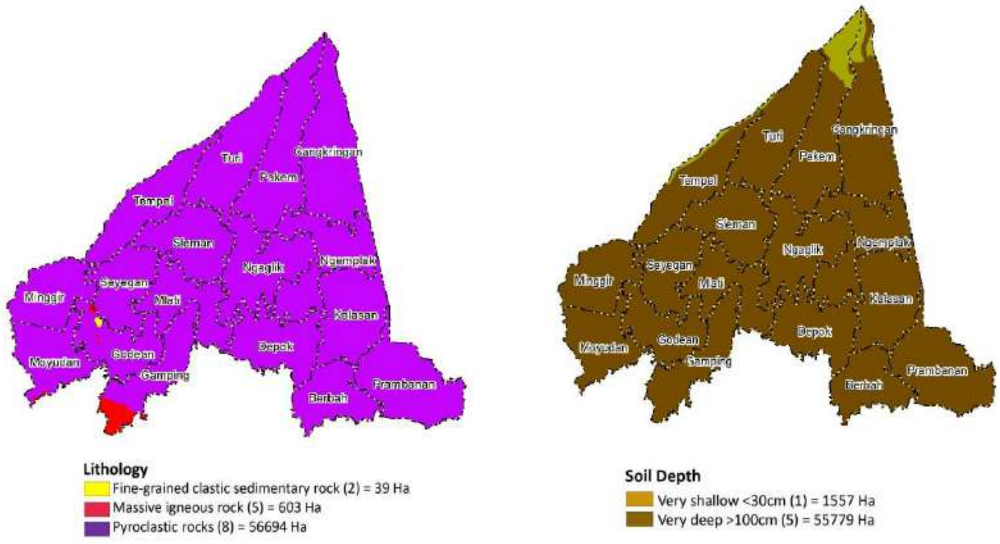

Figure 4. Lithology and soil depth of Sleman District, Yogyakarta, Indonesia

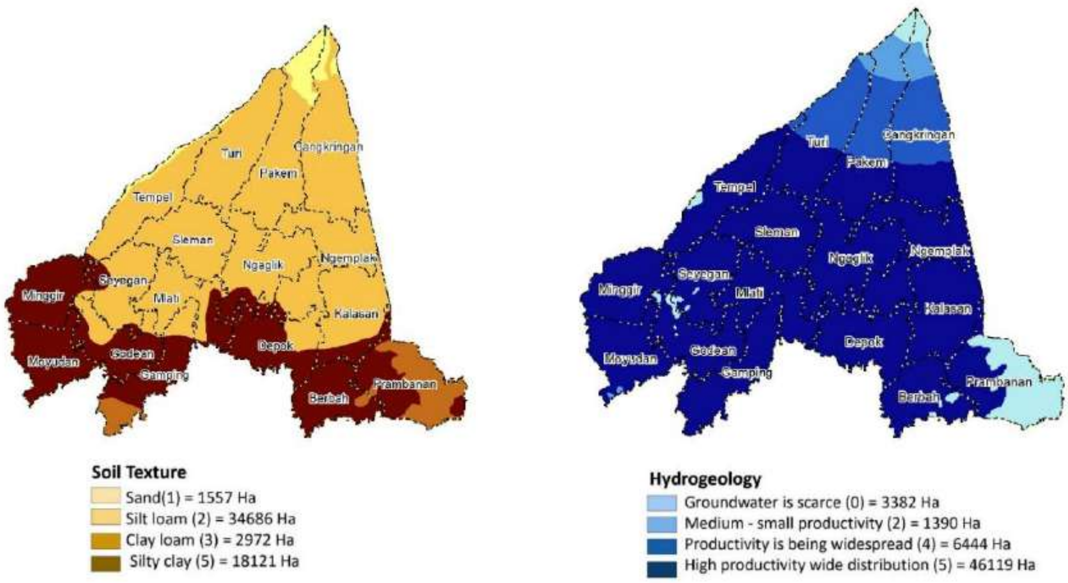

Figure 5. Soil texture and hydrogeology of Sleman District, Yogyakarta, Indonesia

Table 12. Hydrogeological class of Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
Rare groundwater	3382	5.89%	Pakem, Cangkringan, Prambanan, Sayegan
Medium-small productivity	1390	2.42%	Cangkringan, Pakem, Turi
Medium productivity-widespread	6444	11.23%	Cangkringan, Pakem, Turi
High productivity-wide distribution	46119	80.43 %	Spread across all sub-district areas

Erosion disasters and land potential index

Erosion can remove nutrient-rich soil layers and reduce the fertility of agricultural land. This can reduce crop yields and plant productivity. In addition, sedimentation caused by erosion can clog irrigation canals and rivers, cause flooding, and damage aquatic ecosystems. The conditions of erosion disaster classes in Sleman District can be illustrated in Table 13, where the majority are in a severe condition, spread throughout the entire region of Sleman District, covering an area of 28,765 hectares or 50.16%. Additionally, an area of 19,010 hectares or 33.15% falls into the class where no erosion disaster occurs. This distribution is also depicted in Figure 6, which shows the spread throughout the entire region. Next in sequence are the medium class with a land area of 8855 Ha or 15.44%, the light class with a total area of only 67 Ha or 0.11%, then the very heavy class with a total area of 640 Ha or 1.11%.

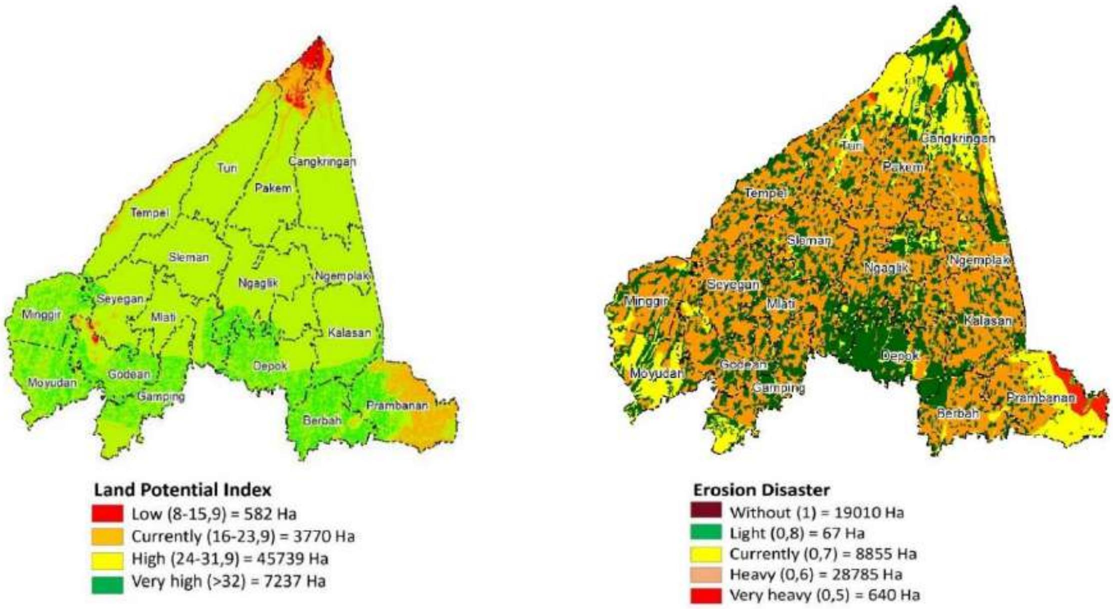

Figure 6. Erosion disasters and land potential index of Sleman District, Yogyakarta, Indonesia

Table 13. Sleman District erosion disaster class

Class	Area (Ha)	Percentage	Region
Without (1)	19010	33.15%	Spread across all regions
Light (0.8)	67	0.11%	Spread across all regions
Medium (0.7)	8855	15.44%	Spread across all regions
Weight (0.6)	28765	50.16%	Spread across all regions
Very Heavy (0.5)	640	1.11%	Spread across all regions

Erosion removes important nutrients such as nitrogen, phosphorus, potassium and other micronutrients from the soil. Plants on organic farms depend on the natural nutrients of the soil to grow without the use of chemical fertilizers. If nutrients are lost due to erosion, plants can become weak and susceptible to pest and disease attacks. Basically, every soil has a different level of sensitivity to erosion, depending on the physical properties and rocks that form it. Thus, apart from being related to land form, erosion conditions are also related to soil properties and rock types Plants can become weak and susceptible to pest and disease attacks. Basically, every soil has a different level of sensitivity to erosion, depending on the physical properties and rocks that form it. Thus, apart from being related to land form, erosion conditions are also related to soil properties and rock types Plants can become weak and susceptible to pest and disease attacks. Basically, every soil has a different level of sensitivity to erosion, depending on the physical properties and rocks that form it. Thus, apart from being related to land form, erosion conditions are also related to soil properties and rock types (Stellacci et al. 2021).

Discussion

Determination of land criteria class

Based on the data presented in Table 14, which represents the overall results of the Land Potential Index parameters in Sleman District, there are four classes of land potential with varying distributions. The low land potential class covers 583 hectares or 1.01% of the area, spread across Seyegan, Godean, and Cangkringan Sub-districts. The medium land potential class has an area of 3,772 hectares or 6.57% of the area, completely shown in Figure 7 which includes the sub-districts of Moyudan, Minggir, Seyegan, Godean, Gamping, Berbah, Prambanan, Kalasan, Tempel, Turi, Pakem and Cangkringan. The high land potential class is the most dominant with an area of 45,741 hectares or 79.77%, spread evenly across all sub-districts. Meanwhile, the very high land potential class covers an area of 7,239 hectares or 12.62%, found in Moyudan, Minggir, Seyegan, Godean, Gamping, Mlati, Depok, Berbah, Prambanan, Kalasan, Ngemplak, Ngaglik, Tempel, Turi, and Pakem.

Table 14. Land potential index class for Sleman District, Yogyakarta, Indonesia

Class	Area (ha)	Percentage	Region
Low	583	1.01%	Seyegan, Godean, Cangkringan
Currently	3772	6.57%	Moyudan, Minggir, Seyegan, Godean, Gamping, Berbah, Prambanan, Kalasan, Tempel, Turi, Pakem and Cangkringan
Tall	45741	79.77%	Spread throughout the sub-district
Very high	7239	12.62%	Moyudan, Minggir, Seyegan, Godean, Gamping, Mlati, Depok, Berbah, Prambanan, Kalasan, Ngemplak, Ngaglik, Temple and Pakem

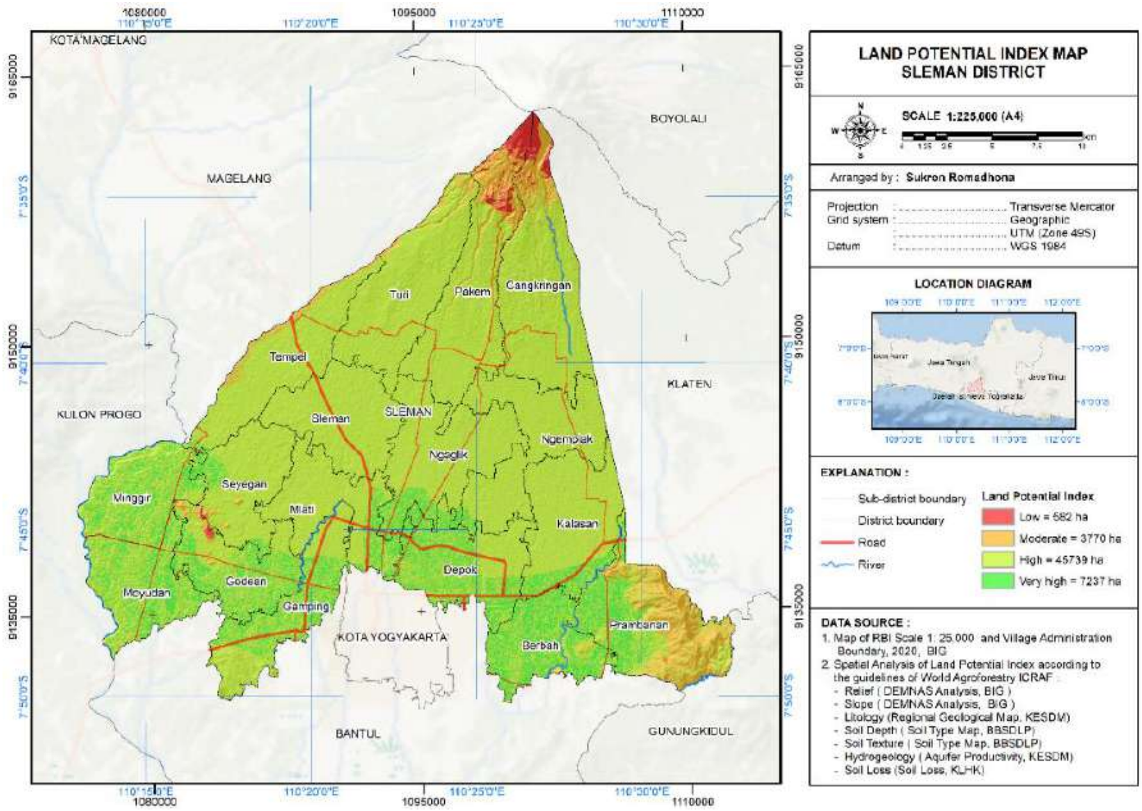

Figure 7. Map of Land Potential Index distribution classes in Sleman District

Analysis of this data shows that most of Sleman District has high land potential, which is evenly distributed across all sub-districts. This indicates excellent opportunities for developing the agricultural sector in this region. Sub-districts with very high land potential, such as Moyudan, Minggir, and Godean, have more significant opportunities for intensive sustainable agricultural development, including organic crops. Although more limited, areas with low and medium potential still have essential contributions to make in land use diversification and agricultural risk mitigation. The implication is that the government and stakeholders in Sleman District must focus on optimizing high and very high-potential land through sustainable agricultural practices and advanced agricultural technologies. In addition, adaptive and sustainable land management strategies are needed to address environmental challenges and support the economic welfare of local farmers.

Land potential index provides information regarding land use directions in accordance with land potential and resources. Agricultural land potential data is important data because it is used to evaluate the suitability of agricultural land and determine good land use. The condition of the land potential index in the Sleman District area is mostly in the high class based on the potential index data of Land Potential Index Level (IPL) which is produced from the overlay of slope parameters, lithology, soil type, groundwater productivity and disaster vulnerability erosion.

Figure 7 related to the land potential index class distribution map can explain that in the high class with a total land area of 45,741 Ha or 79.77% of the high class area is spread throughout the sub-district area. Next is the very high Land Potential Index (IPL) class with a total land area of 7239 Ha or 12.62% of the very high class spread across the areas of Moyudan, Minggir, Seyegan, Godean, Gamping, Mlati, Depok, Berbah, Prambanan, Kalasan, Ngemplak, Ngaglik, Temple and Pakem. In sequence, the land potential index class is medium with a total land area of 3772 Ha or a percentage of 6.57%, the next most rarely found is the low class with a total land area of 583 Ha or a percentage of 1.01%. The level of land potential can be determined from several supporting parameters, the higher the land potential index, the greater the potential for land use.

Distribution and development potential of food crops and horticulture lands

The map of potential distribution of food and horticultural crops in Sleman District can be shown in Figure 8 shows that certified organic rice plants are scattered in Berbah District (Sido Rukun Farmer Group) and Pakem (Rukun Farmer Group). Figure 9 shows the distribution of salak farmer groups in the Sleman District area, in more detail the distribution includes in Turi Sub-district (Gemilang Farmer Group), certified organic salak is a leading horticultural commodity.

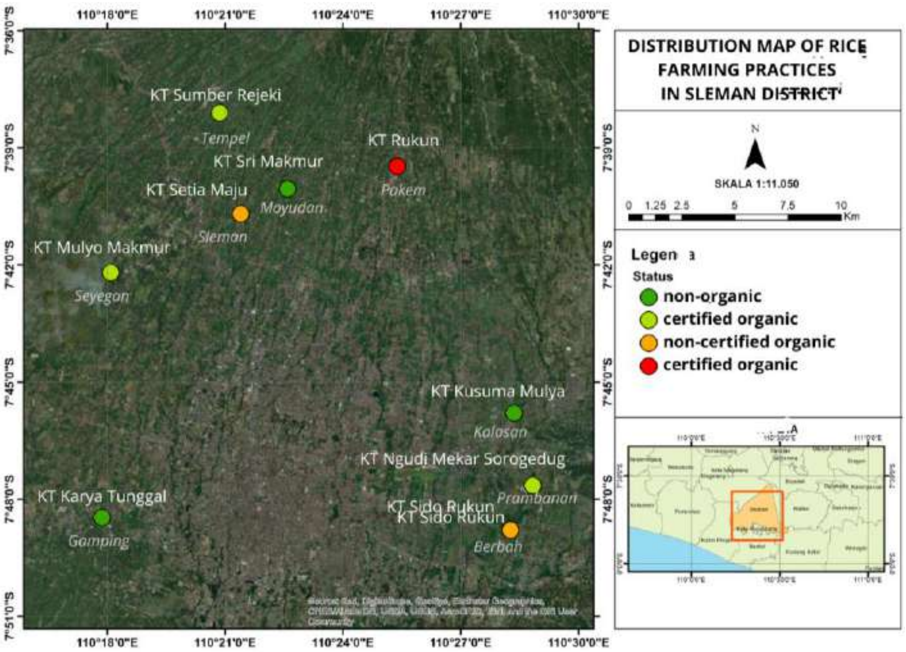

Figure 8. Distribution of rice fields with different farming practices in Sleman District, Yogyakarta, Indonesia

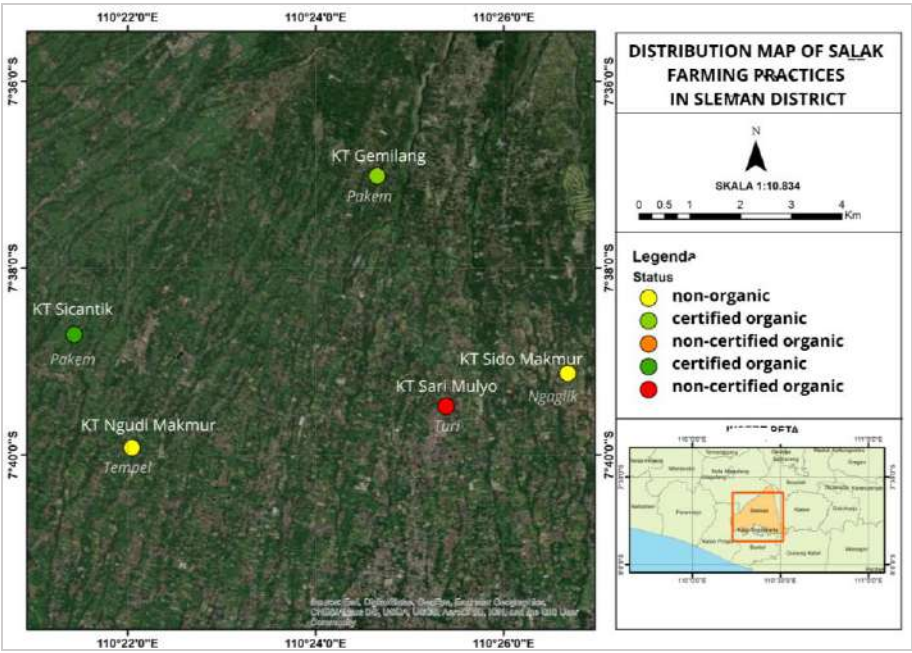

Figure 9. Distribution of *salak* fields with different farming practices in Sleman District, Yogyakarta, Indonesia

Figure 10. Farmland for rice crops. A. Organic rice farmland; B. Non-organic rice farmland

Figure 11. Salak farmland. A. Salak farmland with organic practices; B. Salak field with non-organic practices

Meanwhile, non-organic rice is dominated in Gamping (Karya Tunggal Farmer Group) and Kalasan (Kusuma Mulya Farmer Group) Sub-districts. Non-organic salak is found in Tempel Sub-district (Ngudi Makmur Farmer Group). In addition, non-certified organic rice is scattered in Prambanan (Farmer Group Ngudi Mekar Sorogedug Lor) and Seyegan (Farmer Group Mulyo Makmur) Sub-districts, while non-certified organic salak is found in Pakem Sub-district (Farmer Group Mandiri). This map reflects the diversification of agricultural commodities in various sub-districts in Sleman District, with differences in certification status indicating variations in agricultural practices and the potential for local commodity development.

Figure 10 shows the area of rice farmland in Sleman District. Certified organic rice farming areas in Sleman District have physical characteristics that support sustainable agricultural practices. The soil in this area is generally fertile with a sandy loam texture that is good for water absorption and air circulation, allowing plants to grow optimally. The land is located in the low to medium plains with altitudes between 100-500 meters above sea level, providing ideal climatic conditions for organic farming. The availability of sufficient irrigation water sources from rivers and boreholes also ensures the sustainability of water supply for crops. Non-organic farmland is often located in areas with easier access to infrastructure and markets, but tends to face challenges of soil degradation due to intensive use of chemical fertilizers and pesticides. Soil structure on non-organic land is often denser, with lower organic matter content and soil pH that can vary more widely. The sustainability of these lands depends on more intensive soil management practices to maintain productivity.

Figure 11 shows the area of salak farmland in Sleman District. Certified organic salak farmland areas in Sleman District show better physical characteristics compared to non-organic land. Certified organic land generally has a looser soil structure, higher fertility levels, and better biodiversity due to the implementation of sustainable agricultural practices that avoid the use of synthetic chemicals. In contrast, non-organic salak land tends to experience soil degradation due to excessive use of chemical fertilizers and pesticides, which can lead to decreased soil and water quality, and reduced biodiversity. In addition, certified organic land is often managed with better soil and water conservation techniques, thus retaining soil moisture and reducing erosion. Overall, certified organic farming practices in Sleman District have a positive impact on land physical characteristics compared to non-organic practices.

Linkages between organic farming and SDGs in Sleman District

Organic farming promotes environmentally friendly practices that improve soil fertility, reduce water pollution, and increase biodiversity. Determining the right land potential for organic farming is crucial in achieving these SDGs, as suitable land will support sustainable productivity without harming the environment. Land suitability evaluation based on ecological and socio-economic criteria ensures that agricultural practices can support food security, farmer welfare, and climate change mitigation. Thus, organic farming carried out on suitable land not only contributes to the production of healthy and safe food but also to the achievement of holistic, sustainable development.

SDG 2: End Hunger. Organic farming in Sleman can increase food production and food security through sustainable techniques such as crop rotation, crop diversity, and agroforestry. Crop rotation can prevent soil degradation and maintain soil fertility, while crop diversity helps reduce the risk of crop failure due to certain diseases or pests. Agroforestry, which combines agriculture with forestry, increases land productivity while maintaining environmental sustainability. SDG 3: Healthy and Prosperous Lives Organic farming promotes healthy diets by producing nutrient-rich foods without harmful pesticides and chemical fertilizers. This improves consumer health and reduces farmers' exposure to harmful chemicals. Using organic materials and natural farming techniques helps retain nutrients in the soil and plants, resulting in healthier and more nutritious food products. SDG 9: Industry, Innovation, and Infrastructure: Innovation and technology in organic agriculture, such as precision farming, can increase productivity and reduce environmental impact. In Sleman, using modern technology and innovative practices in organic farming can improve production efficiency and environmental sustainability. Developing infrastructure supporting organic farming is also essential to strengthen local agricultural systems.

In conclusion, land potential (IPL) is in the high class with a total land area of 45741 Ha or 79.77% of the high class area spread across all sub-district areas. Next is the very high Land Potential Index (IPL) class with a total land area of 7239 Ha or 12.62% of the very high class spread across the areas of Moyudan, Minggir, Seyegan, Godean, Gamping, Mlati, Depok, Berbah, Prambanan, Kalasan, Ngemplak, Ngaglik, Temple and Pakem. In sequence, the land potential index class is medium with a total land area of 3772 Ha or a percentage of 6.57%, the next most rarely found is the low class with a total land area of 583 Ha or a percentage of 1.01%. The distribution and land development potential of food crops and horticulture in Sleman show significant variations related to geographical conditions, soil quality, and infrastructure support. Based on the land potential analysis, the northern and central parts of Sleman have high suitability for the development of food crops such as rice and secondary crops, especially in areas with moderate elevation and adequate irrigation systems. Research and observations show that organic farming in Sleman contributes to the SDGs by reducing hunger through increased sustainable food production (SDG 2), promoting health by providing food free of harmful chemicals (SDG 3), and encouraging technological innovation for efficiency and sustainability (SDG 9). This practice provides positive results and a new outlook on the importance of organic farming in the region.

ACKNOWLEDGEMENTS

Researcher would like to express his gratitude because this research was fully funded by the Indonesian Education Scholarship (BPI), Higher Education Financing Centre (BPPT), through the Education Financing Service Center (Puslapdik), the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, and the Management Institution Education Fund, with the following statement letter (LPDP) 1189/J5.2.3./BPI.06/10/2021.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 84-93

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090109

Determinants of women's participation in income generating activities in western Ethiopia

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Manuscript received: 19 September 2024. Revision accepted: 9 December 2024.

Abstract. *Yaried AA, Bullo MS. 2025. Determinants of women's participation in income generating activities in western Ethiopia. Asian J Agric 9: 84-93.* Participating in various income generating activities can improve women's living standards by providing additional income, increasing agricultural productivity, reducing poverty and improving food security. This study focuses on assessing determinants of women's participation in income generating activities in Itang special district of Gambella region. Primarily, Itang Special District was selected purposively among thirteen districts and 168 respondents were selected by using simple random sampling method. The data for this study was collected from both primary and secondary data sources. The descriptive analysis revealed that 62 (36.9%) of the households were farm participants, 86 (51.2%) were non-farm, and 20 (11.9%) were off-farm participants, respectively. Correspondingly, the multinomial logit model indicated that education status, household size, land size, livestock holding, access to credit, access to extension contacts, distance to main road, access to training, access to infrastructures, and access to market information is enormously significant variables that affect women's participation in income generating activities. In conclusion, women's participation in income generating activities has a greater role on improvements of their means of living. Therefore, government agencies, policymakers, and NGOs should pay attention to strengthening rural women households' participation in various income generating activities to improve their means of living.

Keywords: Income generating activities, Itang Special District, livelihood, multinomial logit model

INTRODUCTION

Globally, agriculture is the main fiscal activity practiced by the rural community. Likewise, it is main sources of income for the majorities of rural people in Ethiopia (Wendimu and Moral 2021). Due to this, 83% of the population is engaged in agricultural sector (ILO 2014), and it contributes 39% of national GDP (UNDP 2018). Accordingly, only 27% of the population participated in non-farm economic activities (Tesfaye and Nayak 2022). However, agriculture as a primary source of income has failed to guarantee an adequate livelihood for most farming households in sub-Saharan African countries like Ethiopia (Babatunde 2013). This is because the agricultural sector is highly characterized by decreasing farm sizes, low output levels per land size, and a high degree of own consumption (Bekabil 2023).

In developing countries, women are less empowered than men among several dimensions, including sociocultural, economic, legal and political dimensions of development (Peterman et al. 2021). Thus, achieving the goal of reducing the level of poverty without effective involvement of women in different income generating activities (farm, non-farm and off-farm activities) is impossible to achieve comprehensive development of Ethiopian economy (Bogale and Lemma 2023). Therefore, their participation in income generating activities is more essential to improve their livelihood in developing countries by creating employment opportunities, production of locally needed commodities, income generation, growth of farm activities and reducing poverty (Tesgera et al. 2024). In addition to this, income generating activities can improve the living standards of the poor by increasing income, improving nutrition, savings, education, and fulfill basic necessities of the rural people (Mengistu and Belda 2024).

In rural society, rural women usually play a greater role in the household by performing several types of income generating activities, including cultivation of crops and vegetables, livestock and poultry rearing, small business, and handicraft (Vimefall and Levin 2023). However, women walk against their male's guardian decision because they are depended on their husband's income (Kuma and Godana 2023). So, the participation of women in income generating activities can contribute to enabling households to cope with income shocks, ensure food security, reduce the level of poverty and bring economic growth and sustainable development (Bryan et al. 2023). However, rural women participation in different income generating activities has been limited by education, resources, and support for their pursuit of various income generating activities and this leads them to engage in a low-profit livelihood activity (World Bank 2015; Chekol 2024). Additionally, a study by Alemu et al. (2021) described that education, total land holding, amount of credit and average time spent in domestic work are the main factors that condense the level of women's participation in income generating activities. In most cases, still there is gender economic inequality is associated with disadvantages for women in access to cash, credit, and other forms of wealth compared to men, and there is a restriction on earning and saving money from income-generating opportunities (Haley and Marsh 2021).

Agriculture is the main fiscal activity for the rural people in Gambella region, specifically in Itang special district. But the study area is mostly characterized by unpredictable rainfall and relatively higher temperature (Dangia and Dara 2020). Therefore, rain-fed dependent agriculture alone cannot reduce the high level of poverty; hence, participating in other income generating activities is equally importance (Asmah 2011; Sisay 2024). In rural Ethiopia, if there is no other source of income away from agricultural production in order to generate additional income and minimize agricultural risks, it is impossible to feed and fulfill household needs (Woleba et al. 2023). However, women's participation in various livelihood diversification strategies in Gambella region is very low (Addisu 2017). Therefore, the main objectives of this study is to assess the major determinants of women's participation in income generating activities in Itang special district of Gambella region.

MATERIALS AND METHODS

Study area

The study was conducted in Itang special district which is 45 km away from Gambella regional city, Ethiopia (Figure 1). It encompasses 24 villages with an area of 2,188 km^2. Itang is one of the special districts found in Gambella region of Ethiopia and it is bordered on the northwest by South Sudan, the south and southeast by the Anuak Zone, on the north by the Oromia Region, and on the west by the Nuer Zone. The district is located at 8015'N latitude & 34010'E longitude in the region. The Itang Special District landscape is normally flat with the altitude ranging from 350 to 480 meters above sea level. The total population of Itang Special District is around 42,000 people (Gatdet et al. 2024). Within these populations, approximately 20,589 are females whereas 21,411 are males. While 5,958 or 16.7% are urban inhabitants, a further 278 or 0.78% are pastoralists. The main ethnicities of this district are the Nuer (63.96%), Anuak (25.17%), and foreigners from Sudan (4.62%), Shita (2.66%), and all other ethnic groups 3.59%. Languages spoken in this district include Nuer (68.72%), Anuak (25.75%), and Opuuo (2.66%). The religion with the largest number of believers is Protestant, with 81.63% of the population, while other groups with sizable followings are traditional beliefs (7.54%), Orthodox Christian 6.27%, and Roman Catholic 2.62%.

Like other rural areas of Ethiopia, the area's main economic activity is agricultural production. Cereal crops (sorghum and maize) vegetables, animal herding and fishing are the main activities implemented in the district. However, it is one of the districts that has been highly affected by floods. Hence, in addition to farm activities, the area is also suitable for construction of various income generating activities. Due to this reason, this district was selected from Gambella region to identify the determinants of women's participation in income generating activities in Itang special district of Gambella region.

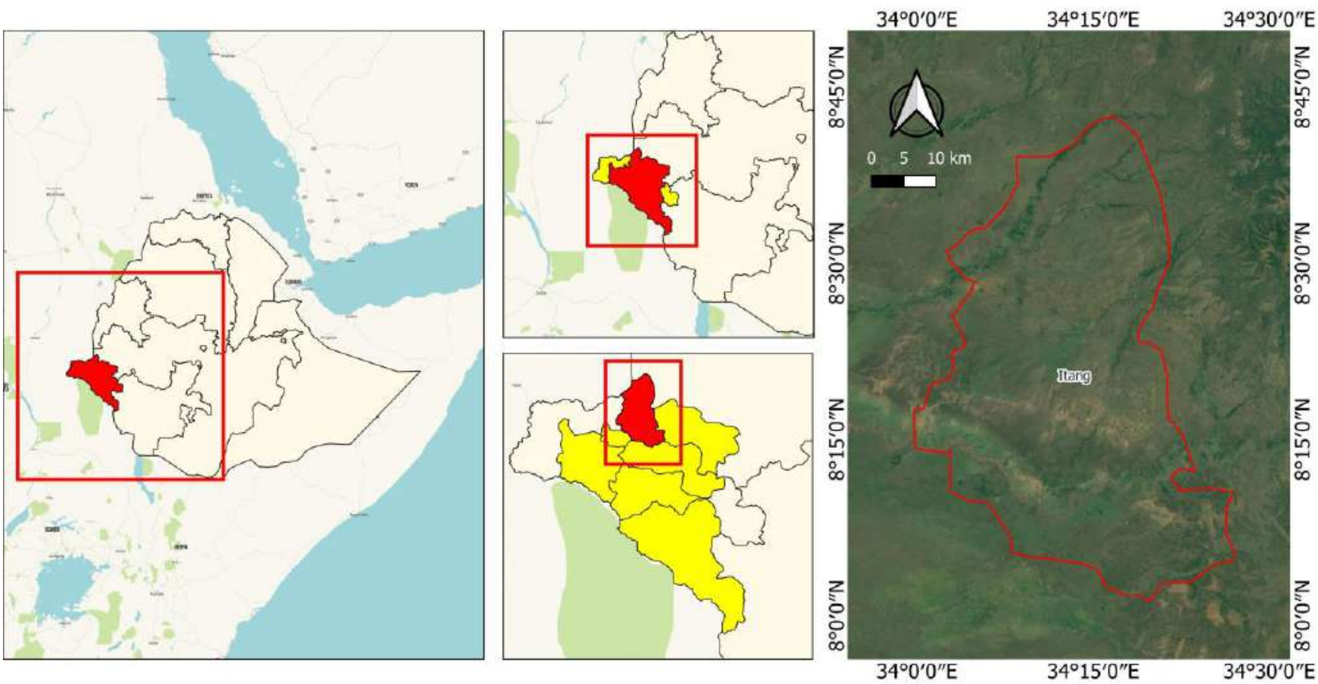

Figure 1. Maps of the study area in Itang special district, Gambella region of Ethiopia

Data collection and sampling techniques

A cross-sectional research design was employed to collect the data from sampled households at a single point in time. Both qualitative and quantitative data collection methods were used to obtain primary and secondary data. The primary data were collected through different data collection methods such as field observation, household survey, and focus group discussion. Whereas a secondary source of data was collected from various documents available in districts and villages, different books, journals, articles, and academic research papers to understand more about the determinants of women's participation in income generating activities. The study employed multi-stage sampling techniques. At the first stage, Itang special district was selected among thirteen districts using purposively sampling method because of its potential for income generated activities such as agricultural production, petty trade, and charcoal making. Similarly, at the second stage, three villages (such as Pulkode, Pilwall, and Achua villages) were selected from 24 villages in the district using purposive sampling technique because of its great potential and suitability for construction of different income generating activities. In the third stage, in order to select the sample respondent from each village, the study used a simple random sampling technique. Due to this, 168 sample respondents were selected among 946 households found in the selected villages and to calculate the sample size (Yamane 1967) formula was used:

$n = N/(1+Ne^2)$

$$\mathbf{n} = \frac{\mathbf{946}}{\mathbf{1 + 946(0.07)^2}} = \mathbf{168}$$

Where:
n: sample size required
N: number of people in the population
e: allowable error

Data analysis

Descriptive statistics (frequency, percentage, means and standard deviation) were used to analyze the socioeconomic characteristics of the households. Likewise, multinomial logit models were employed to analyze the determinant factors that affect women's participation in income generating activities in the study area (Table 1). Nevertheless, before running the multinomial logit model, variance inflation factor and contingency coefficient were employed to check multicollinearity among explanatory variables. The Variance Inflation Factor method was employed to distinguish the problem of multicollinearity for continuous variables. When the VIF value is greater than 10, there is more significant multicollinearity among explanatory variables. The contingency coefficient is calculated to see the degree of association between the dummy or discrete variables (Gujarati 2004). The values of VIF for continuous explanatory variables were found small (that means VIF values of a continuous variable are less than 10). Therefore, based on the VIF result, the data have no serious problem of multicollinearity. Due to this, all seven continuous explanatory variables were taken and entered into a multinomial logit model analysis.

Similarly, the contingency coefficients measure the association among discrete or dummy variables. The value of contingency coefficient ranges between 0 and 1. Hence, with the values of zero show no association between variables, and values close to 1 indicate a high degree of association. Accordingly, the results of the contingency coefficient disclose that there was no serious problem of association among discrete or dummy explanatory variables. Therefore, all six discrete or dummy explanatory variables were entered into multinomial logit model analysis.

Table 1. Description of variables used in the multinomial logit model

Name of dependent variable	Types of variables	Definition and measurement
Income Generating Activities	Polychromous	0 for farm activity 1 for non-farm activity 2 for off-farm activity
Name of independent variable	**Types of variables**	**Definition and measurement**
Ages of the household	Continuous	Age in years
Education status of household	Categorical	0 for illiterate, 1 for grade (1-4), 2 for grade (5-8), 3 for grade (9-12), 4 for diploma, and above
Marital status of the household	Categorical	0 single 1 for married 2 for divorced 3 for widowed
Land size of household	Continuous	Total land size in ha.
Distance to the nearest market	Continuous	Market distance in km.
Livestock holding	Continuous	Number of livestock in TLU
Access to credit	Dummy	1 for users and 0 non-users
household size	Continuous	Numbers of family members
Access to extension services	Continuous	Number of contacts with DAs
Distance to main road	Continuous	Road distance in km
Access to training	Dummy	1 for yes 0 for no
Access to infrastructures	Dummy	1 for yes 0 for no
Access to market information	Dummy	1 for yes 0 for no

The multinomial logit (MNL) model is the most commonly used model for the analysis of discrete choice data that is often used when the dependent variable has more than two nominal categories (Cameron and Trivedi 2005; Anas and Hiramatsu 2012). As explained by Greene and Hensher (2003), either multinomial logit or multinomial probit regression model can be utilized when there are more than two dependent variables. However, multivariate normal distributions and multinomial probit models are rarely used in empirical studies Alemu et al. (2021). Accordingly, in this study, multinomial logit model was employed to analyze the determinants of women participation in income generating activities. The model specification is given as follows:

$$U_{ij1*} = \beta_{1Xi'} + \varepsilon_{i1}$$
$$U_{i2*} = \beta_{2Xi'} + \varepsilon_{i2}$$
$$U_{i3*} = \beta_{3Xi'} + \varepsilon_{i3}$$

Where:

U_{i1}: If households are involved in farm activity

U_{i2}: If households involved in non-farm activity

X_i: Vector of explanatory variable that affects income generating activities

U_{i3}: If household involved in off-farm activity

β: A parameter to be estimated and

ε_i: Is the error term

To express the MNL model, Y denotes a random variable which takes the values of {0, 1, 2...J} for J, a positive integer, and X denotes a set of explanatory variables. In this case, Y denotes income generating activities like farm, non-farm, and off-farm activity and X contains household attributes like ages of the household, educational status, marital status, and others. Since the probabilities must sum to unity, $P(Y = j/x)$ is determined the probabilities for j=2...J. Let x be a 1× K vector with first unit. The MNL model has response possibilities:

$$P(Y = j/x) = \frac{\exp(X\beta_j)}{[1 + \sum_{h=1}^{j} \exp(X\beta_j), = 1 \ldots \ldots . j}$$

RESULTS AND DISCUSSION

Socio-demographic characteristics of the households

As stated in Table 2, about 54.8% of the respondents were Illiterate, while 30.6% were enrolled from grade 1-4, whereas 8.1% completed their grades from 5-8, and 6.5% were attended their educational status from grade 9-12 from farm households. Although, about 18.6% of the respondents were Illiterate, while 23.3% were enrolled from grade 1-4, whereas 44.1% completed their grades from 5-8, 10.5% completed 9-12 grades, and the remaining 3.5% were found above diploma from non-farm households. Similarly, 30.0% of the respondents were Illiterate, while 45.0% were enrolled from grade 1-4, whereas 15.0% completed their grades from 5-8, and 10.0% attend their educational status from grade 9-12 from off-farm households. The chi-square test (Table 2) reveals that the educational status of the households was statistically significant at a 10% significance level,and this shows there is a significant connotation between the educational status of the households and their probability of participating in different income generating activities in the study area. This study was similar to the study of Alemu et al. (2021) and Bogale and Lemma (2023). They stated that when the schooling year of the women increases their confidence to participate in income generating activities would be also increased.

As stated in Table 2, 8.0% were single, 67.7% were married, 13.0% were widowed, and 11.3% were divorced from households' who participated in farm activities. While, 32.6% were single, 45.4% were married, 12.7% were widowed, and 9.3% were divorced from households' who participated in non-farm activities. Correspondingly, 25.0% were single, 60.0% were married, 5.0% were widowed, and 10.0% were divorced from households' who participated in off-farm activities. As chi-square test in Table 2 reveals that, marital status of the households was not statistically significant and this shows there is no association between the marital status of the households and their probability of participating in different income generating activities in the study area. The focus group discussants also argued that married households have more household members in the rural areas, and this led them to participate in different income generating activities. This result was consistent to the study of Alemu et al. (2021).

Access to credit plays a greater role in improving the livelihoods of farm, non-farm, and off-farm households. As stated in Table 2, about 29.0% of the farm households access a credit service from informal institutions, family, friends, and neighbors, and 71.0% of farm households did not get credit access due to several challenges such as lack of collateral, limited education, high transaction cost, and lack of information. On the other hand, 33.7% of non-farm households have credit access, and 66.3% of non-farm households did not get credit access. Similarly, 20.0% of off-farm households have credit access, and 80.0% of off-farm households did not get credit access due to the same challenges of farm households. The chi-square test (Table 2) reveals that credit access of the households was statistically significant at 10% significance level and this shows there is a significant association between credit access and their probability of participating in different income generating activities in the study area. This result was in line with the study of (Alene 2020). He indicated that women who have access to credit could participate in various livelihood activities to maximize their profit than credit non-users women households.

About 30.6% of the farm households have access to training, which can increase their agricultural productivity and 69.4% did not get a training access. On the other hand, 19.8% of non-farm households have training access, and 80.2% of non-farm households did not get training access. Similarly, 10.0% of off-farm households have training access, and 90.0% of off-farm households did not get training access. The chi-square test (Table 2) reveals that the training access of the households was statistically significant at a 10% significance level, and this shows there is a significant association between training access and their probability of participating in different income generating activities in the study area. A study by Akter et al. (2022) indicated that women who had access to training opportunities had a greater probability to participate in various income generating activities and earned more income.

Access to infrastructure plays an energetic role in determining women's participation in varies income generating activities. As the survey result shown in Table 2, 25.8% of the farm households have access to infrastructural facilities such as roads and electricity, and 74.2% did not get training access. On the other hand, 76.7% of non-farm households have access to infrastructures, and 23.3% of non-farm households did not get access to infrastructures. Similarly, 20.0% of off-farm households have infrastructural access, and 80.0% of off-farm households did not get infrastructural access. As chi-square test reveals that, infrastructural access of the households was statistically significant at 10% significance level and this shows there is a significant association between infrastructural access and their probability of participating in different income generating activities in the study area. This result was consistent with the study of Kassie et al. (2017). They argued that households who have an access to infrastructural facilities i.e., road, have a greater probability to participate in non-farm and off-farm livelihood activities.

This is measured by whether a household can access available market information to enhance their income generating activities. Thus, the survey results revealed that 35.5% of farm households have accessed and used market information through different channels, and 64.5% have no access to market information. Likewise, 75.6% of non-farm households have accessed and used market information through different channels, and 24.4% have no access to market information. Correspondingly, 56.0% of off-farm households have accessed and used market information through different channels, and 44.0% have no access to market information. The channels of market information for all farm, non-farm, and off-farm households were neighbors, radio, TV, mobile phone, and marketplaces. A chi-square test reveals that, access to market information was statistically significant at 1% significance level and this shows there is a significant association between access to market information and their probability of participating into different income generating activities in the study area (Table 2). This result was in line with the study of Argaw et al. (2021). They stated that households who have access to market information can get enough information to sell and buy the different outputs that can be produced from non-farm and off-farm income generating activities.

Table 2. Descriptive statistics results for dummy explanatory variables by income generating activities

Variables	Response	Women's participation in income generating activities						X^2 –value
		Farm activity (N=62)		Non-farm activity (N=86)		Off-farm activity (N=20)		
		Freq.	%	Freq.	%	Freq.	%	
Educational status	Illiterate	34	54.8	16	18.6	6	30.0	39.08
	Grade 1-4	19	30.6	20	23.3	9	45.0	
	Grade 5-8	5	8.1	38	44.1	3	15.0	
	Grade 9-12	4	6.5	9	10.5	2	10.0	
	Diploma and above	0	0	3	3.5	0	0	
Marital status	Single	5	8.0	28	32.6	5	25.0	14.02
	Married	42	67.7	39	45.4	12	60.0	
	Widowed	8	13.0	11	12.7	1	5.0	
	Divorced	7	11.3	8	9.3	2	10.0	
Access to credit	Yes	18	29.0	29	33.7	4	20.0	1.52
	No	44	71.0	57	66.3	16	80.0	
Access to training	Yes	19	30.6	17	19.8	2	10.0	4.50
	No	43	69.4	69	80.2	18	90.0	
Access to infrastructure	Yes	16	25.8	66	76.7	4	20.0	46.25
	No	46	74.2	20	23.3	16	80.0	
Access to market information	Yes	22	35.5	65	75.6	7	56.0	27.55
	No	40	64.5	21	24.4	13	44.0	

Table 3. Descriptive statistics results for continuous explanatory variables by income generating activities

Variable	Women's participation in income generating activities						F-Value
	Farm (62)		Non-Farm (86)		Off-Farm (20)		
	Mean	Std. Err	Mean	Std. Err	Mean	Std. Err	
Ages of the household	32.73	7.78	29.69	5.38	30.8	5.79	1.17
Household size	2.63	1.50	4.03	2.44	2.75	2.71	1.47
Land size of household	1.37	.59	.51	.49	1.05	.49	6.63
Livestock holding (TLU)	3.37	2.70	.55	1.14	.85	1.30	2.79
Access to extension contacts	.53	.88	.30	.65	.3	.80	1.32
Distance to the nearest market	5.02	2.36	2.95	1.96	4.85	1.60	2.31
Distance to main road	4.19	2.33	2.25	1.85	4.3	1.56	1.03

Age is one of the demographic characteristics that influence the participation of women's in varies income generating activities. As shown in Table 3, the average age of women households who participated in farm, non-farm and off-farm activities was 32.73, 29.69, and 30.8, with a standard deviation of 7.78, 5.38, and 5.79 respectively. A one-way ANOVA was also conducted to investigate the impact of age on the women's participation in income generating activities (F=1.17, P=0.27). This shows that older women are less likely to participate in diverse income generating activities. Previous research results indicated that, as the ages of women gets older their probability to actively participating in various economic activities become declines (Alemu et al. 2021; Tesgera et al. 2023).

Women's participation in income generating activities differs with their variation in landholding size. The survey result in Table 3 shown that women who are participating in farm, non-farm and off-farm activities have an average land size of 1.37, .51, and 1.05 ha with standard deviations .59, .49, and .49, respectively. This implies that women who have more cultivable land size highly participated in farm activities and moderately in off-farm activities. Hence, having higher farm size lowers their concentration to participate in other income generating activities (non-farm and off-farm). A one-way ANOVA was also conducted to investigate the impact of land size on the women's participation on income generating activities and was statistically significant at a 1% significant level (F=6.63, p=0.000). This result was similar to the study ofAlemu et al. (2021). They stated that as the size of land increases, the income obtained from agricultural production increases and the possibilities of women to search additional livelihood activity declines.

Household size in this study is considered as the number of individuals who live together in the respondent's home. The survey result in Table 3 shown that women who are participating in farm, non-farm and off-farm activities have an average mean household size of 2.63, 4.03, and 2.75 ha with standard deviation of 1.50, 2.44, and 2.71, respectively. A one-way ANOVA was also conducted to investigate the effect of household size on the women's participation on farm and off-farm activities and was statistically significant at 5 and 10% significant level, respectively (F=1.47, p=0.072). This is in line with the study of (Lechmann and Schnabel 2012). They indicated that, as the size of family increases the workload for women associated with children care becomes increased and this consumes their time that could have been invested in income generating activities.

Livestock is among the most important economic assets for the livelihoods of rural people. As shown in Table 3, the average means of livestock holding of women households who participated in farm, non-farm and off-farm activities were 3.37, .55, and .85, respectively. This implies that those households who participated in farm activities owned more TLU as compared to the rest groups. The minimum and maximum numbers of livestock in TLU for farm, non-farm and off-farm households were (0) (9.8), (0) (7.09) and (0) (5), respectively. A one-way ANOVA was also conducted to investigate the impact of livestock holding on the women's participation on income generating activities and was statistically significant at a 1% significant level (F=2.79, p=0.002). This result contradicts with the study of Sariyev et al. (2022). They indicated that increased number of livestock limits the probability of women's involvement market-oriented activities.

For this study, extension contact is the number of contact that a household has with an extension agent within a year to get agricultural extension service. Based on the survey result shown in Table 3, women who are participating in farm, non-farm and off-farm activities have an average mean of extension contacts with extension agent of .53, .30, and .3 with standard deviation of .88, .65, and .80, respectively. A one-way ANOVA was also conducted to investigate the impacts of extension contacts on the women's participation on farm and non-farm activities and was statistically significant at 1 and 10% significant level, respectively. This indicates that women households' who participated in farm and non-farm activities were more frequently contacted by extension agents and had more potential to get more information about their livelihood activity. As indicated by Eneyew and Bekele (2012) households who have gained frequent extension contact with an extension agent can choose diverse income generating activities than their counterparts.

It is obvious that access to road is very important infrastructure to transport easily from place to place and to implement different income generating activities. The survey result presented in Table 3 shown that, the average distance to the main road of women households who participated in farm, non-farm and off-farm activities was 4.19, 2.25, and 4.3 km with a standard deviation of 2.33, 1.85, and 1.56, respectively. A one-way ANOVA was also conducted to investigate the impacts of distance to the main road on the women's participation in income generating activities and was statistically significant at 10% significant level (F=1.03, p=0.098). This result is in line with the study of Minyiwab et al. (2024).

The survey result presented in Table 3 shown that, the average distance to the nearest marketplace of women households who participated in farm, non-farm and off-farm activities was 5.02, 2.95, and 4.85 km with a standard deviation of 2.36, 1.96, and 1.60, respectively. A one-way ANOVA was also conducted to investigate the impacts of distance to the nearest market on the women's participation to income generating activities, and it was not statistically significant (F=2.31, p=0.023). This means there is no relationship between distance to the nearest market and women's participation in income generation activities (farm, non-farm and off-farm activity). This is in agreement with (Gecho 2017; Montanari and Bergh 2019). They described that, when the market place is far away from their home to buy inputs for their business and sell their output, they are less likely to be participate in non-farm and off-farm activities.

Determinants of women's participation in income-generating activities – Explanation of significant variables

Education status of household (EDUSTAT)

As the multinomial logit model result indicated in Table 4, educational status of the household had a negative relationship with women's participation in farm activities, and was statistically significant at 5% significance level. Despite this fact, the educational status of the household was also statistically significant at a 10% significance level and had a positive relationship with women's participation in non-farm income generating activities. This implies the women household who are illiterate involved in farm activities and women who are attending the formal education have more knowledge and skill to perform in different types of non-farm activities. It means that, as the level of education increased, the probability of women household participation in non-farm or farm income generating activities would be increased or decreased by -.011, and .228, respectively. Similarly, Demissie and Legesse (2013), and Bogale and Lemma (2023) indicated that education increases the skill and knowledge of a person thereby, increases the productivity of income generating opportunity to engage in diversification.

Household size (HHSIZE)

Household size has a negative association with women's participation in farm and off households, and positive relationship with non-farm activities, and it was statistically significant at a 5 and 10% significant level, respectively (Table 4). This implies that small household size enables rural households to choose farm and off-farm activity as a source of income. The possible reason was that the household head having large household size invest capital for food, health and clothing to fulfill the needs of this large household size. To do this, the income gained from agriculture only is not enough rather choose non-farm income generating activities. According to the result of multinomial logit model, keeping the influence of other variables constant, as the numbers of household increased by one person, the probability of women to participate in farm, and off-farm activities would be decreased by .105 and -.160 in farm and off-farm activities, respectively and increased by .054 for non-farm activities. This is in line with the study of Lechmann and Schnabel (2012). They indicated that, as the size of family increases the workload for women associated with children care becomes increased and this consumes their time to invest in income generating activities.

Land size of household (LANDSIZE)

Land size has a positive association with women's participation in farm households, and negative relationship with non-farm and off-farm activities, and it was statistically significant at 1, 5 and 10% significant level, respectively. As the model result revealed that, as the land size of the household increases by one hectare, women participation in farm activity would be increased by 2.23 units, and decreased by .339 and 1.89 unit for non-farm and off-farm activities, respectively (Table 4). This shows that, as the size of land increase the income gained from agricultural production increases, and the probability women to involve in other income generating activities would be decreased, because they have no income problem as they can get it from farm activities Alemu et al. (2021).

Livestock holding (TLU)

Livestock holding determine women probability to participate in farm income generating activities positively and negatively with off-farm activities, and was statistically significant at 1% significance level. That means, as the livestock holding of the households' increases by one unit of TLU, the probability of women to be engaged in farm activities increases by .348 units, and decreased by .432 units for off-farm activities by keeping the other factors constant (Table 4). This result contradicts with the study of Sariyev et al. (2022). They indicated that increased number of livestock limits the probability of women's involvement market-oriented activities.

Access to credit (ACCREDIT)

In the study area, access to credit determines the likelihoods of women household to participate in non-farm income generating activities negatively and significantly at 10% significance level (Table 4). As the multinomial logit model result shows that, the probability women households' participation in non-farm activities is less by a factor of .272 units for those who did not have access to credit service. This shows that women households who have no credit access are less likely to participate in income generating activities such as non-farm activities. This result is similar to the study of Anshiso and Shiferaw (2016).

Access to extension contacts (EXCONTAC)

Access to extension contacts has a negative association with women's participation in farm activities, and positive relationship with farm activities, and it was statistically significant at a 1 and 10% significant level, respectively. As the multinomial logit model result shows that, the probability women households' participation in farm activities is less by a factor of 1.145 units for those who did not have an access to extension service. Similarly, the probability women households' participation in non-farm activities could be increased by .075 units for those who have an access to extension service (Table 4). Similar research outputs were revealed by Asfaw et al. (2017). They showed that access to extension contact with an extension agent provides important information related to agricultural production and technical assistance on agricultural activities and this makes the women farmers give more attention to agricultural works.

Distance to main road (DMROAD)

According to the multinomial logit model result, distance to main road has a positive association with women's participation in off-farm income generating activities, and statistically significant at 10% significance level. This shows that women households who are near to the main road can easily get transportation service. Keeping the influence of other variables constant, as distance to the main road increase in one km, the probability of women's participation in off-farm income generating activities would be increased by .282 km (Table 4). This result is in line with the study of Minyiwab et al. (2024). They stated that proximity to the main road has a greater relationship with households' participation in various livelihood activities.

Access to training (ACCTRAIN)

Access to training has a negative relationship with women's participation in farm activities and has a positive association with women's participation in off-farm activities. Access to training was also among statistically significant explanatory variable for farm, and off-farm activities at a 5 and 10% significant level, respectively (Table 4). As the multinomial logit model result shows, the probability women households' participation in farm activities is less by a factor of 1.173 units for those who did not have an access to training. Likewise, the probability women households' participation in off-farm activities would be increased by 2.058 units for those who have a training access. A study by Akter et al. (2022) indicated that women who had access to training opportunities had a greater probability to participate in various income generating activities and earned more income.

Access to infrastructures (ACCINFRA)

A multinomial logit model result revealed that access to infrastructures has a positive association with women's participation in off-farm income generating activities, and statistically significant at 10% significance level (Table 4). This shows that women households who have an access to infrastructures such as road can easily get a transportation service to implement their income generating activity (off-farm activity). That means the probability women households' participation in off-farm activities would be increased by .223 units for those who have an access to infrastructures. This result was consistent with the study of Kassie et al. (2017). They argued that households that have access to infrastructural facilities, i.e., road, have a greater probability of participating in non-farm and off-farm livelihood activities.

Access to market information (MARKINFO)

Access to market information has a positive association with women's participation in farm households, and negative relationship with non-farm and off-farm activities. It was statistically significant at 1 and 5% significant level, respectively. As the model result revealed that women households who have an access to market information can improve agricultural production and productivity to meet their family necessities. That means the probability women households' participation in farm activities would be increased by 1.778 units for those who have an access to market information. Alike, the probability women households' participation in non-farm and off-farm activities would be decreased by .160 and 1.617 units, respectively for those who did not have an access to market information (Table 4). This result was in line with the study of Argaw et al. (2021).

In conclusion, largely, income-generating activities have a greater role in the improvements of sustainable livelihood outcomes in the study area by increasing income the household, improving food security, for provision of basic necessities, by reducing vulnerability of risks, and by reducing poverty level of women. The findings of the study show that, from the total sampled women households 36.9% participated in farm activities (included crop production, livestock production, vegetable and fruit production, and poultry production), 51.2% participated in non-farm activities (including petty trade, wage laborer from constructions, hairdressing, selling of food and drinks, and selling of tea and coffee) whereas 11.9% of women household participated in off-farm income generating activities (included charcoal making or selling, and selling of firewood). Factors such as education status of household, household size, land size of household, livestock holding, access to credit, access to extension contacts, distance to main road, access to training, access to infrastructures, and access to market information were statistically significant and affects women's participation in different income generating activities. Whereas, ages of the household, marital status of the household, and distance to the nearest market were not statistically significant. Hereafter, Education, Access to training, access to Land, and Availability of infrastructures have a greater role on the participation of women households in various income generating activities. Therefore, the government should encourage women households to participate in various types of income generating activities by non-formal education programs, trainings, and building different infrastructural facilities like a road to increase women's participation in various income generating activities in the study area. Finally, this study was conducted only in one district (Itang Special District). Therefore, similar studies may be conducted in other parts of the district found in Gambella region in particular and in different parts of Ethiopia in general to get clear idea about the participation of women in various income generating activities.

Table 4. Multinomial logit model result on women's participation in income generating activities

Variables	Women's participation in income generating activities											
	Farm-activities				Non-farm activities				Off-farm activities			
	Marginal effect	Coeff	Standard error	P-value	Marginal effect	Coeff	Standard error	P-value	Marginal effect	Coeff	Standard error	P-value
Agehh	.014	-.059	.165	0.722	-.011	.089	.157	0.571	-.003	-.031	.111	0.785
Edustat	.228	-1.253	.632	0.047**	-.042	.979	.586	0.095*	-.185	.273	.471	0.562
Maristat	.283	-1.400	1.006	0.164	-.122	1.459	1.017	0.151	-.161	-.059	.638	0.926
Hhsize	.105	-.747	.354	0.035**	.054	.196	.349	0.575	-.160	.552	.308	0.073*
Landsize	-2.232	12.445	3.893	0.001***	.339	-9.338	3.697	0.012**	1.893	-3.111	1.864	0.095*
Tlu	-.348	2.250	.718	0.002***	-.084	-.977	.637	0.125	.432	-1.273	.412	0.002***
Accredit	-.887	4.636	2.931	0.114	.272	-4.192	2.508	0.095*	.615	-.445	1.890	0.814
Excontac	1.145	-6.951	2.502	0.005***	.075	3.922	2.181	0.072*	-1.219	3.029	2.048	0.139
Distmark	-.177	1.116	1.059	0.292	-.029	-.546	1.011	0.590	.206	-.570	.718	0.427
Dmroad	.104	-1.020	.975	0.296	.179	-.246	.925	0.790	-.282	1.265	.764	0.098*
Acctrain	1.173	-8.956	4.438	0.044**	.886	1.184	3.392	0.727	-2.058	7.772	4.608	0.092*
Accinfra	-.130	-.031	1.465	0.983	.352	-1.707	1.342	0.203	-.223	1.738	1.024	0.090*
Markinfo	-1.778	10.162	3.314	0.002***	.160	-7.054	2.923	0.016**	1.617	-3.108	1.569	0.048**
_cons		-6.166	5.177	0.234		11.085	5.644	0.050		-4.919	4.740	0.299

Note: Log-likelihood: -41.02, Number of observations: 168, LR chi^2 (26): 241.87, Prob>chi^2 = 0.000***, Pseudo R^2: 0.75, ***, **, * indicates significant at < 1, 5, and 10% probability levels, respectively

ACKNOWLEDGEMENTS

The authors highly acknowledge Gambella University, Ethiopia for providing all the necessarily materials and granting the research fund for the study. Finally, we would like to thanks Itang special district agricultural staff workers, respondents, and village administrates who facilitate and share their insights and actively participated in the data collection process.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 94-102

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090110

Effects of iron supplementation rates and time of application on iron biofortification and macronutrient uptake in radish (*Raphanus sativus*) microgreens

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Manuscript received: 25 June 2024. Revision accepted: 20 December 2024.

Abstract. *Leal JS, Pabiona MG, Cristobal JU, Melencion AB, Labajo JRN. 2025. Effects of iron supplementation rates and time of application on iron biofortification and macronutrient uptake in radish (*Raphanus sativus*) microgreens. Asian J Agric 9: 94-102.* This study investigates strategies to enhance iron biofortification in radish (*Raphanus sativus*) microgreens through various iron supplementation rates and application timings. The primary objectives were to evaluate fresh weight at harvest and iron absorption efficiency under different treatment conditions. Results demonstrated significant variations in fresh weight, with microgreens treated with SNAP + 10 ppm iron chelate six days post-blackout (A1B3) yielding the highest fresh weight (149.38 g), while those treated four days post-blackout showed the lowest weight (113.54 g). In terms of iron content, treatment A2B1 exhibited the highest concentration (247.78 ppm), whereas A1B2 recorded the lowest (185.03 ppm). The study highlights the intricate relationship between iron supplementation levels (Factor A) and application timing (Factor B) concerning growth and nutrient uptake dynamics in radish microgreens. Overall fresh weight did not differ significantly across iron levels, based on iron concentration x application timing. Moderate iron concentrations initially supported fresh weight, but excess levels hindered growth, emphasizing the delicate balance required in iron supplementation strategies. Nitrogen uptake benefited from moderate iron levels and delayed application, while phosphorus and potassium assimilation were optimized with timely nutrient supply ($p<0.05$). Notably, total potassium content varied significantly ($p<0.05$), peaking at 20 ppm iron, suggesting iron's facilitative role in potassium uptake up to a threshold. Moreover, iron content in microgreens remained stable across tested concentrations but significantly increased with specific application timings, underscoring the importance of precise nutrient management for effective iron biofortification. These findings contribute to advancing tailored nutrient management practices to optimize growth, nutrient uptake dynamics, and iron biofortification in radish microgreens. Future research directions should explore underlying physiological mechanisms to refine strategies for sustainable crop production and enhance nutritional quality.

Keywords: Iron, EDTA, nutrient uptake, radish microgreens, *Raphanus sativus*

Abbreviations: DAB: Days After Blackout, DMY: Dry Matter Yield, SNAP: Simple Nutrient Addition Program

INTRODUCTION

Although required in minute quantities, micronutrients are indispensable for plants and humans, and their deficiency can lead to severe health problems. Currently, one-third of the global population suffers from micronutrient deficiency, with pregnant women and children being the most affected groups Thompson and Amoroso (2014) and Harding et al. (2017). Iron deficiency, a significant cause of hidden hunger, leads to conditions such as anemia and impaired mental development (Yoneyama et al. 2015). This global health issue has persisted for years (Zielińska-Dawidziak et al. 2016). Iron is essential for producing hemoglobin, which plays a vital role in oxygen transport and respiration in humans (Morrissey et al. 2015). In plants, iron is crucial for photosynthesis, redox reactions, electron transport, leaf pigmentation, and respiration (Yoneyama et al. 2015; Morrissey et al. 2015). Furthermore, iron is believed to modulate defense mechanisms against invaders in plants and animals (Aznar et al. 2015).

Radish (*Raphanus sativus* L.) microgreens have been identified as a suitable candidate for this process. Microgreens, defined as young and tender vegetables (Di Bella et al. 2020), are emerging as a functional food with the potential to address human nutritional deficiencies (Pannico et al. 2020). These microgreens contain higher concentrations of nutrients compared to their mature counterparts (Ebert 2022) and possess properties that promote health and prevent diseases (Kowitcharoen et al. 2021).

Microgreens, often referred to as superfoods or functional foods, are young and tender vegetables from families such as Brassicaceae, Asteraceae, and Fabaceae, among others (Di Bella et al. 2020; Li et al. 2021a). Their popularity is due to their concentrated bioactive compounds and higher nutrient content than mature vegetables (Chandra et al. 2012; Ebert 2022). This makes microgreens particularly suitable for biofortification efforts aimed at enhancing human health.

Iron's critical functions in human and plant biology underscore the importance of ensuring adequate iron intake. Vegetables, (which can be grown locally and come in various sizes, textures, shapes, and flavors), can significantly combat hunger and malnutrition (Fan 2016). One promising approach to addressing iron deficiency is the biofortification of vegetables, particularly through agronomic biofortification (Dias et al. 2015). Biofortification involves increasing the concentration of specific nutrients in the edible parts of plants (Giordano et al. 2019).

Despite iron's importance, its concentration must be carefully managed. In both plants and humans, excessive iron can lead to the generation of harmful reactive oxygen species, resulting in cellular damage and impaired nutrient absorption (Przybysz et al. 2016; Buturi et al. 2021). Safe levels of iron intake for humans are as follows: 0.27 mg day^{-1} for infants 0-6 months, 11 mg day^{-1} for infants 7-12 months, 7 mg day^{-1} for children 1-3 years, 10 mg day^{-1} for children 4-8 years, 8 mg day^{-1} for adolescent 9-13 years both male and female, 11 mg day^{-1} (male) and 15 mg day^{-1} (females) for adolescent 14-18 years, 8 mg day^{-1} for adult males 19+ years, 18 mg day^{-1} for adult females 19-50 years, 8 mg day^{-1} for 51+ adult females, 27 mg day^{-1} for pregnant women, and 9-10 mg day^{-1} for lactating women (Turck et al. 2024). While for radish, the optimal iron concentration ranges from 5-20 mg kg^{-1} (Rout and Sahoo 2015). Potential mitigation strategies include the use of Biochar (Dad et al. 2020), plant natural detoxification mechanism, and soil pH management (Lapaz et al. 2021). In humans, toxic levels of iron can accumulate in vital organs, causing damage (Zhang et al. 2019), while in plants, high iron levels can interfere with the absorption of essential minerals (Zhang et al. 1999).

Conversely, iron deficiency is the most common form of malnutrition globally, leading to severe health consequences such as anemia, impaired mental development, cardiomyopathy, and heart failure in humans (Jankowska et al. 2010; Yoneyama et al. 2015; Lewis et al. 2016; Zhang et al. 2018). In plants, iron deficiency manifests as chlorosis in younger leaves, reduced crop growth, yield, and nutritional quality (Ghasemi et al. 2012).

Given the critical balance required for iron in biological systems, this study proposes the biofortification of radish microgreens as a solution to iron deficiency. The objectives of this study are (i) to determine the fresh weight of radish microgreens at harvest, (ii) to compare the effects of different treatments on iron absorption, and (iii) to compare the effects of the treatments on iron absorption. By focusing on microgreens, which are recognized for their dense nutrient content and health-promoting properties (Kowitcharoen et al. 2021; Ebert 2022), this research aims to find a feasible means to increase iron concentrations in these plants, thereby offering a potential strategy to alleviate iron deficiency in humans. The study will explore the potential of radish microgreens to serve as a nutrient-dense food source that can be easily integrated into diets to combat iron deficiency and improve overall health outcomes.

MATERIALS AND METHODS

Study area

The study was conducted as a pot (10”×20” trays) experiment, arranged according to the study lay-out in two five-tier shelves, inside a non-controlled-environment room with the light supplied by two 36W T8 LED lights per shelf with natural ventilation utilizing the existing wind at Purok 3A Kahaponan, Valencia City in the Province of Bukidnon Philippines (Figure 1). The area is situated at an elevation of 317.8 meters above sea level receiving an average of 2101 mm of rainfall annually, and temperatures ranging from 28°C to 30°C during the conduct of the study.

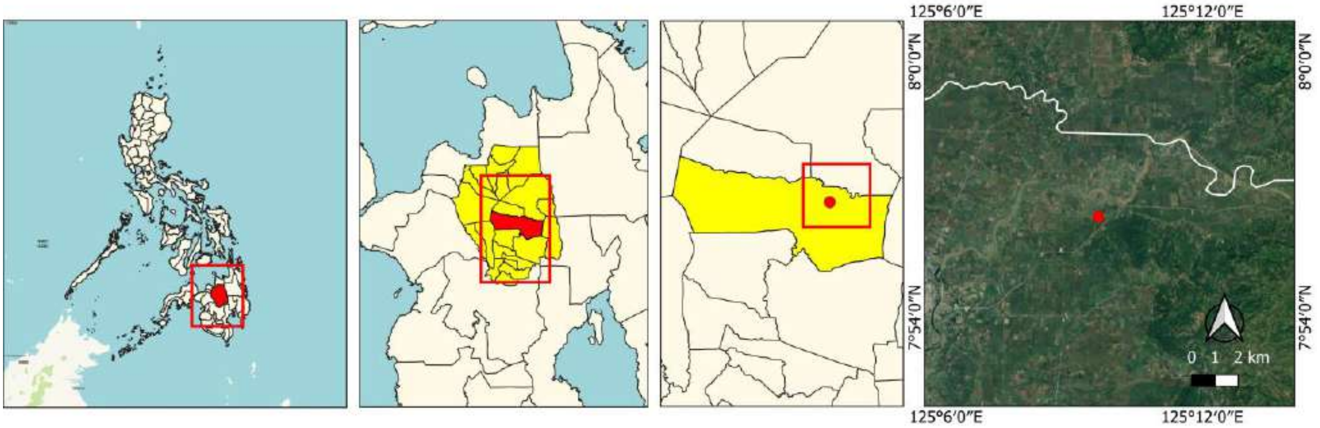

Figure 1. Location of Purok 3A, Kahaponan, Valencia City, Bukidnon, Philippines (7°56′22.01″N, 125°9′34.63″E)

Table 1. Treatments utilized during this study

Treatment	Description
Factor A	
A1	SNAP + 10 ppm iron chelate (Fe^{3+} EDTA)
A2	SNAP + 20 ppm iron chelate (Fe^{3+} EDTA)
A3	SNAP + 30 ppm iron chelate (Fe^{3+} EDTA)
Factor B	
B1	Nutrient solution applied at 2 DAB period
B2	Nutrient solution applied at 4 DAB period
B3	Nutrient solution applied at 6 DAB period
Combination	
A1B1	SNAP + 10 ppm iron chelate applied at 2 DAB period
A1B2	SNAP + 10 ppm iron chelate applied at 4 DAB period
A1B3	SNAP + 10 ppm iron chelate applied at 6 DAB period
A2B1	SNAP + 20 ppm iron chelate applied at 2 DAB period
A2B2	SNAP + 20 ppm iron chelate applied at 4 DAB period
A2B3	SNAP + 20 ppm iron chelate applied at 6 DAB period
A3B1	SNAP + 30 ppm iron chelate applied at 2 DAB period
A3B2	SNAP + 30 ppm iron chelate applied at 4 DAB period
A3B3	SNAP + 30 ppm iron chelate applied at 6 DAB period

Note: *SNAP: Simple Nutrient Addition Program; **DAB: Days After Blackout Period

Experimental design and treatments

The study was conducted using the factorial concept in a Completely Randomized Design (CRD) with two factors as follows: (A) the rate of iron supplementation and (B) the time of iron supplementation. Factor A had three levels: SNAP* + 10 ppm iron, SNAP + 20 ppm iron, and SNAP + 30 ppm iron, with Iron coming from an iron chelate source. On the other hand, Factor B also had three levels: two Days After the Blackout (DAB)** period, four days after the blackout period, and six days after the blackout period. The study had nine treatments replicated three times, thus having 27 samples. The treatments and their descriptions are presented in Table 1.

Procedures

Preparation of the nutrient solution

According to Rajan et al. (2019), seeds do not need nutrient supplementation to germinate. However, seeds require nutrients for better growth and development. Thus, we adopted the utilization of 25% commercial c, as also adopted by Kyriacou et al. (2019) and Corrado et al. (2021), with the formulation of the nutrient solution as follows: 2.0 mM NO_3- N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4- N, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20 µM B, and 0.3 µM Mo. For the iron treatment, 10 ppm, 20 ppm, and 30 ppm Fe from an iron chelate source (i.e. specifically Fe (III)-EDTA), were added to the treatments according to the factorial layout.

Care, maintenance, and harvest

After the microgreens emerged, the 10”×20” trays that were used to cover each microgreen tray for blackout were detached, allowing the microgreens to be exposed to light for 12 hours each day. The illumination was supplied by two 36-watt T8 LED lights per shelf. The microgreens were monitored daily, specifically at 6 am and 4 pm. Irrigation of the microgreens was carried out following the bottom watering technique, administering 250 mL of distilled water every day, at 6 o’clock in the morning till harvest, excluding a day scheduled for application of treatments. Thus, the biofortification of each treatment was implemented through fertigation following the designated biofortification schedule i.e., 2 DAB period, 4 DAB period, and 6 DAB period.

The microgreens' harvest was carried out using a sterilized scissor, soaked for at least 10 minutes in 70% isopropyl alcohol, cutting the hypocotyl as close to the growing media as possible. After acquiring the fresh weight of every treatment, the microgreens were stored in a designated paper bag per treatment.

Sample preparation and analysis

After harvest, the microgreen samples were stored in paper bags and prepared in the Soil and Plant Analysis Laboratory (SPAL) as follows: They were washed with distilled water, air-dried, and placed in an a force draft oven to further remove excess moisture from the plant samples. Once moisture-free, the samples were ground and subjected to dry-ashing before chemical analysis. The microgreens were analyzed for total nitrogen (N), total phosphorus (P), total potassium (K), and total iron (Fe) content at the Soil and Plant Analysis Laboratory (SPAL), College of Agriculture, Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. The methods used were as follows: Total Nitrogen was determined using the Micro-Kjeldahl Method; Total Phosphorus was analyzed using the Dry Ashing/Vanado-Molybdate Method with a UV-Vis Spectrophotometer; Total Potassium was determined by the Dry Ashing/6N HCl Extraction Method with a Flame Photometer; and Total Iron was assessed through the Dry Ashing Method (6N HCl Extraction Method) with an Atomic Absorption Spectrophotometer (PCARRD 1991).

Data analysis

The data collected were analyzed using the Analysis of Variance (ANOVA) following a Factorial in Completely Randomized Design (CRD) procedure available in the Statistical Tool for Agricultural Research STAR IRRI software. If the F computed value is significant or highly significant, a post hoc test using the LSD test will follow.

RESULTS AND DISCUSSION

Fresh weight and nutrient concentrations

The fresh weight of radish microgreens at harvest showed different responses to varying iron concentrations and nutrient application timings (Table 2), there were no significant differences in the fresh weight among the different levels of Factor A (iron concentration) as well as Factor B (timing of nutrient application). Specifically, increasing iron concentration from A1 (131.34 g) to A2 (131.65 g) resulted in a slight increase in fresh weight, followed by a decline at the highest concentration (A3, 123.09 g). This suggests that moderate iron supplementation can be beneficial. However, excessive amounts may hinder growth due to potential phytotoxicity by the production of Reactive Oxygen Species (ROS) that damage plant cells (Sharma et al. 2012).

The timing of nutrient application (Factor B) showed a clear pattern for the effects of nutrient concentration. Delaying the application from B1 (128.19 g) to B2 (121.93 g) led to a decrease in fresh weight, yet further delay to B3 (135.97 g) increased the fresh weight. This implies that the timing of nutrient availability is critical, potentially aligning with the plants' developmental stages and nutrient uptake efficiency. However, the interaction between Factors A and B did not produce statistically significant differences in the fresh weight, suggesting that while trends are present, they require more robust statistical power to confirm. Despite the lack of statistical significance, the interaction trends suggest that combinations of higher iron concentrations and delayed nutrient application may either mitigate or amplify the effects on fresh weight, depending on specific environmental conditions, plant growth stages, and other underlying factors.

The study found no significant differences in nitrogen (N), phosphorus (P), and potassium (K) levels across different treatments, suggesting that these nutrients were not substantially influenced by the iron concentrations or nutrient application timings under the conditions tested. However, the observed variations in fresh weight were predominantly influenced by the iron concentration and timing of nutrient application. Factor A2 demonstrated the highest iron concentration (230.62 ppm), indicating that moderate iron supplementation enhances iron uptake, which is crucial for processes such as nitrogen and potassium metabolism. Iron is essential for nitrate assimilation and ion transport, where moderate levels can enhance nitrate reductase activity, facilitating nitrogen assimilation. Conversely, excessive iron can induce oxidative stress, impairing nitrogen metabolism and root architecture (Mitra 2017). Iron also plays a critical role in potassium uptake by supporting energy production in the roots, which is essential for active potassium transport. However, excessive iron can disrupt this balance by altering redox reactions, impairing potassium transport (Duck and Connor 2016; Kobayashi et al. 2019). Interestingly, phosphorus uptake is less affected by iron levels, likely due to the involvement of different transporters and biochemical pathways (Verbon et al. 2017).

While, no significant correlations were found between nitrogen, phosphorus, potassium, and iron concentrations (iron vs. nitrogen: r=-0.0800, p=0.6915; iron vs. phosphorus: r=-0.1493, p=0.4574; iron vs. potassium: r=0.1008, p=0.6170), the correlation analysis did reveal a significant negative correlation between total nitrogen and potassium content (r=-0.4147, p=0.0315) presented in Table 3. This suggests that, although iron did not directly influence these nutrients, increased nitrogen concentrations might be associated with reduced potassium uptake. These results imply that while iron enrichment may not directly alter the uptake of these nutrients, its effects on plant growth and development are likely due to its role in supporting energy production, enzyme activity, and metabolic pathways rather than direct nutrient interactions. Therefore, iron's influence on microgreens may be independent of the levels of nitrogen, phosphorus, and potassium, highlighting the potential for targeted iron enrichment to enhance the nutritional content of crops without adversely affecting the uptake of other essential nutrients. This selective influence underscores the importance of iron in biofortification strategies, particularly in addressing iron deficiency in human diets.

Table 2. Fresh weight (g) of radish microgreens and their nutrient concentrations at harvest

Treatment	Fresh weight (g)	N (%)	P (%)	K (%)	Fe (ppm)
Factor A					
A1	131.34	3.44	0.63	2.07b	194.76
A2	131.65	3.28	0.60	2.86a	230.62
A3	123.09	3.31	0.65	2.50ab	201.41
F-test (p<0.01)	ns	ns	ns	*	ns
Factor B					
B1	128.19	3.37	0.58	2.50	214.93
B2	121.93	3.32	0.60	2.39	196.31
B3	135.97	3.34	0.69	2.55	215.56
F-test (p<0.01)	ns	ns	ns	ns	ns
Factor A x Factor B					
A1B1	131.10	3.33	0.59	2.48	209.68
A1B2	113.54	3.52	0.60	1.25	185.03
A1B3	149.38	3.47	0.69	2.44	189.58
A2B1	134.26	3.34	0.56	2.79	247.78
A2B2	128.32	2.96	0.57	3.12	205.37
A2B3	132.38	3.55	0.64	2.67	238.72
A3B1	119.20	3.44	0.58	2.22	187.32
A3B2	123.92	3.48	0.64	2.80	198.53
A3B3	126.16	3.00	0.72	2.49	213.37
F-test	ns	ns	ns	ns	ns

Note: **: Highly significant, *: Significant, ns: Not significant, means followed by the same letter are not significantly different at a 5% level of significance

Table 3. Pearson correlation analysis between nutrient concentration of NPK and Fe

Variable	Total nitrogen content	Total phosphorus content	Total potassium content
Total nitrogen content			
Total phosphorus content	r=-0.0232; p=0.9086		
Total potassium content	r=-0.4147; p=0.0315	r=-0.1198; p=0.5518	
Total iron content	r=-0.0800; p=0.6915	r=-0.1493; p=0.4574	r=0.1008; p=0.6170

Average height at harvest, dry matter yield, and nutrient uptake

Average height at harvest and dry matter yield

The height of radish microgreens at harvest varied significantly depending on the timing of nutrient application (Factor B) ($p<0.05$). Microgreens that received the nutrient solution during the B3 growth stage were the tallest, with an average height of 13.49 cm, which was notably higher than those treated at the B2 stage (11.82 cm) and slightly taller than those treated at B1 (12.46 cm). These results are consistent with findings by Li et al. (2021b), who observed increased height in radish, kale, broccoli, cabbage, and mustard microgreens when nutrient supplementation was provided during later growth stages. The greater height observed at B3 could be attributed to the plants' increased nutrient demand during this advanced vegetative stage, supporting Bodale et al. (2021), who highlighted the importance of timely nutrient application for maximizing growth during critical stages of development.

Considering Factor A (Fe concentration), no significant differences in plant height were observed among the treatments. Heights ranged from 12.35 cm at 10 ppm Fe (A1) to 12.98 cm at 20 ppm Fe (A2), and 12.45 cm at 30 ppm Fe (A3), indicating that within this concentration range, Fe did not significantly influence the height of radish microgreens. This finding contrasts with some literature, including the study by Mitra (2015), which suggested that higher concentrations of Fe could lead to toxicity and negatively affect plant growth. In this study, however, Fe levels up to 30 ppm did not demonstrate any detrimental effects on the height of microgreens.

Dry Matter Yield (DMY) showed no significant differences across the treatments, indicating that neither Fe concentration nor the timing of nutrient application substantially influenced biomass production. DMY values ranged from 0.836 g kg^{-1} in B3 to 0.879 g kg^{-1} in B2. The minor increase in DMY at 20 ppm Fe (A2) to 0.893 g kg^{-1}, and the subsequent decrease at 30 ppm Fe (A3) to 0.839 g kg^{-1}, equating to the DMY at 10 ppm Fe (A1) suggest a possible threshold beyond which Fe may not enhance, and might even inhibit, biomass accumulation. This finding is consistent with the findings of Di Gioia et al. (2019), who noted that Fe concentrations above optimal levels could negatively impact dry matter content in *Brassica* species, reflecting potential Fe toxicity effects.

These results align with previous studies, such as Park et al. (2014), who reported no significant differences in dry matter yield of microgreens across various nutrient treatments, highlighting the intricate nature of nutrient interactions and their influence on plant growth. Similarly, Filho et al. (2015) and Giordano et al. (2019) reported that Fe levels could significantly influence dry matter yield in hydroponically grown chicory and lettuce, with higher concentrations potentially leading to reduced biomass due to toxicity.

The timing of nutrient application significantly affects the height of radish microgreens, with delayed application (B3) producing the tallest plants. However, Fe concentration within the studied range did not significantly impact plant height. On the other hand, dry matter yield remained unaffected by both Fe concentration and nutrient application timing, highlighting the intricate balance required in nutrient management to optimize growth without inducing toxicity.

Nitrogen uptake

Fe concentration did not reveal significant differences in nitrogen uptake with values ranging from 0.185 g pot^{-1} to 0.195 g pot^{-1} as shown in Table 4. This indicates that variations in Fe concentration within the tested range (10 ppm to 30 ppm) had no significant impact nitrogen assimilation, supporting the broader understanding that nitrogen uptake in plants can be influenced by multifaceted factors beyond Fe levels alone, as noted by Reitra et al. (2017).

Conversely, Factor B (timing of nutrient application) significantly affected nitrogen uptake ($p<0.05$). Notably, microgreens treated at the B3 stage exhibited the highest nitrogen uptake at 0.210 g pot^{-1}, followed by B2 at 0.191 g pot^{-1}, and the lowest at B1 with 0.187 g pot^{-1}. This emphasizes the crucial role of nutrient application timing during vegetative growth stages in promoting root development and improving nutrient absorption efficiency, that highlight the importance of optimal nutrient timing for maximizing crop yield and nutrient utilization. Further exploring the interaction between Fe concentration and nutrient application timing, synergistic effects were observed. For instance, at the B3 stage, microgreens receiving A1 (10 ppm Fe) exhibited a nitrogen uptake of 0.195 g pot^{-1}, whereas A3 (30 ppm Fe), with nitrogen uptake of 0.038 g pot^{-1}. This interaction highlights how higher Fe concentrations can boost nitrogen assimilation when combined with optimal timing, such as in the B3 application. Conversely, treatments like A3B3, where high Fe concentration coincided with nutrient application at B3, resulted in lower nitrogen uptake, potentially due to Fe-induced limitations on nitrogen utilization. To maximize nitrogen uptake and overall plant productivity in microgreen growth, certain nutrient management strategies are required, as these findings highlight the complexity of nutrient dynamics in radish microgreens.

Table 4. Average height at harvest (cm), dry matter yield (g kg^{-1}), and nutrient uptake, in terms of nitrogen, phosphorus, potassium (g pot^{-1}), and iron (mg kg^{-1}), of radish microgreens after a 14-day growing cycle

Treatment	Average height at harvest (cm)	Dry matter yield (g kg^{-1})	N (g pot^{-1})	P (g pot^{-1})	K (g pot^{-1})	Fe (mg kg^{-1})
Factor A						
A1	12.35	0.839	0.189	0.035	0.117	10.717
A2	12.98	0.893	0.193	0.035	0.170	13.236
A3	12.45	0.839	0.185	0.036	0.138	10.975
F-test (p<0.05)	ns	ns	ns	ns	ns	ns
Factor B						
B1	12.46ab	0.856	0.190	0.033	0.139	12.019
B2	11.82b	0.879	0.191	0.035	0.145	11.379
B3	13.49a	0.836	0.185	0.038	0.140	11.530
F-test (p<0.05)	*	ns	ns	ns	ns	ns
Factor A x Factor B						
A1B1	11.86	0.896	0.195	0.034	0.144a	12.432
A1B2	11.31	0.699	0.161	0.028	0.058b	8.444
A1B3	13.88	0.923	0.210	0.042	0.148a	11.275
A2B1	13.02	0.857	0.188	0.032	0.157a	13.835
A2B2	12.50	0.934	0.183	0.034	0.195a	12.557
A2B3	13.41	0.887	0.207	0.038	0.159a	13.417
A3B1	12.50	0.816	0.187	0.032	0.117a	9.889
A3B2	11.66	1.004	0.229	0.042	0.183a	13.137
A3B3	13.18	0.698	0.138	0.033	0.113a	9.898
F-test	ns	ns	ns	ns	*	ns

Note: **: Highly significant, *: Significant, ns: Not significant, means followed by the same letters are not significantly different at a 5% level of significance

Phosphorus uptake

Table 4 presents the phosphorus uptake patterns in radish microgreens influenced by varying iron supplementation rates and nutrient application timings. Factor A (Fe concentration) did not result in significant differences in phosphorus uptake, with mean values consistently around 0.035 g kg^{-1} across the tested concentrations (10 ppm to 30 ppm). In contrast, Factor B (timing of nutrient application) significantly affected phosphorus assimilation.

Microgreens treated at the B3 stage had the highest phosphorus uptake at 0.038 g kg^{-1}, followed by B2 at 0.035 g kg^{-1}, and the lowest uptake occurred at B1 with 0.033 g kg^{-1}. These results suggest that delaying nutrient application to the later vegetative growth stages improves phosphorus absorption, likely due to enhanced root development and increased nutrient demand during these phases. This finding aligns with previous research, such as Bodale et al. (2021), which emphasizes the importance of nutrient application timing in optimizing nutrient uptake efficiency. The interaction between Fe concentration and nutrient application timing further explains phosphorus uptake dynamics. At the B3 stage, phosphorus uptake reached its highest value of 0.042 g kg^{-1} under the highest Fe concentration (A3), indicating a synergistic effect where increased Fe levels and delayed nutrient application significantly boost phosphorus assimilation. In contrast, the lowest phosphorus uptake, 0.028 g kg^{-1}, occurred in the A1B2 treatment, where the lowest Fe concentration (A1) was paired with nutrient application at the B2 stage. This interaction highlights the critical role of Fe availability and nutrient timing in optimizing phosphorus uptake efficiency in radish microgreens. These findings align with studies like those by Di Gioia et al. (2019), emphasizing the importance of managing nutrient levels and application timing to maximize phosphorus uptake in crops.

Potassium uptake

Potassium uptake in radish microgreens, influenced by varying Fe concentrations and the timing of nutrient application, provides critical insights into nutrient management strategies. According to the results from Table 4, Factor A (Fe concentration) did not yield significant differences in potassium uptake (p>0.05), with mean values varying from 0.117-0.170 g kg^{-1}. This result suggests that potassium uptake in radish microgreens may not be highly sensitive to Fe concentration variations within the tested range (10 ppm to 30 ppm). These findings align with previous studies by Przybysz et al. (2016) and Giordano et al. (2019), indicating that potassium uptake in plants can be influenced by factors other than Fe concentration alone.

However, Factor B (timing of nutrient application) did impact potassium uptake significantly (p<0.05). Radish microgreens receiving the nutrient solution at the B2 stage exhibited the highest potassium uptake at 0.145 g kg^{-1}, followed closely by B3 at 0.140 g kg^{-1}, and the lowest at B1 with 0.139 g kg^{-1}. This suggests that nutrient application timing during the vegetative growth stage can enhance potassium absorption, likely due to increased root activity and nutrient demand during this phase. This observation is consistent with findings by Przybysz et al. (2016), emphasizing the critical role of timing in optimizing nutrient uptake and utilization in crops.

The relationship between Fe content and the nutrient delivery time further clarifies the potassium uptake rate. At the B2 stage, potassium uptake peaked at 0.170 g kg^{-1} with Fe concentration A2, indicating a synergistic effect where higher Fe concentrations coupled with timely nutrient application enhance potassium assimilation. The lowest potassium uptake was observed in the A1B2 treatment, combining the lowest Fe concentration and nutrient application at B2, resulting in 0.117 g kg^{-1}. This interaction underscores the interplay between Fe availability and nutrient timing, affecting potassium uptake efficiency. These results highlight the complex connections between nutrients in plant physiology and growth, especially concerning potassium uptake in radish microgreens. Optimizing potassium assimilation and overall plant productivity requires effective nutrient management strategies considering both Fe concentration and application timing.

Iron uptake

Iron (Fe) uptake in radish microgreens is crucial to nutrient management, particularly in enhancing nutritional quality and plant growth. According to the results from Table 4, Factor A (Fe concentration) did not yield significant differences in iron uptake ($p>0.05$), with mean values ranging from 10.717 mg kg^{-1} to 13.236 mg kg^{-1}. This indicates that within the tested range of Fe concentrations (10 - 30 ppm), iron uptake in radish microgreens remained relatively stable, suggesting a limited impact of Fe concentration alone on iron assimilation. These findings align with previous studies by Park et al. (2014), which also reported no significant increase in iron uptake with varying forms of Fe chelates in radish and broccoli sprouts. Factor B (timing of nutrient application) significantly influenced iron uptake ($p<0.05$). Radish microgreens receiving the nutrient solution at the B1 stage exhibited the highest iron uptake at 12.019 mg kg^{-1}, followed by B2 at 11.379 mg kg^{-1}, and the lowest at B3 with 11.530 mg kg^{-1}. This highlights the importance of nutrient application timing during the vegetative growth stage in maximizing iron assimilation, potentially due to enhanced root activity and nutrient demand during this phase. This observation is consistent with studies emphasizing the critical role of timing in optimizing nutrient uptake and utilization in crops.

The interaction between Fe concentration and the timing of nutrient application further explains iron uptake dynamics. At the B1 stage, iron uptake peaked at 13.835 mg kg^{-1} with Fe concentration A2, indicating a synergistic effect where higher Fe concentrations coupled with timely nutrient application enhance iron assimilation. The lowest iron uptake was observed in the A3B3 treatment, combining the highest Fe concentration and nutrient application at B3, resulting in 9.898 mg kg^{-1}. This interaction underscores the intricate relationship between Fe availability and nutrient timing, affecting iron uptake efficiency. These results show the intricate relationships that control iron uptake in radish microgreens and emphasize the necessity of integrated nutrient management into consideration both the time and concentration of iron. Subsequent investigations may delve into the fundamental physiological processes propelling these processes to maximize iron absorption effectiveness in microgreen cultivation. Growers can improve radish microgreens' iron biofortification and address iron deficiency in human diets by enhancing their nutrient management techniques.

The interaction between Dry Matter Yield (DMY) and nutrient uptake in radish microgreens reveals intriguing insights into how nutrient management influences both plant growth and nutrient assimilation. From Table 4, it is evident that DMY was not significantly affected by Fe concentration (Factor A) or the timing of nutrient application (Factor B). However, the intricate interplay between these factors and nutrient uptake deserves attention. Studies by Filho et al. (2015) and Di Gioia et al. (2019) highlight that nutrient availability, including Fe, can influence DMY by affecting physiological processes such as photosynthesis and nutrient translocation. Although Fe concentration in the study alone did not show a direct impact on DMY, the interaction with nutrient uptake, particularly of essential nutrients like nitrogen (N), phosphorus (P), potassium (K), and Fe itself, suggests a nuanced relationship.

Fe uptake did not show a direct correlation with Dry Matter Yield (DMY); however, the nutrient uptake patterns across various treatments offer valuable insights. Treatments that demonstrated higher nutrient uptake, particularly those with optimal Fe concentrations and timely nutrient applications, were generally associated with improved DMY outcomes. This observation is consistent with studies indicating that balanced nutrient uptake—especially during key growth phases—enhances overall plant health and productivity (Kutman et al. 2011; Di Gioia et al. 2016). By integrating insights from DMY and nutrient uptake research, growers can optimize their nutrient application strategies to enhance both yield and nutritional quality in radish microgreens. Understanding these relationships not only improves agricultural practices but also supports sustainable microgreen production systems that effectively meet yield and nutritional requirements

Furthermore, the study underscores the intricate relationship between iron concentration (Factor A) and the timing of nutrient application (Factor B) in influencing nutrient uptake dynamics, particularly for nitrogen, phosphorus, potassium, and iron. While nitrogen assimilation benefited from moderate iron concentrations and delayed application, phosphorus and potassium uptake thrived with timely nutrient supply, highlighting the critical role of synchronized nutrient management in optimizing nutrient absorption and utilization (Smolik et al. 2013; Park et al. 2014).

Additionally, the results clarify the complexity of iron intake and how it affects overall nutritional quality, confirming stability at different iron concentrations but significant improvement at particular application timings. This emphasizes the key balance required for effective iron biofortification in radish microgreens, which is critical for nutritional content optimization (Filho et al. 2015; Di Gioia et al. 2019).

This research contributes valuable insights into refining nutrient management strategies tailored to maximize growth, nutrient uptake, and nutritional quality in radish microgreens. By elucidating the intricate interactions between iron concentration, nutrient application timing, and nutrient uptake dynamics, the study paves the way for future advancements in sustainable microgreen production systems to address nutritional deficiencies and enhance food security through nutrient-dense crops. Further investigations into the underlying physiological mechanisms are recommended to bolster agronomic practices and foster resilient agricultural systems.

ACKNOWLEDGEMENTS

The author extends heartfelt gratitude to God, family, mentors, and all contributors for their invaluable support and guidance throughout this study.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 103-111 DOI: 10.13057/asianjagric/g090111

A comparative study of electricity, irrigation and cropping pattern in three villages of Uttar Pradesh, India

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Manuscript received: 27 August 2024. Revision accepted: 23 December 2024.

Abstract. *Gupta R, Tripathi J. 2025. A comparative study of electricity, irrigation and cropping pattern in three villages of Uttar Pradesh, India. Asian J Agric 9: 103-111.* India's economy, particularly Uttar Pradesh, one of its most agriculturally dependent states, heavily relies on agriculture. In the modern world, electricity plays a crucial role in every aspect of the economic and social life of the masses, and it became more vital in an agriculturist economy like India. Agriculture production and other agriculture-related activities have become increasingly dependent on the supply of electricity in recent times. Policymakers have expressed a strong desire to improve farming's certainty and reliability with improved electricity supply and by reducing reliance on monsoons for irrigation. The present work highlights this issue by elucidating how the electricity supply influences agriculture production, investment in agriculture, employment structure, and the living standards of women within an economy or community. The present study, based on a comparison of three villages in Pratapgarh District, Uttar Pradesh, India, has explored several deeper questions associated with the impact of electrification on irrigation facilities, groundwater access, and cropping patterns. The study is mainly based on primary data that has been collected during the agricultural crop year 2019-2020. Major findings of the study indicate that villages with healthier electricity supplies had higher investment in irrigation, profitable cropping patterns, and reasonable water prices. Villages with a healthier electricity supply had a higher number of groundwater irrigation sources as well as two to three times more investment in irrigation facilities than villages with a poorer electricity supply. The healthier electricity supply also has a positive impact on the employment structure of the surveyed villages and the living conditions of women.

Keywords: Agricultural production, cropping pattern, electricity supply, irrigation, water prices

INTRODUCTION

Agriculture is considered the backbone of an economy like India. Two-fifths of Indians spend over two-thirds of their earnings on food and allied commodities, which indicates the importance of the agrarian sector in India (Jha 2020). The agriculture sector has also occupied a prominent position in the Indian economic system since independence (Yadav and Gupta 2021), to which an agrarian state like Uttar Pradesh contributes more. Uttar Pradesh, being a populous state in India and having an agricultural economy, places greater importance on this sector. The agriculture sector contributes about 24% of Uttar Pradesh's Gross State Domestic Product (GSDP), and around 65% of its population depends on it for their livelihood. Uttar Pradesh is considered the top producer of food grains in the country (RBI 2023). The provision of reliable, sufficient, and affordable electricity not only plays a crucial role in agriculture production and productivity but also influences crop diversity and cropping patterns, ultimately affecting the nation's food security. Large-scale crop diversification significantly impacts the micro- and macro-food security of the rural and urban poor (Chakrabarti 2014). The debate over electricity supply and groundwater management in the farming sector is quite rich. Land and water are considered key factors for the sustainable agricultural development of a nation (Osama et al. 2017).

A number of studies can trace water charges, power reform, and water management in farming. Energy as a resource and electricity as an amenity are no exception (Nathan 2014). Studies indicate that irrigation and agriculture production are strongly associated with electricity supply. An increase in the cost of electricity adversely affects the agricultural land types (Sridharan et al. 2020) and production. Electricity subsidies have increased the production and productivity of the agriculture sector in Punjab state (Sarkar and Das 2014). Since the adoption of the green revolution policy, the government of India at the national level and the governments of different states at the local level have promoted groundwater irrigation facilities, free electricity, and subsidized irrigation equipment to increase agriculture production and productivity. The implementation of the electricity reform has led Punjab's cropping pattern to shift toward water-intensive crops such as rice and wheat (Sarkar and Das 2014).

Uttar Pradesh's cropping pattern exhibits a wide variety due to its geographical location in the fertile Gangatic plain and its ample monsoon rainfall zone. For a long time, agricultural irrigation in the state was largely dependent on the river's monsoon. However, in recent times, the state has transitioned from relying on the monsoon to groundwater irrigation, with the development of more dependable and adaptable irrigation sources. The Uttar Pradesh government has given considerable importance to the development of groundwater irrigation; therefore, groundwater extraction has rapidly increased since the adoption of the green revolution policy. Initially, the state promoted the groundwater extraction policy without any electricity reform, forcing farmers to use diesel engines. Over time, the expansion of the power sector and the rapid increase in diesel rates have led to the replacement of diesel engines with submersible engines. In Uttar Pradesh, there has been a significant shift from diesel engines to submersible engines for both irrigation and domestic purposes. In addition to irrigation, many other agricultural activities, such as sowing and harvesting, have become dependent on electricity in recent times. Many states of India have adopted a free or flat electricity policy, which has increased agriculture production and crop diversity in the respective states. Literature indicates a strong association between electricity supply, water charges, irrigation facilities, cropping patterns, and rural electrification. These factors not only enhance agricultural production and productivity but also enhance non-agricultural variables, leading to an increase in employment opportunities, household per capita income, expenditure, and living conditions (Chakravorty et al. 2014; Khandker et al. 2014; Sarkar and Das 2014; Chatterjee 2015; Dev 2016; Fishman et al. 2016; Osama et al. 2017; Xie and Su 2019). Numerous studies have mapped the relationship between electricity supply, water prices, irrigation sources, cropping patterns, and the positive impact of electricity reform policy on agricultural production and productivity as well as non-agricultural variables at the macro level, i.e., state level or national level. However, studies at the micro level, i.e., village level, are scarce, which is crucial for a large and populus state like Uttar Pradesh. Therefore, the present study aims to provide a micro-level perspective, which is crucial for developing bottom-up policies.

MATERIALS AND METHODS

Sample selection and data collection

The study is mainly based on the comparison of three villages in Uttar Pradesh, India (Figure 1). Villages have been selected using a purposive sampling method. Given our understanding, soil type and other factors such as distance from urban areas and groundwater depth significantly influence agriculture production and cropping patterns, so the selected villages for the survey have the same soil type, nearly equal groundwater depth, and are within a 5 km radius from the urban area. Three survey villages (Tardaha, Bijemau, and Pure Keshavrai) are in the Pratapgarh District of Uttar Pradesh. It is the south-eastern part of Uttar Pradesh and lies on the Allahabad-Faizabad highway. According to the Central Statistical Organization, India, the district has an area of 3730 square kilometers. The district primarily relies on agriculture as its primary source of livelihood. There are three cropping seasons in this area. The Khareef season is the first of the year. Main crops of the first season are paddy, *arhar*, maize, *till*, *chari*, black gram (*urad*), and millet. The second season is considered the rabi season, and the main crops are wheat, mustard, gram, and *matar*. Farmers primarily sow vegetables during the third season, known as *zayed*. *Arhar* and sugarcane are annual crops of the district.

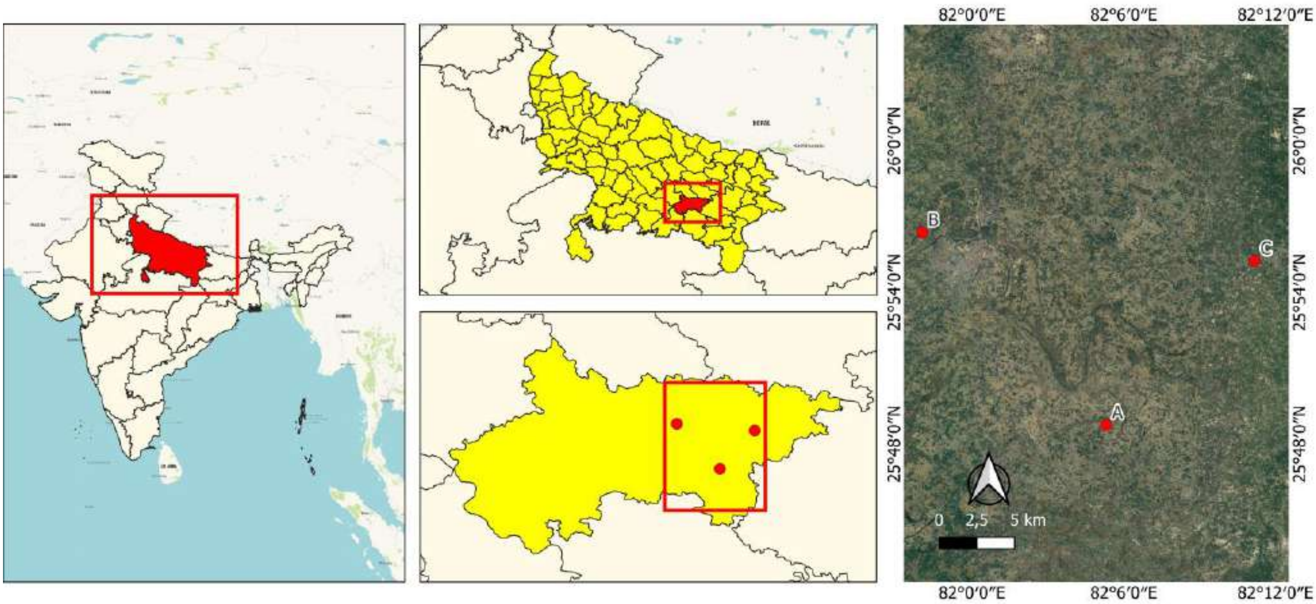

Figure 1. Study area of three villages in Uttar Pradesh, India. A. Bijemau Village, B. Pure Keshavrai Village, C. Tardaha Village

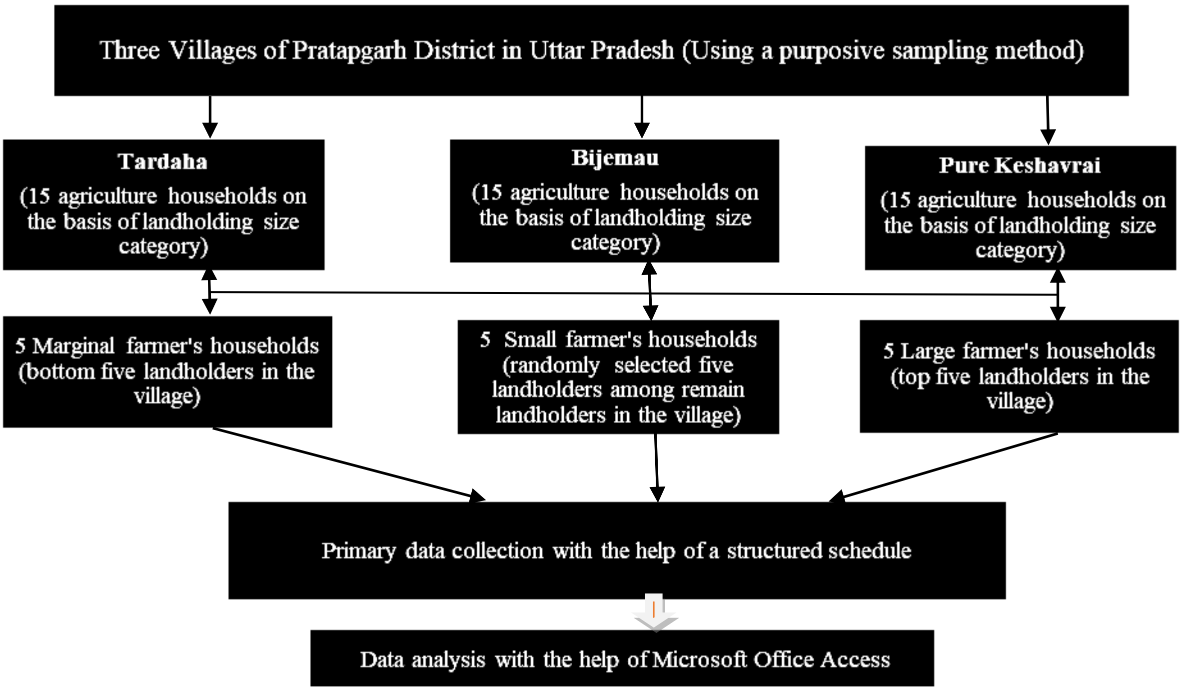

Figure 2. Summary of the primary data collection method

One of three selected villages, Tardaha, has sufficient electricity supply hours with full voltage. The second village, Bijemau, provides a 15-18 hours electricity supply, albeit at a very low voltage. At the time of survey, Pure Keshavrai, the third village, lacked full electrification. A rapid rural survey has been conducted to identify the sample villages, interviewing key informants about their electricity supply and irrigation sources. Furthermore, total 15 agriculture households have been selected from each village, representing various landholding size categories, and five from each landholding size category at the household level. All agriculture households of the village have been classified into three categories: marginal, small, and large farmer's households based on the size of their land holdings. The top five landholders in the village were considered large farmers, the bottom five as marginal farmers, and the remaining as small farmers. Among the small farmers, randomly selected five of them. For this research, primary data has been collected with the help of a structured schedule. The primary survey has been conducted during the agricultural crop year 2019-2020. A simple cross-tabulation method has been used for data analysis with the help of Microsoft Office Access. The following flowchart summarizes the primary data collection method (Figure 2).

RESULTS AND DISCUSSION

Basic information of the three sampled villages

Table 1 displays basic information about each of the three villages that were surveyed, including the number of households, their distance from the powerhouse, the number of electrified households, the depth of the groundwater, the type of soil, and the main occupation of households. This information comes from the primary survey and information gathered from the Gram Panchayats in those villages. Table 1 indicates that all three surveyed villages have almost the same (alluvial) soil type and nearly the same level of groundwater depth.

Tardaha is considered a small village with a population of only 1000 people (Table 1). The Brahmin caste, a general caste category, dominates the village. All the large farmers of the village belong to the Brahmin caste. Village has electricity connection with high voltage. Only 55 of the village's 158 households have access to electricity. The main occupations of the village are salaried jobs and self-employment; agriculture is considered a secondary occupation in the village. The village is located one kilometer away from the urban area. The village clearly demonstrates the benefits of its location. The village boasts a good school, college, hospital, petrol pump, and a well-connected road (*chakroad*) to the market. It has built a new power house in front of the village.

Bijemau is also considered a small village, with 371 inhabitants. The Brahmin caste also dominates this village (Table 1). The village has electrified 17 out of 35 households. The village has a low voltage supply. The main occupation of the village is salaried jobs, and agriculture is considered a secondary occupation in the village. The village is located 5 km from the main market, or *tahseel*, but has poor connectivity. An old powerhouse is situated in an urban area far away from the village, and only one transformer is in running condition in the village.

Table 1. Basic information of the three sampled villages in Uttar Pradesh, India, i.e. Tardaha Village, Bijemau Village, Pure Keshavrai Village

Information	Villages		
	Tardaha	Bijemau	Pure Keshavrai
Number of households	158	35	264
Electrified household	55	17	1
Voltage	High	Low	High
Distance from powerhouse	0 km	5 km	3 km
Number of transformers	1	1	0
Distance from urban area	1 km	5 km	5 km
Groundwater depth	120	165	165
Source of water	Groundwater	Groundwater	Groundwater & River
Prevalent soil type	Alluvial	Alluvial	Sandy Alluvial
Population	1000	371	3391
Main occupation	Salaried Job & Self Employment	Salaried Job	Agriculture
Secondary occupation	Agriculture	Agriculture	Labour
Dominant caste	Brahmin	Brahmin	Thakur

Pure Keshavrai can be considered a large village with a 3391 population and 264 households. The village is located on the banks of the Sai River. The river separates the village from the main city, which is only 5 kilometers away. The main occupation of the village is agriculture, and labour work is considered a secondary occupation in the village. The village has a high voltage supply but only one household has an electricity connection in the village (Table 1). Connectivity of village to urban area is very good, but other facilities are inadequate in the village. In Pure Keshavrai, there are no scheduled caste farmer's households. Only *thakur* (general caste category) and *vaishya* (backward caste category) households are in the village. None of the respondents from the all three surveyed village has reported any role of social caste or category for the quality and quantity of electricity supply.

Irrigation sources

Tardaha Village has 7 water extraction engines for irrigation purposes; Bijemau Village has 3 water extraction engines, one of which is a government electric engine for irrigation purposes, and Pure Keshavrai Village has only one water extraction engine for irrigation purposes. Surface water primarily feeds irrigation, while electricity powers it (Berardy and Chester 2017). The irrigation facility was appropriate in the Village of Tardaha. There was no government water supply in the village, but a reliable electricity supply provided irrigation source to the farmers. Each farmer had either his own diesel or electric engine or purchased power to buy water. Out of the 15 samples, 7 have their own personal water extraction source. Even marginal farmers, earning a monthly income of Rs 5,000 and living in poverty, possess their own water extraction engine. According to Master Data from Prayagraj Zone Uttar Pradesh Power Corporation, three large farmers in the village have their own agricultural electricity connection and personal tubewells. After constructing a new power house in the village, the farmers opted to use a 1-HP, 3-inch pipe machine known as a "submersible" for irrigation instead of purchasing water. Farmers in Tardaha Village reported that the cost of a 1 HP engine is approximately 9000-10000 rupees, which is comparable to the irrigation expenses for three khareef seasons.

Bijemau's Village condition appears slightly worse. The village has a government electric engine for irrigation, which runs on three-phase voltage. The village is located 5 km from the powerhouse, and it has a single operational transformer. The same engine also supplies electricity to nearby villages. The village reported approximately 15-18 hours of electricity supply but experienced significant voltage problems. In the survey year, there was not a single day when the engine delivered water. Among the 15 samples, only 2 have their own personal engine (a submersible engine that does not need a separate connection), and the rest of the farmers depend on the government electric engine. None of the farmers had an agriculture electricity connection. During the survey, voltage was so low that the 1 HP engine could not run. But in the rabi season (winter crop), voltage becomes normal and farmers get sufficient water.

The irrigation sources in Pure Keshavrai have been observed to be extremely bad. Neither government nor private irrigation sources were available in the village. Only one farmer out of the 15 had their own electricity connection and engine facility for irrigation, and there were no reports of an agriculture connection. The house of the farmers who had an electricity connection and an irrigation source was located on the main road of the village, while the remaining farmers resided within the village itself. So, it is tedious and near impossible to get water for irrigation from this engine inside the village. In spite of the village being located on the banks of the Sai River, no irrigation ways have developed to use river water as an irrigation source. The majority of farmers in Pure Keshavrai Village lack both a personal irrigation source and the ability to purchase water, so they mainly depend on rainfall for irrigation.

Investment on irrigation sources

The study indicates that power supply controls the investment in irrigation projects. In the Village of Tardaha, where power supply was good, farmers invested more in water extraction machines. Even the cost of maintenance was higher in Tardaha than in other villages. Despite the disparity in investment between small and large farmers, the study estimated the maintenance cost of old machines and investment in new equipment during the last 25 years. Among the marginal farmers in different villages, investment in water extraction machines was higher in Tardaha Village (Table 2). In Pure Keshavrai, only one farmer has invested 40,000 rupees in an engine. In Bijemau, there has been a notable lack of investment in irrigation projects. Farmers use government engines during periods of adequate electricity supply, but their personal engines become useless during periods of low voltage. Therefore, wealthy farmers who can afford large investments typically install only 1 or 5 horsepower (3-inch) pipe machines.

Groundwater access and water rate

Water is a common pool resource, and everyone should have the right to access groundwater. Equity in water resources can be measured in two ways. First, how many farmers get water, and second, how much they pay for it. For headcount measurement, cropping pattern is a useful indicator. If we compare the cropping pattern among farmers of different landholdings within a village, the same cropping pattern will show equality in water accessibility. But if resource-rich farmers choose water-intensive crops and marginal farmers cultivate dry crops, it will indicate unequal water distribution.

The study has observed a positive correlation between a reliable electricity supply and accessibility to groundwater. Tardaha was a well-developed and fully electrified village. All the farmers in the village, regardless of their size, were receiving irrigation water. Even marginal farmers prefer to set up a 1-hp small electric engine instead of purchasing water. If we compare the cropping pattern among the farmers of Tardaha Village, none of the farmers have found who had not shown the water-intensive crop paddy (Table 3). The survey reports indicate that Tardaha Village adopts the same cropping pattern not only due to cost-benefit considerations but also because animals play a significant role. Animals damage crops, leading farmers to choose the paddy cropping pattern, which is less susceptible to animal damage. However, the availability of ample irrigation water made this possible. To protect the crop from animals, some small farmers cultivated 100% paddy in the khareef season of the survey year. In Bijemau, only two farmers had personal water extraction machines. A scarcity of water prevented many farmers from cultivating paddy during the survey period. When comparing the cropping patterns of marginal, small, and large farmers in a village, it was found that the marginal farmers cultivated more dry crops than the small and large farmers. In Pure Keshavrai, the sole water seller resided on the village's main road, 500 meters away from the main tola. It became increasingly expensive and challenging to obtain water within the village. Marginal farmers in the Pure Keshavrai Village completely abandoned water-intensive farming practices. Only during the khareef season and only when the monsoon was good did the few farmers in the Pure Keshavrai Village engage in water-intensive farming. *Till*, *urad*, and millet were the main crops of the khareef season in the village. Only five farmers cultivated paddy in the survey year (Table 3).

Table 3 displays the number of farmers per category for various crop production. In Tardaha Village, all 15 farmers cultivated paddy. None of the small farmers has cultivated any other dry crop in the khareef season. Large farmers have diversified their cropping patterns more than small farmers. In Bijemau, the situation was slightly different. Large farmers were primarily responsible for paddy cultivation. Only one farmer was involved in paddy farming. In the winter season, the cropping pattern was the same in all landholding categories in both villages. In Pure Keshavrai, not a single farmer cultivated paddy during the khareef season. During the winter season, the crops produced by small and large farmers varied significantly. Water charges also measure groundwater accessibility. The irrigation water price is an indispensable element for the modernization of agriculture (Xiqin et al. 2022). Water charges across different categories and villages represent the accessibility of water in their own right. The study found that water charges were consistent across different categories of farmers but varied between villages. Table 4 attempts to capture the affordability of farmers in purchasing water. To maintain simplicity, we have excluded the cost of a self-user engine and only accounted for the water market prices. Tardaha Village holds a developed water market. The water charge for a 4-inch pipe with 5 horsepower was 120 rupees per hour for an electric engine and 150 rupees for a diesel engine. In Bijemau, only two farmers had a personal water extraction machine. They charged 100 rupees per hour for a 5-hp, 3-inch pipe engine. The water charges in Bijemau Village were significantly higher than those in Tardaha Village. In the Village of Pure Keshavrai, there existed a complete monopoly. In Pure Keshavrai, a solitary farmer operated as a seller, charging Rs. 210 per hour for a 5-horsepower, 4-inch pipe electric engine. The water charge in Pure Keshavrai Village was just 1.5 times that of Bijemau and Tardah. In Bijemau, the primary method of irrigation was through a government engine but with uncertainty. Farmers purchase water for irrigation only when power supply is not available. Therefore, the water charge in Bijemau was not particularly high. However, in Pure Keshavrai, farmers have limited options. This leads to a marked increase in the cost of water. Because of the high cost of water, poor farmers in Pure Keshavrai Village rely solely on the monsoon. Therefore, only a few farmers cultivate during the khareef season.

Table 2. Average investment on water extraction machines in the three sampled villages category-wise (in Rs.) in Uttar Pradesh, India, i.e. Tardaha Village, Bijemau Village, Pure Keshavrai Village

Category	**Villages**		
	Tardaha	**Bijemau**	**Pure Keshavrai**
Marginal	20,000	0	0
Small	40,000	0	0
Large	91,000	45,000	50,000
Total	1,51,000	45,000	50,000

Table 3. Category-wise number of farmers produce different crops in the three sampled villages in Uttar Pradesh, India, i.e. Tardaha Village, Bijemau Village, Pure Keshavrai Village

Crops	Tardaha			Bijemau			Pure Keshavrai		
	Marginal	Small	Large	Marginal	Small	Large	Marginal	Small	Large
Paddy	5	5	5	1	4	4	0	2	3
Till	0	0	3	3	0	4	5	4	3
Urad	0	1	3	4	5	4	1	2	4
Millet	0	0	2	4	5	4	0	3	5
Wheat	4	5	5	5	5	5	1	4	5
Mustard	0	2	5	4	4	5	5	4	3

Table 4. Number of water extraction machines and water rate in the three sampled villages in Uttar Pradesh, India, i.e. Tardaha Village, Bijemau Village, Pure Keshavrai Village

Indicator	Villages		
	Tardaha	Bijemau	Pure Keshavrai
Number of water extraction machines	7	3	1
Water rate (in Rs. Per hour)	120	100	210
Type of machine	4 inch/5 hp	3 inch/5 hp	4 inch/5 hp
Number of water sellers	5	2	1

Cropping pattern

Factors such as cost, benefit, and natural resources determine the cropping pattern. Natural resources directly influenced the cropping pattern in Tardaha Village. A full-voltage supply facilitates the extraction of water. Farmers have been cultivating paddy, a crop that is becoming increasingly profitable. Paddy is a more profitable crop, and animals inflict less damage on it than other crops, posing the biggest challenge in agriculture today. Each of the 15 farmers has cultivated paddy during the khareef season. Large farmers sow paddy on about 25% of their total agricultural land. Even small farmers cultivated only paddy during the survey year, despite the monsoon being late. The study encompassed 17.65 hectares of agricultural land, with approximately 57% of it dedicated to paddy cultivation in khareef season. Only the cost-return ratio influences cropping patterns in the rabi season. Farmers cultivate wheat, mustard, and pulses. Farmers cultivated wheat on approximately 58% of the total land and mustard on approximately 21%. On the remaining land, pulses are cultivated.

In Bijemau, the cropping pattern closely resembles that of the Village of Tardaha during normal years. But in the survey year, monsoon was late, and electricity supply was poor in the village. Farmers could not get sufficient water for irrigation. Due to the low groundwater level in the village, diesel engines were unable to extract sufficient water for irrigation. Large farmers cultivated paddy with *moong*, *urad*, *till*, and *arhar*, while marginal farmers who could not manage water for irrigation cultivated *urad*, *moong*, and *till*. The study encompassed 11.42 hectares of land in the village, with approximately 31% dedicated to paddy cultivation. The village cultivated about 20.13% *urad*, 8.40% *till*, and 18% *bajra*. According to the farmers' information, the cropping pattern during the last rabi season resembled that of the Village of Tardaha.

The cropping pattern in Pure Keshavrai Village was notably unique. Farmers were totally dependent on the monsoon for their agriculture. Among the 15 sampled farmers, only 5 had sown paddy. All the paddy farmers were considered marginal farmers. *Till*, *bajra*, and *urad* were the main crops during the khareef season. The study covered 4 hectares of village land, of which 10.12% contained *urad*, 16.17% *till*, 16% *bajra*, and only 29% paddy. During the rabi season, wheat and mustard were the main crops. The cropping pattern for winter crops was almost identical to that of Bijemau. Some farmers were farming only in the khareef season/rainy season (Figure 3).

Impact on employment structure and living conditions of women

In the modern world, electricity touches every aspect of life, directly or indirectly. It affects not only luxuries or electronic goods, but it also plays an important role in structuring society and the economy. The employment structure primarily impacts a society or economy. The electricity supply significantly influences the restructuring of employment opportunities. Electricity enhances business and self-employment opportunities in rural areas within the primary sector and other sectors. The quality of electricity increases the non-agricultural incomes of rural households (Chakravorty et al. 2014).

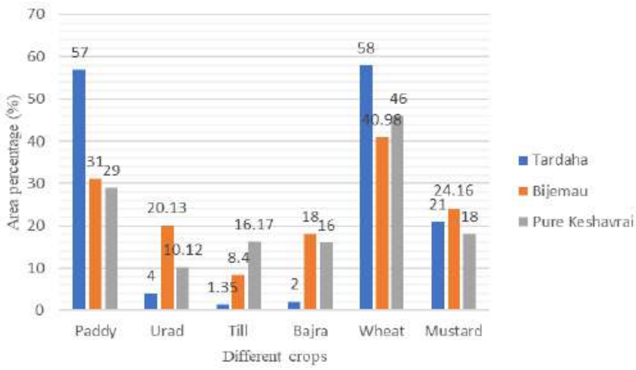

Figure 3. Area percentage (%) under different crops in the three sampled villages in Uttar Pradesh, India

In the Village of Tardaha, the employment pattern is diversified. Agriculture is a secondary source of income in the village. Among the 15 sampled households, 9 have either a regular job in the government or private sector, or they are self-employed. Many farmers owned rice mills, flour mills, and oil extraction machines, while some larger farmers also sold water. One farmer even owned a small cafeteria. Among large farmers, only one's primary occupation was farming. However, he was also involved in various agriculture-related businesses, such as cultivating straw and operating tractors. The overall analysis shows that the availability of adequate electricity has given villagers opportunities to explore self-employment. The occupational structure in Bijemau bears a striking resemblance to that of Tardaha. Bijemau is almost similar to Tardaha. The availability of electricity has significantly enhanced the employment opportunities in the village of Bijemau. The Agarbatti and Dhoopbatti factories were operating efficiently in the village, offering approximately 6-7 workers employment opportunities. Rich and large farmers earn approximately Rs 10,000 per annum from the sale of water. Two farmers operate a flour mill and a rice mill in the village. However, in Pure Keshavrai, the occupational pattern differed significantly. The majority of the farmers were engaged in agriculture. Among 15 sampled households, 11 households' main occupation was farming. The remaining households primarily engage in labor in urban areas of Uttar Pradesh or other states. Not a single farmer was associated with business or self-employment. Income levels among the villagers were very low compared to Tardaha and Bijemau Villages. Rural electrification increases household per capita income and expenditure (Khandker et al. 2014).

Electricity has a significant impact on women. In the Village of Tardaha, women recounted their experiences after the installation of a water extraction engine. The women from affluent families expressed that they no longer rely on male family members to fetch water from outside their homes. Households that had installed a handpipe within their premises reported that women were solely responsible for fetching water from the handpipe—a task that required significant time and energy. However, the installation of a water extraction engine has saved their time and energy. Women from scheduled castes have expressed that they no longer have to carry water from others homes, which can sometimes be embarrassing. Therefore, we can easily assert that a proper electricity supply contributes to the improvement of women's living standards and saves time and energy. In the Village of Bijemau, the situation was nearly identical. Only in the summer season, when electricity supply becomes poor, do women face the problem of water drawing from handpipes or wells. In Pure Keshavrai, only one household has a water extraction engine. None of the farmers has a personal hand pump, as the water table is low in the village. The village only has two government handpipes, known as *'Indiamarka'*, installed among 20-25 families. Needless to say, the water burden falls either on women or female children of the family.

Discussion

A brief comparison of three villages in Uttar Pradesh having a limitation of very small sample size, only three villages and fifteen households from each village, using purposive sampling, provides insight that different stages of electricity indicate the profound impact of electricity on the agriculture sector and non-agriculture variables like employment structure and living standard of women. The study found that electricity supply is highly associated with capital investment in irrigation sources, water prices, and cropping patterns. Villages with a healthier electricity supply had a higher number of groundwater irrigation sources, like Tardaha Village. Tardaha Village, with a healthier electricity supply, invests two to three times more in irrigation sources than Bijemau and Pure Keshavrai Villages, with a poorer electricity supply. In Tardaha Village, which has a healthier electricity supply, the water charges are also relatively low, further reducing the variable cost of farming. The study also demonstrates that government intervention in Bijemau Village maintains control over the water price market, but the supply of irrigation water remains uncertain due to inadequate electricity. In Bijemau, the primary irrigation source relied on a government engine, but it was fraught with uncertainty. The power house is located 5 km away and is shared with another village, resulting in a very poor quality of electricity supply in Bijemau Village. Due to the uncertainty of irrigation water, marginal farmers in Bijemau Village are less engaged in water-intensive crops such as paddy. Additionally, the area covered by paddy crops in Bijemau Village is smaller than that of Tardaha Village, which enjoys a healthier electricity supply. Bijemau Village is not experiencing better outcomes due to socio-political factors, but rather due to its poor electricity supply. Therefore, a bottom-up electricity reform policy necessitates that the government implement measures to regulate water prices and enhance irrigation sources to ensure a sufficient supply of water. This will protect marginal and small farmers from excessive irrigation expenses. An improvement in irrigation facilities can increase agricultural production and productivity (Yadav and Gupta 2023), and the impact of this is also evident in the cropping pattern. Villages with a healthier electricity supply, like Tardaha, tend to cultivate more profitable and water-intensive crops such as wheat and paddy, which provide farmers with significant benefits in terms of agricultural production due to their minimum support price and year-round demand.

The present study also found that not only does a healthier electricity supply benefit the agriculture sector, but it also positively impacts non-agricultural variables like employment opportunities and the living condition of women. Free electricity supply has a positive income effect in Andhra Pradesh, which may lead to increased expenditure on education and consumption rather than investment in agriculture (Chatterjee 2015). High production and productivity, with the help of healthier electricity supply, have a long-term impact. Villages like Tardaha, which enjoy a healthier electricity supply and higher income, actively explore new self-employment and business opportunities within their village. Whereas villages have poor electricity supply like Pure Keshavrai, people are forced to explore employment opportunities in the urban areas of the state or in other states. In the Village of Tardaha, women shared their experiences following the installation of a water extraction engine. They reported a reduction in their reliance on male family members for water, as well as a reduction in the time they spent fetching water.

Therefore, we can assert that a healthier and more efficient electricity supply plays a crucial role in agricultural production and productivity as well as non-agricultural variables like employment structure and the living conditions of women, but unregulated electricity supply also poses several challenges. Overuse of water for crop production is one of the main causes of water scarcity in major economies of South Asia (Mitra et al. 2017). Due to overexploitation, Punjab and Haryana states are facing severe water crises (Rosencranz et al. 2021; Gupta 2023; Khara and Ghuman 2024). The negative effects of the free electricity policy on underground water raise several questions about the effectiveness of both the free electricity policy and its reform. Research suggests that sustainable agriculture development requires electricity reform, but it also necessitates proper water management. The problem is not a shortage of water but the absence of proper mechanisms, distribution, and efficient use (Dev 2016). Efficient pricing of water and energy can lead to a more efficient allocation of water in developing countries, such as India (Fishman et al. 2016). Many studies indicate that in recent years, the adoption of proper water mechanisms, efficient water use through water-saving irrigation methods, and reasonable water pricing has increased the groundwater level. Due to the introduction of paddy as the main kharif crop, Punjab and Haryana became water-scarce states after the Green Revolution, but improvements in water use efficiency have increased the water level (Gill 2016). Due to a severe groundwater overexploitation problem in Hebei, the province of China has adopted a water-saving agricultural irrigation system and improved water use rights and water pricing systems (Lilia et al. 2020). The reform of agricultural water prices is essential for social stability and serves as a primary tool to encourage the rational use of water resources (Xie and Su 2019). The drip irrigation method, a water delivery method that slowly drips water into the soil or directly into the roots of plants, can be considered the most water-efficient for irrigation if managed properly (Reddy 2017). A number of studies also indicate that reasonable water pricing and monetary incentives reduce electricity consumption (Sudarshan 2017) and enhance water conservation. The reasonableness of the water price has a direct influence on people's attitude, behavioral decision regarding willingness to pay, and motivation for water conservation in China (Mu et al. 2019). Farmers of Punjab prefer the policy with a reward for consuming less electricity rather than the free and unmetered electricity policy (Kaur and Pollitt 2024).

Uttar Pradesh is a populous state of India, and the agricultural production demand, mainly for main and water-intensive crops like paddy and wheat, is not going to decline in recent times; even their demand will increase day by day. Therefore, the entire study emphasizes the need for a bottom-up policy (a policy from micro unit) that not only provides an efficient and reliable irrigation source along with a proper electricity supply and reasonable water prices but also equips farmers with proper irrigation management knowledge and training to prevent future water crises in the state.

In conclusion, the study concludes with the finding that the quantity and quality of electricity supply, water prices, and reliable irrigation sources play a vital role in increasing production and productivity of the agriculture sector with the help of groundwater extraction. It also helps to promote employment opportunities at the local level and improve the living conditions of women. Many of the agricultural states of India, like Uttar Pradesh, have used power reform policy as a tool to increase agricultural production, manage the cropping pattern, and control water prices. However, overextraction of underground water can lead to a water crisis. Therefore, it's crucial to use water efficiently to boost agricultural production and productivity, which can be achieved through appropriate bottom-up electricity reform policy, water-saving irrigation technology, and government intervention.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 112-121 DOI: 10.13057/asianjagric/g090112

Assessment of local wisdom biofertilizer formulas on enhancing microbial diversity and photosynthate allocation in acid-stressed maize

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Manuscript received: 29 October 2024. Revision accepted: 31 December 2024.

Abstract. *Irwandhi, Kamaluddin NN, Khumairah FH, Prihatiningsih N, Simarmata T. 2025. Assessment of local wisdom biofertilizer formulas on enhancing microbial diversity and photosynthate allocation in acid-stressed maize. Asian J Agric 9: 112-121.* The use of biofertilizers derived from local wisdom practices presents a promising sustainable agricultural strategy for enhancing crop resilience in acidic soils. This study aimed to assess the effectiveness of the Local Wisdom Biofertilizer (LWB) formula by examining the chemical and biological characteristics of various LWB formulations and their impact on growth-promoting microorganisms (GPMs) diversity (bacteria, fungi, and actinomycetes) and maize growth characters. A Randomized Complete Block Design (RCBD) was employed, testing six LWB formulations (F1 to F6) at four dosage levels (0, 2, 4, and 6%) to assess their effects on key growth traits of maize grown in suboptimal soils. Results revealed significant variation in organic carbon, nitrogen, phosphorus, and potassium across formulations, with F3 demonstrating the highest organic carbon (14.80%) and potassium (0.08%). In comparison, F2 exhibited the highest total nitrogen (4.07%), and F5 had the highest phosphorus content (0.03%). Formulation F6 demonstrated the highest concentrations of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) among the treatments. The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively enhanced maize plant height, with F3K3 and F5K3 having a particularly significant effect on chlorophyll content. Additionally, F1K1 led to the largest bacterial population, F5K1 supported the greatest fungal population, and F6K2 exhibited the highest actinomycetes population. Correlation analysis indicated a negligible relationship between chlorophyll content and other agronomic traits, with values ranging from -0.02 to 0.01. Principal Component Analysis further analyzed the influence of LWB on various plant traits. These findings underscore the potential of integrating local wisdom biofertilizers into sustainable soil management practices, especially in regions grappling with soil acidity. Future research should delve into the microbial and biochemical mechanisms underlying these benefits to optimize biofertilizer formulations for broader agroecological applications.

Keywords: Acid soil, eco-friendly fertilizer, GPMs, local microorganism, maize

INTRODUCTION

Maize (*Zea mays*) holds significant economic value due to its wide range of uses, including food for humans and livestock and as a raw material for various industries (de Matos Nascimento et al. 2020). In 2023, maize production in Indonesia decreased by 12.5% compared to 2022, mainly due to a 10.03% reduction in the area harvested that year (BPS 2023). This condition is exacerbated by Indonesia's corn production system, which still needs to improve production techniques and farming status (Fitriatin et al. 2017). It is also due to the change of corn land into land for other food commodities and the conversion of agricultural land to non-agricultural land (Khumairah et al. 2019).

The enhancement of maize productivity in Indonesia can be achieved by expanding cultivated land (extensification). One potential area for development is suboptimal land subjected to acidic stress. More than 30% of the global land area comprises acidic soils, with 50% of the potentially arable land classified as acidic (Basak and Biswas 2016). Most of Indonesia's land is in the Inceptisols order, and 52.0 million ha of Inceptisols have the potential to be developed (Fitriatin et al. 2021). In tropical areas with high rainfall, Inceptisols are susceptible to loss of nutrients and organic matter, resulting in degraded soil (Hindersah et al. 2022).

Cultivation of maize in Inceptisols faces several challenges due to the inherently low soil fertility and organic matter content and the acidic soil reaction of these soils (Sofyan and Sara 2019). Low availability of nutrients in the growth phase will inhibit several plant metabolic processes, thereby inhibiting flower formation and reducing crop yields (Wei et al. 2017). This decrease in crop yields occurs on acidic soils due to the limited availability of nutrients such as phosphate (P), molybdenum (Mo), and magnesium (Mg) in the soil, as well as the toxicity of aluminium (Al) and manganese (Mn) to plants (Shi et al. 2017).

Enhancing nutrient availability in acidic soils can be achieved through fertilization efforts (Simanjuntak and Setiawan 2021). Fertilizers are applied to increase the nutrients required for optimal plant growth (Hamid and Tanweer 2021). In recent years, the reliance on inorganic fertilizers has risen sharply (Muktamar et al. 2016). Excessive use of inorganic fertilizers causes a decrease in soil fertility (Gawęda et al. 2020). High doses of inorganic fertilizer in the long term can increase soil compaction, reduce organic matter, and disrupt the balance of nutrients, thereby reducing soil fertility. Inorganic fertilizers are expensive and have limited availability (Yang et al. 2022). However, there is hope in the form of local wisdom biofertilizer, a promising alternative technology that could potentially replace or reduce the dependence on inorganic fertilizers.

Local Wisdom Biofertilizer (LWB) is a fermented liquid from natural materials rich in growth-promoting microorganisms (GPMs). These microorganisms can break down organic matter, serving as biofertilizers, decomposers, and organic pesticides (Roeswitawati and Ningsih 2018). LWB is a biofertilizer that can improve plant leaf health and promote growth. Materials that can be used include cow urine, vegetable waste, fruit waste, banana tubers, leaves, and banana stems (Retnowati and Katili 2021). Other materials, such as seaweed waste, molasses, and earthworm castings, can also be used (Arfarita et al. 2022). The organic materials in LWB contribute to soil health restoration, increased fertilizer efficiency, and improved crop productivity (Simarmata et al. 2016). LWB fertilizers contain a variety of plant growth-promoting rhizobacteria (PGPR), such as *Azospirillum* sp., *Azotobacter* sp., and *Bacillus* sp. (Retnowati and Katili 2021). Each of these PGPRs can mobilize, facilitate, and enhance nutrient availability by transforming nutrients from forms that are not readily accessible into those that are through biological processes (Simarmata 2013). Applying PGPR as biofertilizers in maize cultivation can increase yields while reducing inorganic fertilizer use by up to 50% (Fitriatin et al. 2014, 2015). Given this potential, the current research is aimed at developing an LWB formula by examining the characteristics of the LWB formula, the diversity of GPMs in each formulation, and the effect of LWB fertilizers in enhancing the growth and development of maize on suboptimal soils, as well as to identify the best formula.

MATERIALS AND METHODS

Location

The formulation and analysis of the Local Wisdom Biofertilizer (LWB) were conducted at the Soil Biology, Soil Fertility, and Plant Nutrition Laboratory, Faculty of Agriculture, Universitas Padjadjaran, Sumedang, West Java, Indonesia from March to May 2024. Subsequently, the bioassay of LWB was carried out from June to August 2024 in a greenhouse located at the Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran (6°54'58.46"S 107°46'18.1"E) (Figure 1). The experimental site is located in a tropical region about 752 meters above sea level. The research location has an annual temperature of around 19-30°C, with a relative humidity level of 39-84%. The soil used is included in the Inceptisols soil classification obtained from Jatinangor. This soil has the characteristics of a slightly acidic pH of 5.83 with low available phosphorus (1.23 ppm) and an organic carbon content of 1.66% (Fitriatin et al. 2024).

Selection and formulation of LWB

The LWB formula was developed based on an existing biofertilizer formulation known as Jakaba (*Jamur Keberuntungan Abadi*), which was discovered by farmers in Kedung Dowo Village, Arjasa Sub-district, Situbondo District, East Java, Indonesia (7°45'30"S 114°09'10"E), as illustrated in Figure 1. This formulation was further refined into six LWB formulas. Each material used in the LWB formulations, as detailed in Table 1, was placed into a 60 L fermentor equipped with an air outlet. Initially, 25 L of water was added, and the materials were homogenized using a turbo mixer. Additional water was added until the final volume reached 30 L. Each formula was fermented for 60 days and monitored by observing aroma and microbial activity. After the fermentation process is complete, the solution is filtered using an 80-100 mesh sieve and transferred to a jerry can for storage until there is a decrease in microbial activity.

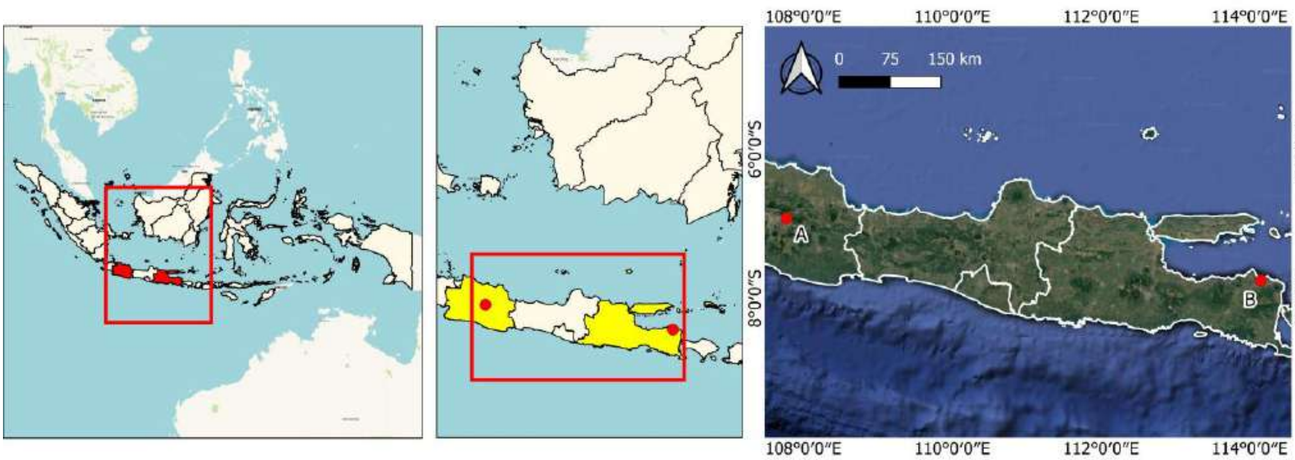

Figure 1. A. Locations of the development and bioassay of the local wisdom biofertilizer in Ciparanje Experimental Farm, Sumedang, West Java, Indonesia; B. The discovery of the Jakaba formula in Kedung Dowo Village, Situbondo District, East Java, Indonesia

Table 1. Composition of materials in each local wisdom biofertilizer formulation

Formulation	Composition
F1	Seaweed (2.5 kg), *Azolla* (2.5 kg), molasses (300 mL), rice husk charcoal (1.25 kg), and yeast (30 g)
F2	Snail shell extract (2.5 kg), crab shell extract (2.5 kg), shrimp shell extract (2.5 kg), eggshell extract (2.5 kg), and rabbit urine 600 mL
F3	Seaweed (1.25 kg), *Azolla* (1.25 kg), molasses (300 mL), rice husk charcoal (625 g), yeast (30 g), snail shell extract (1.25 kg), crab shell extract (1.25 kg), shrimp shell extract (1.25 kg), eggshell extract (1.25 kg), and rabbit urine 600 mL
F4	Rice bran (2 kg), MSG (20 g), shrimp paste (10 g), brown sugar (200 g), lime powder (3 tbsp), bamboo root soaking solution (2 L), and bean sprouts (200 g)
F5	Seaweed (1.25 kg), *Azolla* (1.25 kg), molasses (300 mL), rice husk charcoal (625 g), yeast (30 g), rice bran (1 kg), MSG (10 g), shrimp paste (5 g), brown sugar (100 g), lime powder (1.5 tbsp), bamboo root soaking solution (1 L), and bean sprouts (100 g)
F6	snail shell extract (1.25 kg), crab shell extract (1.25 kg), shrimp shell extract (1.25 kg), eggshell extract (1.25 kg), rabbit urine 600 mL, rice bran (1 kg), MSG (10 g), shrimp paste (5 g), brown sugar (100 g), lime powder (1.5 tbsp), bamboo root soaking solution (1 L), and bean sprouts (100 g)

Note: F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

Chemical analysis of LWB

Nitrogen total (%)

The nitrogen content analysis of the fertilizer was conducted using the Kjeldahl method, which involves several stages, including sample digestion, distillation, and titration (Gani et al. 2023). A completely homogenized sample of LWB, weighing precisely 250.0 mg, was cautiously transferred into a Kjeldahl flask. Subsequently, 3 mL of H_2SO_4 and 0.25-0.50 g of selenium were added to the mixture, shaken until completely homogenized. The mixture was then allowed to stand for 2 to 3 hours. The digestion was performed incrementally at temperatures between 150 and 350°C until a clear solution was obtained. After the solution was cooled, a small volume of distilled water was added to inhibit crystallization.

The distillate solution was poured into a boiling flask to a total volume of 250 mL, and deionized water was added until the flask reached the halfway. The distillate reservoir was set up in a 100 mL Erlenmeyer flask, which contained 10 mL of 1% boric acid and three drops of Conway indicator. The distillation procedure involved the addition of 20 mL of 40% sodium hydroxide (NaOH). The distillation was regarded as complete once the solution volume in the Erlenmeyer flask reached 75. The resulting solution was then titrated with 0.05 N H_2SO_4 until a transition from green to pink occurred. The volume of H_2SO_4 used was measured, and the procedure was repeated to obtain the blank sample. The nitrogen content was calculated using the following formula:

$$\text{Nitrogen (\%)} = (Vc - Vb) \times N \times 14 \times fk$$

Where:
Vc: volume H_2SO_4 titration (mL)
Vb: volume H_2SO_4 blank (mL)
N: normality of raw solution H_2SO_4
14: atomic weight of nitrogen
100: % conversion
fk: moisture content correction factor

Organic carbon (%)

According to Gani et al. (2023), the analysis of Organic-C content in LWB can be conducted in several stages. Initially, a sample of LWB weighing 50 to 100 mg is transferred into a 100 mL volumetric flask. Then, 5 mL of a 2N $K_2Cr_2O_7$ solution is introduced, and the mixture is shaken well. Subsequently, 7 mL of H_2SO_4 is added to the solution and left to stand for 30 minutes. To prepare a 250 ppm C standard solution, 5 mL of a 5000 ppm C standard solution is pipetted into a 100 mL volumetric flask. Next, 5 mL of H_2SO_4 and 2N $K_2Cr_2O_7$ solution are added, following the same method used for the 250 ppm C standard solution. Each solution is then diluted to the 100 mL mark and mixed thoroughly. The absorbance of each solution is measured with a spectrophotometer at a wavelength of 651 nm. A standard curve is constructed to derive the linear equation, which allows for the determination of the x-value of the solution. The organic-C content is calculated using the following formula.

$$\text{Organic-C (\%)} = \text{ppm curve} \times \text{mL} \times fk$$

Where:
ppm curve: the sample rate was derived from the regression curve showing the relationship between the standard series rate and the reading, adjusted by subtracting the blank value.
fk: moisture correction factor = 100 / (100 - % moisture content)

Phosphate and potassium analysis (%)

Phosphorus content was measured using spectrophotometry, while potassium was determined using flame photometry. A 500 mg sample of LWB from each formula was weighed and placed into a digestion flask. Then, 0.5 mL of $HClO_4$ and 5 mL of HNO_3 were thoroughly mixed into the solution. The mixture was heated in a reactor, starting at 100°C. After the yellow vapors had dissipated, the temperature was raised to 200°C. The digestion process was considered complete when white vapors were observed, and approximately 0.5 mL of liquid remained in the flask. The solution was then cooled and diluted with deionized water to a final volume of 50 mL. After mixing, the mixture was filtered using W-41 filter paper (Extract A). Next, 1 mL of Extract A was transferred into a 20 mL test tube, and then 9 mL of deionized water was added and mixed until the solution became homogeneous, resulting in a 10-fold dilution (Extract B).

The absorbance of potassium, and the absorption values of extract B and the potassium standard solution, were documented. Each potassium standard solution received 1 mL of extract B, and the mixtures were then placed in individual 20 mL test tubes. Next, 9 mL of phosphate coloring reagent and standard solution were added to each sample. The mixtures were thoroughly mixed using a vortex mixer and allowed to sit for 15 to 25 minutes. The absorbance of each sample and potassium standard solution was measured and recorded at a wavelength of 693 nm. Standard curves for both potassium and phosphate samples were created to formulate linear equations, enabling the calculation of concentrations (ppm).

Biological analysis of LWB

The isolation of nitrogen-fixing bacteria was carried out using selective media, namely Jensen's medium (sucrose; 20 g L^{-1}, $CaCO_3$: 2 g L^{-1}, $MgSO_4$: 0.5 g L^{-1}, Na_2MoO_4: 0.0005 g L^{-1}, NaCl: 0.5 g L^{-1}, K$_2$HPO4: 1 g L^{-1}, $FeSO_4$: 0.1 g L^{-1}, Agar: 15 g L^{-1}) and Pikovskaya agar medium (dextrose: 10 g L^{-1}, $Ca_3(PO_4)_2$: 5 g L^{-1}, (NH_4)2SO$_4$: 0.5 g L^{-1}, $MgSO_4.7H_2O$: 0.1 g L^{-1}, yeast extract: 0.5 g L^{-1}, $FeSO_4.7H_2O$: 0.0001 g L^{-1}, KCl: 0.2 g L^{-1}, $MnSO_4.7H_2O$: 0.0001 g L^{-1}, Agar: 15 g L^{-1}, pH 7). The cultures were then incubated at room temperature for 48 hours.

Biological assay of LWB on maize

This study utilized a Randomized Complete Block Design (RCBD) with treatments comprising six LWB formulations (F1, F2, F3, F4, F5, and F6) and three LWB dosage levels (2%, 4%, and 6%) as well as a control group (without LWB application). The experiment involved a total of 19 treatments, each replicated twice. Maize seeds were sown in polybags filled with 1 kg of soil, and 10 mL of LWB was applied according to each treatment specification. Plant maintenance included daily watering based on field capacity and was continued until 28 days after planting (DAP). Plant height, measured from the base to the plant tip, was the growth parameter. Chlorophyll content was assessed using an Opti-Sciences Chlorophyll Meter CCM 200 Plus. At 28 DAP, the maize plants were harvested, and root length was measured from the base to the longest root. Shoots and roots were separated using scissors, and fresh weights were recorded with a digital balance. Samples from each treatment group were transferred to paper envelopes and dried in an oven until they reached a stable weight. The final dry weights of both the shoots and roots were documented. The total biomass for each treatment was calculated by summing the dry weights of shoots and roots (Fitriatin et al. 2020). The rhizosphere microbial population was analyzed using the Serial Dilution Plate Method, as described by Ben-David and Davidson (2014). Soil samples were cultivated on various media: Potato Dextrose Agar for fungi, Nutrient Agar for bacteria, and Starch Casein Agar for actinomycetes.

Data analysis

The impacts of various treatments on the measured variables were assessed using ANOVA with SPSS (Statistical Package for the Social Sciences) software. Significant differences were identified through Duncan's test, with significance established at $P<0.05$. Correlation analysis was conducted using Pearson correlation testing, and Principal Component Analysis (PCA) was also performed. Data visualization of the research findings was accomplished using Prism 9 software.

RESULTS AND DISCUSSION

Chemical properties of LWB

The results of the chemical analysis for each LWB formula, as illustrated in Figure 2, indicate that each formula exhibits distinct levels of organic carbon, nitrogen, phosphorus, and potassium. The organic carbon content ranges from 0.40 to 14.80%, with formula F3 exhibiting the highest concentration at 14.80%. Nitrogen levels vary between 0.94 and 4.07%, with formula F2 containing the greatest amount at 4.07%. Phosphorus concentrations range from 0.01 to 0.03%, with formula F5 demonstrating the highest phosphorus content at 0.03%. Potassium levels vary from 0.01 to 0.08%, with formula F3 again showing the highest potassium content at 0.08%. These variations in nutrient content can be attributed to the different materials utilized in each formula. The nutrient composition in each formula plays a crucial role in supporting plant growth and development. Organic fertilizers containing organic matter, nitrogen, phosphorus, potassium, and other essential elements can serve as a valuable source of plant nutrition (Ma et al. 2022).

Biological properties of LWB

Biological analysis of LWB shows that each formula contains growth plant microorganisms (GPM) with different populations (Figure 3). Formulation F6 has the highest population of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) compared to other formulas. This microbial population plays an essential role in supporting plant growth and development. NFB enhances nitrogen availability to plants, while the high population of PSB increases phosphate availability. The diversity of GPM populations in LWB also acts as a decomposer agent, aiding in the breakdown of organic matter in the biofertilizer (Batara et al. 2016). Furthermore, the LWB formula can also contain growth hormones such as Indole-3-acetic acid (IAA), cytokinin (zeatin), cytokinin (kinetin), and gibberellin, which can be used to support plant growth (Sodiq et al. 2019). Research by Sodiq et al. (2021) showed that LWB contains GPMs such as *Bacillus cereus* and *Lysinibacillus* sp., which can be used as a biofertilizer inoculant consortium.

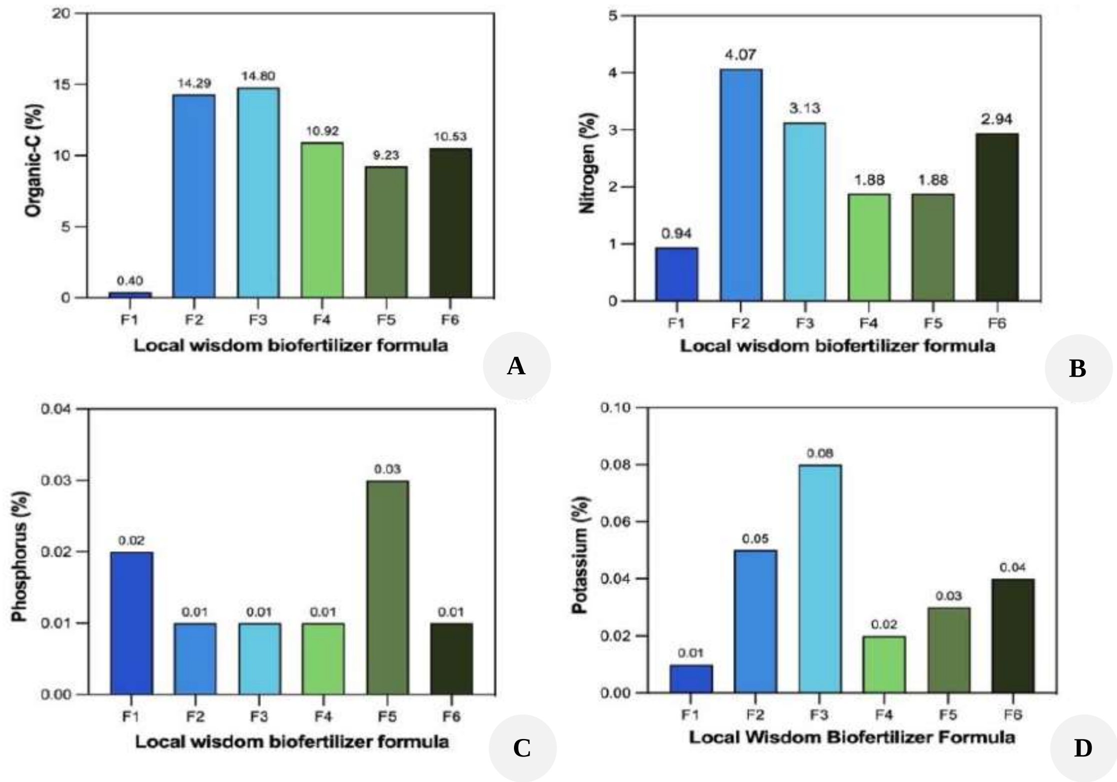

Figure 2. Macronutrient content of local wisdom biofertilizer. A. Organic-C; B. Nitrogen; C. Phosphorus; D. Potassium. F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

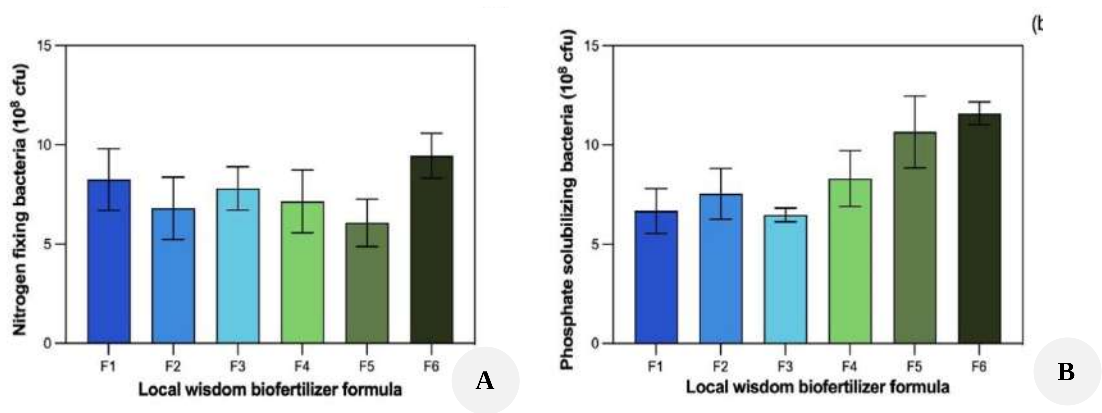

Figure 3. Diversity of growth-promoting microorganism populations in the LWB formula. F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Nabati Jakaba-based LWB, F6: Animal Jakaba-based LWB

The influence of formulation and dosage of LWB on the growth traits of maize

The application of local biofertilizer formulas and dosages influenced the morphological characteristics of maize (Table 2). The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively increased maize plant height. F3K3 and F5K3 treatments significantly affected the chlorophyll content in corn plants. This condition may be related to an increase in the microbial population in the corn rhizosphere. Adding organic fertilizer increases nutrient absorption by roots through the proliferation of beneficial bacterial groups. This finding is consistent with Lidbury et al. (2019), who demonstrated that the application of organic fertilizers significantly increased bacterial community biomarkers, particularly Bacteroidetes. Bacteroidetes are abundant members of the plant microbiome that suppress pathogens and play a critical role in rhizosphere phosphorus mobilization, a nutrient that often limits plant growth.

Table 2. Morphological traits of maize by different formulas and dosages of local wisdom biological fertilizers

Treatment	PH	Cl	WWS	DWS	WWR	DWR	BM
F0K0	37.5a	6.0a	2.0	0.27	2.27	1.24	1.52
F1K1	37.5a	6.9a	2.1	0.22	3.34	1.73	1.94
F1K2	43.5a	6.7a	2.6	0.31	5.51	3.01	3.32
F1K3	44.5a	6.0a	2.5	0.36	3.16	1.74	2.09
F2K1	41.5a	7.2a	2.7	0.35	5.26	2.96	3.31
F2K2	45.5a	6.0a	2.7	0.23	4.53	2.62	2.84
F2K3	47.5a	6.5a	3.5	0.51	6.48	3.78	4.29
F3K1	43.0a	6.8a	2.2	0.20	3.70	2.16	2.36
F3K2	47.5a	6.6a	2.5	0.36	4.49	2.59	2.95
F3K3	49.5b	9.4b	3.1	0.37	3.05	1.74	2.11
F4K1	43.5a	7.85a	2.4	0.34	3.69	2.00	2.34
F4K2	45.0a	7.7a	2.5	0.31	2.90	1.62	1.92
F4K3	50.0b	6.8a	3.2	0.40	3.06	2.14	2.53
F5K1	48.5b	5.6a	3.2	0.34	4.50	2.64	2.98
F5K2	43.0a	7.4a	2.4	0.25	2.49	1.30	1.55
F5K3	48.5b	8.2b	2.4	0.27	4.66	2.78	3.05
F6K1	49.5b	5.9a	3.0	0.46	5.29	3.02	3.47
F6K2	48.5b	7.3a	3.2	0.48	8.34	5.00	5.48
F6K3	44.0a	7.1a	2.1	0.22	3.09	1.86	2.07

Note: Numbers followed by the different letters in a column are significantly different based on the Duncan test at $p \leq 0.05$. PH: Plant height, Cl: Chlorophyll, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass

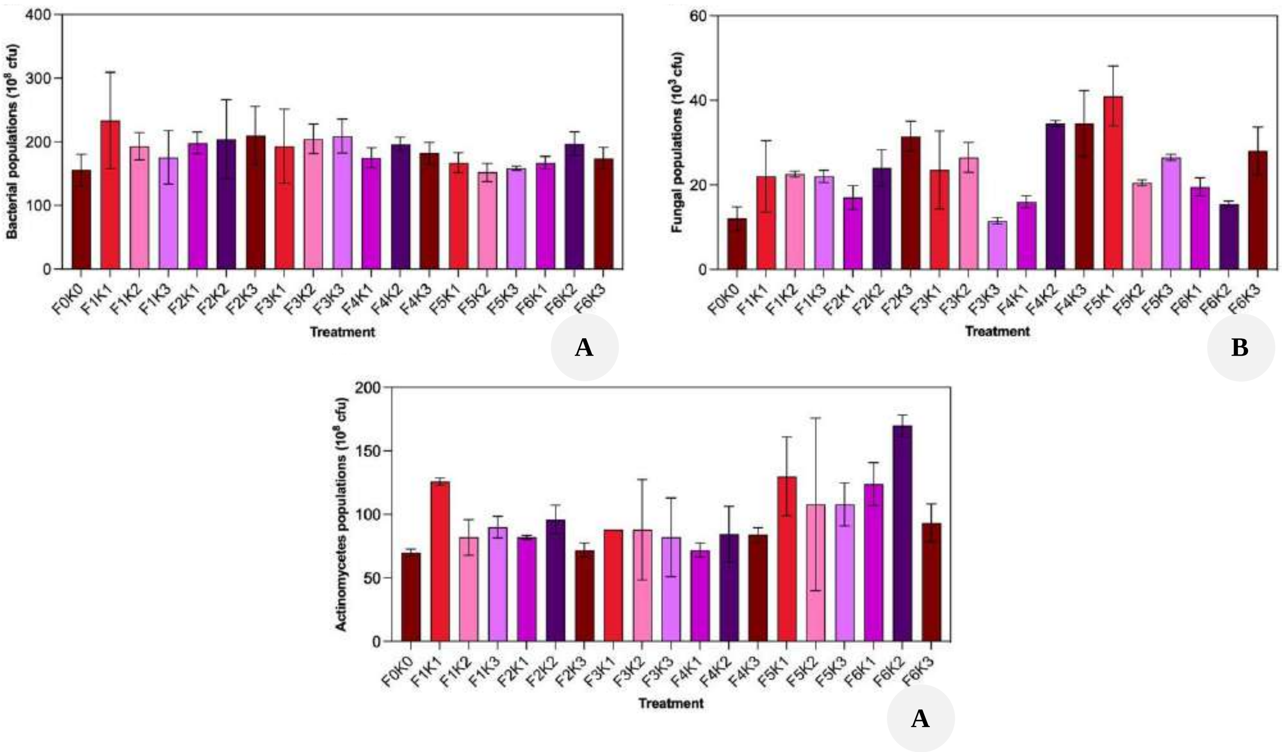

Figure 4. Microbial population the rhizosphere of maize after application of LWB with a combination of formula (F) and dosage levels (K). F1: Plant-based LWB, F2: Animal-based LWB, F3: Plant and animal-based LWB, F4: Jakaba, F5: Jakaba vegetable-based LWB, F6: Animal Jakaba-based LWB. K0: without LWB, K1: LWB dose 2%, K2: LWB dose 4%, K3: LWB dose 6%

Microbial population of local wisdom biofertilizer formulation

The use of LWB on maize plants significantly impacted the microbial populations in the rhizosphere. The F1K1 treatment produced the highest bacterial population, while the F5K1 formulation produced the largest fungal population. Meanwhile, F6K2 produced the highest actinomycete population (Figure 4). This is in line with Fitriatin et al. (2017), which shows that the addition of biofertilizer with P fertilizer can increase the bacterial population in the soil. The use of organic fertilizers can diminish the growth of pathogenic bacteria like Acinetobacter while promoting greater diversity within rhizosphere bacterial communities (Ma et al. 2022). Microbial communities in the rhizosphere are essential in maintaining soil fertility and supporting plant growth. Microbes in the rhizosphere play a role in the decomposition of organic matter, pathogen management, and nutrient cycling. The microbial content in biofertilizers added to the soil also helps provide essential nutrients for plant growth (Melini et al. 2023). Biofertilizers consist of various microorganisms, including bacteria, that can dissolve phosphate to be available to plants. The presence of phosphate-solubilizing bacteria in the soil allows them to adapt to their environment and compete with native microbes found in the soil (Janati et al. 2023).

The increase in microbial population due to the addition of LWB can occur due to the influence of organic material content in each formula. Organic materials can increase microbial activity, thereby encouraging rhizosphere microbial diversity. Increasing organic matter in the soil also improves soil structure, nutrient retention, and water retention capacity (Kamaa et al. 2011). Apart from that, it also influences the structure of bacterial and fungal communities in the rhizosphere. Changes in microbial community structure can enhance the symbiotic relationship between plants and microbes. This increased interaction can support plant growth and resistance to environmental stress. In addition, rhizosphere microbial diversity also increases resistance to pathogens and reduces the potential for crop failure due to unfavorable soil conditions (Ma et al. 2022).

Table 3. Morphological traits variance revealed by principal components

	PC1
Eigenvalue	4.158
Variability (%)	59.40%
Cumulative %	59.40%

Table 4. Principal component analysis of morphological traits in maize

	PC1
PH	0.199
Cl	0.004
WWS	0.857
DWS	0.805
WWR	0.946
DWR	0.951
BM	0.968

Note: PH: Plant height, Cl: Chlorophyll, WWC: Wet weight of the shoot, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass

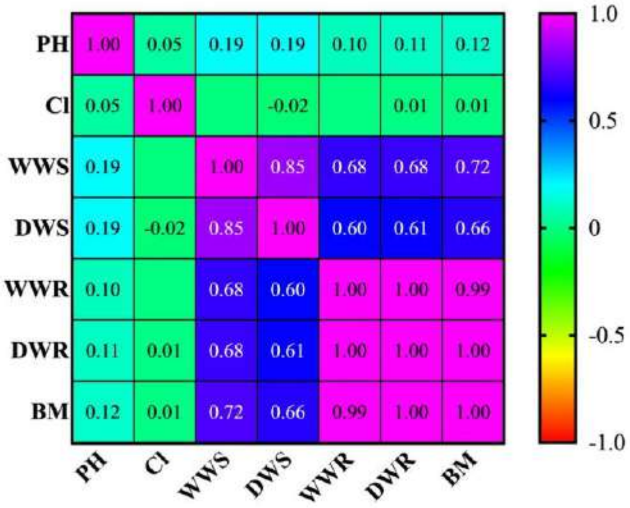

Figure 5. Correlation matrix between morphological characteristics of corn plants and the effect of biofertilizer application. Color reflects the value of the correlation coefficient (r) on a scale from -1 to 1. The traits analyzed include PH: Plant height, Cl: Chlorophyll content, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the roots, DWR: Dry weight of the roots, and BM: Overall biomass. High positive correlation values are shown in dark blue to purple, while values close to zero are shown in bright green to light blue

Pearson's Correlation Analysis of growth traits in maize

Correlation analysis was conducted to assess the relationships among various morphological traits of maize, including plant height, chlorophyll content, wet and dry weights of the shoots, wet and dry weights of the roots, and overall biomass. The pairwise Pearson's correlation analysis in Figure 5 shows that each plant character has a different degree of association. Chlorophyll (Cl) is a pigment in leaves essential to photosynthesis. In the photosynthesis process, Cl plays a role in capturing and converting sunlight into energy in the form of glucose. However, the results of correlation analysis show that chlorophyll has a very weak or almost negligible correlation with other characters, with correlation values ranging from -0.02 to 0.01. This weak correlation indicates that although Cl is essential in photosynthesis, Cl does not directly influence all plant growth characteristics. Characteristics such as root development, disease resistance, or water use efficiency are likely influenced by other factors, such as environmental conditions or microbial interactions (Li et al. 2018). The strength of the correlation can be interpreted using the absolute value of r, very strong (0.90-1.00), strong (0.70-0.89), moderate (0.40-0.69), weak (0.10-0.39), and negligible (0.00-0.10) (Pengphorm et al. 2024). The near-zero correlation values indicate that changes in chlorophyll content do not necessarily correspond to changes in other traits. This condition is likely caused by the adaptation of corn plants to environmental stress, which causes fluctuations in chlorophyll concentration so that it does not have a significant effect on other characteristics (Talebzadeh and Valeo 2022). This condition can occur due to the response expressed by genes in plant leaves influenced by the LWB formula application. Genes expressed differently in chlorophyll and chloroplast biogenesis are the biomolecular reasons for the formation of green or yellow colors in leaves (Mei et al. 2022). However, to find out the deeper mechanism of this finding, further research is needed on the mechanism of LWB fertilizer in influencing chlorophyll content so that it does not damage other characteristics. The traits, including plant height, wet and dry weights of the shoots, wet and dry weights of the roots, and total biomass, show positive correlations, ranging from moderate to strong positive relationships. This positive association suggests that increases in the others often accompany an increase in one trait. High total biomass in corn is associated with greater wet weight and dry weight of roots, as well as with higher wet weight and dry weight of shoots (Walne et al. 2021).

Morphological traits diversity based on PCA

The use of biofertilizers on maize plants can affect a range of plant characteristics, and the variability of these traits can be thoroughly assessed using Principal Component Analysis (PCA) (Zulchi et al. 2024). PCA helps to describe the variation in variables (plant traits), reveal trait diversity, and explain potential data variance (Acquaah 2012). When applied to maize traits, PCA identified a significant first principal component with an eigenvalue of 1, contributing to 59.40% of the observed variation, as shown in Table 3. This first principal component (PC1) contributes primarily to variations in biomass. Broad trait diversity is generally assessed using a significant dissimilarity coefficient or an Euclidean distance exceeding 1 (Karuniawan et al. 2021). Principal Component 1 (PC1), which primarily represents plant height (PH) with a loading of 0.199 (see Table 4), also captures the effects of other traits. With an eigenvalue of 4.158, PC1 explains 59.40% of the overall trait diversity in maize, serving as a crucial component in conveying information regarding trait diversity and the relationships among superior traits in the biplot diagram.

The biplot shown in Figure 6 accounts for 59.40% of the total variance in the characters, as represented by PC1. In the PC1 biplot, variables with shorter vectors from the origin, such as Cl (chlorophyll) and PH (plant height), demonstrate minimal variation. In contrast, variables with longer vectors, including WWS (wet weight of the shoots), WWR (wet weight of the root), DWC (dry weight of the shoots), DWR (dry weight of the root), and BM (biomass), demonstrate a higher degree of variability. These findings align with previous research, which indicates that vector lengths in PCA biplots reflect the magnitude of variability in specific traits and their influence on the overall variation of the dataset (Jolliffe and Cadima 2016).

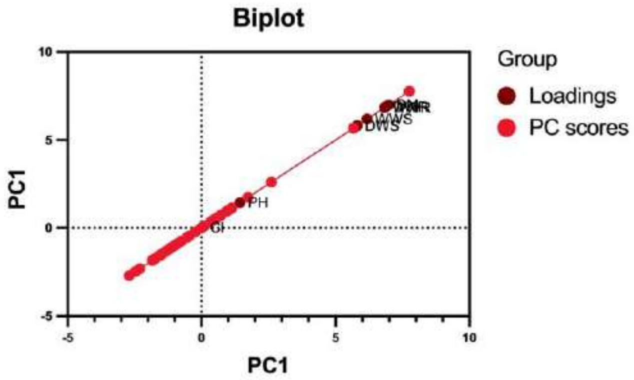

Figure 6. Principal Component Analysis (PCA) biplot showing the distribution of principal component scores (PC scores) and loadings on morphological traits of maize plants. Variables such as PH: Plant height, Cl: Chlorophyll, WWS: Wet weight of the shoots, DWS: Dry weight of the shoots, WWR: Wet weight of the root, DWR: Dry weight of the root, BM: Biomass appear to contribute differently to the principal component. The relationship between variables is described by the direction and length of the loading vector, where variables with the same direction of length vectors have a high positive correlation

In Figure 6, the variation in maize plant traits is indicated by vector lengths, suggesting that the diversity in plant response is likely influenced by individual traits and the availability of nutrients due to biofertilizer application. Previous studies have shown that biofertilizers enhance nutrient uptake efficiency, leading to significant variation in plant growth traits (Bhardwaj et al. 2014). The pattern of variation identified in this study underscores the importance of nutrient-driven physiological responses in maize plants, particularly in traits such as biomass and root shoot weight. These results further support the role of biofertilizers as sustainable agricultural inputs, driving variability in growth traits while enhancing overall crop resilience and productivity (Prasad et al. 2019).

In conclusion, formulation F3 exhibited the highest levels of organic carbon (14.80%) and potassium (0.08%), while F2 recorded the highest total nitrogen content (4.07%), and F5 had the greatest phosphorus content (0.03%). Formulation F6 demonstrated the highest concentrations of nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB) among the treatments. The treatments F3K3, F4K3, F5K1, F5K3, F6K1, and F6K2 effectively enhanced maize plant height, with F3K3 and F5K3 having a particularly significant effect on chlorophyll content. In addition, F1K1 led to the largest bacterial population, F5K1 supported the largest fungal population, and F6K2 demonstrated the highest actinomycetes population. Correlation analysis shows a minimal relationship between Cl content and other characters, with correlation values ranging from -0.02 to 0.01.

ACKNOWLEDGEMENTS

The author would like to express his appreciation to the Indonesian Education Fund Management Institute (LPDP), which has sponsored the author's master's education, and the Padjadjaran University Academic Leadership Grant (ALG) for their valuable support and funding of the publication of this article.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 122-130 DOI: 10.13057/asianjagric/g090113

The feasibility insights of organic rice farming from Central Java and Yogyakarta, Indonesia

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Manuscript received: 10 October 2024. Revision accepted: 10 January 2025.

Abstract. *Rozaki Z, Al Hadi SS, Rahmawati N, Triyono, Ardila RA, Pamungkas HW, Fathurrohman YE. 2025. The feasibility insights of organic rice farming from Central Java and Yogyakarta, Indonesia. Asian J Agric 9: 122-130.* Agricultural practices are increasingly moving towards environmentally friendly and health-conscious approaches, including organic rice farming. This is also supported by the increasing demand for organic rice among consumers who care about health and the environment. Therefore, this study aimed to evaluate the feasibility of organic rice farming in Yogyakarta and Central Java considering costs, income, profitability, and sustainability among 150 organic rice farmers randomly selected from five districts. The results showed that the average cost incurred for organic rice farming was IDR 11,832,023 per hectare per year, while the income generated reached IDR 14,943,216 per hectare per year. Based on the data, the net income of farmers was IDR 3,111,193 per hectare per year. The feasibility analysis using the R/C ratio showed a value of IDR 1.26, indicating that for every IDR 1.00 spent, farmers earn IDR 1.26, with a profit of IDR 0.26. This implies that organic rice farming is not only environmentally friendly but also financially feasible, with favorable cost-benefit ratios, as it reduces the costs incurred by farmers. In conclusion, the development of organic rice farming has great potential, specifically since consumers, through their demand for organic rice, play a crucial and influential role in its growth.

Keywords: Environmental issue, feasibility, organic farming, sustainability

INTRODUCTION

Organic farming is becoming increasingly popular and widely practiced across various agricultural commodities, including rice. Generally, rice is a staple food for the majority of the Indonesian population, making the presence of organic variety very important (Panjaitan et al. 2020; Bado et al. 2021). Organic rice is crucial in supporting more sustainable and environmentally friendly agricultural practices. It has many advantages compared to conventionally grown rice, including being safer for consumption due to the absence of pesticide residues, having a softer and smoother texture, a longer shelf life, and appearing whiter when cooked (Sujianto et al. 2022). The growing trend of health-conscious consumption has increased the demand for organic rice among the public (Rozaki et al. 2020).

The rising demand for organic rice motivates farmers to transition from conventional to organic farming (Yanakittkul and Aungvaravong 2020). However, this transition is not easy, as the non-usage of synthetic chemicals in organic rice farming requires considerable effort. Many farmers are shifting from conventional to organic farming, particularly in rice production on the island of Java, specifically in Central Java and Yogyakarta (Sujianto et al. 2022). This shift is not only due to the growing organic rice market but also deteriorating soil fertility due to excessive chemical use (Methamontri et al. 2022; Baird 2024). By adopting organic farming practices, farmers are confident to restore soil fertility or at least prevent further degradation (Bado et al. 2021). Organic farming is environmentally friendly and provides economic and social benefits to local communities. From a financial perspective, it provides a more stable income for farmers, helps maintain agricultural sustainability, and reduces community dependence on harmful chemicals.

The sustainability of organic rice farming is often evaluated based on the ability to provide economic, social, and ecological benefits (Bado et al. 2021; Bhatt and John 2023; Bottazzi et al. 2023). From an economic standpoint, the financial feasibility serves as an important indicator of whether this farming practice is self-sustaining in the long term (Nematollahi et al. 2021; Methamontri et al. 2022). Meanwhile, social and ecological benefits may take longer to fully realize, as soil recovery and environmental quality improvement processes require years (Lu and Cheng 2023).

Most farmers who practice organic rice farming rarely keep detailed records of production costs and seldom document the income received from sales (Seufert et al. 2023). Therefore, it is difficult to determine the actual costs and revenues from farming operations (David and Ardiansyah 2017). Many farmers have never calculated farming practices' revenue-cost (R/C) ratio, leading to unawareness of whether organic rice farming is financially viable (Indrasti et al. 2021).

Central Java and Yogyakarta are characterized by significant organic rice production compared to other areas in Indonesia (Rozaki et al. 2020; Apriyani et al. 2021). These two areas play an important role in supplying organic rice to local and national markets. With the support of various active farming groups and government and non-governmental organizations, sustainable farming practices are being developed. Moreover, many farmers in these areas are realizing the importance of restoring soil fertility through organic methods, specifically after the land has suffered degradation due to continuous chemical fertilizer use (Bado et al. 2021). Magelang District in Central Java is the area with the largest organic rice production in Indonesia (Provinsi Jateng 2024), underscoring the significant potential for organic rice development in Central Java and Yogyakarta. The fertile soil characteristics and favorable climate in this area are highly suitable for the growth of organic rice. The ease of access and the availability of data are also practical reasons supporting the selection of this area.

Therefore, this study aimed to address the gap in understanding the feasibility of organic rice farming, which has not been extensively explored. The main contribution is the provision of comprehensive data on the costs, income, and profits of organic rice farming in Central Java and Yogyakarta. The increasing demand for organic rice, supported by the (Wang 2023) study, is a testament to consumers' growing awareness of healthier and environmentally friendly lifestyles and their recognition of the long-term health benefits of organic rice. This study also emphasizes the importance of organic farming as a solution for preserving the environment and improving farmers' welfare. The significance lies in the ability to provide clear information to farmers about the feasibility of farming operations and promote the transition toward more sustainable agricultural practices.

MATERIALS AND METHODS

Study area

This study was conducted using a purposive or deliberate sampling method based on several considerations, such as the number of organic rice farmer groups, the size of organic rice farmland, and the availability of organic certification. The selected locations in Central Java Province, Indonesia, include three districts: Magelang District, Karanganyar District, and Sragen District. Meanwhile, in the Special Region of Yogyakarta Province, Indonesia, two districts were selected: Bantul and Sleman (Figure 1).

Sampling procedure and data collection

This study used a descriptive method, and data were collected through interviews using a questionnaire. The samples were selected using proportional stratified random sampling, where the areas were divided into five districts, and the sample size for each area was determined according to the proportion of households. The number of respondents was proportional to the number of households in each district, with 30 respondents per district, resulting in a total of 150. Data collection was focused on assessing the feasibility of organic rice farming in Central Java and the Special Region of Yogyakarta. Generally, the data collected includes farmer characteristics (age, education level, number of family members, farming experience) and all activities or inputs associated with the farming process, such as labor, fertilizers, seeds, agricultural tools, and other related aspects.

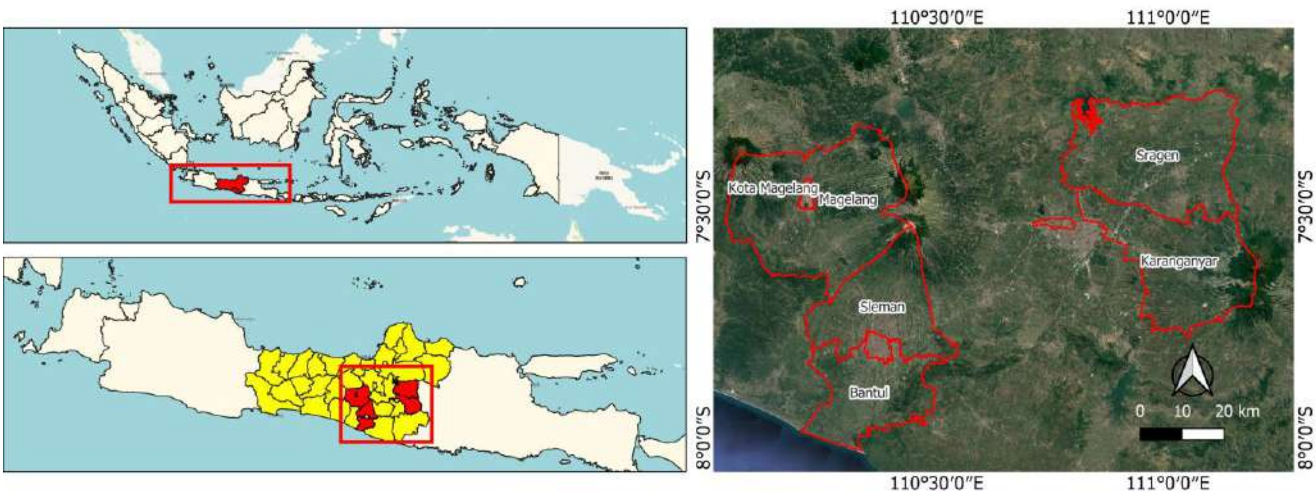

Figure 1. Study sites in Magelang, Karanganyar, and Sragen districts of Central Java Province, Indonesia and Bantul and Sleman districts of Special Region of Yogyakarta Province, Indonesia

Analytical technique

Data were analyzed using descriptive-analytical technique and the characteristics of the respondents were analyzed using percentages. Meanwhile, the feasibility of organic rice farming was assessed using the R/C ratio analysis, described as follows:

Cost analysis

TC= FC + VC

Where:
TC: Total Cost
FC: Fixed Cost
VC: Variable Cost

Revenue

TR= Y × Py

Where:
TR: Total Revenue
Y: Production Obtained
Py: Product Unit Price

Income

R= TR - TC

Where:
R: Revenue
TR: Total Revenue
TC: Total Cost

Revenue cost (R/C) ratio analysis

R/C= Total Revenue/Total Cost

Where:
Revenue: The Amount of Revenue Obtained
Cost: The amount of Costs Incurred

When the R/C ratio has a score greater than one, organic rice farming is considered feasible, but when the score is below one, it is deemed not feasible. The higher the value above one, the greater the feasibility of organic rice farming.

RESULTS AND DISCUSSION

Farmers characteristics

Age

According to Ackerl et al. (2016), age is the span of life or the period since a person is born and is measured in units of time or years. Age plays a crucial role in a person's decision-making ability, which directly contributes to work productivity and impacts the success of farming activities. Farmers within the productive age range tend to perform better compared to those who are no longer in the productive age bracket due to stronger physical abilities and broader perspectives. The productive age range was found to be between 15-64 years old.

Table 1 shows the age distribution of organic rice farmer respondents, with the majority (112 or 74.67%) falling within the productive age range of 15-64 years. Among farmers, there were 76 males and 36 females, showing the majority were still active in working and had the physical strength for farming. Meanwhile, 38 (25.33%) were over the age of 64, consisting of 34 males and four females. At this age, farmers are considered no longer physically productive. From this data, it can be concluded that the average age of organic rice farmers remains within the productive age range, with most respondents being male.

Education level

Education is a process of learning that enables an individual to develop potential and acquire necessary skills. It also plays a role in shaping the mindset, while the level of education refers to the formal stages that must be completed by a person based on the highest obtained diploma. Individuals with higher levels of education tend to have broader perspectives, both in terms of knowledge and insight (Etshekape et al. 2018; Guo et al. 2021; Baga et al. 2023).

Table 2 shows the educational achievements of organic rice farmers. Among the 150 farmers, 60 (40%) only completed 0-6 years of education, failing to continue schooling after elementary school. Additionally, 40 farmers (26.7%) received 7-9 years of education, equivalent to junior high school, and 46 (30.7%) completed 10-12 years of education, graduating from high school or an equivalent institution. Only 4 farmers (2.6%) had education beyond 12 years, reaching a level higher than senior high school. Therefore, a significant number of organic rice farmers have limited educational achievements, with the majority only completing 0-6 years of education. This may impact the method of conducting farming practices, including the understanding of organic farming techniques and the potential to improve agricultural yields. Education is crucial in enhancing farmers' skills and knowledge, which can influence agricultural success.

Table 1. Age

Age (years)	Gender		Total	Percent
	Male	Female		
15-64	76	36	112	74.67
>64	34	4	38	25.33
Total	110	40	150	100.00

Table 2. Education level

Duration of education (year)	Freq.	Percent
0-6	60	40.0
7-9	40	26.7
10-12	46	30.7
>12	4	2.6
Total	150	100.0

Table 3. Family dependents

Number of family dependents	Freq.	Percent
2	39	26.0
3	40	26.7
4	37	24.7
5	20	13.3
>5	14	9.3
Total	150	100.0

Table 4. Farming experience

Farming experience (years)	Freq.	Percent
0-7	15	10.0
8-14	20	13.3
>15	115	76.7
Total	150	100.0

Table 5. Land area of organic rice farmers

Land area (hectare)	Freq.	Percent.
<0.5	139	92.7
0.5-2	11	7.3
>2	0	0
Total	150	100

Table 6. Organic rice farmers land tenure

Land tenure	Total (ha)	Percent.
Own Land	22.7	60.2
Swap Land	13.7	36.3
Rental Land	1.3	3.5
Total	37.7	100

Number of family dependents

The number of dependents in a family consists of all members whose needs are supported by the head of the household (Ruhyana et al. 2020; Wulandari et al. 2021; Sulistyo et al. 2022). Family members may include fathers, mothers, children, siblings, or other individuals who are still dependent on the head of the household. The number of dependents can significantly influence household expenses and overall well-being (Utari et al. 2022P). Dependents represent farmers' responsibility for livelihood and significantly affect family income and expenses. Farmers with a larger number of dependents tend to have a greater availability of family labor.

Table 3 shows the number of family dependents of organic rice farmers. From the data, 39 farmers (26.0%) have two dependents, and 40 farmers (26.7%) have three. Additionally, 37 farmers (24.7%) have four dependents, 20 (13.3%) have five dependents, and 14 (9.3%) have more than five dependents. This shows that the majority of farmers have between 2 to 4 family dependents. Farmers with more dependents face challenges in meeting basic needs, such as education and healthcare, as well as greater risk regarding food security.

Farming experience

Farming experience can be categorized into three levels, namely inexperienced (less than 7 years), moderately experienced (8-14 years), and experienced (more than 15 years) (Triyono et al. 2021; Methamontri et al. 2022). Table 4 shows the distribution of organic rice farmers based on their experience level. Among the respondents, 15 (10%) fall into the category of less experienced (0-7 years), while 20 farmers (13.3%) were classified as moderately experienced (8-14 years). The majority, namely 115 farmers (76.7%), were in the experienced category (more than 15 years). Therefore, most farmers were considered experienced in organic rice farming. Farmers with more than 15 years of experience have typically faced various challenges, such as weather changes, pests, and plant diseases, and have developed strategies to address these issues. The expertise possessed enables a better understanding of agricultural practices and the application of techniques that can enhance productivity as well as the sustainability of farming enterprises.

Land area

According to Nkomoki et al. (2019) and Santoso et al. (2023), the area of farmland owned by farmer groups can be classified into three categories: large-scale (owning more than 2 hectares), medium-scale (owning 0.5 to 2 hectares), and small-scale (owning less than 0.5 hectares).

Table 5 shows that 139 farmers (92.7%) have small landholdings (less than 0.5 hectares), while 11 (7.3%) have moderate-sized landholdings (0.5 to 2 hectares). Specifically, no farmers (0%) were categorized as having large landholdings (more than 2 hectares). This data underscores the prevalence of small landholdings among organic rice farmers, with 139 managing less than 0.5 hectares. The limited land can significantly influence farming practices. However, by implementing good soil management and embracing sustainable farming techniques, small-scale farmers can play a crucial role in producing healthy and high-quality organic food, all while preserving environmental sustainability. This highlights the responsibility and commitment of farmers to maintain good soil health for sustainable farming practices.

Land tenure

Land tenure is classified into two main categories, namely owned and not owned. Ownership refers to the hereditary rights that an individual has over a piece of land. Non-owned land tenure represents a temporary form of possession, entailing the transfer of cultivation rights from the landowner to another party. Tenure arrangements based on profit-sharing have positive effects on production but can also contribute negatively to risks (Rokhani et al. 2020; Achmad et al. 2022; Rao et al. 2022).

As shown in Table 6, organic rice farmers own 22.7 hectares of land, accounting for 60.2% of the total. Possessing individual land implies that there is no need to pay rent, thereby increasing agricultural profits. Farmers cultivate 13.7 hectares, or 36.3%, of land through sharecropping or using land owned by others without rental costs. In sharecropping, farmers can manage land belonging to another individual in exchange for a share of the harvest or profits. This system is beneficial as it facilitates larger areas of cultivation without incurring costs to purchase land. Meanwhile, those who rent land occupy 1.3 hectares, contributing 3.5%. This rental system is considered less favorable because farmers must adhere to certain rules set by the landowners, which can limit farming methods used. Renting land can also be a financial burden, specifically when the rental costs are relatively high. Therefore, on average, organic rice farmers own individual land for farming. With more farmers managing personal land, there is a significant opportunity to increase organic rice production in the area, thereby supporting environmental sustainability.

Cost

Farming input cost

Production costs refer to the expenses incurred during the production process before yielding a product. These costs include production inputs such as fertilizers, pesticides, seeds, labor, and equipment depreciation Hartono et al. (2019). According to Table 7, the production costs for organic rice farming include IDR 3,047,462 for seeds, which is a significant expense as high-quality seeds can improve crop yields and ensure healthy plant growth. There was also an expenditure of IDR 2,270,752 for animal manure, which is a good source of nutrients for plants as it helps enhance soil fertility. Approximately IDR 54,050 was spent on solid organic fertilizer, for improving soil structure and water retention. At the same time, IDR 46,021 was allocated for liquid organic fertilizer to provide direct nutrition for plants and increase the availability of nutrients. Finally, IDR 103,635 was spent on organic pesticides to control pests and diseases without harming the environment or endangering human health. The highest facility cost in organic rice farming was associated with seed purchases. The proper use of inputs and costs is a factor in achieving optimal productivity in organic rice farming.

Labor

Labor costs refer to the specific expenses paid to workers in the production segment (Resdiana 2022). These costs are divided into two categories, namely family labor (using labor from within the family) and external labor (hiring workers from outside). In addition to human labor costs, labor expenses also include machinery costs incurred for each production cycle. Organic rice farmers still heavily rely on labor, specifically from within the family, as it can reduce the costs incurred. As shown in Table 8, the total family labor was 111,040 days. In comparison, 32,111 days of labor were attributed to external sources outside the family, with the highest requirement occurring during the harvest season, amounting to IDR 1,537,218. This shows that although farmers use labor outside the family, there is a significant dependence on family labor to carry out most agricultural activities.

Depreciation cost

Depreciation costs for agricultural equipment refer to the expenses incurred for the tools used, calculated in Indonesian Rupiah (IDR) per year. The amount of depreciation for agricultural equipment was determined using the straight-line method. The salvage value is the worth of the equipment at the point it can no longer be used or considered to be zero (Nurmala et al. 2017). Table 9 shows that the depreciation costs of agricultural tools include hoe IDR 24,052 (11.21%), sickle IDR 12,233 (5.70%), sprayer IDR 7,587 (3.54%), scale IDR 4,158 (1.94%), shovel IDR 4,145 (1.93%), rake IDR 2,130 (0.99%), tractor IDR 158,596 (73.92%), and other harvesting tools IDR 1,661 (0.77%). Therefore, it can be concluded that the tractor, a crucial tool in farming, has the highest depreciation cost compared to other agricultural tools, amounting to IDR 158,596. This reflects the tractor's importance and the high maintenance costs associated with farming activities.

Table 7. Average of input cost per year

Description	Unit (Kg)	Price/Unit (IDR)	Total (IDR)
Seeds	102.36	29,772	3,047,462
Fertilizer			
a. Manure Fertilizer (kg)	2,674.62	849	2,270,752
b. Solid Organic (kg)	360.33	150	54,050
c. Liquid Organic (Liter)	114.48	402	46,021
Pesticide			
a. Organic (kg)	2.55	40,641	103,635
Total			5,521,919

Table 8. Average of labor cost per year

Description	Average Human Labor			Machine (IDR)	Total (IDR)
	Fam. Lab. (WH)	Non-Fam. Lab. (WH)	Total (IDR)		
Land Processing	5,550	274.56	951,000	1,206,936	2,157,936
Planting	1,876.95	2200.77	1,008,951	19,089	1,028,040
Weed Clearing	44,654.34	128.67	96,621	762	97,383
Harvest	15,773.34	28123.86	1,305,756	231,462	1,537,218
Post - Harvest	28,710.45	443.58	61,023	0	61,023
Transport	14,475.39	939.24	79,860	46,032	125,892
Total	111,040	32,111	3,503,211	1,504,281	5,007,492

Note: WH: Working Hour

Table 9. Average of depreciation cost per year

Types of farm tools	Depreciation costs (IDR)	Percentage (%)
Hoes	24,052	11.20
Sickle	12,233	5.70
Sprayer	7,587	3.54
Scales	4,158	1.94
Shovels	4,145	1.93
Rakes	2,130	0.99
Tractor	158,596	73.92
Harvesting equipment	1,661	0.77
Total	214,562	100.00

Table 10. Other cost

Cost types	Total (IDR)	Percent
Land tax	45,061	4.36
Certification fee	550,847	53.36
Farmer group membership fee	32,525	3.15
Land rent	282,231	27.34
Farm equipment repair	121,694	11.79
Total	1,032,358	100.00

Table 11. Average of revenue per year

Revenue	Total
Production (Kg)	1,530.44
Price (IDR)	9,764
Total	14,943,216

Table 12. Average of on-farm income per year

Description	Total (IDR)
Revenue (A)	14,943,216
Costs:	
Farming	5,521,919
Labor	5,007,492
Tool depreciation	214,562
Other costs	1,032,358
Total cost (B)	11,832,023
Income (A-B)	3,111,193

Table 13. Average non-farm income per year

Non-farm income	Total (IDR)	Percent
Breeders	2,538,384	15.20
Trade	3,115,152	18.66
Laborer	6,618,586	39.64
Odd jobs	727,273	4.36
Pensions	484,848	2.90
Employee	1,236,364	7.40
Services	1,975,758	11.83
Total	16,696,364	100.00

Other cost

The last component of expenses is categorized as other costs, which include land tax, certification fees, membership fees for farmer groups, land rental, agricultural equipment repairs, loan interest, and transportation costs incurred by farmers over a year (Susanawati et al. 2021). Table 10 shows that the various costs incurred by farmers include taxes IDR 45,061 (4.36%), certification IDR 550,847 (53.36%), membership fees for farmer groups IDR 282,231 (27.34%), and land rental IDR 121,694 (11.79%). Therefore, the largest cost faced is certification, reflecting the importance of accreditation in maintaining organic farming standards. Certified organic farms can enhance consumer trust, meet quality standards, and improve access to broader markets.

Revenue

Farmers aim to achieve high production yields in farming activities (Rahayu et al. 2019). When rice harvest is abundant, a portion of the yield will be sold, but when the harvest is not substantial, all will be consumed by the household. Table 11 shows the average physical production achieved over one year, totaling 1,530.44 kg, with each kilogram of rice sold at an average price of IDR 9,764. Therefore, the total income generated amounted to IDR 14,943,216. Farmers' income is significantly influenced by the quantity of production and the selling price of rice per kilogram; hence, the higher the production and selling price, the greater the income.

Income

On farm income. Farm income is the difference between revenue and all costs, is a testament to the resilience and dedication of organic rice farmers. The total funding divided by gross income reflects the overall production value before deducting production costs (Sin et al. 2023). Table 12 shows that the production value achieved by organic rice farmers over one year was IDR 14,943,216, with total costs amounting to IDR 11,705,461. This includes agricultural costs IDR 5,521,919, labor IDR 5,007,492, depreciation IDR 214,562 and other expenses IDR 1,032,358. After all costs were deducted from the total revenue, organic rice farmers obtained a net income of IDR 3,293,447. This shows that, despite the various costs incurred, farmers still profit substantially from organic farming efforts.

Non-farm income. Income outside of farming refers to additional earnings obtained from work unrelated to farming activities. When a farmer has supplementary income from sources other than farming, it is referred to as off-farm income. Table 13 shows the average off-farm income earned by farmers over one year including livestock farming IDR 2,538,834 (15.20%), trade IDR 3,115,152 (18.66%), labor IDR 6,618,586 (39.64%), odd jobs IDR 727,273 (4.36%), pensions IDR 484,848 (2.90%), employment IDR 1,236,364 (7.40%), and services IDR 1,975,758 (11.83%), Therefore, the largest off-farm income was from labor, which totaled IDR6,618,586.

Table 14. Average total income of organic rice farmers per year

Revenue	Value (IDR)	Percentage (%)
Farming (Organic Rice)	3,111,193	15.71
Non-farm	16,696,364	84.29
Total	19,807,557	100.00

Total income. Total income is the sum of yield from agricultural and non-agricultural activities (Ruhyana et al. 2020; Santoso et al. 2023). According to a previous study, the total income of a farmer is the sum of all agricultural and non-agricultural ventures undertaken (Abadega 2021; Sulistyo et al. 2022). Table 14 shows that the total income earned by organic rice farmers in one year was IDR 19,807,557, coming from agricultural and non-agricultural activities, IDR 3,111,193 and IDR 16,696,364, respectively. This shows that farmers earn significantly more from non-agricultural sources than agricultural activities, underscoring the importance of income diversification, as it can help improve economic well-being. Although agriculture is the primary source of income, additional income from other activities is crucial in supporting daily living expenses.

R/C analysis

The analysis of agricultural feasibility aims to determine whether a farming venture is worth pursuing. The feasibility of organic rice farming was calculated using the R/C ratio, which shows the relationship between the income generated and the total costs incurred. Based on the results, the obtained R/C ratio was 1.26, showing that for every IDR 1.00 spent on production costs, farmers will receive a profit of IDR 1.26, resulting in a profit of IDR 0.26. A higher R/C value signifies greater profitability. This high R/C ratio signifies the profitability of organic rice farming, providing reassurance and confidence to farmers and investors (Indrasti et al. 2021).

Discussion

Organic rice farming is economically promising. Farmers can earn a net income of IDR3,111.193 per hectare per year, with an average cost of IDR 11,832,023 per hectare per year and a gross income of IDR 14,943,216 per hectare per year, indicating an income of IDR 1.26 for every IDR 1.00 spent. This R/C ratio not only shows the feasibility of organic rice farming but also instills confidence in its potential for further development. The growing public awareness of the importance of consuming organic food further supports this potential.

The demographic profile of the respondents supports the sustainability of organic farming. Strong physical ability is important for farming activities; hence, most farmers (74.67%) were within the productive age range of 15-64 years. Although only 40% completed six years of schooling, this does not necessarily prevent farmers from adopting new farming techniques. The condition underscores the importance of educational programs that improve farmers' skills and knowledge about organic farming practices. Sustainable farming practices can enhance environmental quality and maintain land conditions. Furthermore, organic farming improves soil and the overall ecosystem by reducing harmful chemicals. Government support in education and input assistance, such as seeds and fertilizers, is crucial for empowering farmers to continue practicing organic farming.

Among the respondents, there were different levels of family dependents: 26% had two, 27% had three, 25% had four, and 22% had five or more. Many families rely heavily on farm income to meet daily needs. Hence, farmers with many dependents often prioritize sustainable farming practices to ensure a steady income. Additionally, this study shows that small-scale farming dominates, with nine out of ten farmers (92.7%) owning less than half a hectare of land. Despite the limited land, small-scale farmers can produce high-quality organic crops while contributing to environmental conservation by applying sustainable techniques; through their high-quality organic crops and sustainable techniques, they make a significant contribution to environmental conservation. Land ownership also plays an important role, as 60.2% of the surveyed land is privately owned, providing stability and control over resource use. This arrangement promotes responsible land management and facilitates continued investment in sustainable farming practices.

The results are in line with previous studies underscoring the economic feasibility of organic rice farming. For example (Panjaitan et al. 2020; Bado et al. 2021) showed similar profit margins. Similarly, Rozaki et al. (2020) found increased consumer demand for organic products, emphasizing the importance of sustainable farming practices. Studies focusing on the socio-economic implications of transitioning to organic farming also support the results. Bado et al. (2021) and Panjaitan et al. (2020) discussed soil fertility restoration through organic methods, underscoring environmental benefits. Lu and Cheng (2023) also emphasized the long-term ecological benefits of sustainable farming practices in Ecological Applications, reinforcing the observations about the sustainability of organic rice farming.

In this study, the production costs of organic rice farming were analyzed to identify the potential cost saving for farmers. A study by Dat et al. (2023) found that the average production cost for non-organic farming is IDR 19,994,600 per hectare. In contrast, our findings show that the average production cost for organic rice farming is IDR 10,646,915 per hectare. This indicates that farmers using organic methods can save around IDR 9,347,655 per hectare compared to non-organic methods. Although the initial costs of organic farming may be higher due to the use of organic inputs, in the long term, farmers can achieve significant cost savings by reducing the use of chemical fertilizers and pesticides. Therefore, this comparison confirms that organic rice farming offers greater economic benefits for farmers in the long run.

This study shows that organic rice farming in Central Java and Yogyakarta has positive economic feasibility. However, it is important to delve deeper into the implications and limitations associated with the feasibility. One significant implication of the results is the potential for increased income in transitioning to organic farming. Although the results show promising outcomes, challenges such as market price fluctuations and weather uncertainties must be considered. Limitations in farmers' knowledge and skills regarding organic farming practices could hinder the effective implementation of this method. Further studies are needed to understand how social and economic factors may influence the success of organic rice farming, as well as how support from the government and related institutions can help overcome existing challenges.

The connection between the results on the feasibility of organic rice farming and the broader literature on sustainability policies needs to be strengthened. This study shows that organic rice farming in Central Java and Yogyakarta is not only economically viable but also contributes to environmental sustainability. In this context, the results are in line with previous studies emphasizing the importance of sustainable agricultural practices in supporting food security and preserving ecosystems. By integrating these results into broader agricultural policies, governments, and stakeholders can formulate more effective strategies to promote the adoption of organic farming practices. This would help raise public awareness about the benefits of organic agriculture while promoting investment and support for farmers transitioning to more environmentally friendly farming systems.

This study shows the benefits of transitioning to organic rice farming in Central Java and Yogyakarta. The results show economic feasibility and sustainable farming practices that support environmental preservation as well as improve farmer welfare. It is important to engage all stakeholders in developing these practices. Further studies are needed to create more comprehensive strategies for supporting farmers. With collaboration between farmers, the government, and academics, organic rice farming can continue to grow and positively contribute to healthy food production as well as environmentally friendly agriculture. Therefore, to ensure successful implementation, concrete recommendations are needed from policymakers. First, the government should provide incentives for farmers transitioning to organic farming practices, such as subsidies for purchasing organic inputs and access to training on sustainable farming techniques. Second, a better marketing network for organic rice products should be established to help farmers reach consumers more effectively. Another recommendation is to increase public awareness of the benefits of organic rice through educational campaigns, which can help drive market demand. Finally, there is a need to acquaint farmers with modern technologies, such as digital platforms, to help record and analyze financial data, as well as innovation capabilities to increase agricultural yields with reduced costs.

ACKNOWLEDGEMENTS

This study was funded by the Directorate of Research, Technology, and Community Service - Directorate General of Higher Education, Research and Technology - Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia (Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat- Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi - Kementerian Pendidikan, Kebudayaan, Riset, Dan Teknologi Republik Indonesia), Contract Number 107/E5/PG.02.00.PL/2024 (0609.7/LL5-INT/AL.04/2024, 63/KP-LRI/VI/2O24) under the program of DRTPM 2024. The opinions expressed in this study are those of the authors and do not necessarily reflect the views of the funding agency.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 131-139 DOI: 10.13057/asianjagric/g090114

Exploring indigenous *Bacillus* spp. as a biostimulant to enhance the growth and yield of rice under glasshouse conditions

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Manuscript received: 10 October 2024. Revision accepted: 16 January 2025.

Abstract. *Shultana R, Zuan ATK, Rana MM, Naher UA, Paul PLC, Akter M, Shupta SA, Roy TK. 2025. Exploring indigenous* Bacillus *spp. as a biostimulant to enhance the growth and yield of rice under glasshouse conditions. Asian J Agric 9: 131-139.* Plant growth-promoting rhizobacteria (PGPR) appeared as an emerging approach to sustainable agriculture because of their ability to reduce the need for synthetic fertilizers. This study evaluated five potential *Bacillus* strains isolated from rice-growing areas in northern Malaysia for their ability to enhance rice growth parameters under laboratory and glasshouse conditions. Among the five strains, two UPMRB9 (*Bacillus tequilensis* 10b) and UPMRE6 (*Bacillus aryabhattai* B8W22) exhibited all the desired traits. These strains were subsequently inoculated into three potential rice plants, resulting in improved morphological (plant height, leaf area), physiological (photosynthesis, transpiration, stomatal conductance, dry biomass, and grain yield), and biochemical (total chlorophyll, total proline content, total soluble sugar, and uptake of N, P, K, Ca, and Mg) attributes. Inoculation of Putra-1 plants with UPMRB9 showed a 10.76% increase in plant height, a 50.82% increase in grain yield, a 12.32% increase in colonization, and a 56% increase in stomatal conductance. Additionally, inoculation of BRRI dhan67 with UPMRB9 reduced the transpiration rate by 100.89%. MR297 plants treated with UPMRB9 demonstrated an 86.58% increase in leaf area, a 125.81% increase in photosynthesis, a 222.19% increase in dry biomass, a 56.03% increase in total chlorophyll, a 62.93% increase in proline content, and a 62.93% increase in total soluble sugar (TSS). Therefore, UPMRB9, as a multiple plant growth promoter, could be an alternative to synthetic fertilizers for rice cultivation.

Keywords: *Bacillus*, biofertilizer, nutrient uptake, PGPR, proline

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial dietary staple worldwide, providing sustenance and nutrition to billions. Estimates indicate that global rice production reached 517 million tons in 2023 (FAO 2023). Rice feeds more than half of the worldwide population, making a significant energy contribution to low-income families as well. In tropical areas, rice is commonly grown as a staple crop (Abbas et al. 2019). The utilization of cultivars and management techniques like fertilization have a major impact on rice yield. Many nations' contemporary agricultural production systems mainly depend on synthetic fertilizers and pesticides. However, worries over the harmful effects on human health and the environment have been raised by the extensive usage of these synthetic agrochemicals. Furthermore, over-reliance on synthetic fertilizers may increase the demand for inputs to sustain current yield levels. Natural ecosystems, on the other hand, provide nutritional advantages, insect pest resistance (Roy et al. 2024a), and more sustained yields. Overuse of synthetic fertilizers may change the physical, chemical, and biological properties of the soil. Using beneficial microbes that enhance crop growth is a sustainable substitute (Ríos-Ruiz et al. 2020). Furthermore, rice is widely distributed across different soils and climates globally in the face of changing climate scenarios, so it has significant strategic value (Fahad et al. 2019; Ríos-Ruiz et al. 2020). But, over the past few decades, the use of beneficial soil microorganisms, such as Plant Growth-Promoting Rhizobacteria (PGPR), for sustainable agriculture has expanded significantly due to the negative environmental impact and rising prices of synthetic fertilizers (Ikhajiagbe and Osazee 2019). *Bacillus* spp., commonly found in soil, serves as PGPR by forming robust endospores that resist high temperatures and chemical concentrations (Kashyap et al. 2019). *Bacillus* spp. promote plant growth by improving seed emergence, increasing biomass, and enhancing grain yield (Egamberdieva et al. 2017; Weemstra et al. 2022). PGPR inoculants offer a natural remedy by facilitating advantageous biological processes in agricultural ecosystems with little adverse effects. As a result, there is increasing interest in creating and utilization of biofertilizers for different crops based on PGPR.

The production of Auxin (especially indole-3-acetic acid, IAA) for root development, nutrient uptake facilitation, atmospheric nitrogen fixation (Mashabela et al. 2022), siderophore production for iron uptake (Singh et al. 2022), phosphorus solubilization (Dhole et al. 2023), ACC deaminase synthesis (which cleaves ethylene, essential in response to stress), and biofilm production (which improves bacterial adhesion to root tissue and nutrient uptake) are some of the mechanisms by which PGPR promotes plant growth. The PGPR consortia are typically used as biofertilizers since individual strains frequently lack the necessary mechanisms to support host growth (Redondo-Gómez et al. 2023). Still, there is limited research for analyzing how PGPR affects rice growth (Shultana et al. 2020).

Several previous studies have demonstrated that mung bean inoculation with the rhizobacterial strain *Bacillus drentensis* significantly increased stomatal conductance, transpiration rate, and total chlorophyll content in comparison to the uninoculated control (Mahmood et al. 2016). According to a previous study, the inoculation of holy basil plants with *Pseudomonas* sp. and *Bacillus lentus* effectively reduced the effects of salinity by increasing the levels of photosynthesis, minerals, and the antioxidant system (Golpayegani 2011). Proline and total soluble sugar concentration are sensitive physiological indexes that act as osmolytes to shield plant growth from oxidative stress. Plants may resist osmotic and salt strains by controlling proline, which stabilizes proteins and membranes, scavenges free radicals, and buffers cellular redox potential in salt stress (Hayat et al. 2012). Higher total soluble sugar (TSS) content in plants also serves as a defense mechanism against salinity. An increase in proline and TSS in wheat plants is a significant PGPR contribution to osmotolerance (Upadhyay et al. 2012). Therefore, it was hypothesized that the inoculation of the indigenous PGPR strain, *Bacillus* spp., could increase the growth and yield of rice. In this study, efforts were made to explore the ability of potential bacterial isolates to promote rice growth through enhancing physiological characteristics and the regulation of biochemical metabolites and their influence on rice yield under glasshouse conditions.

MATERIALS AND METHODS

Plant growth-promoting traits of the selected strains (in vitro screening)

The five bacterial strains were previously isolated and identified as salt-tolerant strains (Shultana et al. 2020). Among them, two potential strains, UPMRB9 (*Bacillus tequilensis* 10b) (accession number: NR 104919.1) and UPMRE6 (*Bacillus aryabhattai* B8W22) (accession number: NR 115953.1), were further inoculated into three rice varieties to observe their effects on growth and yield. The Indole-3-acetic acid (IAA) production was quantified using the colorimetric method of Gordon and Weber (1951) with some amendments of salinity concentrations. The selected isolate was cultured in tryptic soy broth (TSB) modified at different concentrations of NaCl (0, 0.5, 1, 1.5, and 2 M) and supplemented with 5 mL of L-tryptophan which acts as the precursor of IAA, and allowed shaking for 24 h. After incubation, approximately 1.5 mL of the culture was transferred into sterile Eppendorf tubes and centrifuged at 7000 rpm for 7 min. The IAA production was then measured by adding 2 mL of Salkowski reagent to the bacterial culture supernatant, and the absorbance was recorded at 535 nm using a spectrophotometer. The ability of the bacteria to fix atmospheric nitrogen, phosphate and potassium solubilization ability and siderophore production was estimated following the method of (Tan et al. 2014).

The plant inoculation study under glasshouse conditions

The soil samples were collected from Tanjong Karang, Selangor, Malaysia, allowed to dry, and mixed well. The soil series is bernam; pH 4.23. The available nutrients of the initial soil were 0.62% of N, 0.08 mg/kg of P, 0.29 mg/kg of K, 0.23 mg/kg of Ca and 0.33 mg/kg of Mg. Each plastic pail, with dimensions of 30 cm in diameter and 46 cm in height, was filled with 20 kg of soil. Following the fertilizer recommendations of the Department of Agriculture, Kuala Selangor, Malaysia, urea, triple super phosphate (TSP), and muriate of potash (MOP) were applied @170, 80, and 150 kg ha^{-1}, respectively. Urea fertilizer was used in three equal splits at 15, 45, and 60 days after transplanting (DAT), while TSP and MOP fertilizers were applied as basal doses. The treatments were: A. Rice variety: i. BRRI dhan67; ii. Putra-1; and iii. MR297; B. PGPR strains: i. No inoculum; ii. UPMRB9; and iii. UPMRE6. The seeds of three rice varieties, namely BRRI dhan67, Putra-1, and MR297, were initially submerged in ethanol (95%) for 10 s. After removing the ethanol, seeds were submerged again in 3% NaOCl for 1 min (ChloroxTM) followed six times by rinse with sterilized dH_2O water. The glass Petri dishes covered with moistened filter papers (3 layers) were used to germinate 20 surface-sterilized seeds. To keep the filter paper moist (Whatman No 1), sterile dH_2O was added regularly and the seed germination percentage was recorded daily for up to 4 days. After 5 days, the seedlings were transplanted. Twenty surface-sterilized seeds were germinated on three layers of moistened filter papers (Whatman No. 1) with distilled water added regularly to keep the papers moist. After five days, the germinated seeds were transplanted. The fully grown selected bacterial strains were cultured in Tryptic Soy Broth (TSB) medium and shaken for 24 hours before their cells were collected through centrifugation. Bacterial cells (approximately 10^8-10^9 CFU mL^{-1}) and sterile distilled water (as a control) were inoculated onto five-day-old rice seedlings and kept to settle for one hour. A single seedling of each rice cultivar was transplanted into the soil of each plastic pail. The roots of each rice plant were re-inoculated at 14 days after transplanting (DAT), with sterile distilled water added as an uninoculated control. The leaf area was measured at the maximum tillering stage, calculated following the formula stated by Shultana et al. (2013). The photosynthesis, transpiration, and stomatal conductance rates data were estimated at the flowering stage for each rice variety using the LI-COR (LI-6400XT) portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA). Mature leaves were randomly selected, plucked, sealed in plastic bags, and transported to the laboratory. For additional biochemical analysis, the leaves were crushed with liquid nitrogen, wrapped in plastic bags, and kept in a freezer at -80°C.

The content of proline in leaves was measured by considering the protocol as stated by Bates et al. (1973) and Jin et al. (2019) with a slight adjustment of chemical concentrations. An amount of 0.1 g of fresh leaves was homogenized with 2 mL of 5% (w/v) sulfosalicylic acid and was allowed for centrifugation (Sigma 3K30) at 10,000 rpm for 10 minutes. One milliliter of supernatant was taken in a test tube holding 1 mL of glacial acetic acid and 1 mL acid ninhydrin (including the standard). The test tubes were kept in a water bath (95°C) for 1 hour following cool in an ice bath. Toluene (2 mL) was mixed on each tube including the standard. The proline concentration was determined by taking the absorbance reading at 520 nm using the microplate reader (BioTek 800 TS).

The total soluble sugar was determined by adopting the method of Yemm and Willis (1954) and Johnson et al. (2023). Leaf samples (100 mg) were homogenized in 10 mL of 80% ethanol following centrifugation (Sigma 3K30) at 4,000 rpm for 20 min. After the collection of supernatants, the residue was re-submerged with 10 mL of 80% ethanol following centrifugation at 4,000 rpm for 20 min. Then the supernatant was collected again. The two times collected supernatant was mixed with a known amount of ethanol extract (0.1 to 0.2 mL). Further, the test tubes were placed in a water bath at 100°C to make it dry through evaporation. Subsequently, the test tubes were allowed to cool in room temperature (27°C). Afterward, in each test tube, 1mL of distilled water was added and thoroughly mixed. Anthrone reagent (4 mL) was added to each test tube along the wall of the test tube and mixed gently. The test tube was heated again in a water bath at 100°C for 10 min and rapidly cooled on an ice bath. The absorbance of the prepared sample was measured by spectrophotometer (UV-3101PC, Shimadzu) at 620 nm against a reagent blank.

The plant tissue samples were oven-dried for a full 72 hours at 70°C. Using a plant grinder, the samples were grounded, and standard nutrient analysis procedures were used to measure the amounts of the nutrients (Awang et al. 2009). N, P, K, Ca, and Mg concentrations were measured using an Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer 400). By multiplying the concentration values by the plant dry weight, the uptake of N, P, K, Ca, and Mg was calculated (Goteti et al. 2013).

Yield data components, including filled grains, 1000-grain weight, and grain yield, were recorded after the terminal harvest. After the grains acquired a moisture content of 14% through air drying, they were weighed and counted (Tan et al. 2014).

Statistical analysis

Using SAS 9.4 software, the recorded data were subjected to Analysis of Variance (ANOVA). Tukey's Studentized Range (HSD) test compared means at a 0.05 probability level. Correlation coefficient analysis was performed using R Studio (version 2023.12.1) and the "metan" package.

RESULTS AND DISCUSSION

Screening of selected bacterial isolates for plant growth-promoting traits

The plant growth-promoting (PGP) traits of the five selected bacterial isolates varied widely. UPMRB9 exhibited significantly higher IAA production (16.33 µg mL^{-1}), followed by UPMRE6 and UPMRG1. UPMRE6 was recorded to solubilize the highest amount of phosphorous resulting in the highest concentrations of available phosphorus (41.50 µg mL^{-1}) followed by UPMRB9 and UPMRE3 while the highest available potassium concentration (15.79 mg L^{-1}) was measured by UPMRE6. High atmospheric nitrogen fixation and siderophore production were observed in strains, UPMRB9 and UPMRE6 (Table 1).

Effect of bacterial inoculation on the morphological characters of three rice cultivars

Bacterial inoculations significantly increased the plant height of the tested rice varieties. The rice variety, Putra-1 and MR297 responded positively with UPMRB9. However, the inoculation of Putra-1 showed the greatest increase in plant height (117.55 cm), followed by UPMRE6 (111.50 cm) which is a 10.76% and 5.06% increase compared to the uninoculated plant. BRRI dhan67 equally responded to UPMRB9 and UPMRE6 inoculations. However, regardless of rice variety, the lowest plant height was measured without bacterial inoculation (Figure 1.A). The UPMRB9 inoculations enlarged the leaf areas of the Putra-1 and MR297 rice varieties (482.23 cm). Further, the leaf areas of BRRI dhan67 were increased by UPMRE6 inoculation. The highest significant value was recorded by the inoculations of UPMRB9 to MR297 rice plant (482.23 cm) which is an 86.58% increase over the uninoculated plant (Figure 1.B).

Table 1. Plant growth-promoting traits of different bacterial strains

Bacteria strains	IAA (µg mL^{-1})	Available P (µg mL^{-1})	Available K (mg L^{-1})	N fixation	Siderophore production
UPMRA4	7.00b	22.11c	5.72de	-	-
UPMRB9	16.33a	40.49a	13.82b	++	++
UPMRE3	7.13b	40.14a	6.90cd	+	+
UPMRE6	13.06a	41.50a	15.79a	++	++
UPMRG1	12.49a	35.53b	7.76c	+	+

Note: Means having the same letter (capital) in a column does not differ significantly at alpha 0.05 level by Tukey (HSD)

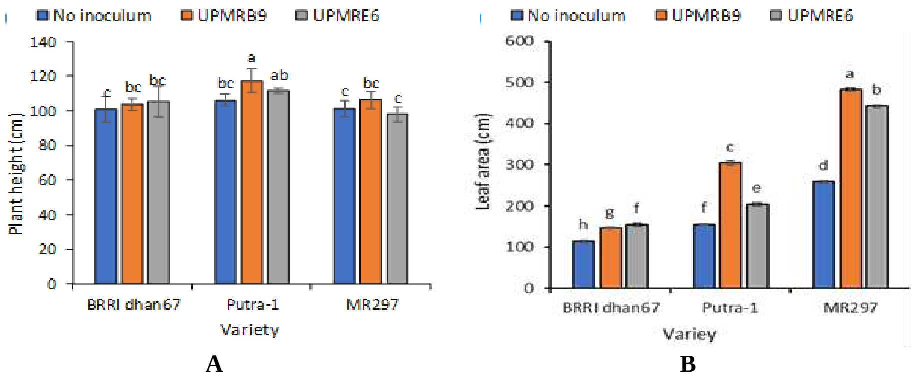


Figure 1. Effect of UPMRB9 and UPMRE6 inoculation on: A. plant height and B. leaf area of tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

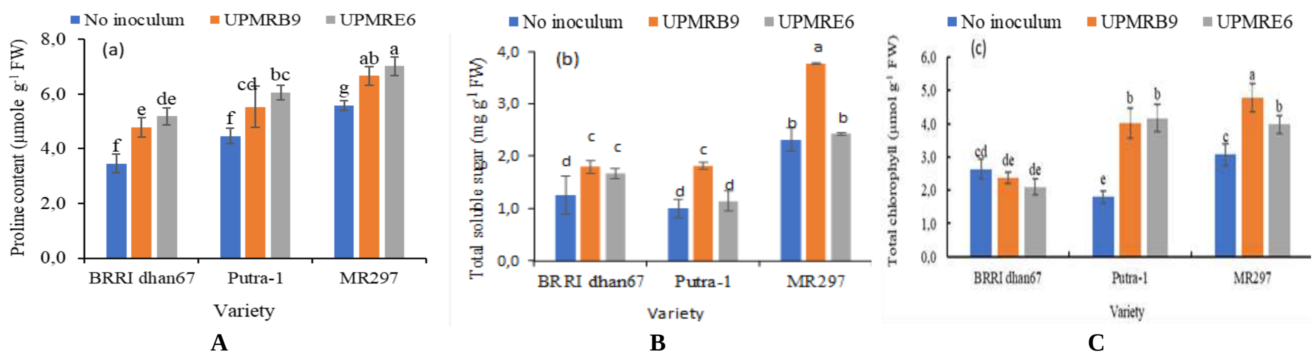


Figure 2. Effect of UPMRB9 and UPMRE6 inoculation on: A. The tested rice plants' proline; B. Total soluble sugar; and C. Total chlorophyll content. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

Effect of bacterial inoculations on biochemical properties of three rice plant

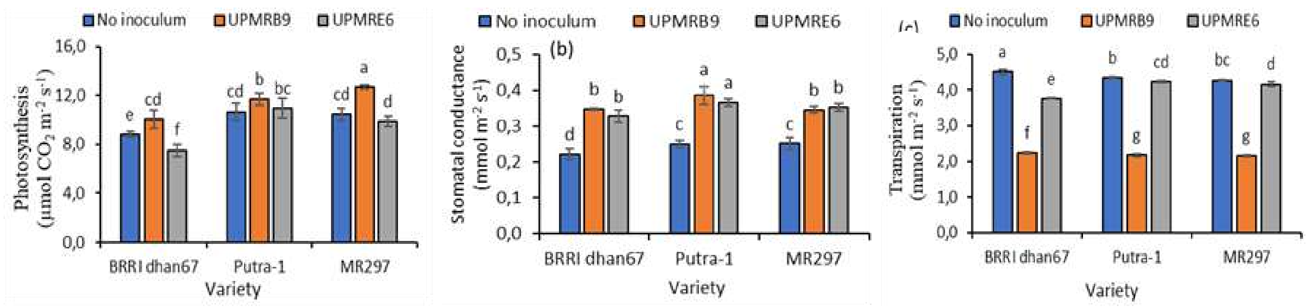
The biochemical properties of the three rice varieties followed similar trends with bacterial inoculations. The highest proline regulation was observed in the MR297 variety inoculated with UPMRE6 (7.02 µmole g^{-1} FW), followed by UPMRB9 (6.66 µmole g^{-1} FW) which is 25.81 and 19.35% increase over uninoculated plant. Besides, the lowest proline regulation was observed in BRRI dhan67 regardless of bacterial strains (Figure 2.A). Similarly, the highest total soluble sugar content was found in the MR297 variety inoculated with UPMRB9 (3.78 mg g^{-1} FW) which is 62.93% higher than the uninoculated plant (Figure 2.B). The total soluble sugar was found highest treated with UPMB9 in all three varieties. A similar trend was observed for total chlorophyll content, with MR297 rice variety inoculated with UPMRB9 (4.79 µmole g^{-1} FW), a 56.03% increase compared to the uninoculated plant. Besides, the lowest total chlorophyll content was measured in BRRI dhan67 regardless of bacterial strains (Figure 2.C).

Effect of bacterial inoculations on physiological traits of the tested rice plants

The physiological properties of the tested rice cultivars significantly varied with bacterial inoculations. Regardless of the type of rice, the photosynthesis rate increased dramatically with the inoculation of UPMRB9. However, the highest rate of photosynthesis was measured in the MR297 variety inoculated with UPMRB9 (12.70 µmol CO_2 m^{-2} s^{-1}) which is a 28.41% increase over the UPMRE6 inoculated MR297 rice variety (Figure 3.A). Additionally, enhanced stomatal conductance was recorded in the Putra-1 variety inoculated with UPMRB9 (0.39 mmole m^{-2} s^{-1}) which is 56% of the increase over the uninoculated plant (Figure 3.B). An accelerated rate of transpiration was estimated in BRRI dhan67 without bacterial amendment (4.50 mmole m^{-2} s^{-1}) (Figure 3.C).

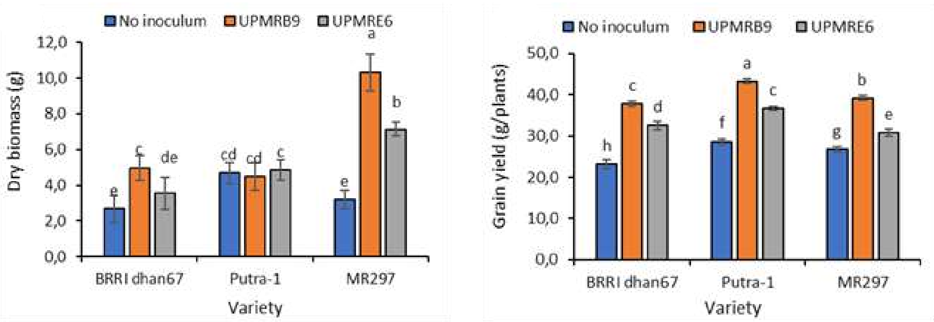
Effect of bacterial inoculations on dry biomass and grain yield of the tested rice plants

The dry biomass and grain yield of the tested rice cultivars were significantly enhanced with the assimilation of the tested bacterial strains. The MR297 rice variety accumulated the highest dry biomass with UPMRB9 inoculation (10.31 g) (Figure 4.A). The strain, UPMRB9 inoculation significantly improved the grain yield of three rice varieties**.** However, the highest grain yield was measured in Putra-1 rice inoculated with UPMRB9 (43.21 g/plants) which is a 50.82% increase over the uninoculated plant. Besides, the lowest grain yield was recorded in BRRI dhan67 without bacterial inoculation (23.15 g/plant) (Figure 4.B).



A B C

Figure 3. Effect of UPMRB9 and UPMRE6 inoculation on: A. Photosynthesis; B. Stomatal conductance; and C. Transpiration of the tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05



A B

Figure 4. Effect of UPMRB9 and UPMRE6 inoculation on: A. The tested rice plants' dry biomass and B. grain yield. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

The total bacterial population in the rhizosphere of tested rice plants

Bacterial colonization differed among the rice varieties. UPMRB9 showed higher abundance regardless of the rice variety. However, the highest abundance of UPMRB9 was counted in Putra-1 rice (7.20). Besides, the lowest abundance of UPMRB9 was counted in the MR297 rice variety (6.59). The colonization pattern of UPMRE6 was slightly higher with BRRI dhan67 and was not markedly varied between Putra-1 and MR297 (Figure 5).

The uptake of nutrients in PGPR-inoculated rice plants

Bacterial inoculation significantly upgraded nutrient uptake in the rice plants. UPMRE6 inoculation in the Putra-1 variety triggered the uptake of N (3.93 g plant^{-1}), P (7.18 g plant^{-1}), Ca (5.62 g plant^{-1}), and Mg (3.73 g plant^{-1}), while UPMRB9 stimulated higher uptake of K uptake in BRRI dhan67 (32.84 g plant^{-1}). The increments in N, P, Ca, and Mg content in the Putra-1 variety were 112.43, 152.82, 195.79, and 74.30%, respectively, while K content in BRRI dhan67 increased by 135.08% (Figures 6.A-E).

Correlation between yield and other traits among the three rice varieties

There were various associations observed between PGPR inoculation and rice varieties (Figure 7). Positive and significant correlations were found between grain yield and CFU g^{-1} dry soil, plant height, photosynthesis, stomatal conductance, transpiration, proline content, dry weight and leaf area. However, negative and non-significant correlations were observed with tiller number and total soluble sugar content by UPMRB9 inoculation (Figure 7.A). In UPMRB9 inoculation, significant positive correlations were found between grain yield and plant height, photosynthesis, stomatal conductance, and transpiration, but negative and non-significant correlations with tiller number (Figure 7.B). Without inoculation, positive and significant associations were found between grain yield, plant height and photosynthesis, while negative and significant associations were observed with CFU g^{-1} dry soil, tiller number, and soluble sugar content. Negative and non-significant associations were found with proline content, dry weight, and leaf area (Figure 7.C).

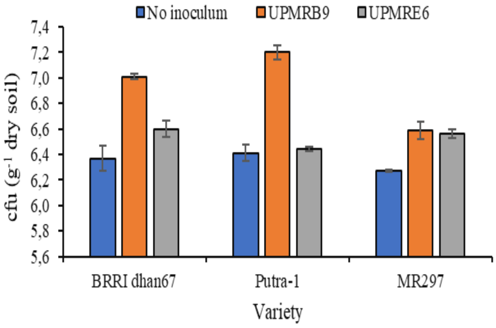


Figure 5. The total population of UPMRB9 and UPMRE6 in the rhizosphere of the tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

Discussion

It has been widely accepted that *Bacillus* spp. could promote plant growth through biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and the synthesis of siderophores and phytohormones (de Souza et al. 2015). In our study, two bacterial strains, *Bacillus tequilensis* (UPMRB9) and *Bacillus aryabhattai* (UPMRE6) confirmed higher IAA production, increased phosphate and potassium availability, nitrogen fixation, and siderophore production. Earlier, Bahadir et al. (2018) identified four *Bacillus* strains that exhibited multiple plant growth-promoting characteristics. Further, Abdullahi et al. (2022) isolated and identified three *Bacillus* strains (*Bacillus subtilis*, *B. niacini*, and *B. cereus*) from the rice rhizosphere, which exhibited various plant growth-promoting properties. These potential strains are reported to produce ammonia which acts as a source of nitrogen to the plant. Besides, the phosphate solubilizing ability of the strains solubilizes the insoluble form of phosphate into soluble form and makes it available to the plants. Moreover, the indole 3 acetic acid (IAA) is one of the prominent features of plant growth-promoting rhizobacteria which manipulates the growth of their host through cell elongation and root-shoot development.

The vegetative growth phase is critical for any crop, characterized by increased plant height, a greater number of tillers and leaves, and higher root and shoot dry biomass. In this study, *Bacillus tequilensis* inoculation in Putra-1 rice significantly accelerated root growth and stem elongation. Additionally, this inoculation greatly enhanced the leaf area in the MR297 variety. Putra-1 rice, a tall-statured plant, benefited from the high IAA production of the bacterial strain and promotes robust growth of roots. Furthermore, the strain's ability to fix atmospheric nitrogen and solubilize phosphorus and potassium contributed to the extensive leaf area in MR297. Earlier reports have also indicated that bacterial inoculation increased plant height, leaf number, tiller number, root dry mass and shoot dry mass (Sharma et al. 2014). The two *Bacillus* strains significantly stimulated total soluble sugar and proline content in the tested rice cultivars, indicating their capability to induce plant resistance to various stresses. Previous reports suggest that *Bacillus* species, major inhabitants of the rhizosphere and epiphytic microorganisms, exhibit plant growth-promoting activities and stress tolerance (Aswathy et al. 2017). Similarly, under nutrient-scarce conditions, *Bacillus amyloliquefaciens* SN13 and its consortium enhanced proline and total soluble sugar content in rice (*Oryza sativa* L. var. IR-36) (Bisht et al. 2020).

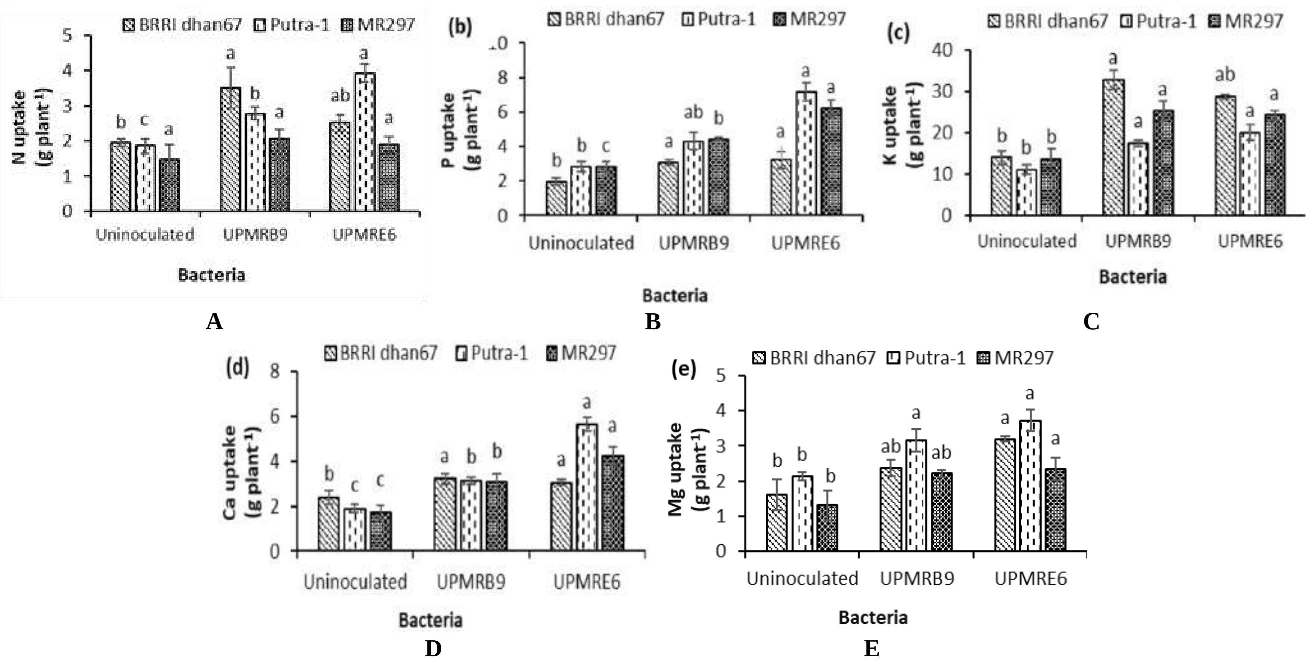


Figure 6. Effect of UPMRB9 and UPMRE6 inoculation on the uptake of: A. Nitrogen; B. Phosphorous; C. Potassium; D. Calcium and E. Magnesium in tested rice plants. Means having the same letter in each variety do not differ significantly using Tukey test at p>0.05

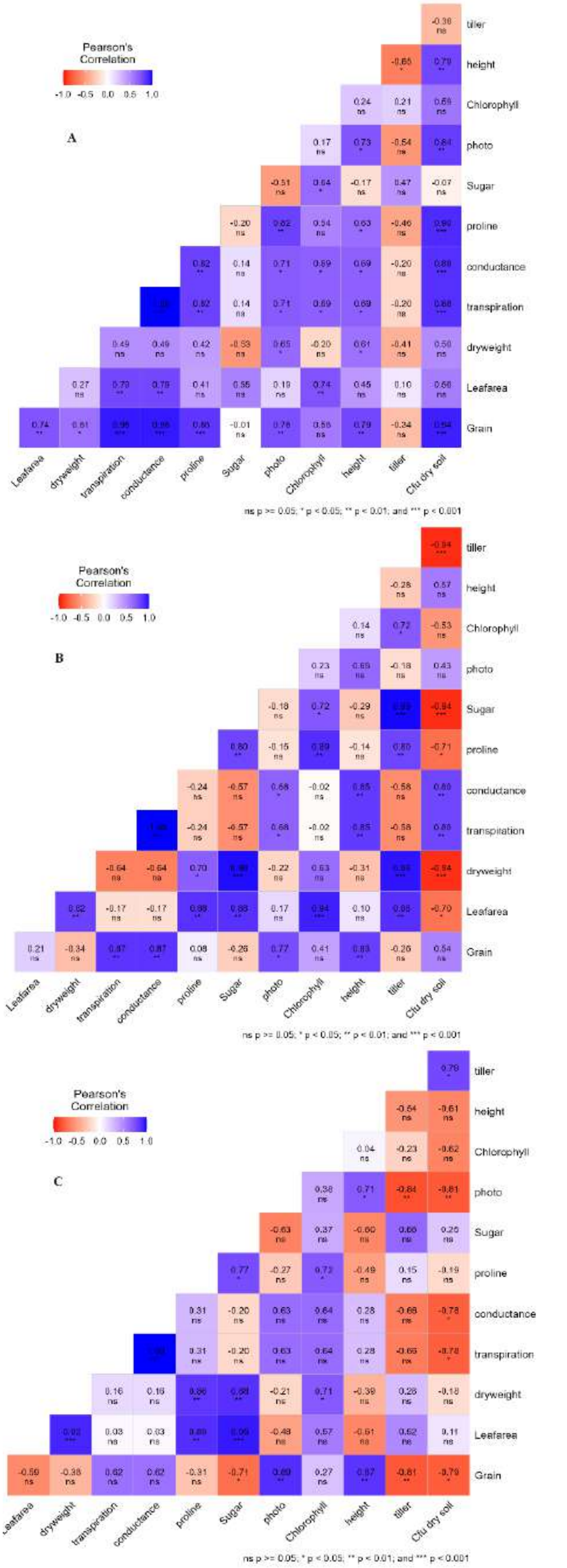


Figure 7. Correlation among yield and other traits. A. Association of UPMRE6 inoculation with three rice varieties; B. Association of UPMRB9 inoculation with three rice varieties; and C. Association of no inoculation with three rice varieties

The physiological properties of the three rice varieties varied significantly with bacterial inoculations. The MR297 variety inoculated with UPMRB9 exhibited the highest rate of photosynthesis. Putra-1 variety inoculated with UPMRB9 was recorded to enhance stomatal conductance. However, an accelerated rate of transpiration was observed in BRRI dhan67 without bacterial amendment. Previous investigations reported that by raising gas exchange parameters and chlorophyll fluorescence, inoculating plants with *B. subtilis* and *B. amyloliquefaciens* improved the photosynthetic performance of pepper plants. The bacteria were shown to facilitate an increased electron transfer rate in the thylakoid membranes, which in turn promoted photosynthetic capability (Samaniego-Gámez et al. 2016). The UPMRB9 strain could be beneficial for utilization in sustainable agricultural cultivation. A substantial amount of exopolysaccharide production by UPMRB9 aids in better colonization with Putra-1 roots.

Additionally, the higher photosynthetic ability in putra-1 results in the secretion of photosynthates (sugars) through the roots, fostering a stronger host-microbe relationship. Several researchers reported that the root colonization efficiency of PGPB is intimately linked to microbial competition and survival in the soil, as well as the regulation of gene expression and cell-cell communication via quorum sensing (Egamberdieva et al. 2017; Gamalero et al. 2022; Wang et al. 2022; Weemstra et al. 2022). Plant-growth-promoting rhizobacteria (PGPR) such as *Bacillus* spp. are widely dispersed in soil and produce strong endospores that are resistant to high temperatures and chemical concentrations (Bhat et al. 2023). *Bacillus* species improve biomass, yield, and seed emergence to promote plant growth. This is because of the synthesis of phytohormones like cytokinin, IAA, and gibberellin, and the increased uptake of available minerals, phosphorus, and nitrogen in the soil (Xie et al. 2014; Egamberdieva et al. 2017). Rice plants inoculated with PGPR exhibited profuse vegetative growth, with a wider root surface area, facilitating greater nutrient uptake, including Ca and Mg (Kaur et al. 2023). Through symbiotic relationships with plants, PGPR contributes to vigorous root growth by providing essential nutrients while utilizing root exudates as a food source (Vimal 2018; Weemstra et al. 2022).

In UPMRB9 inoculated conditions, positive and significant associations were observed among grain yield and parameters such as plant height, photosynthesis, conductance, and transpiration (Roy et al. 2024b). They also observed similar interactions between grain yields and other traits. This indicates that UPMRB9 inoculation positively influences these aspects, potentially leading to improved plant growth and productivity. The positive correlation with grain yield suggests that these factors contributed to increased yield under inoculated conditions. Interestingly, non-significant and negative associations indicated that inoculation might influence these parameters in a manner not conducive to higher grain yield. Multilocational field experiments addressing different climatic conditions could provide a better understanding of its activity under diverse natural conditions. However, several challenges could arise during its adoption under field conditions such as the shelf life of PGPR inoculum, selection of an appropriate carrier material, inoculum application techniques, climatic conditions and soil characteristics. Overcoming all the factors decides the successful establishment of a biofertilizer inoculum in a particular area.

In conclusion, the two potential indigenous strains, *Bacillus tequilensis* 10b and *Bacillus aryabhattai* B8W22 exhibited multiple plant growth-promoting properties. These strains significantly enhanced the growth of three rice varieties by improving morphological properties like plant height and leaf areas, physiological properties like photosynthesis, stomatal conductance and transpiration and biochemical properties like proline, total soluble sugar and total chlorophyll content which eventually triggered increased biomass and grain yield production of three rice varieties. Hence, to improve the existing rice production systems these potential isolates may be utilized as a source of biofertilizer. This glasshouse study serves as an initial basis for future large-scale field applications. However, substantial field experiments should be conducted before providing general recommendations.

ACKNOWLEDGEMENTS

The authors sincerely acknowledged the Universiti Putra Malaysia (UPM) for providing research facilities and OWSD (Organization for Women in Science for the Developing World) and the Swedish International Development Cooperation Agency (SIDA) for the fellowship award.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 140-148 DOI: 10.13057/asianjagric/g090115

Assessment of climate-smart agricultural practices of plantain farmers in Anambra State, Nigeria

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Manuscript received: 18 November 2024. Revision accepted: 20 January 2025.

Abstract. *Emmanuel OE, Nnenna OM, Nkiruka B-CG, Anulika UO, Thankgod EK, Angela ON, Nwuguru NR, Ekunyi UN, Ighokpozi OE, Ajah OE, Chinenyenwa T-AA, Olivia OC, Marylilian OO, AGU OP, Rhoda IT, Ojochenemi I, Chukwuka CC. 2025. Assessment of climate-smart agricultural practices of plantain farmers in Anambra State, Nigeria. Asian J Agric 9: 140-148.* The study examined the climate-smart agricultural practices of plantain farmers in Anambra State, Nigeria. The study used a multistage sampling technique to select 76 plantain farmers. A structured questionnaire was used for primary data collection, and mean, descriptive statistics, multivariate probit regression, and factor analysis models were employed in the analysis. The result shows that the plantain farmers were middle-aged (46 years), females (59.2%), married (53.9%), with a household size of 7 persons and farming experience of 18 years. The result further shows that 75% of the plantain farmers were aware of climate change. Climate-smart agricultural (CSA) practices adopted include mixed farming (80.3%), movement to different sites (68.4%), planting of early maturing variety (86.8%), crop rotation (88.2%), and application of farm yard manure (71.1%). Age, gender, level of education, household size, farm size, farming experience, extension contacts, credit use, and victim of climate events statistically influenced the adoption of CSA practices. Problems of capital, soil fertility, lack of technical know-how, poor extension access, lack of timely information on weather conditions, instability in the planting calendar, high cost of inputs, and limited lands constrained plantain production and adoption of CSA practices. Efforts should be made to provide farmers with training and workshops on climate-smart agricultural practices; this will enhance their knowledge to make informed decisions in adopting CSA practices in mitigating climate change.

Keywords: Agricultural practices, assessment, climate-smart, plantain farmers

INTRODUCTION

Plantain (*Musa paradisiaca* L.) has occupied a strategic position in agricultural production across the continents over the years (Adi 2024). It represents the world's second-largest fruit crop, with Cameroon being the world's leading country in plantain production with 4.3 million tons, followed by Ghana with 4 million tons, Uganda with 3.7 million tons, Colombia with 3.5 million tons and Nigeria with 3.1 million tons (FAOSTAT 2023). The crop ranks as the fourth most important starchy food commodity after rice, wheat, and maize (FAOSTAT 2023). About 70 million people in Africa depend on plantain for more than 25 and 10% of their carbohydrate and calorie intake, respectively (FAO 2022). The plantain farming sector in Nigeria is small. However, its production contributes to the national gross domestic product and remains significant in terms of food production, food value chain, and improvement of rural livelihoods. According to Ayeh et al. (2023), it is an important source of revenue for many smallholder farmers. Thus, sustaining livelihoods in sub-Saharan African countries, including Nigeria, is important. Despite these gains from plantain production, its cultivation relied primarily on climate continuously changing over time (Ojo et al. 2024). The agricultural sector is expected to produce food for the global population that will reach 9.1 billion people in 2050 and over 10 billion by the end of the century (WorldBank 2023).

The sector's reliance on climate is faced with several challenges that impede food production and frustrate the farmers efforts and commitment to agricultural activities. Climate change has led to significant changes and adverse impacts on the economic livelihoods of local plantain household farmers (Fofana et al. 2024). These impacts are envisaged to worsen over the coming years due to livelihood pressures and frequent occurrences of natural hazards such as extreme temperature, drought, and flood (Yahaya 2019). Extreme climate events, such as high-temperature stress, drought, and flooding, severely reduce plantain production. Plantain crops require much water to germinate, develop, and grow evenly. However, with consistent shortfalls in rainfall and its abnormal variations, plantain production suffers distorted growth and development, leading to decreased yield, loss in income, and economic market value (Mohammadi et al. 2023). Climate changes determine the pattern of vegetation, types, and yields of crops and animals. The length of cropping seasons causes a considerable yield loss in plantain crops, thereby adversely affecting smallholder plantain farmers (modification of plantain production and food systems, introducing uncertainty and vulnerability risks) and food security (Mall et al. 2017; FAO 2023; Fofana et al. 2024).

Considering these negative consequences of climate change, various international institutions are working together with the World Bank Group and the Food and Agricultural Organization (FAO) to devise agricultural systems that will enhance and boost food production at all levels. According to Bai et al. (2024), agricultural systems need to be transformed to increase the productive capacity and stability of smallholder farmers in the wake of climate change. In this context, a global shift to Climate-Smart Agriculture (CSA) has been applauded by various institutions, stakeholders, researchers, policymakers, and agro-investors and across private, public, and civil society sectors (Ma and Rahut 2024). According to FAO (2024), the practice of CSA sustainably increases productivity, reduces climate change risks and vulnerability, enhance adaptation, and reduces emissions that cause climate change (mitigation), while protecting the environment against degradation and denudation.

Studies (Keil et al. 2021; Waaswa et al. 2022; Mohammadi et al. 2023; Omotoso and Omotayo 2024; Ojo et al. 2024; Cuni-Sanchez et al. 2025) have x-rayed the impacts of climate change on important food crops such as cassava, rice, yam, millets, cowpea, maize, vegetables, etc., which is commendable but however, studies on climate change and plantain cultivation are quite scanty and poorly documented, hence the rationale for the study. The study assessed the coping strategies of plantain farmers in the face of climate change in Anambra State, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in Anambra State, Nigeria (Figure 1). It is located between longitude 6°36'E to 7°21'E and latitude 5°38'N to 6°47'N with a land mass of 4415.54 square kilometers. The state has fertile land holdings rich for agricultural production. Farming and trading are the most predominant occupation of the people, and majority of the farmers are smallholders. The area belongs to the densely populated rainforest zone of the country with two vegetation seasons, which are notably the rainy and dry seasons. The climate varies with temperatures around 25 to 27°C and rainfall around 1200 mm. The soil types include loamy, sandy, humus, and clay, while agricultural activities are carried out on the dark loamy and humus soil. The major crops produced are plantain, yam, cassava, rice, maize, cocoyam, cowpea, tomatoes, and vegetables, while the livestock produced in the state include poultry, sheep, and goats.

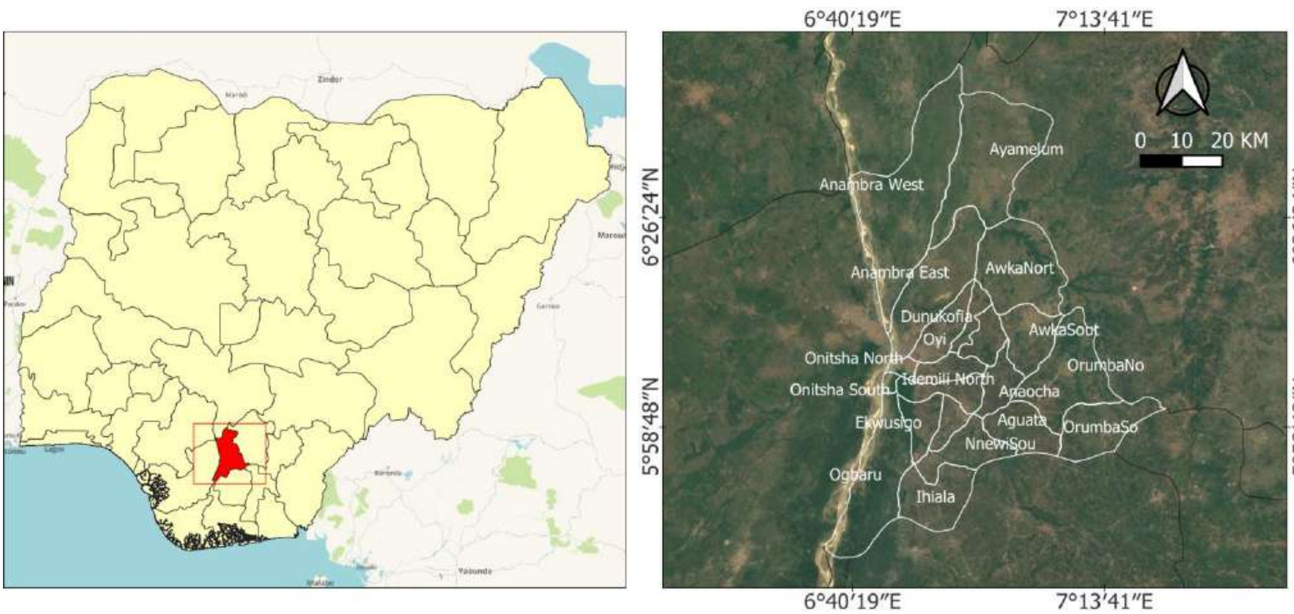

Figure 1. Map of study site in Anambra State, Nigeria

Table 1. Sample distribution of plantain crop farmers

Zones	No. of registered plantain farmers	No. of plantain farmers Sampled	No. of farmers who provided valid responses
Aguata	50	30	18
Anambra	53	32	24
Awka	48	29	20
Onitsha	41	25	14
Total	192	116	76

Sample selection

Multistage sampling technique was used for this study. Multistage sampling is a statistical method that involves selecting samples from a population in multiple stages. It has been used widely in several studies (Emmanuel et al. 2024; Osuji et al. 2024; Igberi et al. 2025). In the first stage, two Local Government Areas (LGAs) were purposively selected from each of the four agricultural zones of the state, namely Aguata, Anambra, Awka, and Onitsha, making 8 LGAs based on their involvement in plantain farming and agricultural activities. The second stage involved a random sample selection of plantain crop farmers from the list of registered farmers in each of the selected LGAs across the four zones of the state. Aguata zone has 50 registered plantain farmers; Anambrahas 53, Awka 48, while Onitshahas 41 registered farmers. This shows that there are unequal numbers and representations of the plantain farmers across the four zones of the state. Hence, an equal sample representation was made from a proportion of 60 percent of the total population from each zone. The sample size for each of the zones were; Aguata 30, Anambra 32, Awka 29, and Onitsha 25. This gave a total of 116 plantain sampled crop farmers across the zones. The list of registered plantain farmers, the proportion of the population sampled, and the valid sample used for the study are shown in Table 1.

Method of data collection

The study was based on primary data collection. Data were obtained with the aid of a structured questionnaire administered to the sampled 116 plantain crop farmers across the four zones of the State. The questionnaire consists of the core objectives of the study, to which the respondents were requested to respond objectively. However, out of the 116 plantain crop farmers, only 76 provided helpful information in line with the study's objectives.

Method of data analysis and model specification

Data were analyzed using descriptive statistical tools such as the mean, frequency, and percentage and econometric tools such as the multivariate probit regression and factor analysis models. The multivariate probit regression model analyzed the factors influencing plantain farmers adoption of climate-smart agricultural practices. It is a statistical and econometric model that simultaneously estimates multiple correlated binary outcomes. It has an advantage over other econometric models in that its application is straightforward and flexible in handling a variety of correlation structures and the interpretability of its parameters. In this study, the multivariate probit regression modeled the dichotomous dependent variable, the various CSA practices adopted by the plantain farmers, and the independent variables. The model is estimated using the maximum likelihood method. The model is expressed as follows:

$Z_{1i} = \propto_1 R_{1i} + \beta_1 H_{1i} + \delta_1 C_{1p} + \varepsilon_{1i}$
$Y_{1i} = 1$ if $Z_{1i} > 0$; $Y_{1i} = 0$ if $Z_{1i} \leq 0$
$Z_{2i} = \propto_2 R_{2i} + \beta_2 H_{2i} + \delta_2 C_{2p} + \varepsilon_{2i}$
$Y_{2i} = 1$ if $Z_{2i} > 0$; $Y_{2i} = 0$ if $Z_{2i} \leq 0$
$Z_{ji} = \propto_j R_{ji} + \beta_j H_{ji} + \delta_j C_{jp} + \varepsilon_{ji}$
$Y_{ji} = 1$ if $Z_{ji} > 0$; $Y_{ji} = 0$ if $Z_{ji} \leq 0$

Empirically, the model is specified as:

$Z_i = \alpha_i X_i$

$Z_i = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_4 + \alpha_5 X_5 + \alpha_6 X_6 + \alpha_7 X_7 + \alpha_8 X_8 + \beta_9 X_9 + \beta_{10} X_{10} + \delta_{11} X_{11} + \delta_{12} X_{12} + \varepsilon_1$

Where:

$\mathbf{Z_i}$ = a latent decision variable from which the plantain farmers make choices.

Note that about 11 climate-smart agricultural practices were engaged by the plantain farmers, and this formed the dependent variables and includes:

MF = Mixed farming (Adopted = 1, Not adopted = 0)

MDS = Movement of different sites (Adopted = 1, Not adopted = 0)

PMV = Planting of early maturing variety (Adopted = 1, Not adopted = 0)

CR = Crop rotation (Adopted = 1, Not adopted = 0)

AFM = Application of farm yard manure (Adopted = 1, Not adopted = 0)

ILS = Increased land size cultivated (Adopted = 1, Not adopted = 0)

CPP = Changes in planting period (Adopted = 1, Not adopted = 0)

DNF = Diversification into non-farm activities (Adopted = 1, Not adopted = 0)

CHD = Change in harvesting date (Adopted = 1, Not adopted = 0)

LF = Lengthened fallow (Adopted = 1, Not adopted = 0)

UWF = Use of weather forecast (Adopted = 1, Not adopted = 0)

X_i = represents the independent variables and this includes; $X_{1:}$ Farmer's age (Years); $X_{2:}$ Farmer's gender (1: Male; 0: Female); $X_{3:}$ Farmer's level of education (Years spent in school); $X_{4:}$ Household size (number of persons in a household); $X_{5:}$ Farm size (Ha); $X_{6:}$ Farming experience (Years); $X_{7:}$ Labor (Man-days); $X_{8:}$ Number of extension contacts with the farmer in the cropping year; $X_{9:}$ Total income (Naira); $X_{10:}$ Credit used (Amount borrowed in Naira); $X_{11:}$ Cooperative membership (Yes: 1, No: 0); $X_{12:}$ Victim of climate events (Yes: 1, No: 0); ei: error term

Table 2. Socio-economic characteristics of plantain farmers

Variable	Frequency	Percentage
Age		
21-30	13	17.1
31-40	10	13.2
41-50	28	36.8
51& above	25	32.9
Mean	46	SD=0.06
Sex		
Male	31	40.8
Female	45	59.2
Marital status		
Single	11	14.5
Married	41	53.9
Divorced	12	15.8
Widowed	12	15.8
Level of education		
Primary	30	39.5
Secondary	22	28.9
Tertiary	14	18.4
Non formal	10	13.2
Household size		
1-4	24	31.6
5-8	52	68.4
Mean	7	SD=1.05
Farm size		
0.1-1.0	51	67.1
1.1-2.0	17	22.4
2.1-3.0	08	10.5
Mean	0.8	SD=1.08
Extension contacts		
1-2	34	44.7
3-4	38	50.0
5-6	04	5.3
Mean	3	SD=0.09
Cooperative membership		
Yes	51	67.1
No	25	32.9
Participation in workshop/training		
1-2	27	35.5
3-4	47	61.8
5-6	02	2.6
Mean	3.3	SD=1.02
Farming experience		
1-10	17	22.4
11-20	48	63.2
21-30	11	14.5
Mean	18	SD=1.07
Source of capital		
Banks	08	10.2
Friends/relatives	19	25.0
personal savings	27	35.5
Cooperatives society	12	15.8
Other	10	13.2

RESULTS AND DISCUSSION

Socio-economic characteristics of plantain farmers

Table 2 presents the socio-economic characteristics of plantain farmers. The result shows that 17.1% of the respondents were 21-30 years, 13.2% were 31-40 years, 36.8% were 41-50 years, and 32.9% were 51 years and above. The average age of the respondents was 46 ± 0.06 years. This implies that majority of the plantain farmers were middle-aged, indicating that plantain farming is predominantly carried out by individuals in their prime working years (Zizinga et al. 2022). The sex distribution of plantain farmers shows that 40.8% of the respondents were male while 59.2% were female, indicating that plantain farming is dominated by females, highlighting the significant role of women in the agricultural sector (Zhou et al. 2023). It was observed that the majority, 53.9% of the respondents, were married, while 14.5% were single and the divorced and widowed accounted for 15.8%. This implies that the married farmers provided the needed family labor required to practice climate-smart agriculture in the study area. The result shows that 39.5% of the respondents had primary education, 28.9% had secondary education, 18.4% had tertiary education. However, 13.2% of the respondents had no formal education. This implies that most plantain farmers in the area were literate, significantly influencing their adoption of CSA practices (Zakaria et al. 2020). The study shows that most respondents had a household size of 5-8 persons while the mean household size was 7±1.05 persons. This indicates that majority of the plantain farmers had good number of family members who assisted in plantain farming and implementation of CSA practices. Result indicates that 81.6% of the respondents had farming solely as their occupation, while 18.4% combined farming with other economic activities. This shows that most respondents were farmers, who cultivated plantain using CSA practices (Waaswa et al. 2022).

The farm size shows that majority, 67.1% of the respondents had 0.1-1.0 hectares of land, followed by 22.4% that had 1.1-2.0 hectares of land. The mean farm size was 0.8±1.08 hectares. This indicates that the plantain farmers had limited farm sizes, which can affect their productivity, economies of scale, and access to better inputs and markets (Zhou et al. 2023). Extension contacts result shows that majority, 50% of the respondents had 3-4 extension contacts, and 44.7% had 1-2 extension contacts. The mean extension contacts were 3±0.09, indicating low extension contacts among plantain farmers. This invariably shows limited access to modern innovations and trends disseminated by extension agents. Result shows that majority 67.1% of the plantain farmers belong to a cooperative society, while 32.9% did not associate themselves with any cooperative society. This implies that those that belong to cooperative societies are better off because they obtain various sources of information and variable inputs than those that did not belong to any cooperative society (Vatsa et al. 2023). It was observed that 61.8% of the plantain farmers have participated in workshop/training 3-4 times, while 35.5% have participated 1-2 times. However, only 2.6% of the farmers participated 5-6 times. The mean participation in workshop/training was 3.3±1.02 times. This implies that there has been a low participation in workshop/training among plantain farmers. The majority 63.2% of plantain farmers had 11-20 years of experience in plantain farming. A 22.4% had 1-10 years of experience, while 14.5% had 21-30 years of experience.

The mean farming experience was 18±1.07 years. This shows a high experience level among plantain farmers (Tran et al. 2020). The majority 35.5% of the respondents had personal savings as their major source of capital followed by 25% who agreed that their source of capital was from friends/relatives. It was also observed that there was a low access to credit from banks by plantain farmers in the area.

Level of awareness of farmers to climate smart agricultural practices

Figure 2 shows the level of awareness of farmers to climate smart agricultural practices. The result shows that 39.5% of the farmers were moderately aware of climate smart agricultural practices, 35.5% are sufficiently aware, while 25% of the farmers were not aware of climate-smart agricultural practices. Overall, the study implies that most farmers (75%) have good knowledge of climate smart agricultural practices. Good knowledge and understanding of climate-smart agricultural practices aid farmers in its adoption because most crop farmers are conservative and do not easily respond to new practices unless they are convinced or have seen where such practices were used (Tabe-Ojong et al. 2023). Furthermore, being aware of a particular technique and its adoption/utilization enhance crop yields, outputs and land productivity, improving crop farmers' economic livelihood (Waaswa et al. 2022; Zhou et al. 2023; Yuan et al. 2024).

Climate-smart agricultural practices adopted by plantain farmers

Table 3 shows the CSA practices adopted by the plantain farmers. The farmers mostly adopted the application of farm yard manure. This involves the application of green manure and other domestic wastes to the plantain field; this allows the farmers to sustain higher plantain yield during unfavorable weather conditions such as erratic and destructive winds. Diversification into non-farm activities was ranked second. This practice involves engaging in alternative income-generating activities alongside agriculture. By diversifying their income sources, farmers reduce their dependence on a single crop and mitigate the risks associated with climate change impacts on agricultural production. It provides a buffer against climate-related shocks and contributes to overall household resilience (Khatri-Chhetri et al. 2020; Abigaba et al. 2024).

Mixed farming was ranked third. This involves the practice of crop cultivation and animal husbandry simultaneously. This allows for the sustenance of farmers livelihood in case of any eventuality arising from changes in climate and extreme weather conditions (Shikuku et al. 2017). The planting of early maturing varieties ranked fourth. Early maturing varieties have a shorter growing period, allowing farmers to harvest their plants earlier. This practice helps mitigate the impacts of climate change by reducing exposure to unfavorable weather conditions such as droughts, floods, or extreme temperatures. Early maturing varieties also offer opportunities for better market timing, allowing farmers to take advantage of market demands and achieve higher profits (Omotoso and Omotayo 2024).

The movement to different sites ranked fifth. This involves periodically shifting plantain cultivation to new locations. This practice helps to mitigate the accumulation of pests, diseases, and soil-borne pathogens that can reduce crop productivity. By moving to different sites, farmers can break pest and disease cycles, reduce the reliance on chemical inputs, and maintain the long-term productivity of their plantain farms (Radeny et al. 2022). Changes in harvesting date ranked sixth. This involves adjusting the timing of plantain harvest to adapt to changing climatic conditions. This practice aims to avoid adverse weather events, optimize fruit quality, and minimize post-harvest losses. By shifting the harvest date, farmers can align their activities with favorable weather patterns and reduce the risk of losses due to climate-related factors (Vatsa et al. 2023). The use of weather forecasts ranked seventh. This involves using weather trends to monitor planting and harvesting timelines. This reduces climatic risks, shocks, and other harmful risks associated with climate change.

Lengthened fallow ranked eighth. This involves extending the period between plantain cropping cycles to allow the land to rest and regenerate. Extended fallow periods improve soil fertility, reduce erosion, and promote natural ecosystem functions. This practice helps restore the health of the land and enhances the long-term sustainability of plantain cultivation (Zhou et al. 2023).

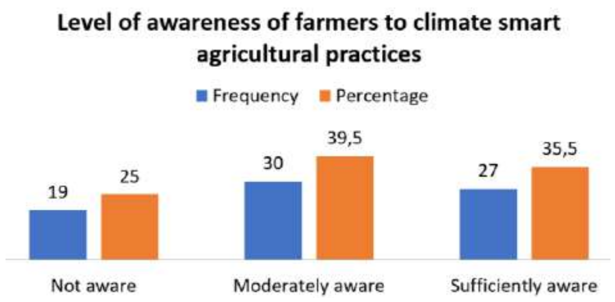

Figure 2. Level of awareness of farmers to climate smart agricultural practices

Table 3. Climate-smart agricultural practices adopted by plantain farmers

CSA practices adopted	Mean	Rank
Application of farm yard manure	93.5	1st
Diversification into non-farm activities	89.1	2nd
Mixed farming	88.7	3rd
Planting of early maturing variety	83.2	4th
Movement to different site	78.1	5th
Changes in harvesting dates	74.6	6th
Use of weather forecasts	72.8	7th
Lengthened fallow	69.8	8th
Change in planting periods	61.4	9th
Crop rotation	58.4	10th
Increased land size cultivated	45.2	11th

Note: Multiple responses

Changes in the planting period tanked ninth. This refers to adjusting the timing of plantain planting to adapt to changing climate conditions. This practice involves planting earlier or later than usual to avoid extreme weather events and optimize plant growth and development. By adapting to changes in the planting period, farmers can enhance plantain production and minimize climate risks such as drought or flooding (Kifle et al. 2022). Crop rotation ranked tenth. This essential CSA practice involves systematically alternating the types of crops grown in a particular field over time. This practice helps to improve soil fertility, reduce pest and disease buildup, and enhance nutrient cycling. By rotating crops, plantain farmers can break pest and disease cycles and optimize soil health, increasing productivity and sustainability in the long run (Mirón et al. 2023). Increasing the land size cultivated ranked least. Expanding the land area under cultivation allows farmers to diversify their production and potentially increase their overall yield. However, land expansion must be done sustainably, considering ecological factors and land management practices to avoid negative impacts on soil health and biodiversity (McNunn al. 2020).

Factors influencing the adoption of climate-smart agricultural practices of plantain farmers

Table 4 shows the factors influencing the adoption of CSA practices of plantain household farmers. The wald chi^2 produced a value of 211.10, which was significant at 1% level and showed the model goodness of fit. The result showed that age, gender, level of education, household size, farm size, farming experience, extension contacts, credit use, and victim of climate events statistically influenced the choice and adoption of various CSA practices. Age was statistically significant and negatively signed for shifting cultivation, crop rotation, and use of weather forecast at 5, 10, and 1% significance levels respectively. This implies that as farmers advance in age, their adoption and use of the various CSA strategies diminishes. Age plays a significant role regarding the adoption of CSA practices. Studies have shown that younger farmers are more likely to adopt CSA practices than aging farmers (Ayeh et al. 2023). The gender coefficient was negatively signed at 5%, 1%, and 10%, respectively for mixed farming, crop rotation, expansion of cultivation and change of harvesting date respectively. This implies that female plantain farmers adopted more CSA practices than male farmers. This is because female farmers are easily persuaded to adopt agricultural practices relative to male farmers, which exhibit indecisiveness when adopting a technology (Martey et al. 2021).

The coefficient of education was also positively signed for planting of early maturing varieties, expansion of cultivation, and change in planting period at 1 and 5% significance levels. This result suggests that as the farmers advance in education, the likelihood of adoption and usage of these practices increases over time. Education is important for the adoption of CSA practices because as farmers acquire more education, they become more exposed to and open-minded to innovations and technologies. Education plays a crucial role in increasing farmers' awareness and understanding of climate change, helping them access relevant information and knowledge about sustainable farming techniques, and encouraging their willingness to embrace innovative technologies (Tabe-Ojong et al. 2023). The coefficient of household size was positively signed at 5, 1, and 10%, respectively for mixed farming, crop rotation, expansion of cultivation, diversification into non-farm activities and the use of weather forecast. This implies that plantain farmers with larger households who provide labor will have higher tendencies to adopt the CSA practices. Studies reveal that large households enhance the adoption and implentation of CSA practices unlike small households (Omotoso and Omotayo 2024). The coefficient of farm size was positively signed at 10 and 5% for mixed farming, application of organic manure and change in planting period respectively. This implies that the larger the farm size, the higher the adoption and use of the CSA practices. Larger farm holdings encourage and support the adoption and usage of CSA practices. This allows for greater input and output resource optimization (Kifle et al. 2022).

The coefficient of farming experience was also positive at 5 and 10% for shifting cultivation, crop rotation and expansion of cultivation, change in planting period and change in harvesting date respectively. This implies that experienced farmers are more likely to adopt these strategies relative to inexperienced farmers. Farming experience plays an important role in knowing the best practices to adopt, when to adopt them, and the resultant benefits of such adoptions (Shikuku et al. 2017). The coefficient of extension visits was positively signed for shifting cultivation, planting of early maturing varieties, application of organic manure, change in planting period and lengthened fallow at 10, 5, and 1% respectively implying that plantain farmers that had extension contacts were more inclined to the adoption and usage of the CSA practices. Extension contacts are very important because farmers are educated on the best practices to use within their available resources. Farmers who had regular extension contacts are more likely to adopt best climate practices which improves yield (Radeny et al. 2022).

The coefficient of credit use was positively signed for mixed farming, planting of early maturing varieties and diversification into non-farming activities at 10% level of significance. This implies that the use of credit resources encourages the adoption of CSA practices. No doubt, these CSA practices might be capital demanding in nature and hence the need for credit support. Credit access plays a crucial role in facilitating the adoption of climate-smart technologies among smallholder farmers (Keil et al. 2021). The coefficient of victim of climate change shows that mixed farming, planting of early maturing varieties, expansion of cultivation, diversification into non-farming activities, lengthened fallow and use of weather forecast were significant at 10, 5, and 1% respectively. This implies that farmers who have experienced negative effects of climate change are most likely to adopt CSA practices unlike farmers who had not experienced climate risks. Studies reveal that farmers who experience adverse climate change effects, such as extreme weather events, are more likely to adopt best adaptive practices to mitigate future climate risks (Waaswa et al. 2022).

Table 4. Factors influencing the adoption of CSA practices of plantain farmers

Variable	MF	MDS	PMV	CR	AFM	ILS	CPP	DNF	CHD	LF	UWF
X_1	-0.9453	-0.6733	-12.7383	-14.8456	-12.9045	-9.9465	-19.0733	-0.0647	-0.0454	-9.0463	-0.0833
	(-0.899)	(-2.752)**	(-0.833)	(-1.853)*	(-0.933)	(-0.451)	(-1.083)	(-1.023)	(-1.235)	(-0.022)	(-4.712)***
X_2	-0.0564	-12.8454	-0.7489	-0.9546	-0.9404	-0.8462	-0.9352	-0.9562	-0.0945	-0.8933	-0.9420
	(-2.7441)**	(-0.954)	(-1.423)	(-3.932)***	(-1.091)	(-1.671)*	(-1.231)	(-1.390)	(-3.213)***	(-0.342)	(-1.222)
X_3	10.8531	0.5673	0.7489	0.7552	8.0464	0.7452	9.4642	0.0534	0.9531	0.8342	0.0734
	(1.002)	(0.344)	(4.032)***	(1.401)	(0.542)	(2.922)**	(3.933)***	(0.921)	(1.423)	(0.721)	(1.023)
X_4	4.0992	0.7489	0.9462	23.9462	0.9648	0.5489	8.0353	2.8421	0.7343	0.9352	3.9347
	(2.941)**	(1.041)	(0.531)	(1.851)*	(1.191)	(2.123)**	(1.423)	(3.634)***	(1.231)	(0.521)	(1.812)*
X_5	0.4674	0.7452	0.8595	0.8451	0.8576	0.3672	0.7342	0.9452	0.0454	0.8351	0.0632
	(1.942)*	(0.642)	(0.621)	(1.201)	(2.621)**	(1.001)	(1.512)*	(1.301)	(0.821)	(1.101)	(0.732)
X_6	9.7843	10.6484	0.0452	0.6758	0.9464	9.3546	0.9341	12.9301	0.8342	2.0733	0.0763
	(1.031)	(2.921)**	(0.233)	(2.122)**	(0.522)	(2.833)**	(1.933)*	(0.521)	(2.481)**	(1.022)	(1.111)
X_7	8.9342	0.74841	0.8462	0.6598	11.9404	0.7489	4.0464	0.0647	0.9453	0.8342	0.0533
	(0.833)	(1.292)	(0.922)	(0.523)	(1.632)	(0.521)	(1.190)	(0.002)	(0.903)	(1.200)	(0.822)
X_8	7.6381	8.9464	0.6490	21.9046	0.9453	3.9454	0.0478	2.9451	3.0533	0.9421	0.8331
	(0.456)	(1.932)*	(2.821)**	(1.026)	(1.920)*	(1.002)	(2.811)**	(0.022)	(0.633)	(3.921)***	(0.422)
X_9	0.5476	0.8453	12.9461	0.8946	5.9464	4.0713	0.0353	0.9523	0.8312	4.9511	0.9422
	(0.961)	(1.099)	(0.099)	(0.061)	(0.056)	(0.410)	(0.821)	(1.311)	(0.521)	(0.612)	(0.001)
X_{10}	0.8657	13.9454	9.0464	0.9468	0.9546	0.7454	0.5562	1.0833	0.0621	0.7331	0.3464
	(1.671)*	(0.833)	(1.900)*	(1.292)	(0.922)	(1.062)	(0.062)	(1.841)*	(0.232)	(0.422)	(0.002)
X_{11}	13.8453	0.8563	0.9357	0.9845	0.6473	0.8321	0.9044	0.0644	0.9533	0.8452	0.9241
	(1.002)	(0.021)	(0.811)	(0.712)	(1.211)	(0.391)	(1.091)	(0.922)	(0.122)	(1.032)	(0.009)
X_{12}	0.7442	0.7842	0.6590	0.5390	9.0341	0.6547	0.0621	0.9452	1.0264	0.0525	0.9532
	(1.934)*	(0.942)	(2.921)**	(1.012)	(0.921)	(1.999)*	(0.123)	(2.021)**	(1.201)	(5.901)***	(1.678)*

Note: Wald chi^2: 211.10***. values in parenthesis are z-values and significant at ***1%, **5% and *10%

Constraints militating against plantain production and CSA practices

Table 5 shows that the major constraints militating against plantain production and CSA practices. These includes problem of capital, problem of soil fertility, lack of technical know-how, poor extension acces, lack of timely information on weather conditions, instability in planting calendar, high cost of inputs and limited lands. The lack of capital was identified as a major constraint against plantain production and adoption of CSA practices. Insufficient financial resources limit farmers ability to practice large scale plantain production and purchase of improved planting materials, organic fertilizers, and other needed inputs, thereby reducing their agricultural productivity. It also frustrates investments in climate-smart agricultural practices in mitigating climate risks (FAO 2022; Fofana et al. 2024). Declining soil fertility can result in reduced crop yields and increased vulnerability to pests and diseases.

Soil fertility management practices, such as organic matter incorporation and balanced nutrient management are crucial for sustaining the long-term productivity of plantain farms. Thus, poor soil fertility can negatively impact plantain production and limit farmers ability to adopt climate-smart agricultural practices (Keil et al. 2021). Limited knowledge and skills in climate-smart agricultural practices can hinder farmers ability to implement appropriate techniques and strategies for climate change adaptation and mitigation. The lack of technical know-how can impede the adoption of CSA practices among plantain farmers, limiting their capacity to effectively respond to climate change challenges (Waaswa et al. 2022). Improving access to extension services is crucial for disseminating knowledge, providing technical support, and promoting the adoption of CSA practices among plantain farmers.

However, limited interaction with extension agents reduces farmers' access to information, training, and guidance on climate-smart agricultural practices, and constraining their ability to adopt and implement CSA practices effectively (Vatsa et al. 2023). Lack of timely information on weather conditions and climate trends limits farmer’s ability to make informed decisions regarding their crop cultivation and adoption of appropriate climate-smart agricultural practices. Access to timely information is crucial for farmers to adapt to climate change, make informed decisions, and effectively implement climate-smart strategies. Climate variability and changing weather patterns can disrupt traditional planting schedules, making it difficult and challenging for farmers to determine the optimal time for planting. This constraint affects plantain farm planning, management, and overall productivity.

Consequently, the instability in planting calendar alters plantain production, reducing its market value and frustrates the adoption of CSA practices (Omotoso and Omotayo 2024). The expenses associated with purchasing improved planting materials, fertilizers, pesticides, and other inputs can be a significant barrier for farmers, particularly those with limited financial resources. The high cost of inputs hinders the adoption of climate-smart agricultural practices among plantain farmers. It poses a financial burden and reduces the purchase of necessary inputs. Inadequate land makes the implementations of CSA practices difficult, since most of these practices require large farm holdings to implement, more so, limited land holdings limits large scale plantain production (Zakaria et al. 2020; Ndudzo et al. 2024).

In conclusion, the study findings shows that the plantain farmers were in their productive age, more of female, married, had primary education and household size of 7 persons and relatively. The study revealed a high level of awareness among plantain farmers regarding climate-smart agricultural practices. The most adopted CSA practices included diversification into non-farm activities, crop rotation, planting of early maturing varieties, and changes in harvesting and planting periods. The result showed that age, gender, level of education, household size, farm size, farming experience, extension contacts, credit use and victim of climate events statistically influenced the choice and adoption of various CSA practices. Constraint such as limited capital, poor soil fertility, technical know-how, poor extension access, and lack of timely information on weather conditions hindered plantain production and the adoption of CSA practices. Efforts should be made to provide farmers with training and workshops on climate-smart agricultural practices; this will enhance their knowledge and skills in adopting CSA practices. Research institutions and agricultural agencies should collaborate to provide plantain farmers with up-to-date information on climate change and its variations; this will enhance timely adoption of the CSA practices by the plantain farmers to mitigate adverse effects of climate change.

Table 5. Constraints militating against plantain production and CSA practices

Factorized variable	FC 1	FC 2	FC 3	FC 4
Problem of soil fertility	0.675*			
Instability in planting calendar		0.863*		
Drying of sucker after harvest due to high temperature			0.373	
Lack of timely information on weather conditions				0.678*
Problem of capital	0.783*			
Poor extension access		0.563*		
Inadequate farming land			0.548*	
Erosion occurrence and wind storm				0.274
Shortage of labor force	0.488			
Lack of technical know-how		0.892*		
High cost of fertilizers			0.492	
High cost of inputs				0.784*
Poor attitude of plantain farmers to climate change	0.353			

Note: Extraction Method: Factor component analysis. Rotation Method: Varimax with Kaiser Normalization. *: signifies components with score of 0.5 and above and selected factors

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 149-159 DOI: 10.13057/asianjagric/g090116

Diversity of insect pollinators in the buffer zone of Mount Merapi National Park, Indonesia

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[2]Department of Environmental Science, School of Graduate, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
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Manuscript received: 31 March 2024. Revision accepted: 3 February 2025.

Abstract. *Herdananta BY, Charsyah C, Putra CDH, Mumpuni CHY, Nofitasari H, Setyawan AD. 2025. Diversity of insect pollinators in the buffer zone of Mount Merapi National Park, Indonesia. Asian J Agric 9: 149-159.* Pollinating insects have a significant impact on plant reproduction on land, accounting for almost 90% of their function. Both the environment and vegetation influence the presence of pollinating insects. This study aims to analyze the diversity of pollinating insects in several habitat types in the buffer zone of Mount Merapi National Park (MMNP), Tegalmulyo and Sidorejo Villages, Kemalang, Klaten, Indonesia. This research was conducted in March 2024 by taking 3 habitat types, namely forests, agricultural fields, and shrublands, each with 4 replicates. The research method used is a roaming research purposive random sampling technique, and the sampling point was determined at the selected location based on the survey results and accompanied by direct observation. Furthermore, data analysis was carried out on the species ecological index (diversity, richness and evenness), Species Accumulation Curve (SAC) and its relationship with abiotic factors. The results of the study found pollinator insects totaling 121 individuals consisting of 55 species, 26 families, and 5 orders, namely the order Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera. There are several flowering plants infested by pollinating insects; 15 plant species were obtained and divided into 11 different families. The results of the calculation of the pollinator species diversity index were 3.731, a species richness index of 11.26, and a species evenness index of 0.93. Based on the results of the SAC, we recommend to future researchers to make the number of transects more than 12 if conducting research in similar locations until there are no more species additions. The results of measuring abiotic factors from the three habitats have different temperatures, humidity, and wind speeds that can affect the presence of pollinating insects and flowering plants. Based on these results, the ecosystem at the research location is well-maintained, so it is still suitable as a habitat for insect pollinators.

Keywords: Abiotic factors, diversity, insect pollinators, habitat type

INTRODUCTION

Pollination network events in plants arise due to various interactions between thousands of species with their respective needs for survival. The network of interactions between plants and their pollinators is strong evidence of mutualism between them. Pollination is often mentioned from the point of view of ecosystem services that have various benefits for humans (Mathiasson and Rehan 2020). One of the animals included in pollinator animals is the insects because pollinating insects play an important role in the reproduction of almost 90% of plants on land. The existence of various types of host plants will also affect the variety of pollinator species to support their food sources and shelter (Wakhid et al. 2023). Therefore, the ecological role of pollinators carried out by insects is very important for plant biodiversity and all related organisms in the living chain, including maintaining agricultural sustainability and human food security (López-Uribe et al. 2020). Pollination as an ecosystem service (ES) has attracted considerable international attention as a result of the decrease in pollinator populations caused by the widespread use of pesticides and the simplification of landscapes on community farmland. There is ample evidence to suggest that wild bees and other pollinators, such as flies, butterflies, and florivorous bats, may face similar challenges (Ghisbain et al. 2021). Research reports that the decline in pollinating insects is caused by the excessive use of pesticides in pest control on agricultural land (Sponsler et al. 2019). In addition, according to Nurlaila et al. (2021), land fragmentation can cause pollinator insects to lose their habitat because flowers are also less available as food for pollinator insects.

Many studies have evaluated how environmental factors affect pollinating animals. Examples of research conducted by Dyola et al. (2022) that identify various impacts of environmental factors on the presence of pollinating animals can differ greatly between climate/ vegetation zones. According to research by Rahmi et al. (2020), the high diversity of pollinating insects in the buffer zone of Gunung Leuser National Park, which helps agricultural crops pollinate, indicates that the environmental conditions in the area are still natural and not exposed to many disturbances. The diversity and richness of pollinator insect species vary depending on their habitat. Research conducted by Koneri et al. (2021)

explained that the existence of plants as homes for pollinator insects affects the diversity of pollinator insects because pollinator activities in foraging will not be too far from the nest. In addition, the diversity of pollinators is also due to the many variations of flowers that feed pollinator insects (Katumo et al. 2022).

Mount Merapi National Park is a conservation area administratively located in 4 districts, namely Sleman District, Klaten District, Boyolali District, and Magelang District. Mount Merapi National Park is also divided into several resorts, namely Dukun Resort, Srumbung Resort, Pakem-Turi Resort, Cangkringan Resort, Kemalang Resort, Musuk-Cepogo Resort, and Selo Resort. Kemalang Resort is a resort that has hiking trails, namely the Sapuangin Hiking Trail, an area with many tourism opportunities, including camping, tracking, and plant education tours (Maghfiroh et al. 2023). The area around the Sapuangin Hiking Trail consists of various habitats, such as natural forest areas, shrublands, and agricultural fields. The fertile agricultural land in the Sapuangin area is due to its location at the foot of Mount Merapi so that various food, fruit, and plantation crops can be planted (Widhiastuti et al. 2023). This makes each region diverse and rich, and there are different species of pollinating insects. Therefore, this study was conducted to analyze the diversity of pollinating insects in several habitat types in Mount Merapi National Park, Tegalmulyo, and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Indonesia.

MATERIALS AND METHODS

Study area

This research was carried out in March 2024, and the sampling location was in the buffer zone of Mount Merapi National Park in Tegalmulyo Village with a coordinate point of 7°34'3.78 "N 110°28'4.47" E at an altitude of 1,350 masl and Sidorejo Village with a coordinate point of 7°34'29.3 "N 110°28'4.79" E at an altitude of 1,218 masl. Tegalmulyo Village and Sidorejo Village, Kemalang Subdistrict, Klaten District, Central Java Province, Indonesia (Table 1; Figure 1). Furthermore, the sampling habitat is in forest areas, agricultural fields, and shrublands (Figure 2). Forest areas are a habitat that contains various types of large woody plants that form a canopy. The shrublands are dominated by clustered undergrowth, and the agricultural fields are a field that is deliberately treated by the community to utilize its results.

Table 1. Location of the three type habitats

Type	Location	Coordinate	Note (Vegetation)
Forest	Basecamp of Sapu Angin, Deles Indah Tourist Area, Pine Forest Deles Indah	7°34'00.9"S 110°27'56.1"E, 7°34'25.0"S 110°27'59.2"E, 7°34'07.5"S 110°28'01.4"E	*Calliandra calothyrsus* Meisn., *Acacia decurrens* Willd., *Schima wallichii* (DC.) Korth. (Trees) and *Imperata cylindrica* L. (land cover)
Shrublands	Forest Basecamp of Sapu Angin and Deles Indah Reservoir	7°34'02.8"S 110°28'04.3"E, 7°34'20.8"S 110°28'01.2"E, 7°34'51.5"S 110°28'05.5"E	*Ageratum conyzoides* L., *Crassocephalum crepidioides* (Benth.) S.Moore, *Imperata cylindrica* L., etc. More detailed species in Table 3. (weeds)
Agricultural fields	Photo Spot of Sapu Angin and Deles Indah Monument	7°34'25.8"S 110°28'03.0"E, 7°34'29.6"S 110°28'07.4"E, 7°34'06.1"S 110°28'02.2"E, 7°33'59.8"S 110°28'12.1"E	*Capsicum annum* L. and *Coffea arabica* L (main crops), *Rosa* sp. and *Pennisetum purpureum* Schumach. (side crops) and weeds

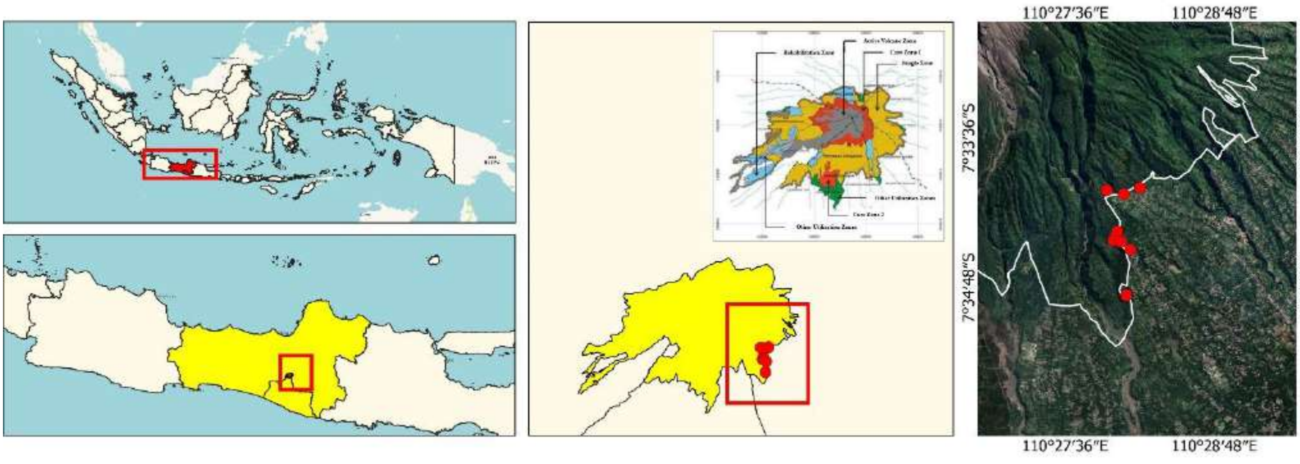

Figure 1. Location in the buffer zone of Mount Merapi National Park, Tegalmulyo and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Central Java, Indonesia (Hermawan et al. 2010)

Figure 2. Photographs of study sites in the buffer zone of Mount Merapi National Park, Tegalmulyo and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Central Java, Indonesia: A. Forest; B. Shrubland; C. Agricultural field

Procedures

The method used in this study was the exploration method purposive random sampling technique was used, and the sampling point was determined at the selected location based on the survey results accompanied by direct observation. The exploration method was carried out by observing along the track and recording each species found (Khoirurrais et al. 2021). The length of the track that became the research location was around 50-100 m in each habitat type in accordance with research conducted by Darsono et al. (2020). The total number of transects made at the research site was 12 transects consisting of 4 agricultural fields habitat type transects (2 morning and 2 noon), 4 shrublands habitat type transects (2 morning and 2 noon), and 4 forests habitat type transects (2 morning and 2 noon). The process of identifying insects obtained from the research site was documented directly. For insects that are difficult to identify, it was necessary to catch them using a sweeping net and photograph for documentation purposes (scan). Insects that have unknown species would be identified using several insect identification books, including Peggie (2006;2011;2014), Ilhamdi et al. (2020), Ruslan (2015), Ruslan and Dwi (2021), Rohman et al. (2019), Aprilia et al. (2018) and other sources from the internet, namely bugguide.net and the plant species identification are carried out based on databases of Plants of the World Online by the Royal Botanic Gardens, Kew (http://www.plantsoftheworldonline.org/) (POWO 2024) and the Global Biodiversity Information Facility by the world's governments (https://www.gbif.org/) (GBIF, 2024).

. The tools needed were an insect net (sweeping net) for the purpose of catching and identification, a mobile camera for documentation, a lux meter to measure light intensity, a hygrometer to measure air humidity in the sampling area, and an insect identification book. The observation time is carried out at 08.00-15.00. This aims to determine the comparison of the abundance of pollinating insects in the morning and evening. This research included quantitative descriptive research.

Data analysis

This study was analyzed in a quantitative descriptive manner. Quantitative descriptive analysis can describe data from calculations or measurements (Arisandy and Triyanti 2020). This study describes the results of the identification and calculation of pollinator insect diversity in several habitats using the diversity index, species evenness index, and species richness index with the following formula:

Shannon-Wiener Diversity Index

Species diversity is determined using the Shannon-Wiener diversity index formula by Odum (Fachrul 2007):

$$H' = -\Sigma \text{ Pi Ln Pi}$$

Where:
H': Species diversity index
Pi: Ratio of the number of individuals of a species for the whole species (ni/N)
Ni: Number of individuals of one species i
N: Number of individuals of all species

The quantitative value of the diversity of a species can be classified in the following range of values:

If the diversity index (H')<1.0 indicates low diversity

If the diversity index 1.0≤(H')≤3.0 indicates moderate diversity

If the diversity index (H')>3.0 indicates high diversity

Evenness Species Index

The Evenness Index is utilized by Baderan (2021) to determine the evenness of each species in each community by using the following formula:

$$E = H'/\text{Ln } S$$

Where:
E: Evenness index (values between 0-1)
H': Shannon-Wiener species diversity index
S: Number of species found

The evenness index value ranges from 0-1 with the following categories:

0<E≤0.4: Small evenness, depressed communities
0.4<E≤0.6: Moderate evenness, labile community
0.6<E≤1.0: High evenness, stable community

Margalef Species Richness Index

The species richness index can be calculated using the 1958 Margalef formula (Kent (2012); Mawazin and Subiakto (2013), in Anjani et al. 2022):

$$R = (S-1)/\text{Ln } N$$

Where:
R: Margalef species richness index
S: Number of species found

N: Number of individuals of all species

Criteria for the value of the Margalef Species Richness Index:
R<2.5: Low species richness
2.5<R<4: Medium species richness
R>4: High species richness

The Species Accumulation Curve (SAC)

The species accumulation curve is used to describe the increase in species with an increase in the number of samples (plots/attempts/transects). The SAC curve is used to assess the adequacy of sampling numbers and estimate species richness (Bevilacqua et al. 2018; Rong et al. 2020). The creation of the SAC curve is by connecting the number of plots/efforts/transects as the x-axis with the number of species as the y-axis so as to form a curve. The minimum number of transects can be determined using the SAC curve. If the increase in the number of transects does not cause an increase in the number of species, then the number of transects already represents the extent of the study (Astuti et al. 2020). According to Munthe (2013), the species accumulative curve is useful to show the representation system of the represented forest so that the biodiversity analysis carried out can represent the forest being studied and from the cumulative curve of the species can be determined the minimum plot/transect area or the minimum area used. The analysis of the species accumulative curve was carried out to determine whether the recorded species had represented the number of species in the study area.

RESULTS AND DISCUSSION

Identification of insect pollinators and their composition

Based on the results of the study, in three habitat types (forests, agricultural fields, and shrublands) obtained, 121 individuals of pollinator insects consisting of 55 species from 26 families and 5 orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera). The pollinator insect species are presented in Table 2. Pollinator insects found in Mount Merapi National Park include 9 species of the order Coleoptera, 8 species of order Diptera, 2 species of order Hemiptera, 4 species of order Hymenoptera, and 32 species of order Lepidoptera. Order Coleoptera consisting of pollinator insects of the families Brentidae (1 species), Buprestidae (2 species), Carabidae (1 species), Chrysomelidae (2 species), Coccinellidae (2 species), and Scarabaeidae (1 species). Furthermore, the order Diptera consists of the families Muscidae (2 species), Polleniidae (1 species), Sarcophagidae (1 species), Syrphidae (2 species), Tephritidae (1 species), and Dolichopodidae (1 species). Then, the order Hemiptera, consisting of the families Cicadidae and Scutelleridae with 1 species, was found. Then, the order Hymenoptera consists of the families Apidae (1 species), Eumenidae (1 species), and Vespidae (2 species). Finally, the order Lepidoptera includes pollinator insects from the families Crambidae (2 species), Erebidae (3 species), Hesperiidae (2 species), Lycaenidae (6 species), Nymphalidae (10 species), Papilionidae (1 species), Pieridae (6 species), Riodinidae (1 species), and Saturniidae (1 species). Several insect pollinator species in three habitat types around Mount Merapi National Park in Tegalmulyo and Sidorejo Villages have been documented (Figure 3).

Based on the results of insect observations, as shown in Table 2, pollinators are relatively more diverse in the morning compared to the afternoon. According to Mustakim et al. (2014), generally, pollinator insects visit in the morning starting from 07.00-10.00 a.m to coincide when the flowers bloom, where the volume or nectar in flowers is still relatively high, but over time towards noon and evening, the nectar content tends to decrease. These changes affect the degree of insect participation in plants in the process of pollinators. From the results of the research conducted, the number of pollinating insects most commonly found in the three habitats is the order Lepidoptera, with a total of 9 families and 32 species. This is due to suitable habitat and the availability of many food sources. Environmental conditions and food availability affect the presence of pollinator insects of the order Lepidoptera. The abundance of the order Lepidoptera is also supported by the presence of vegetation suitable for the survival of these pollinator insects, such as host plants and feed plants, both as a source of food and as a shelter (Yusup et al. 2023). Meanwhile, the least commonly found order is the Hemiptera order with 2 families, namely Cicadidae and Scutelleridae, and the Hymenoptera order with 3 families, namely Apidae, Eumenidae and Vespidae. Even though food is abundant at the location, there are ecological factors that could be more suitable for the life of this order. One of the reasons is the influence of environmental factors, namely when rainfall is observed to be quite high, which has an impact on the existence of these orders. Theoretically, animals will move from one environment to another when there is a temporary environmental change, such as rain. Animals will move towards an environment that better suits their needs. By moving from a changing environment, animals can inhabit environmental conditions that are most optimal for their survival (Pariyanto et al. 2019). Environmental factors in the form of rainfall can also influence the Lepidoptera order. However, the thing that might cause this order to be the most commonly found is the adaptability of the species in this order to environmental conditions. These adaptations may include the ability to shelter from rain, tolerance to high humidity, or flexible foraging behavior. The highest number of species in the Lepidoptera order is from the Nymphalidae family, which has 10 species. Based on Marlisa et al. (2022), the Nymphalidae family is cosmopolitan and widespread in many regions of the world. Species from the Nymphalidae family like to be in bright, open places with diverse vegetation. Therefore, Nymphalidae butterflies actively fly and are easy to find during the day. Apart from that, Nymphalidae butterflies like fruit-bearing plants such as in gardens and forests because they are also polyphagous. That is, they eat more than one type of plant (Ramadhani et al. 2024). This characteristic makes the Nymphalidae family from the Lepidoptera order have a high ability to survive in various types of habitats (Marlisa et al. 2022).

Table 2. Pollinator insect species in three habitat types in the buffer zone of Mount Merapi National Park, Tegalmulyo and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Central Java, Indonesia

Order	Family	Species name	Habitat type			Total individual	%
			Forest	Agricultural field	Shrubland		
Coleoptera	Brentidae	*Perapion punctinasum* (J.B.Smith, 1884)	1	0	0	1	0.83
	Buprestidae	*Chrysochroa fulgidissima* (Schönherr, 1817)	1	0	0	1	0.83
		Chrysochroa fulminans (Fabricius, 1787)	1	0	0	1	0.83
	Carabidae	*Lophyra striolata* (Illiger,1800)	1	0	0	1	0.83
	Chrysomelidae	*Aspidimorpha miliaris* (Fabricius, 1775)	2	0	0	2	1.65
		Aulacophora lewisii (Baly, 1866)	1	0	0	1	0.83
	Coccinellidae	*Coccinella magnifica (*Redtenbacher, 1843)	2	0	0	2	1.65
		Epilachna admirabilis Crotch, 1874	0	0	2	2	1.65
	Scarabaeidae	*Protaetia fusca* (Herbst, 1790)	0	0	1	1	0.83
Diptera	Muscidae	*Musca domestica* (Linnaeus, 1758)	6	1	5	12	9.92
		Musca vetustissima (Walker, 1849)	0	0	3	3	2.48
	Polleniidae	*Pollenia rudis* (Fabricius, 1794)	1	0	0	1	0.83
	Sarcophagidae	*Sarcophaga* sp.	1	0	0	1	0.83
	Syrphidae	*Dideopsis aegrota* (Fabricius, 1805)	2	0	0	2	1.65
		Mesembrius vestitus (Wiedemann, 1821)	1	0	0	1	0.83
	Tephritidae	*Rhagoletis pomonella* (Walsh, 1867)	2	0	0	2	1.65
	Dolichopodidae	*Chrysosoma crinicorne (*Wiedemann, 1824)	3	0	0	3	2.48
Hemiptera	Cicadidae	*Huechys incarnata* (Germar, 1834)	6	0	0	6	4.96
	Scutelleridae	*Chrysocoris stolli* (Wolff, 1801)	1	0	0	1	0.83
Hymenoptera	Apidae	*Apis cerana* (Fabricius, 1793)	2	0	1	3	2.48
	Eumenidae	*Delta campaniforme (*Fabricius, 1775)	1	0	0	1	0.83
	Vespidae	*Ropalidia fasciata* (Fabricius, 1804)	5	0	0	5	4.13
		Vespa tropica (Linnaeus, 1758)	1	0	0	1	0.83
Lepidoptera	Crambidae	*Arthroschista hilaralis* (Walker, 1859)	2	0	0	2	1.65
		Scirpophaga incertulas (Walker, 1863)	0	0	2	2	1.65
	Erebidae	*Calliteara horsfieldii* (Saunders, 1851)	0	1	0	1	0.83
		Nyctemera coleta (Stoll, 1781)	1	0	0	1	0.83
		Nyctemera adversata (Schaller, 1788)	2	0	0	2	1.65
	Hesperiidae	*Ampittia dioscorides* (Fabricius, 1793)	0	1	0	1	0.83
		Potanthus omaha (Edwards, 1863)	0	1	0	1	0.83
	Lycaenidae	*Flos anniella* (Hewitson, 1862)	3	0	0	3	2.48
		Heliophorus epicles subsp. *latilimbata* (Fruhstorfer, 1908)	1	0	0	1	0.83
		Jamides celeno (Cramer, 1775)	4	0	0	4	3.31
		Lampides boeticus (Linnaeus, 1767)	0	0	3	3	2.48
		Udara ceyx (Niceville, 1893)	1	0	0	1	0.83
		Udara dilectus (Moore, 1879)	2	0	0	2	1.65
	Nymphalidae	*Dryadula phaetusa* (Linnaeus, 1758)	1	0	0	1	0.83
		Euploea climena (Stoll, 1782)	2	0	0	2	1.65
		Euploea eunice (Godart, 1819)	0	0	2	2	1.65
		Heliconius cydno (H. Bates, 1864)	1	0	0	1	0.83
		Hypolimnas bolina (Linnaeus, 1758)	2	2	0	4	3.31
		Lethe confusa (Aurivillius, 1897)	1	0	0	1	0.83
		Melanitis leda (Linnaeus, 1758)	3	0	0	3	2.48
		Mycalesis janardana (Moore, 1857)	1	0	0	1	0.83
		*Ypthima pandocus (*Moore, 1857)	1	0	1	2	1.65
	Papilionidae	*Graphium sarpedon* (Linnaeus, 1758)	1	1	0	2	1.65
	Pieridae	*Cepora iudith* (Fabricius, 1787)	2	0	0	2	1.65
		Delias fojaensis (van Mastrigt, 2006)	0	0	1	1	0.83
		Eurema brigitta (Stoll, 1780)	1	1	0	2	1.65
		Eurema hecabe (Linnaeus, 1758)	8	2	0	10	8.26
		Leptosia nina (Fabricius, 1793)	0	2	0	2	1.65
		Pieris brassicae (Linnaeus, 1758)	0	2	0	2	1.65
	Riodinidae	*Zemeros flegyas* (Cramer, 1780)	2	0	0	2	1.65
	Saturniidae	*Cricula trifenestrata* (Helfer, 1837)	0	0	1	1	0.83
Total			83	14	24	121	100.00

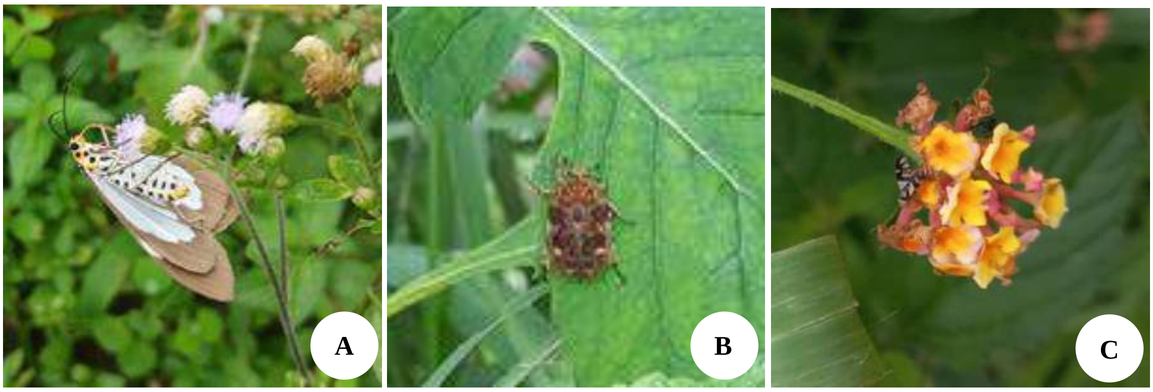

Figure 3. Insect profile of pollinator: A. *Nyctemera coleta*; B. *Protaetia fusca*; C. *Rhagoletis pomonella*

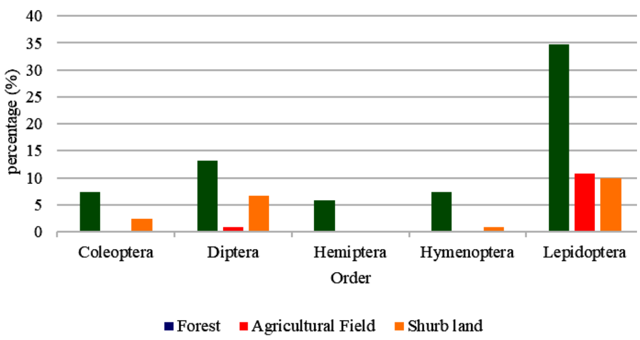

Figure 4. The composition of the order of pollinator insects according to the percentage of individual number

According to Febriyanti et al. (2020), the diversity of the number and type of pollinator insect populations is influenced by the availability of food sources, especially plants that produce fruit. The attractiveness of fruit to certain pollinating insects, which can be influenced by the color and aroma of the fruit, is a factor influencing pollinator diversity. Flowering plants can also affect the presence of pollinating insects. The number and shape of flowers of a plant have a close relationship with the availability of nectar and pollen produced. The more abundant nectar and pollen available on a flower, the higher the chance that pollinator insects will be present to visit the flower. In other words, the availability of nectar and pollen in flowers can be a determining factor in attracting and maintaining the presence of pollinating insects in an ecosystem (Hidayat et al. 2016). Favorable habitat conditions also play an important role, as many host plants support the life of pollinating insects such as honeybees, fruit flies, and their larvae, protecting them from predators and contributing to the survival of the ecosystem. In addition, environmental factors such as temperature, light, and humidity also affect the diversity and abundance of pollinating insects in an ecosystem (Purwantiningsih et al. 2012).

Of the 55 species of pollinator insects found in all habitat types, the highest abundance is a forest habitat type, with species from the order Lepidoptera numbering 42 species, and the least found is a species from the order Hemiptera with 7 individuals found. The agricultural field habitat type is only found in 2 orders: Lepidoptera, which has as many as 13 individuals, and Diptera, which has only 1 individual. The type of shrublands habitat of the order Lepidoptera is the order with the most individuals, with 12 individuals, and the least is the order Hymenoptera, with only 1 individual. Overall, the species found based on the habitat type of the order Lepidoptera became the order with the most individuals, 55.37%. The lowest was the order Hemiptera, with a percentage of only 5.79%, while the other orders, Coleoptera, Diptera, and Hymenoptera, respectively, were 9.92, 20.66, and 8.26% (Figure 4).

Pollinator insects have visited flowering plants

Based on the identification of plants visited by pollinating insects in several habitat types in Tegalmulyo and Sidorejo Villages (Table 3), 15 plant species were obtained and divided into 11 different families. The families are Asteraceae, Balsaminaceae, Commelinaceae, Fabaceae, Gramineae, Malvaceae, Poaceae, Rosaceae, Solanaceae, Theaceae, Verbenaceae. The families Asteraceae and Fabaceae are the largest, each with 3 species. According to Robson in Sukarsa et al. (2022), these two families are among the plant families most visited by pollinating insects because they have small flowers with colors favored by pollinating insects such as bees. The identification results showed that the flowers found had various colors, such as red in *kaliandra* flowers (*Calliandra calothyrsus*) (Figure 5.D), white in *bandotan* flowers (*Ageratum conyzoides* L.) (Figure 5.A), blue in *gewor* grass flowers (*Commelina benghalensis*) (Figure 5.C), yellow in *Acacia* flowers (*Acacia decurrens* Willd.) (Figure 5.E). In addition, the identification results also show that the flowers found have a distinctive and diverse aroma, such as the fragrant aroma of roses (*Rosa* sp.) and the distinctive scent of water henna flowers (*Impatiens walleriana*). The variety of colors and aromas in the flowers found can attract many pollinating insects to perch and directly help the pollinator's process (Syahbanuari et al. 2020).

Table 3. The results of the inventory and identification of flowers visited by pollinator insects in the buffer zone of Mount Merapi National Park, Tegalmulyo and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Central Java, Indonesia

Family	Species name	Local name	T	P	Sp	S	IUCN
Asteraceae	*Ageratum conyzoides* L.	*Bandotan*	-	-	-	●	LC
Asteraceae	*Crassocephalum crepidioides* (Benth.) S.Moore	*Sintrong*	-	-	-	●	NE
Asteraceae	*Tithonia diversifolia* (Hemsl.) Gray	*Paitan*	-	-	-	●	LC
Balsaminaceae	*Impatiens walleriana* Hook.f.	*Pacar air*	-	-	-	●	NE
Commelinaceae	*Commelina benghalensis* L.	*Gewor*	-	-	-	●	LC
Fabaceae	*Calliandra calothyrsus* Meisn.	*Kaliandra*	●	●	-	-	NE
Fabaceae	*Acacia decurrens* Willd.	*Akasia*	●	-	-	-	NE
Fabaceae	*Coffea arabica* L.	*Kopi Arabika*	-	●	●	-	EN
Gramineae	*Imperata cylindrica* L.	*Alang-alang*	-	-	-	●	NE
Malvaceae	*Sida acuta* Burm.fil.	*Galunggang*	-	-	-	●	NE
Poaceae	*Pennisetum purpureum* Schumach.	*Suket Gajah*	-	-	-	●	LC
Rosaceae	*Rosa* sp.	*Mawar*	-	-	-	●	NE
Solanaceae	*Capsicum annum* L.	*Lombok ijo*	-	-	-	●	LC
Theaceae	*Schima wallichii* (DC.) Korth.	*Puspa*	●	●	-	-	LC
Verbenaceae	*Lantana Camara* L.	*Tembelekan*	-	-	-	●	NE

Note: T: Tree, P: Pole, Sp: Sapling, S: Seedling. NE: Not Evaluated, LC: Least Concern, EN: Endangered. ●: Include, -: Not Included

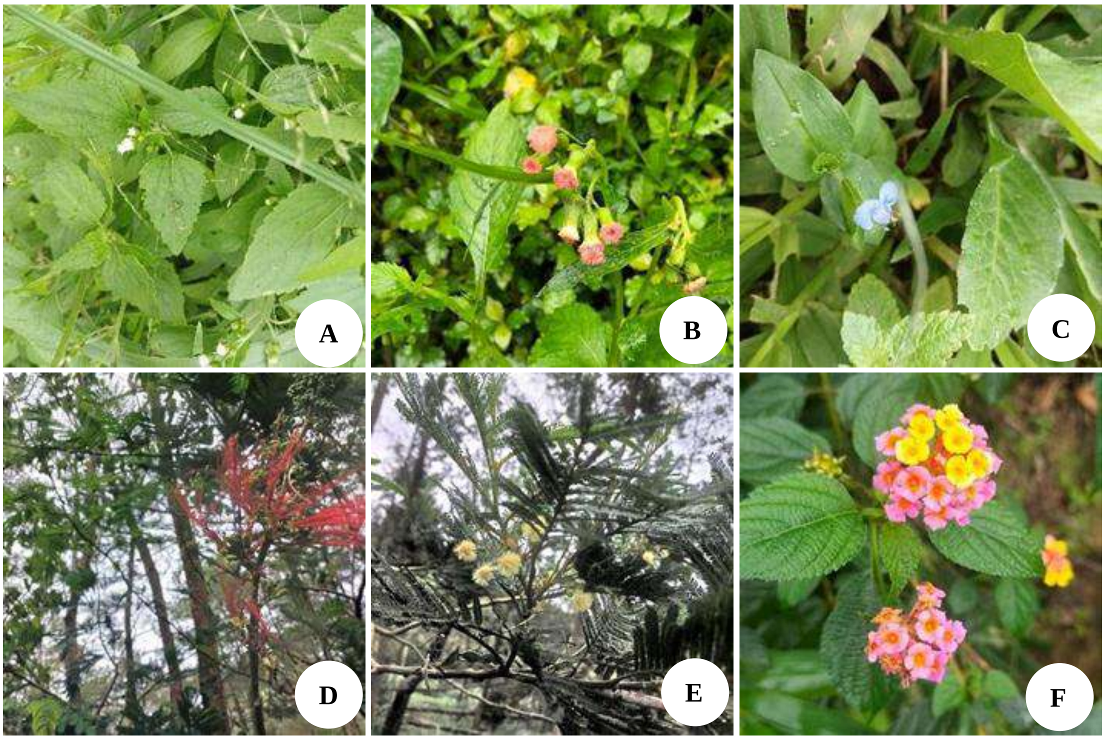

Figure 5. Plant profile infested with pollinator insect: A. *Ageratum conyzoides*; B. *Crassocephalum crepidioides*; C. *Commelina benghalensis*; D. *Calliandra calothyrsus*; E. *Acacia decurrens*; F. *Lantana camara*

Based on the identification results, it is known that the majority of flowering plants found in Tegalmulyo and Sidorejo Villages are seedling groups. This is because the flowering plants in the two villages are mostly flower and shrub plants that grow wild or are planted in fields and yards of houses. Additionally, several flowers have been observed growing in the Tegalmulyo and Sidorejo Villages, with Arabica coffee (*Coffea arabica*) being one of their most prominent crops. Arabica coffee has compound flowers and several white crowns and petals (Kurnia et al. 2023). The pollinator process in arabica coffee can be done alone but can also be assisted by several pollinating insects such as honey bees (*Apis cerana*) (Sitompul et al. 2017). Among all flowering plants, Arabica coffee is the highest ranked IUCN red list category and is also part of the EN or Endangered category. It is crucial to consider all aspects of Arabica coffee plant cultivation, including pollinator insects that aid the pollination process, to ensure sustainability.

The index for diversity, richness, and evenness

The results of the calculation of the diversity index, richness index, and pollinator insect evenness index by order and overall in Tegalmulyo and Sidorejo villages can be seen in Table 4. The species diversity index of all pollinator insects obtained was 3.731. Based on Shannon Wiener's diversity index classification in Hutami et al. (2022), a diversity index of more than 3 belongs to the high diversity group. The value of the species richness index of all species shows that the location has a high species richness, which is 11.26. The criteria for high wealth stated by Margalef in Nisa et al. (2022) states that it must be worth more than 4. While the evenness of existing species is 0.93, it can be said that the existing species are almost evenly distributed. The evenness index value close to 1 means that the distribution of species in the region is evenly distributed (Latumahina et al. 2020). The highest values of diversity, richness, and evenness indices are in the order Lepidoptera. In contrast, the lowest value of the diversity and richness index is the order Hemiptera. Meanwhile, the lowest value of the evenness index is the order Coleoptera.

These three index results are related to each other because, in general, diversity is a combination of aspects of richness and evenness (Rousseau et al. 1999). The higher the diversity there is, indicating the balance of the ecosystem. The formation of ecosystem balance affects the equitable index. The evenness of species indicates the stability of the environment because there is no one species that dominates, and this causes unequal competition between species. Based on the results of the study, although 2 species were obtained with a higher number of individuals than other species, based on the calculation of the evenness index analysis, a relatively high evenness value was obtained, so the existence of each species is in a stable condition. This also means that the uniformity of species in the environment has an even distribution of species (Septiningtyas et al. 2022). The diversity index is also an indicator of pollution because the index will decrease if there is environmental pollution in an increasing area (Hosokawa et al. 2021).

The Species Accumulation Curve (SAC)

The species accumulation curve (SAC) is another popular tool in the analysis of species abundance data. This survey is seen as a data collection process where more and more sampling efforts are being poured in. Individual-based SAC is the number of recorded species expressed as a function of the number of sampling attempts (Li and Li 2022). Using the species accumulation curve to assess whether the number of sampling is sufficient is based on the characteristics of the curve: if the curve has risen sharply, almost in a straight line, this indicates that the number of sampling is insufficient and the number of sampling needs to be increased; if the curve becomes an asymptomatic line after a sharp increase and then increases slowly, This indicates that sampling is sufficient and data analysis can be performed (Rong et al. 2020).

The results of making the SAC of this study are presented in Figures 6 and 7. SAC (Figure 6) is the location of each habitat type, a curve that forms an asymptotic line and increases slowly. The type of forest habitat shows the highest increase in the number of species so that it is drawn on a sharp upward line curve. SAC as a whole habitat is presented in Figure 6. As a result, the curve forms an asymptotic line with a slow increase. The highest increase in the number of species occurred in transects 2, 3, 6, and 9. On the curve this is drawn with a sharp upward line.

Table 4. An index measuring the diversity, richness, and evenness of pollinators insects in the buffer zone of Mount Merapi National Park, Tegalmulyo and Sidorejo Villages, Kemalang Subdistrict, Klaten District, Central Java, Indonesia

Order	Species diversity index	Species richness index	Species evenness index
Coleoptera	0.44	1.67	0.20
Diptera	0.67	1.46	0.32
Hemiptera	0.19	0.21	0.27
Hymenoptera	0.30	0.63	0.22
Lepidoptera	2.13	6.46	0.62
Total	3.731	11.26	0.93

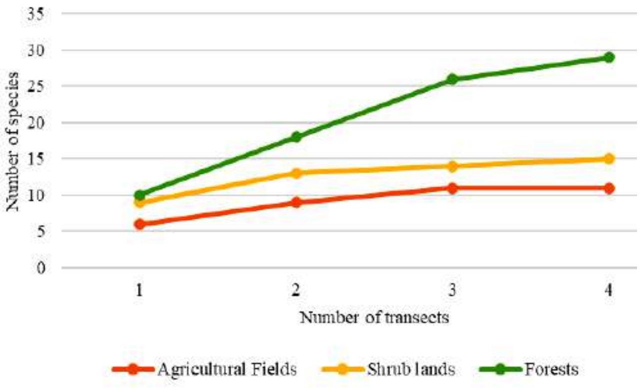

Figure 6. Species accumulation curve based on habitat type

Table 5. Three types of habitats and their environmental factors

Environmental factors	Forest	Agricultural field	Shrubland
Air temperature (°C)	22.1	24.2	24.9
Air humidity (%)	94.5	72	57.5
Light intensity (Cd)	14,750	27,000	28,750

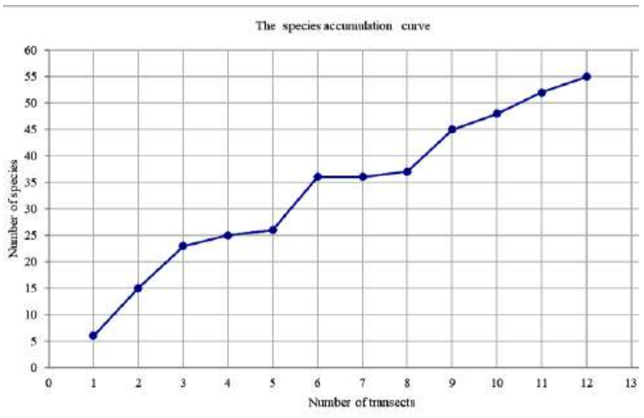

Figure 7. Species accumulation curve (SAC)

The results showed that the two curves drawn had enough sampling to be analyzed. In the curve of Figure 6, the forest habitat type is the location with the highest addition of species, namely transect 1, then shrubland in transect 2 and finally agricultural field that increases continuously 3 species from transects 1, 2, 3, and 4 there is no addition of any more species. This shows that the research location with the type of agricultural field habitat is enough to make a total of 4 transects can represent the habitat type, while for other habitat types, it is recommended to make transects more than 4 because there are still additional species. Overall, SAC the research location is presented in Figure 7. The highest increase in the number of species occurred in transects 2, 3, 6, and 9 respectively, the number of species was 9, 8, 10, and 8. The number of transects 12 made is still an addition of species so that for further research it is recommended to make transects more than 12.

Abiotic environmental factors

Abiotic factors that influence the presence of pollinating insects in the three habitat types measured are air temperature, air humidity, and light intensity (Table 5). Air temperature and light intensity in forest habitat types taken directly at that time were lower than the other two habitat types, namely 22°C and 14,750 Cd. This is because, in forest habitat types, sunlight is blocked by trees that shade the area, so the air temperature is also low. This is also related to the measured air humidity. The air will tend to be more humid in an environment that gets little sunlight. These environmental factors also affect the presence and activity of pollinating insects in the vicinity. Pollinating insects have ecological thresholds that limit their foraging activities (Abrol et al. 2019).

Table 5 shows the result of measuring environmental factors at the research site in the form of air temperature, air humidity, and light intensity. The highest air temperature at the time of measurement of environmental factors was found in shrublands, which was 24.9°C, while the lowest air temperature was 22.1°C, which was found in the forest. According to Bhatnagar et al. (2010), pollinator activity in flowering is strongly influenced by temperature factors. The average air temperature in three habitat types is included in the optimal temperature range for active pollinators, namely 15 to 40°C (Koneri et al. 2021). However, exposure to higher temperatures impacts flowers, so flowers become less attractive to pollinating insects, leading to decreased intensity of pollinator visits (Descamps et al. 2021). The next factor affecting the activity and appearance of pollinators is the intensity of light. Light intensity that was measured in three habitats of this study is in the range of 14,750-28,750 Cd. In addition, sunlight is a crucial need for plants. Exposure to optimal light intensity can increase flower productivity so that it can attract pollinators. In addition, light intensity is important for pollinators such as bees to aid navigation in searching for flowers (Hilário et al. 2000 in Usha et al. 2020). The intensity of sunlight and temperature affect the humidity of the air. The higher the intensity of sunlight and temperature, the lower the air humidity, or it can be said that the air is relatively drier and vice versa. The last environmental factor that was measured was air humidity. In this study, the highest air humidity was 94.5, which was found in the forest, and the lowest air humidity was 57.5, which was found in shrublands. The form of land cover influences the result of the measurement of air humidity. Therefore, the humidity in the air is higher than in the shrublands. Air humidity significantly affects the blooming time of flowers that are appealing to pollinators, as demonstrated by Sukariawan's research (2023). The transpiration process is a factor in flower buds' blooming.

Based on research that has been conducted in three different habitats in Tegalmulyo and Sidorejo Villages, 121 pollinator insects were found consisting of 55 species, 26 families, and 5 orders, namely Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera. The order Lepidoptera is the most common pollinator insect found in all three habitats, consisting of 9 families and 32 species. This is because environmental conditions and food availability in the area are very suitable for the survival of Lepidoptera. Several flowering plants are infested with pollinator insects; 15 plant species were obtained and divided into 11 different families. The families Asteraceae and Fabaceae are the families with the highest number of species visited by pollinating insects because they have small flowers with colors that are attractive to pollinating insects. Based on the calculation of several ecological indices in all habitats, pollinator insects that have been identified have a species diversity index value of 3.731, a species richness index of 11.26, and a species evenness index of 0.93. The results indicate that the identified pollinating insects have high diversity and species richness, with a fairly high level of evenness. Based on the results of the SAC, we recommend to future researchers to make the number of transects more than 12 if conducting research in similar locations until there are no more species additions. Based on the results of measurements of abiotic factors, the three habitats have different temperatures, humidity, and light intensities that can affect the presence of pollinating insects and flowering plants. In addition, the research location has a good ecosystem for pollinator insect habitats so that it can increase agricultural productivity to support food security.

ACKNOWLEDGEMENTS

The authors would like to thank the head of Environmental Science Program, Sebelas Maret University, Indonesia, the head of Mount Merapi National Park (MMNP), and the field guide during the research, i.e. Dhani, Martono and Sarjino, as well as drivers and community groups driving ecotourism in Sidorejo Village, Klaten, Indonesia (Omah Ijo Deles) who helped smooth accommodation during the research.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 160-173 DOI: 10.13057/asianjagric/g090117

Growth vitamin hormone and shading intensity affect the rhizo-caulogenesis of etiolated avocado microclonal rootstocks

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Manuscript received: 3 December 2024. Revision accepted: 7 February 2025.

Abstract. *Esgrina FJO, Tan RJ. 2025. Growth vitamin hormone and shading intensity affect the rhizo-caulogenesis of etiolated avocado microclonal rootstocks. Asian J Agric 9: 160-173.* The propagation of avocado clonal rootstocks is essential for a resilient, productive, and quality commercial cultivation. Optimizing growth conditions, particularly shading intensity and vitamin hormone concentration, plays a critical role in enhancing rhizogenesis, caulogenesis, and other physiological traits. Despite substantial research, the combined effects of these factors remain insufficiently explored. Thus, this study investigated the effects of shading intensity (40, 60, and 80%) and vitamin hormone concentrations (0, 0.1, 0.2, and 0.3 mL/L of water) on key morphological and physiological parameters of etiolated avocado clonal rootstocks, using a split-plot arrangement in randomized complete block design. The results revealed significant interaction effects (1%, Tukeys' HSD) between shading intensity and hormone concentration in all parameters. The highest root number (1.33), root length (0.53 cm), chlorophyll content (61.10 SPAD), fresh weight (25.53 g), dry weight (10.33 g), were observed under 80% shading with 0.2-0.3 mL of vitamin growth hormone/L of water. Moderate shading (60%) combined with 0.1-0.2 mL of vitamin growth hormone promoted stem elongation (25.81 cm), and leaf area (100.99 cm²). These findings underscore the necessity of tailoring propagation protocols to specific environmental and hormonal conditions. The study provides comprehensive insights into optimizing avocado rootstock propagation, contributing to sustainable agricultural practices and supporting the growing global demand for avocados.

Keywords: Avocado clonal rootstocks, rhizogenesis and caulogenesis, rootstock propagation techniques, shading intensity, vitamin hormone concentration

INTRODUCTION

Avocado (*Persea americana* Mill.), known for its exceptional nutritional benefits and economic potential, has become increasingly popular across the globe in recent years (Bhore et al. 2021; Bangar et al. 2022; Sora 2023; Subba et al. 2023). This popularity stems from the fruit's high content of healthy fats, vitamins, and antioxidants, which contribute to its growing demand in both the food industry and health sectors. Despite the widespread recognition of avocado's value, the commercial success of its cultivation heavily relies on the effective management of its propagation, especially the use of clonal rootstocks. These rootstocks play a critical role in improving avocado's resilience to environmental stresses, enhancing fruit quality, and ensuring consistent yield. Clonal rootstocks are integral to ensuring consistent yields and reducing the impact of environmental challenges. Thus, understanding the factors that influence the propagation of these rootstocks is essential for maximizing production efficiency and ensuring the sustainability of the avocado industry.

Rootstock propagation, especially when using clonal methods, is influenced by several important factors, including shading intensity and the use of growth hormones. Shading intensity plays a big role in how well a plant can photosynthesize, which directly affects its growth and overall health. If there's too much or too little shade, the plant struggles to get the right amount of light for photosynthesis, slowing down growth and weakening its roots. On the other hand, growth hormones like auxins and cytokinins help regulate key processes like root development and cell division, making it easier for the plant to grow strong and healthy. By carefully managing both shading and hormone treatments, growers can optimize the propagation process and produce rootstocks that are more resilient and productive (Blakey et al. 2015; Alon et al. 2022; Lahak et al. 2024). Meanwhile, the application of specific growth hormones regulates processes which are vital for successful propagation (Hiti-Bandaralage et al. 2017; Khawas and Upadhyay 2022; Shindre et al. 2023). Many studies - previous and current - have highlighted how these factors influence critical physiological processes, such as photosynthesis (Yang and Li 2017; Müller and Munné-Bosch 2021; Wang et al. 2021; Elango et al. 2023), root development (Miotto et al. 2021; Yoon et al. 2021; Zhou et al. 2022), and auxin distribution (Iglesias et al. 2018; Yang et al. 2018; Xie et al. 2022), which are essential for robust plant growth.

Although there has been considerable research on the asexual propagation of avocado (Barceló-Muñoz and Pliego-Alfaro 2003; Kasana et al. 2024; Li et al. 2024; Williams et al. 2024), the combined effects of shading intensity and hormone concentration on rootstock development remain poorly understood. Despite progress in understanding how individual factors like light and hormones affect plant growth, there is still a gap in knowledge regarding how these variables interact to influence the development of avocado rootstocks. Existing studies have largely focused on these variables in isolation, failing to investigate their potential synergistic interactions (Lovatt and Salazar-García 2005; Tinyane et al. 2018; Supriyanto and Yulianto 2022; Esgrina and Tan 2024; Ibtissem et al. 2024). This research gap has resulted in a lack of comprehensive guidelines for optimizing avocado rootstock propagation protocols, which could otherwise enhance efficiency and success rates in commercial production. Therefore, there is a pressing need to explore how these factors interact, as their combined influence could potentially offer new insights into better propagation practices.

To address this knowledge gap, this study explored shading and vitamin hormone interaction effect on the rhizogenesis (root formation) and caulogenesis (stem development) of etiolated avocado clonal rootstocks. By systematically varying shading and hormone treatments, it seeks to identify the most effective combinations that promote root and shoot development.

The potential impact of this research extends beyond theoretical understanding. By advancing propagation techniques for avocado clonal rootstocks, the findings could contribute to sustainable agricultural practices, enhancing the global supply of high-quality avocados. Moreover, by bridging the current knowledge gap regarding the combined effects of shading intensity and hormone application, this study could offer valuable insights to growers and researchers, enabling them to develop more efficient propagation protocols. Ultimately, the study's outcomes would not only improve the economic viability of avocado cultivation but also contribute to its ecological sustainability, ensuring that future avocado production meets the growing global demand.

MATERIALS AND METHODS

Study area

The study on the interaction of shading intensity and growth vitamin hormone on the shoot and root development of etiolated avocado microclonal rootstocks was conducted at the Horticulture Nursery, College of Agriculture, Central Mindanao University (7.8592°N, 125.0515°E, 311 masl) (googlemap.com 2024) on March to October 2024.

The local climatic data (Figure 1) from Department of Science and Technology Philippine Atmospheric, Geophysical, and Astronomical Administration (DOST-PAGASA) (2024), show a general trend of warm temperatures and fluctuating humidity levels. Minimum temperatures range from 21.93℃ in July to 23.17℃ in May, while maximum temperatures peaked at 34.98℃ in May and dropped to 30.84℃ in September. Humidity levels are relatively high, particularly in July (75.6%) and September (75.9%), with June, August, and October also showing values above 70%. Overall, the data indicate a consistent warm climate with higher humidity towards the mid-year months, particularly during the rainy season, with a slight decrease in humidity during the hotter months of April and May (El Niño phenomenon).

Avocado growth thrives within temperature of 20-30℃ but the recorded maximum temperatures and lower humidity (<70%) suggest conditions slightly above or below the optimal. Hence, the researchers aligned their propagation practices with these environmental factors. There was, in fact, an El Niño phenomenon during this time. Thus, irrigation and misting system were implemented during dryer months to maintain a humid microclimate in an outdoor nursery setting which would be conducive for the clonal rootstocks. The provision of shading nets also helped maintain moderate temperature in the area and reduced the occurrence of plant stress.

Materials and equipment

In this study, the researchers used a range of materials and tools to support the propagation and study process. They employed 'Hass' avocado clonal rootstock and 'Evergreen' nurse seedlings for propagation, provided net shading to the clonal stocks, and applied Hormex® growth vitamin hormone as an exogenous auxin to promote root and shoot development. In an outdoor nursery setup, the soil mix of cocopeat, garden soil, decayed rice hull, and vermicompost was used for planting. They also utilized water sprinklers, misting system, polyethylene bags (8" x 10"), and various working tools like bolos, spades, scissors, and cutters.

For measurement and data collection, they used rulers to take length, SPAD meter (Want® Brand) to measure the amount of chlorophyll, Vernier caliper for taking the diameter, and mobile applications like Easy Leaf® and MunCell® to assess leaf area and leaf color. Statistical analysis was done using Statistical Tool for Agricultural Research (STAR) software ver. 2.0.1, a program developed by the International Rice Research Institute (IRRI), Black Geena pongee cloth was used to control light exposure during etiolation of clonal rootstocks in the boxes. These materials and tools were crucial for assessing the impact of growth conditions on the development of Hass avocado rootstocks.

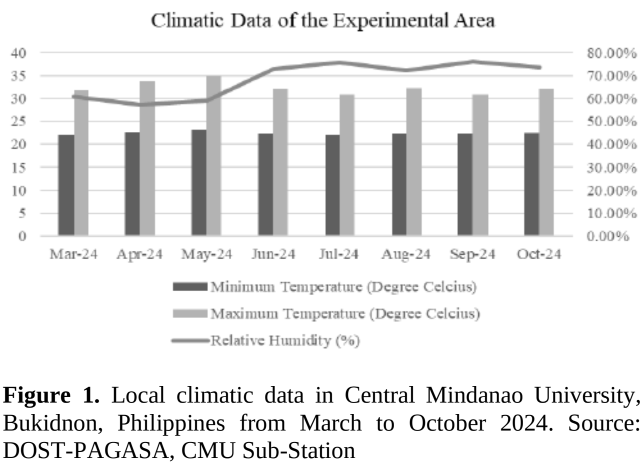

Figure 1. Local climatic data in Central Mindanao University, Bukidnon, Philippines from March to October 2024. Source: DOST-PAGASA, CMU Sub-Station

The researchers utilized an existing outdoor nursery shed as the experimental area, performed necessary repairs and modifications to meet the study's specific requirements and ensure its suitability. They repaired structural damage, improved ventilation, optimized lighting, installed misting systems, and enhanced the drainage system.

'Evergreen' avocado seeds, sourced from local farmers in Barangay Kulaman Valley, Arakan, Cotabato, Philippines, were used as seedling nurses. This approach ensured the use of genetically adapted material that is typically well-suited to the area's environmental conditions. Additionally, clonal rootstocks of the 'Hass' variety were acquired from a private grower in Makilala, Cotabato, Philippines, likely chosen to ensure high-quality rootstock from a trusted supplier.

Experimental design and treatments

The experiment was carried out using a Split Plot arrangement within a Randomized Complete Block Design (RCBD), where the intensity of shading served as the main plot and the concentrations of vitamin hormone were assigned as subplots. Three main plots and four subplots were replicated three times, with each treatment consisting of seven microclones-five of which were used as sample plants and two as buffers. In total, 252 ‘Evergreen’ nurse seedlings and 252 ‘Hass’ clonal rootstocks were used in the study. The researchers used the STAR ver. 2.0.1 to carry out the randomization and field layout of the study.

The treatments applied were as follows: for the main plot, the intensity of net shading included 40% (A1), 60% (A2), and 80% (A3) net shading, while the subplot involved varying concentrations of Hormex growth vitamin hormone: control (B1), 0.5 recommended rate (0.1 mL of vitamin hormone per liter of water, B2), recommended rate (0.2 mL of vitamin hormone per liter of water, B3), and 1.5 recommended rate (0.3 mL of vitamin hormone per liter of water, B4).

Pre-germinating and growing of ‘Evergreen’ seedling nurse

The activity began with pre-germinating 'Evergreen' avocado seeds in beds of 100% cocopeat (Figure 2.A), chosen for its moisture retention and aeration properties. Once germinated, the seeds were transplanted into individual polyethylene bags (8” × 10”) to provide space for root growth and uniform development. The growing medium consisted of 30% garden soil, 30% vermicompost, 30% soil compost, and 10% decomposed rice hull, balancing nutrients, water retention, and drainage. Organic materials enriched the soil, while rice hulls improved aeration. The seedlings were nurtured until their stems reached at least 6 mm in diameter, signaling readiness for grafting, with careful attention to optimal conditions for both stem and root growth.

Grafting of clonal rootstocks

Once the 'Evergreen' stock seedlings reached the desired 6 mm diameter, or "pencil size," they were ready for grafting (Figure 2.B). This occurred about eight weeks after planting the 'Evergreen' seeds in polyethylene bags. The 'Hass' clonal stocks were then grafted onto the 'Evergreen' seedlings, with the aim of establishing a strong connection between the scion and rootstock. By grafting at the optimal growth stage of the stock seedlings, the researchers maximized the chances of successful grafting and healthy plant development.

Etiolating grafted clonal rootstocks

Etiolation boxes were especially designed to create a controlled environment for the experimental plants, using wooden frames for stability and covering them with black Geena pongee cloth (Figure 2.C). This cloth served to block light, inducing etiolation. This setup allowed researchers to observe plant responses to reduced light exposure and monitor the morphological and physiological changes associated with etiolation. After bud break in the clonal rootstocks, the microclones were transferred into the etiolation boxes that provided sufficient ventilation and space for each treatment. The plants were kept in darkness at ambient temperatures, with shoots allowed to grow for 4 weeks post-bud break, representing different stages of shoot development under limited light conditions.

Selecting the most desirable etiolated clonal rootstock

200 to 300 mm (20 to 30 cm) long clonal rootstocks were selected for the rooting experiment (Figure 2.D), as this length is considered optimal for successful rooting. According to the studies by Ernst (1999) and Esgrina and Tan (2024), the ideal length for clonal rootstocks, which allows for better root initiation, the rootstocks were subjected to a period of 2 to 6 weeks of etiolation. By this process, rootstocks were placed in the etiolation boxes covered with Geena pongee cloth to allow low light promote elongation. It helped the clonal rootstocks reach the desired size and characteristics that enhance their ability to form roots once exposed to the different shading intensities and vitamin growth hormone concentrations.

Applying Hormex® growth vitamin hormone

Different concentrations of the growth vitamin hormone were applied to the etiolated ‘Hass’ clonal stocks through an incision made at the base of the etiolated stem (Figure 2.E). The concentrations tested included 0, 0.1, 0.2, and 0.3 mL/L of water. The vitamin hormone formulation consisted of Thiamine Hydrochloride (Vitamin B1) at 25%, Naphthyl Acetic Acid (NAA) at 24%, and Indole Butyric Acid (IBA) at 13%.

Fixing the rooting devices at the base of the etiolated shoot

Spherical rooting devices, each measuring 5 centimeters in diameter, were filled with sterile 100% cocopeat as the rooting media to ensure a clean and controlled environment for root development (Figure 2.F). To further support the growth of the clonal rootstocks, a pinch of slow-release fertilizer (Osmocote) was placed on the surface of the cocopeat within each rooting device. This fertilizer provided a steady supply of nutrients over time, promoting healthy root and shoot growth while reducing the need for frequent fertilization. The combination of cocopeat and Osmocote helped create an optimal rooting medium that encouraged the successful establishment of the clonal rootstocks.

Placing the plants in shade

The plants, with their etiolated shoots and leaves protruding from the media-filled micro-containers, were exposed to varying light intensities of 40, 60, and 80% net shading (Figure 2.G). This controlled light exposure triggered photosynthesis, gradually strengthening and hardening off the plants as they adapted to increasing light conditions. As the plants acclimated, both root initiation and shoot elongation occurred simultaneously, with the roots beginning to form and the shoots continuing to grow and elongate. Sixty days after this, data were gathered by the researchers (Figure 2.H).

Monitoring the daily air temperature and relative humidity

The daily local climate data, including humidity and temperature (minimum and maximum), were obtained from the Department of Science and Technology, Philippine Atmospheric Geophysical and Astronomical Services Administration (DOST-PAGASA) station at Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. Additionally, an outdoor thermometer/ hygrometer was placed in the nursery area to monitor the daily temperature and relative humidity of the immediate environment. Readings were taken at 8:00 a.m. and 2:00 p.m. to record the minimum and maximum temperatures and humidity levels for each day. To mitigate high temperatures and maintain humidity above 70%, the plants were regularly sprinkled, and a misting system was used in the outdoor nursery shed.

Figure 2. Graphical sketch of the study's activities: A. Pre-germinating and growing of seedling nurse; B. Grafting of clonal rootstocks; C. Etiolating grafted clonal rootstocks; D. Selecting the most desirable etiolated clonal rootstocks; E. Applying growth vitamin hormone; F. Fixing the rooting devices at the base of the etiolated shoots; G. Placing the plants in shade; and H. Gathering of data

Statistical analysis

After data collection (Figure 2.H), an Analysis of Variance (ANOVA) was conducted to assess the significance of the treatment differences. This statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 software, which is specifically designed for advanced data analysis in agricultural research. ANOVA allowed the researchers to determine whether the observed variations in the measured parameters were statistically significant. Following ANOVA, a post hoc analysis was done using Tukey's Honestly Significant Difference (HSD) test for parameters that showed significant differences based on the F-value from the ANOVA. Tukey's HSD test enabled pairwise comparisons of treatment means, allowing researchers to pinpoint specific differences between treatment groups. This step was crucial for understanding the nature and extent of differences among treatments, providing more detailed insights into how experimental variables affected the outcomes. In summary, the use of ANOVA and Tukey's HSD test provided a thorough statistical framework for analyzing and interpreting the results, helping researchers draw meaningful conclusions from the data.

RESULTS AND DISCUSSION

Table 1 displays the results of the Analysis of Variance (ANOVA) performed on avocado clonal rootstocks, highlighting the impact of shading intensity, hormone concentration, and their interaction on different root and shoot parameters, except for the stem diameter. Shading intensity independently influenced certain parameters, including root number, root length, and stem diameter. On the other hand, the concentration of growth hormone significantly impacted all the parameters listed in the table. Moreover, Table 2 shows that all parameters, except stem diameter, were significantly affected by the interaction effect of shading intensity and vitamin growth hormone.

Table 1. Summary of the analysis of variance for the effects of shading intensity, hormone concentration, and their interaction on root and shoot parameters of avocado clonal rootstocks

Factors	Root number	Root length (cm)	Stem length (cm)	Stem diameter (cm)	Leaf number	Leaf area (cm^2)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf chlorophyll content (SPAD)
Shading intensity	**	**	**	ns	ns	ns	ns	ns	ns
Hormone concentration	**	**	**	**	**	**	**	**	**
Shading intensity x hormone concentration	**	**	**	ns	**	**	**	**	**

Note: ** = highly significant; ns = not significant (Tukeys' HSD test of significance)

Table 2. Interaction effect of shading intensity and growth hormone concentration on the different parameters of avocado clonal rootstocks

Shading intensity (%)	Growth hormone concentration (mL/L of water)	Root number **	Root length (cm)**	Stem length (cm)**	Stem diameter (cm)ns	Leaf number**	Leaf area (cm^2)**	Fresh weight (g)**	Dry weight (g)**	Leaf chlorophyll content (SPAD)**
40%	Control (0)	0.40b,A	0.90a,A	25.85a,B	0.625	9.07b,AB	85.37b,B	21.47a,AB	8.27a,AB	46.85b,B
	0.1	0.00b,B	0.00b,B	32.07a,A	0.634	10.40a,A	83.59b,B	25.13a,A	10.13a,A	57.04a,A
	0.2	0.00a,B	0.00a,B	18.87b,C	0.623	7.07b,B	110.56a,A	17.27a,B	6.93ab,B	56.18b,A
	0.3	0.00b,B	0.00b,B	20.09a,C	0.626	9.53ab,A	102.78b,A	21.67ab,AB	8.73ab,AB	50.71b,B
60%	Control (0)	0.00c,B	0.00b,B	21.99b,B	0.601	9.53b,B	99.49a,A	20.27a,B	7.60a,AB	53.31a,A
	0.1	0.20[a],A	0.72a,A	25.81b,B	0.678	9.53a,B	100.99a,A	25.47a,A	9.60a,A	56.65a,A
	0.2	0.00a,B	0.00a,B	30.99a,A	0.559	13.40a,A	74.30c,B	19.93a,B	8.93a,AB	47.04c,B
	0.3	0.00b,B	0.00b,B	23.02a,B	0.559	9.13b,B	99.73b,A	19.20b,B	7.47b,B	57.04a,A
80%	Control (0)	1.07a,B	0.887a,A	27.27a,A	0.575	12.80a,A	94.58ab,B	21.87a,A	8.07a,B	49.35ab,B
	0.1	0.00b,C	0.00b,C	27.90b,A	0.665	9.93a,B	75.99b,C	24.20a,A	10.33a,A	59.45a,A
	0.2	0.00a,C	0.00a,C	18.00b,B	0.551	5.13b,C	88.30b,B	12.80b,B	4.87b,C	61.10a,A
	0.3	1.33a,A	0.527a,B	21.53a,B	0.579	11.40a,B	145.12a,A	25.53a,A	10.33a,A	49.91b,B
c.v. (a)%		23.09	8.65	6.42	5.78	10.86	9.00	7.68	17.67	3.95
c.v. (b)%		31.74	13.21	6.98	6.19	10.86	5.28	9.59	10.71	3.81

Note: Lowercase letters represent statistically significant differences among shading intensities (main plot) at each growth hormone concentration level (subplot), while UPPERCASE LETTERS indicate statistically significant differences among growth hormone concentrations (subplot) at each shading intensity level (main plot)

Root number

The number of roots of different clonal rootstocks was measured after a 60-day rooting period, revealing a significant interaction effect (1%, HSD) between shading intensity and hormone concentration (Table 1). The comparison of shading (main plot) at each level of vitamin hormone (subplot) (Table 2) shows that under 80% shade without Hormex hormone, avocado clonal rootstocks produced the highest number of roots, averaging 1.07 roots. This result was significantly greater than the root production under 40% shade (0.40) and 60% shade (0.00) at the same hormone level. At the hormone concentration of 0.1 mL/L, clonal rootstocks under 60% shade yielded the highest root count at 0.20 root, which was greater compared to those exposed to 40% shade (0.00) and 80% shade (0.00). However, all clonal rootstocks subjected to any shading level and treated with 0.2 mL/L of rooting hormone failed to produce any roots. Interestingly, when 0.3 mL/L of rooting hormone were applied, rootstocks under 80% shade achieved the highest number of roots, averaging 1.33 root. This was significantly higher than the root counts under 60% shade (0.00) and 40% shade (0.00).

Moreover, in the detailed comparison of hormone concentration (subplot) at each level of shading intensity (main plot) (Table 2), it was found out that under the control treatment (no rooting hormone), clonal rootstocks placed under 80% shade developed the highest number of roots, with an average of 1.07 roots. This was greater than the root counts under 40% shade (0.40) and 60% shade (0.00). At 0.1 mL of rooting hormone/liter of water, the highest root production was observed under 60% shade (0.20). In contrast, no root was produced under 40% shade or 80% shade. When 0.2 mL of rooting hormone/liter of water was applied, none of the clonal rootstocks developed roots, regardless of shading intensity. Finally, the highest root production, averaging 1.33 roots, was achieved under 80% shade with 0.3 mL of rooting hormone/liter of water. No root was formed under 40 or 60% shade at this hormone level.

Root length (cm)

After a 60-day rooting period (Figure 3) a statistically significant interaction (1%, HSD) was observed in the interaction between shading intensity (main plot) and hormone concentration (subplot) on the root length (cm) of etiolated avocado clonal rootstocks (Table 1). When comparing shading intensity (main plot) at each level of hormone concentration (subplot) (Table 2), it was found out that among the treatments without hormone application, avocado rootstocks grown under 40% shade exhibited the greatest mean root length at 0.90 cm. This was comparable to the root length under 80% shade (0.887 cm) but significantly longer than the roots under 60% shade (0.00 cm). At the 0.1 mL/L hormone concentration, rootstocks grown under 60% shade achieved the longest mean root length of 0.72 cm. In contrast, no root development was observed under either 40 or 80% shade. When treated with 0.2/L hormone, no root was observed under any shading intensity. Under the highest hormone concentration of 0.3 mL/L, rootstocks exposed to 80% shade produced the longest roots, with a mean length of 0.527 cm. However, rootstocks under both 40 and 60% shade failed to develop roots at this concentration.

The comparison of vitamin hormone concentration at each level of shading intensity on the length (cm) of roots of clonal rootstocks (Table 2) reveals the significant interaction (1%, HSD) between rooting hormone concentrations (subplot) and shading intensities (main plot) on the root length (cm) of avocado clonal rootstocks. The results showed that both environmental factors and hormone applications significantly influenced root elongation, with varying effects at different levels of shade and hormone concentration. Under 40% shade, the control group (without vitamin hormone) produced roots with the longest mean length of 0.90 cm, significantly surpassing all other treatments that received rooting hormone applications (0.1, 0.2, and 0.3 mL/L of water), as none of them produced roots. At 60% shade, only the rootstocks treated with 0.1 mL/L of rooting hormone developed roots, with an average length of 0.72 cm. In comparison, all other treatments, including the control group and those treated with higher hormone concentrations (0.2 and 0.3 mL/L), failed to produce roots. For rootstocks placed under 80% shade, the control group (no hormone) once again exhibited the greatest root length, averaging 0.887 cm. This was significantly longer than the roots produced under the same shade intensity with 0.3 mL/L rooting hormone (0.527 cm). Notably, no root was observed for rootstocks treated with 0.1 or 0.2 mL/L of rooting hormone under this shading condition (Figure 3).

Figure 3. Roots and calluses are formed from avocado clonal rootstocks with the interaction of shade intensity and vitamin growth hormone level

Stem length (cm)

The results show that there was a highly significant interaction (1%, HSD) between the main plots and subplots of the experiment. It was, however, only the subplot (hormone concentration) which caused highly significant individual effect on the stem length; shade intensity (main plot) had no significant effect at all (Table 1). In the absence of hormone application, the rootstocks subjected to 80 and 40% shading exhibited the longest stem lengths, measuring 27.27 and 25.85 cm, respectively. These values were statistically superior to those grown under 60% shading, which reached only 21.99 cm. When the plants were treated with 0.1 mL/L hormone, the 40% shade condition yielded the longest stem length at 32.07 cm. This value was significantly higher than those measured under 60% (21.99 cm) and 80% (27.90 cm) shade. Applying 0.2 mL/L of hormone shifted the optimal shade condition. Under this treatment, plants exposed to 60% shade reached 30.99 cm, statistically outperforming those in 40% (18.87 cm) and 80% (18.00 cm) shade conditions. Interestingly, when the hormone concentration was increased to 0.3 mL/L, the stem lengths across the three shading intensities (60, 80, and 40%) showed no statistically significant differences, with values of 23.02, 21.53, and 20.09 cm, respectively.

The comparison of hormone concentration across different levels of shading intensity (Table 2) revealed distinct patterns in stem length development among avocado clonal rootstocks. At 40% shade, rootstocks treated with 0.1 mL/L hormone achieved the longest stem length of 32.07 cm, significantly outperforming the control group (25.85 cm). On the other hand, rootstocks treated with 0.3 and 0.2 mL/L of hormone exhibited the shortest statistically comparable stem lengths of 20.09 and 18.87 cm, respectively. When the shading intensity was increased to 60%, the trend shifted. Rootstocks treated with 0.2 mL/L of hormone produced the longest stems, measuring 30.99 cm, which was statistically superior to all other treatments. Meanwhile, rootstocks treated with 0.1 mL/L of hormone resulted in a stem length of 25.81 cm, statistically similar to both the 0.3 mL/L (23.02 cm) and the control group (21.99 cm). Under 80% shade, the 0.1 mL/L treatment (27.90 cm) yielded the highest stem length, though it was statistically comparable to the control group (27.27 cm). Conversely, the 0.3 mL/L treatment (21.53 cm) and 0.2 mL/L treatment (18.00 cm) resulted in the shortest stem lengths (Figure 4).

Stem diameter (cm)

The stem diameter of etiolated avocado clonal rootstocks was significantly influenced (1%, HSD) by rooting hormone concentration (Table 1). The application of 0.1 mL of vitamin hormone per liter of water resulted in the largest average stem diameter of 0.660 cm. This is the most effective concentration for promoting stem thickening. In contrast, rootstocks that received none (0), 0.2, and 0.3 mL of the hormone per liter of water exhibited statistically comparable but smaller diameters of 0.60, 0.588, and 0.577 cm, respectively. Interestingly, the main plot factor-shading intensity-did not produce a statistically significant individual effect on the stem diameter. Additionally, the interaction between shading intensity and rooting hormone concentration did not yield any significant interaction effects (Tables 1 and 2).

Leaf number

The number of leaves (Table 1; Figure 4) of etiolated avocado clonal rootstocks varied across hormone concentrations and shading intensities (1%, HSD), showing distinct patterns in leaf production. Without the application of rooting hormone, clonal rootstocks exposed to 80% shade produced the most leaves (12.80). In contrast, rootstocks under 60% shade (9.53 leaves) and 40% shade (9.07 leaves) yielded lesser and statistically similar values. When treated with 0.1 mL/L of rooting hormone, all shading levels produced statistically comparable leaf counts: 10.40 leaves under 40% shade, 9.93 leaves under 80% shade, and 9.53 leaves under 60% shade. However, rootstocks treated with 0.2 mL/L of hormone showed a different trend, producing the most leaves (13.40) under 60% shade. This was significantly superior to the leaf counts under both 40% shade (7.07 leaves) and 80% shade (5.13 leaves). Interestingly, when 0.3 mL/L of hormone was applied, the rootstocks under 80% shade produced the most leaves (11.40). However, leaf production decreased under 60% shade (9.13 leaves), and the count under 40% shade (9.53 leaves) was statistically similar to the values obtained under 60 and 80% shade.

Figure 4. Clonal avocado rootstocks are rooted using these circular devices. Their stem length (cm) and diameter (cm), and leaf numbers and area (cm^2) are collected upon termination time

The comparison of hormone concentrations at each level of shading intensity (Table 2) revealed important trends in the leaf production of etiolated avocado clonal rootstocks. At 40% shade, rootstocks treated with 0.1 mL/L of rooting hormone produced the most leaves (10.40). However, this was statistically comparable to those treated with 0.3 mL/L and the control group with no hormone application, having 9.53 and 9.07 leaves, respectively. The 0.2 mL/L treatment produced the fewest leaves of 7.07. At 60% shade, a shift in the trend was observed. Rootstocks treated with 0.2 mL/L of rooting hormone produced the most leaves (13.40), which was statistically superior to all other treatments. In contrast, rootstocks treated with 0.1 mL/L, 0.3 mL/L, and the control group produced statistically similar leaf counts of 9.53, 9.13, and 9.53, respectively. When rootstocks were subjected to 80% shade, those without any hormone application produced the most leaves (12.80), although this result was comparable to the 0.3 mL/L treatment (11.40 leaves). The 0.1 mL/L treatment resulted in a slightly lower count of 9.93 leaves, while the 0.2 mL/L treatment produced the fewest leaves of 5.13.

Leaf area (cm^2)

The leaf area (cm^2) (Figure 4) measurements of avocado clonal rootstocks highlight the significant interaction between shading intensity (main plots) and rooting hormone concentration (subplots), as shown in Table 1. This interaction (Table 2) suggested that both factors significantly influenced (1%, HSD) the growth response of the clonal rootstocks. When no rooting hormone was applied, the largest leaf area (99.49 cm^2) was observed in rootstocks under 60% shade. Rootstocks under 80% shade followed closely with a statistically similar value of 94.58 cm^2, while those under 40% shade had the smallest leaf area (87.37 cm^2). Upon applying 0.1 mL/L of rooting hormone, the highest value was again recorded under 60% shade (100.99 cm^2). It significantly outperformed the rootstocks under 40% (83.59 cm^2) and 80% (75.99 cm^2) shade. The application of 0.2 mL/L of rooting hormone, however, resulted in a shift in optimal shading conditions. In this case, rootstocks under 40% shade exhibited the largest leaf area (110.56 cm^2). This surpassed those under 80% shade (88.30 cm^2). The plants under 60% shade showed a significant decline, producing the smallest leaf area (74.30 cm^2). When 0.3 mL of rooting hormone was applied, a dramatic increase in leaf area was seen under 80% shade (145.12 cm^2), far exceeding the values for 40% (102.78 cm^2) and 60% (99.73 cm^2) shade, which were statistically similar.

Table 2 compares the significant effects (1%, HSD) of different rooting hormone concentrations across various shading intensities on the leaf area of etiolated avocado clonal rootstocks. The data revealed distinct patterns in how hormone application and shading intensity interacted to influence leaf area growth. Under 40% shade, rootstocks treated with either 0.3 mL or 0.2 mL/L of rooting hormone produced statistically similar leaf areas of 102.78 and 110.56 cm^2, respectively. These values were significantly higher than those recorded for the untreated rootstocks (85.37 cm^2) and those treated with 0.1 mL/L of vitamin hormone (83.59 cm^2). When exposed to 60% shade, rootstocks treated with 0.1 mL/L of rooting hormone, 0.3 mL/L, and the control (no hormone) all achieved comparable leaf areas (100.99, 99.73, and 99.49 cm^2, respectively). Interestingly, the application of 0.2 mL/L of rooting hormone resulted in a significantly smaller leaf area of 74.30 cm^2. Finally, for rootstocks under 80% shade, the application of 0.3 mL/L of rooting hormone produced the largest leaf area of 145.12 cm^2, far exceeding the leaf areas of untreated rootstocks (94.58 cm^2) and those treated with 0.2 mL/L of rooting hormone (88.30 cm^2). Rootstocks treated with 0.1 mL/L of hormone exhibited the smallest leaf area (75.99 cm^2).

Fresh weight (g)

The fresh weight (g) of different clonal rootstocks was measured, with results showing a significant interaction (1%, HSD) between shading intensity (main plot) and rooting hormone concentration (sub plot). Table 2 demonstrates that at lower rooting hormone concentrations (0-0.1 mL/L), the fresh weights of the clonal rootstocks were statistically similar across all shading intensities (40, 60, and 80%). However, the application of 0.2 mL/L of rooting hormone significantly affected fresh weight depending on shading intensity. Rootstocks under 60 and 40% shade produced the heaviest and statistically similar weights of 19.93 and 17.27 g, respectively. Furthermore, 0.3 mL/L of rooting hormone yielded the heaviest fresh rootstock weight of 25.53 g under 80% shade. Interestingly, this result was statistically similar to the fresh weight of rootstocks under 40% shade (21.67 g). In contrast, rootstocks under 60% shade produced the lightest fresh weight (19.20 g) under the same hormone concentration.

When comparing the significant effects (1%, HSD) of rooting hormone concentrations (subplot) across different shading intensities (main plot), the results revealed several notable patterns (Table 2). Under 40% shade, clonal rootstocks treated with 0.1 mL/L of rooting hormone achieved the heaviest fresh weight at 25.13 g. This value was statistically comparable to those treated with 0.3 mL/L of hormone (21.67 g) and even the untreated rootstocks (21.47 g). Interestingly, the lightest fresh weight in this shading condition was observed when 0.2 mL/L of vitamin hormone were applied, producing only 17.27 g. Similarly, under 60% shade, the application of 0.1 mL/L of rooting hormone consistently resulted in the highest fresh weight of 25.47 g. It significantly outperformed the untreated rootstocks (20.27 g) and those treated with both 0.2 mL/L (19.93 g) and 0.3 mL/L (19.20 g) of rooting hormone. Under 80% shade, clonal rootstocks treated with 0.3 mL rooting hormone/L of water produced the heaviest fresh weight at 25.53 g. This was statistically comparable to those treated with 0.1 mL hormone/L of water (24.20 g) and the untreated rootstocks (21.87 g). Similar to the other shading intensities, the application of 0.2 mL rooting hormone/L of water resulted in the lightest fresh weight (12.80 g).

Dry weight (g)

The dry weight (g) of avocado clonal rootstocks, as presented in Table 1, showed notable interactions (1%, HSD) between shading intensity (main plot) and rooting hormone concentration (subplot). Rootstocks that were untreated (control) produced statistically similar values across different shading rate: 8.27 g (40% shade), 7.60 g (60% shade), and 8.07 g (80% shade). The same was true for those treated with 0.1 mL of rooting hormone/L of water, with statistically comparable dry weights of 10.13 g (40% shade), 9.60 g (60% shade), and 10.33 g (80% shade). When 0.2 mL of rooting hormone/L of water was applied, the clonal rootstocks exposed to 60% shade achieved the heaviest dry weight of 8.93 g. In contrast, the rootstocks subjected to 40% shade produced a dry weight of 6.93 g. This was statistically similar to those grown under 80% shade (4.87 g). Interestingly, the highest hormone concentration of 0.3 mL of hormone/L of water led to the heaviest dry weight of 10.33 g for rootstocks under 80% shade. However, this result was statistically comparable to rootstocks under 40% shade, which produced a dry weight of 8.73 g. The lightest dry weight (7.47 g) occurred under 60% shade.

When comparing the significant effects (1%, HSD) of rooting hormone concentrations (subplot) across different shading intensities (main plot), the results in Table 2 reveal several key trends regarding the dry weight of avocado clonal rootstocks. Under 40% shade, the application of 0.1 mL of rooting hormone/L of water resulted in the highest dry weight of 10.13 g. This was statistically comparable to rootstocks treated with 0.3 mL of hormone/L of water (8.73 g) and even to the untreated control group (8.27 g). The use of 0.2 mL of rooting hormone/L of water, however, produced the lightest dry weight (6.93 g) of clonal rootstocks. Under 60% shade, rootstocks treated with 0.1 mL of rooting hormone/L of water again showed the heaviest dry weight of 9.60 g. This value was statistically similar to those treated with 0.2 mL of hormone/L of water (8.93 g) and untreated rootstocks (7.60 g). Interestingly, the lightest dry weight (7.47 g) was observed in rootstocks treated with 0.3 mL of hormone/L of water. For rootstocks under 80% shade, the application of 0.3 and 0.1 mL of rooting hormone/L of water resulted in the highest and statistically similar dry weights (10.33 g each). The untreated rootstocks trailed behind with a dry weight of 8.07 g, while the lightest dry weight (4.87 g) was observed in rootstocks treated with 0.2 mL of rooting hormone/L of water.

Leaf chlorophyll content (SPAD value)

The study measured the leaf chlorophyll content (SPAD value) of different avocado clonal rootstocks, focusing on the interaction between shading intensity and hormone concentration levels (Table 1). The results revealed a highly significant interaction (1%, HSD) between these factors, which influenced the chlorophyll content in avocado clonal rootstocks. When no rooting hormone was applied, the highest chlorophyll content (53.31 SPAD) was observed in clonal rootstocks subjected to 60% shade, which was statistically comparable to those under 80% shade (49.35 SPAD). However, when 0.1 mL of rooting hormone/L of water was applied, shading intensity had no significant effect. All plants under 40, 60, and 80% shade showed statistically similar SPAD values of 57.04, 56.65, and 59.45, respectively. When 0.2 mL of hormone/L of water was applied, plants subjected to 80% shade had the highest chlorophyll content (61.10 SPAD). This was followed by those under 40% shade (56.18 SPAD). In contrast, plants under 60% shade had the lowest chlorophyll content (47.04 SPAD). With 0.3 mL of hormone/L of water, plants under 60% shade showed the highest chlorophyll content (57.04 SPAD), while those under 40 and 80% shade attained similar but lower SPAD values of 50.71 and 49.91, respectively.

The comparison of subplots (rooting hormone concentrations) at each level of shading intensity revealed variations in leaf chlorophyll content of avocado clonal rootstocks (Table 2). At 40% shading intensity, the highest chlorophyll content was achieved when 0.1 mL of rooting hormone/L of water was applied, resulting in 57.04 SPAD values. This was statistically similar to the 0.2 mL treatment (56.18 SPAD) but significantly higher than both the 0.3 mL treatment (50.71 SPAD) and the untreated clonal stocks (49.91 SPAD). At 60% shading intensity, the 0.3 mL treatment attained the highest chlorophyll content (57.04 SPAD). This, however, was statistically comparable to the 0.1 mL treatment (56.65 SPAD) and the untreated control (53.31 SPAD). Notably, the 0.2 mL treatment had the lowest chlorophyll content (47.04 SPAD). Under 80% shading intensity, the application of 0.2 mL of rooting hormone/L of water resulted in the highest chlorophyll content (61.10 SPAD). It was statistically similar to the 0.1 mL treatment (59.45 SPAD). The 0.3 mL treatment and the control were both lower, with chlorophyll contents of 49.91 SPAD and 49.35 SPAD, respectively.

Leaf color

Using the Munsell Color Application, the leaf color of different rootstocks was measured. Table 3 evaluates the leaf color of etiolated avocado clonal rootstocks under varying concentrations of growth vitamin hormone (Hormex) and different shading intensities. It used a numerical scale (Esgrina and Tan 2024) from 1 to 6 based on the Munsell color system. The control group (no hormone) showed a leaf color score of 4.60 (40% shading), which increased to 5.00 (at both 60 and 80% shading), with an overall mean of 4.87. In contrast, the 0.1 mL of hormone/L of water maintained a consistent score of 5.00 across all shading intensities, resulting in a mean of 5.00. Similarly, the 0.2 mL treatment also consistently scored 5.00 at all shading levels, matching the overall mean of 5.00. For the 0.3 mL treatment, the score was 5.00 (40% shading), dropped to 4.40 (60% shading), and returned to 5.00 (80% shading), yielding a mean of 4.00, indicating variation in leaf color response based on shading intensity. The mean leaf color scores across the shading levels were 4.90 (40% shading), 4.85 (60% shading), and 5.00 (80% shading).

Table 3. Leaf color of etiolated avocado clonal rootstocks as affected by growth vitamin hormone and shading intensity

Subplot (Hormex hormone concentration)	Main plot (shading intensity)			Mean
	40%	60%	80%	
Control (no hormone)	4.60	5.00	5.00	4.87
0.1 mL/L of water	5.00	5.00	5.00	5.00
0.2 mL/L of water	5.00	5.00	5.00	5.00
0.3 mL/L of water	5.00	4.40	5.00	4.00
Mean	4.90	4.85	5.00	

Scale	Description
1	5 YR (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma) to 7.5 YR (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma)
2	10 YR (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma) to 2.5 Y (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma)
3	5 Y (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10 (Chroma) to 7.5 Y (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10 (Chroma)
4	10 Y (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma) to 2.5 GY (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma)
5	5 GY (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma) to 7.5 GY (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12, 14 (Chroma)
6	10 GY (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 (Chroma) to 2.5 G to (Hue); N1-N9 (Value); 1, 2, 3, 4, 6, 8, 10, 12 (Chroma)

Discussions

The results of this study highlight the significant interaction between shading intensity and hormone concentration in influencing the rhizogenesis and caulogenesis of avocado clonal rootstocks. The effects of shading and growth hormone applications were observed across various morphological and physiological parameters, supporting previous studies on plant growth regulation under different environmental conditions (Brini et al. 2022; Ghorbel et al. 2023).

Optimal hormone concentrations under specific shading levels

Higher shading intensity (80%) in combination with 0.2-0.3 mL/L of hormone resulted in the highest root number, root length, chlorophyll content, fresh weight, and dry weight of avocado seedlings. These findings align with previous studies suggesting that reduced light intensity enhances root initiation by lowering transpiration rates and improving auxin stability (Hossain and Kamaluddin 2005; Pacholczak et al. 2017). This is consistent with findings by Aly et al. (2019), who reported that reduced light intensity positively influences root formation and overall biomass in ginger plants. The results also align with studies on shading effects in strawberries, where Cordoba-Novoa et al. (2022) found that shading reduced plant water deficits, thereby improving vegetative growth. Moderate shading (60%) with 0.1-0.2 mL/L hormone concentration was most effective for stem elongation and callus formation, highlighting the role of intermediate light exposure in promoting cell expansion and differentiation (Štefančič et al. 2005; Kanmegne et al. 2017; Dev et al. 2018; Jiang et al. 2020; Khandaker et al. 2022; Feng et al. 2023). Conversely, at 40% shading, root formation of avocado clonal stocks was significantly limited, particularly when 0.2 mL/L of hormone was applied. This suggests that excessive light exposure may reduce endogenous auxin accumulation (Lee et al. 2022; Xin et al. 2022), counteracting the exogenous application of hormones (Sun et al. 2023). The balance between endogenous and exogenous hormone levels is crucial for successful rhizogenesis and caulogenesis (Hunt et al. 2011; Muttaleb et al. 2017; Chen et al. 2023; Khan et al. 2024). Similar trends were observed by Dewi et al. (2022), who found that excessive light exposure reduced the efficacy of growth regulators in hybrid corn. This suggests that light-induced auxin degradation may have inhibited root formation, which has also been observed in studies on tomato and cotton seedlings under shading conditions (Echer et al. 2019; Zhang et al. 2020; Liphan and Detpiratmongkol 2020).

Physiological mechanisms underlying the observed interactions

The differential responses observed in avocado rootstocks can be explained by key physiological mechanisms influenced by shading and hormone application:

Auxin and root development

The role of auxin in root initiation is well established, but its effectiveness on avocado root initiation and development depends on light conditions (Pantoja-Guerra et al. 2023; Calatrava et al. 2024). Under high shading (80%), reduced photodegradation of auxin may have contributed to higher root proliferation and elongation (Tanimoto 2005; Zhao et al. 2016; Olatunji et al. 2017). The data support findings that moderate-to-high shade (40-80%) enhances root development in woody species (Arévalo-Gardini et al. 2021; Xue et al. 2023) including avocados. Studies by Hersch et al. (2014) and Brini et al. (2022) indicate that light intensity regulates auxin transport and signaling pathways, thereby affecting shade avoidance responses. The positive effect of shading on auxin stability and root proliferation has also been noted in previous work on shade-tolerant Mediterranean species (García-Pérez et al. 2021; Tivendale and Millar 2022).

Shading and stem growth

Stem elongation of avocado scions was most pronounced under moderate shading (60%) with 0.1-0.2 mL/L hormone, resulted in the longest stem lengths, suggesting that intermediate light exposure optimizes stem elongation (Kaur 2017; Hussain et al. 2019). Shading has been shown to increase internodal elongation by altering auxin transport (Abdel-Mawgoud et al. 1996; Collins and Wein 2000; Zhiyu et al. 2007; Wu et al. 2017a; Mishra et al. 2020; Formisano et al. 2022; Luo et al. 2023). Our findings align with previous reports indicating that moderate shading enhances shoot elongation in various crops (Wu et al. 2017b; Sosnowski et al. 2023). Similar results were found in chili pepper studies, where moderate shading improved biomass accumulation (Hariyono et al. 2021). The enhanced shoot elongation observed under 60% shading may be attributed to a shift in hormonal regulation, as shading has been shown to increase gibberellin activity while modifying auxin distribution (Khan and Nabi 2023). A study by Mroue et al. (2017) further supports the role of auxin in integrating environmental cues to regulate plant development.

Chlorophyll content and shading

The highest chlorophyll content in the leaves of avocado seedlings was recorded under 80% shading with 0.2 mL/L hormone, supporting related literatures that shading reduces chlorophyll degradation and enhances pigment synthesis (Duan et al. 2018; Chen et al. 2021; Esgrina and Tan 2024). It is also consistent with findings in basil, where increased shading and light regulation enhanced pigment accumulation (Eghbal et al. 2024). Hormone application further stabilized the chlorophyll levels of avocado seedling leaves, likely by improving nitrogen assimilation and delaying senescence (Mazzoni-Putman et al. 2021; Huang et al. 2022; Feng et al. 2023; Mason 2023; Trösch 2023). Studies in kalmegh also indicate that shading improves chlorophyll stability, which aligns with the observed increase in SPAD values in avocado seedlings (Valio 2001; Liphan and Detpiratmongkol 2020). The role of auxins in maintaining chlorophyll content and delaying leaf senescence has been documented in work on tomatoes (Yuan et al. 2018) and other horticultural crops (Guimarães et al. 2020; Khan and Nabi 2023).

The findings of this study are consistent with earlier research demonstrating that shading and hormone treatments interact synergistically (Khajehpour et al. 2014; Chaiwanon et al. 2016; de Wit et al. 2016; Ibukun 2016; Lymperopoulos et al. 2018; Sulaiman et al. 2020) to enhance propagation success. Auxin-based hormones have been shown to enhance root biomass and shoot development, as observed in both avocado and other tree crops. For instance, similar studies on tree seedlings have shown that moderate-to-high shading improves root initiation while excessive hormone concentrations can have inhibitory effects (Oumahmoud et al. 2023; Xue et al. 2023). Moreover, auxin-based hormones have been linked to improvements in both root and shoot biomass, as observed in this study (Takahashi 2013; Pacheco-Villalobos et al. 2016; Zain et al. 2022; Wang et al. 2024). However, deviations from expected outcomes-such as the limited root formation of avocado clonal stocks at 40% shading despite hormone application-suggest that additional environmental factors, such as growing media, temperature and humidity, may influence hormone activity. Similar variations have been noted in experiments where light intensity fluctuations affected tomato seedling growth (Zheng et al. 2023). Future studies should consider incorporating additional physiological assessments, such as hormonal quantification and gene expression analysis, to further elucidate the underlying mechanisms.

This study underscores the importance of tailoring propagation protocols to specific environmental conditions. The interaction between shading intensity and hormone concentration significantly affects key growth parameters. Specifically, 80% shading with 0.2-0.3 mL/L hormone optimized root formation, leaf area, chlorophyll content, and biomass, while 60% shading with 0.1-0.2 mL/L hormone was most effective for stem elongation. Based on these findings, it is recommended that propagation strategies be adjusted depending on the desired growth outcome. For maximizing overall plant growth, 80% shading with 0.3 mL/L hormone is ideal, while for better shoot elongation and chlorophyll retention, 60% shading with lower hormone concentrations (0.1-0.2 mL/L) is preferable. These insights contribute to refining avocado rootstock propagation techniques, supporting both commercial cultivation and sustainable agricultural practices.

ACKNOWLEDGEMENTS

The scholarship grant to FOEsgrina was provided by the Philippines' Commission on Higher Education (CHED) through its Support to Staff and Instructors' Knowledge Advancement Program (CHED-SIKAP). Additionally, Central Mindanao University extended material and financial support through its research on Avocado, identified by Research Number R-0294. Annie Lee C. Alagos and Maybelyn Pedroso performed the Statistical layout and analyses of this research endeavor, and Reuel C. Pedroso was the plant propagator of this study.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 174-184 DOI: 10.13057/asianjagric/g090118

Tuber diversity of *Dioscorea hispida* from Muna Island, Indonesia, for potential microencapsulated bioherbicide agents

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Manuscript received: 1 November 2024. Revision accepted: 3 March 2025.

Abstract. *Putri WE, Johannes E, Sjafaraenan. 2025. Tuber diversity of* Dioscorea hispida *from Muna Island, Indonesia, for potential microencapsulated bioherbicide agents. Asian J Agric 9: 174-184.* Dioscoreaceae family tubers scattered across Muna Island have diverse characteristics. This research aims to determine the diversity of *Dioscorea* tubers and their potential as bioherbicides using the microencapsulation method. The study was conducted in 6 villages, namely Tampo, Wakumoro, Oelongko, Lasehao, Napano Kusambi, and Napalakura, by collecting uwi (*Dioscorea alata* L.) and gembili (*Dioscorea esculenta* L.), which are utilized by the local community as staple food to substitute rice, made into chips. Gadung (*Dioscorea hispida* Dennts.) is also used as a natural herbicide. This research uses a Factorial Randomized Block Design (RBD). The treatments were *D.hispida* extract at the concentration of 20%, 30%, and 40%. Other factors were 4 species of weeds, i.e., *Thunbergia alata*, *Centrosema pubescens*, *Phyllanthus amarus*, and *Calopogonium mucunoides*. Every treatment has 4 replications. The data were analyzed using ANOVA. The tuber flesh of *D. alata*. and *D. hispida* was orange-white, while *D. esculenta* was purple-white. Applying a 40% microencapsulated D. hispida tuber extract effectively retard the root length of the weed.

Keywords: Bioherbicide, *Dioscorea hispida, kolope*, microencapsulation, Muna Island, tuber diversity

Abbreviations: ANOVA: Analysis of Variance, SEM: Scanning Electron Microscope

INTRODUCTION

Weeds are currently high enough to cause significant losses to farmers. Weed control can be done chemically or biologically to population levels that are not economically detrimental. The continuous use of herbicides may result in adverse environmental effects, namely the poisoning of non-target organisms, water pollution, soil damage, and poisoning due to herbicide residues in agricultural products. According to Ammar et al. (2023), the alternative to weed control in food crops and plantations is using bioherbicides. Various types of weeds in the plantation, e.g., *Thunbergia alata*, *Centrosema pubescens*, *Phyllanthus amarus*, and *Calopogonium mucunoides,* are broadleaf weeds commonly found in plantation areas. These weeds have different self-defense, which are influenced by various factors (Sahari et al. 2023).

The continuous use of herbicides can negatively impact the environment, poisoning non-target organisms, causing water pollution, soil degradation, and poisoning due to herbicide residues on agricultural products. It raises public awareness of the importance of environmental sustainability, which can increase the demand for environmentally friendly agriculture and safer agricultural products. Therefore, there is a growing need for the use of natural herbicides.

Bioherbicides are natural materials that control weeds because they quickly decompose in the soil without leaving residue. Bioherbicides from tubers can be done by extracting secondary metabolites in liquid form. However, these results are less effective because the extract is easily oxidized, reducing the effectiveness of bioactive compounds. An innovation to overcome this problem is the microencapsulation of secondary metabolites of extracts. Microencapsulation is the process of coating liquid or solid core materials using a special encapsulation to give the core particles the desired physicochemical properties. Allelochemical compounds derived from plants and environmentally friendly are phenolic groups such as alkaloids, tannins, steroids, and other compounds that can be harmful when in contact with germination so that they can inhibit cell division (Kong et al. 2019).

Several species of the *Dioscorea* family are used as staple substitutes for rice. They are low-sugar foods that can be processed into flour, chips, and various products with economic value, specifically from the *Dioscorea alata* L. and *D. esculenta* L. species. Meanwhile, other species of Dioscorea that are not consumed are used as natural herbicides. *Dioscorea hispida* Dennts *is* characterized by creeping tubers, large size, and above-ground growth and might be used as a bioherbicide (Sardar et al. 2022). Its tuber grows wild in lowlands up to an altitude of 50 meters, especially in marginal forests and shrubs, and is cultivated in yards. Besides being used as bioherbicides, tubers from several species of the Dioscoreaceae family can also be used as rice substitutes, such as *D. alata*, *D. esculenta*, *Dioscorea bulbifera*, *Dioscorea nummularia*, *Dioscorea pentaphylla*, and *D. hispida*.

The tuber of *Dioscorea hispida* contains toxins, namely the alkaloid dioscorine (Salehi et al. 2019) and diosgenine (Estiasih et al. 2022). These compounds can be used as bioherbicides to inhibit weed growth; Semwal et al. (2019), the content of alkaloids reaches 71.36%. Alkaloid compounds act as bioherbicides to protect plants from weeds and pathogens. The action of alkaloid compounds involves damaging cell structures through a synergistic mechanism with various active compounds; thus, the activity of the compounds increases in damaging cells (Johannes et al. 2022).

Bioactive compounds maintain their stability during storage by microencapsulation. Bioactive compounds are generally less stable, so they must be protected from physical and chemical degradation due to handling and environmental influences. Microencapsulation generally aims to protect bioactive components, reduce nutrient loss, and transform components from liquid to solid, making them easier to handle and apply in food systems. Microencapsulation using the inclusion complex method with β-cyclodextrin encapsulant has been proven to be the most effective in protecting active ingredients from heat and evaporation (Zabot et al. 2021). Based on the above background, research is needed to determine the diversity and concentration of microencapsulation of *D. hispida* tubers as a bioherbicide agent in controlling broadleaf weeds.

MATERIALS AND METHODS

Research location and sampling

The sampling locations were several villages in Muna Island, Southeast Sulawesi Province, Indonesia, i.e. Tampo (122°41'19.424"E, 4°37'36.028"S), Wakumoro (122°30'41.806"E, 5°416.523"S), Oelongko (122°28'14.546"E, 5°7'50.719"S), Lasehao (122°36'35.661"E, 4°38'50.535"S), Napano Kusambi (122°32'0.217"E, 5°0'36.589"S), Napalakura (122°39'25.285"E, 4°38'10.904"S) (Figure 1). The research was conducted at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar Indonesia. The extraction and preparation of microencapsulation were done at the Phytochemistry Laboratory, Faculty of Pharmacy. The bioherbicide application on weed was conducted in the plantation fields of Tampo Village, Napabalano District, Muna Regency. Interviews with *D. hispida* tuber farmers were done to obtain additional information about the tuber.

Experimental design

This study is an experimental quantitative research using a Randomized Block Design (RBD) with 3 treatments of each extract microencapsulation concentration, namely control, 20%, 30%, and 40%, with 4 replications. Tests were carried out on plantation land. Data were analyzed using ANOVA and continued with variance analysis and the Least Significant Difference (LSD) test at the 5% level. The materials used in this study were *D. hispida* tubers obtained from Muna Island, *T. alata* weed, *C. pubescens, C. mucunoides*, and *P. amarus.*

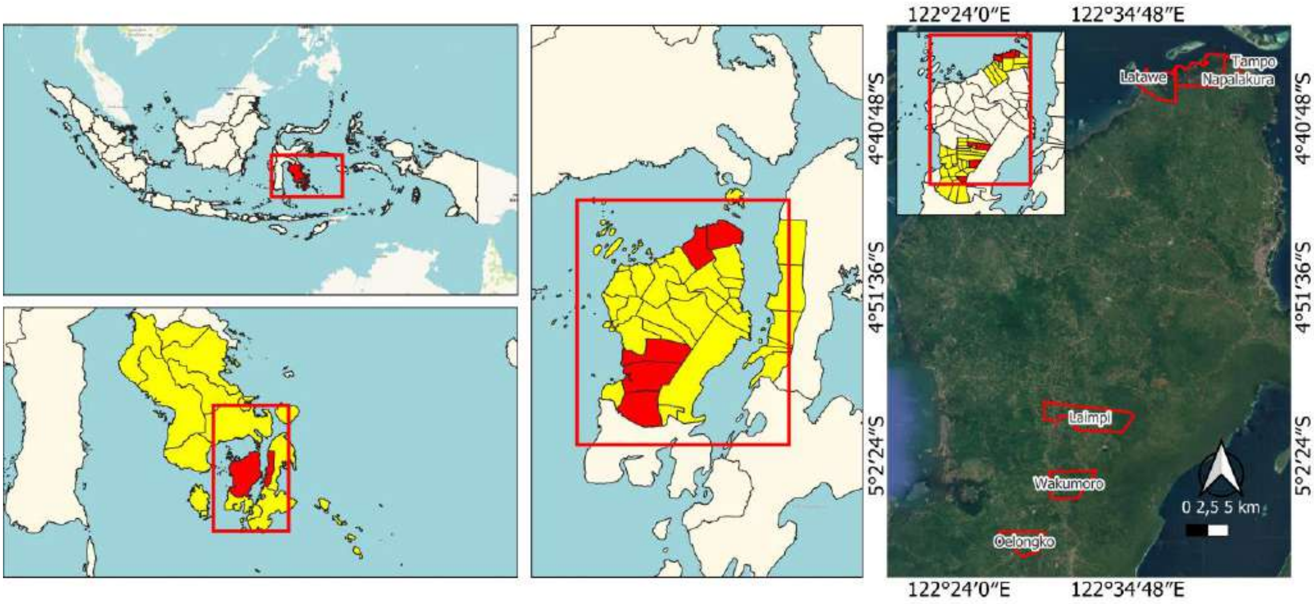

Figure 1. Sampling locations of *Dioscorea* tuber are in Tampo, Wakumoro, Oelongko, Lasehao, Napano Kusambi, Napalakura Villages of Muna Island, Southeast Sulawesi Province, Indonesia

Phenotypic character identification of *Dioscorea* tuber

The identification of phenotypic characters of *Dioscorea* tubers refers to Suleman et al. (2021). The parameters identified are morphometric characters such as habitus, tuber, tuber flesh, stem shape, leaves, roots, and other properties. It is known that the closeness between *Dioscorea* species is based on genetic material that can affect certain phenotypic characters. Taxa below the type can be identified by comparing differences in morphological characters, i.e., the morphological character of the tuber (Davis and Heywood 1973). The observed morphological characters are the shape, the size (length and diameter), the branching, the roots, the location of the root growth, the surface of the tuber skin, the color of the skin inside the tuber, the color of the tuber flesh, and the presence or absence of sap contained in the tuber.

Extraction of *Dioscorea hispida* tuber extract

The tuber of *Dioscorea hispida* was cleaned, sliced thinly, and dried. The dried gadung (*D. hispida*) tubers are ground and then mixed with distilled water at predetermined concentrations, namely 20% (50 g/250 mL), 30% (75 g/250 mL), and 40% (100 g/250 mL), followed by incubation for 3 days at room temperature. Once a day, the bottle is opened to allow the gas inside to escape and close again. After incubation, the extract was filtered using a funnel lined with filter paper. Then, the filtrate was evaporated at 78°C in the water bath to evaporate the solvent. The extract was stored in a vial.

Microencapsulation of *Dioscorea hispida* tuber extract

Maltodextrin was added as much as 5 grams in each treatment of 20%, 30%, and 40% extract concentrations (Salehi et al. 2019) into a 100 mL glass jar, then add 50 mL of distilled water to each treatment. The mixture was heated using a hot plate for 30 minutes. Extracts that have been mixed with an emulsifier, namely tween 80, as much as 1 mL until homogeneous and forming foam was added to the mixture, and then the liquid material was converted into solids using the *Thin-layer drying* method. Then, it was poured into a Petri dish with a thickness of 3 mm and dried in an oven at 70°C for 13 hours until dry. The resulting encapsulations were then pulverized and sieved.

Microencapsulation observation using SEM (Scanning Electron Microscope)

The encapsulated extract of 20%, 30%, and 40% concentrations were observed for their shape by observing their characteristics. The form of microencapsulation is related to its mode of action. The shape of the microencapsulation can vary depending on the packaging material and the mechanism of the microencapsulation formation. The morphological observation of the microencapsulation was carried out using SEM (*Scanning Electron Microscope*).

Applications of microencapsulated extract in plantations

The extract concentrations were 20%, 30%, and 40%, and 4 species of weeds, namely *T. alata*, *C. pubescens, P.amarus,* and *C. mucunoides*. There are 12 combinations of treatments. Every treatment combination had 4 replications. Watering was done in the morning and evening for four weeks following application (Afifah et al. 2024).

Observation parameters in the field

The parameters observed in the field were the degree of (%) of weed damage due to the application of *Dioscorea hispida* extract, which was observed visually using a scoring method of the 2011 Pesticide Commission by Halim (2023) of herbicide efficacy as follows: 0 = no damage 0-5% shape and/or color of leaves and/or abnormal growth, 1 = mild poisoning >5-20% shape or color of leaves and/or abnormal growth; 2 = moderate poisoning >20-50% shape or color of leaves and abnormal growth; 3 = heavy poisoning >50-75% shape or color of leaves and/or abnormal growth; and 4 = very heavy poisoning >75% shape or color of leaves and/or abnormal growth or plant death. Other parameters observed were weed height (cm), measured from the base of the stem to the growing point or tip, observed every week. Other parameters, i.e., root length (cm), weed wet weight (g), and dry weight (g), were observed in the 4th week after application. The bioherbicides are applied around the roots to ease the absorption of the compounds of the microencapsulated extract by plant roots. The wet weight of weeds was observed by pulling out as many weeds down to the roots as the research plots, washing them thoroughly, selecting them according to their type, and weighing them. Weed dry weight is carried out by pulling weeds up to the roots as much as the research plot and washing them thoroughly. Then, the weeds are selected according to their type and dried in an oven for 48 hours at 65°C until they reach a constant weight.

Data analysis

Data obtained from the observations were analyzed using analysis of variance (ANOVA) with the Least Significant Difference (LSD) test at the 5% significance level.

RESULTS AND DISCUSSION

***Dioscorea* species collected in Muna Island, Southeast Sulawesi Province**

Various *Dioscorea* species are growing in several villages on Muna Island, i.e., uwi (*D. alata*), gembili (*D. esculenta*), and gadung (*D. hispida*). There are 3 species of *Dioscorea* found in several villages on Muna Island (Table 1). Two *Dioscorea* species (*D. hispida* and *D. esculenta*) were collected in Tampo village. Three species (*D. hispida, D. alata,* and *D. esculenta)* were collected from Wakumoro. Lasehao village has 1 species (*D. hispida*). Oelongko village has 2 species (*D. hispida* and *D. alata*). Napano Kusambi village has only 1 species (D. *hispida).* The last survey location in Napalakura Village also only has 1 species, *D. alata*.

Morphological characteristics of *Dioscorea* species

*Gadung (*Dioscorea hispida *Dents.)*

Dioscorea hispida has a plant height of 5-10 meters, and a round and spiny stem spread long on the stem and petiole. The stem is green, and the tuber is irregularly round and covered by stiff root hairs. The tuber's skin is brown, and the flesh is white-orange, with the tuber appearing near the surface soil (Figure 2).

*Uwi (*Dioscorea alata *L.)*

Dioscorea alata has appealing characteristics, such as its green and four-winged stems, the distinct green wing (alatus), and the round stem shape. It has a heart-shaped leaf shape, alternate leaf seating at the bottom and facing at the top, and a right-twisting stem direction. The base and tip of the petiole are purple. It has a smooth leaf surface, a broadly notched leaf base, a flat leaf edge, and a tapered leaf tip. The widest leaf position is in the center (Figure 3).

Its tubers have a round shape with a length reaching 15 cm and diameter of 15 cm, moderate rooting, located mainly around the root neck, and a small part is scattered over the entire surface of the tuber, wrinkled skin surface, brown outer skin color. At the same time, the inside is black, purplish white tuber flesh and gummy. Rooting on the surface of the tuber is slightly located where the roots grow around the root neck.

*Gembili (*Dioscorea esculenta *L.)*

Dioscorea esculenta has a green stem with 2 mm spines. Leaves are green with a single variant and alternate leaf; the heart-shaped leaf blade is widened. The variant leaf shape of the *D. esculenta* is shown in Figure 4. The leaf edges of *D. esculenta* are flat, and the leaf tips are pointed. The distance between the lobes is moderate. *D. esculenta* has spines that are bent downward. The bulbs of the *D. esculenta* are cylindrical. The tubers are branched, with more than five tubers per branch. The surface of the tuber has few roots. The inner skin color of the tuber is white, and the flesh color is white-orange. Wrinkles are visible on the surface of the tuber (Figure 4).

Table 1. The initial collection of *Dioscorea* species on Muna Island, Southeast Sulawesi, Indonesia

Location of collection	Collection name	Tuber collection
Tampo Village	*gadung (D. hispida), gembili (D. esculenta)*	●
Wakumoro Village	*gadung (D. hispida), uwi (D. alata), gembili (Dioscorea esculenta)*	●
Oelongko Village	*gadung (D. hispida), uwi (D. alata)*	●
Lasehao Village	*gadung (D. hispida)*	●
Napano Kusambi Village	*gadung (D. hispida)*	●
Napalakura Village	*uwi (D. alata)*	●

Note: ●: Present, -: Absent

Figure 2. The morphology of *Dioscorea hispida*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

Figure 3. The morphology of *Dioscorea alata*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

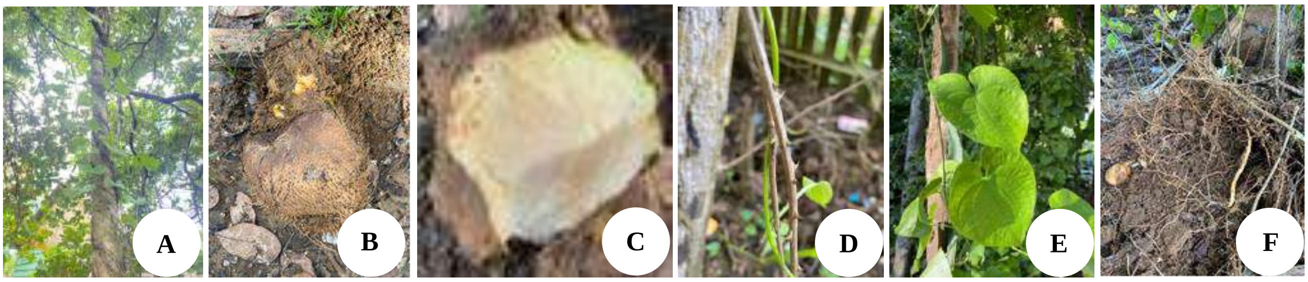

Figure 4. The morphology of *Dioscorea esculenta*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

Comparison of morphological characteristics of *Dioscorea* tubers

The properties and characteristics of the tubers of various species of *Dioscorea* are presented in Table 2. The three species of *Dioscorea* that grow on the island of Muna have different tuber characteristics, such as the appearance of tuber shape, size, inner skin color, and tuber flesh color. The differences in the shape of the tubers observed were irregular shapes in *D. hispida*, oval shapes in *D. alata*, and cylindrical shapes in *D. esculenta*.

Morphology profile of microencapsulated extracts by SEM

The microencapsulation morphology of *D. hispida* was presented through an SEM (Scanning electron m*icroscopy*) analysis with 1000x magnification. The spherical shape consists of two layers, namely a transparent-colored layer and a dark-colored layer. The transparent layer is in the center, an image of the internal layer of the tuber extract. The dark layer is on the outside, the encapsulating layer that envelops the transparent layer as a form of the external layer. The dark layer in microencapsulation has a larger area than the transparent layer, which proves that the tuber extract is encapsulated in the e solution. Although there are some irregular shapes of the microencapsulation (*spherical*), the particle distribution has a uniform size in the 30% and 40% treatments (Figure 5).

Weed height (cm)

Table 3 presents the height of broadleaf weeds treated with microencapsulated *D. hispida* tuber extract as bioherbicide four weeks after application. The findings showed that the weed species influence the weeds' height. The height of *T. alata* and *P. amarus* differs significantly, and the height of *C. pubescens* and *C. mucunoides* was not significantly different. However, the weed type and extract concentration interaction does not significantly impact the weeds' height. The results showed that applying various concentrations of microencapsulated *D. hispida* tuber extract for 4 weeks has significant practical implications. The weed species significantly affected weed height, but the interaction between weed species and *D. hispida* tuber extract concentration does not significantly affect weed height.

Table 2. Comparison matrix of tuber properties and characteristics of *Dioscorea* species

Observed properties and characteristics	*Dioscorea* species		
	D. hispida	***D. esculenta***	***D. alata***
Tubers Shape			
Round	–	–	–
Cylinder	–	–	●
Oval	–	●	–
To finger	–	–	–
Irregular	●	–	–
Tuber length			
5-10 cm	–	–	–
11-15 cm	–	●	–
16-20 cm	–	–	●
21-25	●	–	–
Branching on tubers			
Yes	–	●	–
No	●	–	●
Location of roots on tubers			
Under	–	–	–
Central	–	–	–
Above	–	–	–
Entire surface of the navel	●	●	●
Surface of tuber skin			
Smooth	–	–	–
Finely wrinkled	–	●	–
Roughly wrinkled	●	–	–
Irregular	–	–	–
Porous	–	–	●
Skin color in tubers			
Brown	●	–	–
White	–	●	●
Violet	–	–	–
Dark	–	–	–
Orange	–	–	–
Color of tuber flesh			
White	–	–	–
Orange White	●	–	●
Violet white	–	●	–
Cream	–	–	–
Violet	–	–	–
Tuber latex			
Yes	●	●	●
No			

Note: ●: Present, -: Absent

Root length (cm)

The findings show that the extract concentration significantly affects root length. The root length is also significantly impacted by the weed species. However, the combination of concentration and weed species did not significantly affect root length. The bioherbicide extract application had a tangible effect on inhibiting root length. The 40% bioherbicide concentration effectively inhibited root elongation, as indicated by the average root length. The results showed root elongation was affected by herbicide concentration and weed species. Table 4 shows no interaction effect between the bioherbicide concentration and various species of weeds on root length; however, weed species significantly affect root length. It also shows that the root lengths of *T. alata*, *C. pubescens*, and *C. mucunoides* were not significantly different but different from that of *P. amarus*. Similarly, the extract concentration had a significant effect on root length.

Phytotoxicity level (%)

The treatment of 40% bioherbicide resulted in phytotoxicity on weed after 4 weeks of application (Table 5). The microencapsulated bioherbicide of *Dioscorea* tuber extract significantly affected various broadleaf weeds. The phytotoxicity of broadleaf weeds is presented in Table 6. The results showed that the phytotoxicity of *T. alata* and *C. pubescens* due to the application of *D.hispida* tuber extract were not significantly different, while *C. mucunoides* and *P. amarus* were significantly different. The weed species have a significant effect on the level of toxicity due to the bioherbicide application. The interaction between the weed species and bioherbicide concentration does not significantly affect the level of toxicity. The most sensitive weed against *D. hispida* tuber extract was *P. amarus*, such as changes in the number of leaves and the color and shape of the leaves. These findings emphasize the importance of this research in understanding the physiological effects of controlling weed populations, especially in weed management.

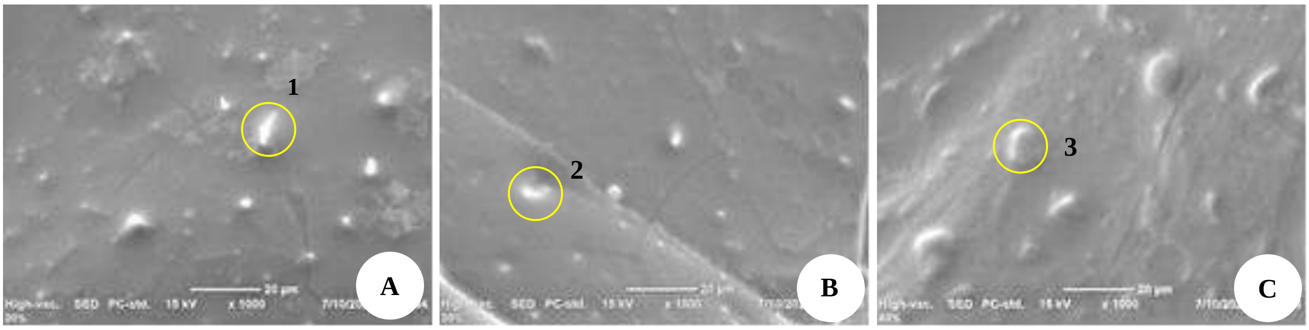

Figure 5. Morphology of microencapsulated extract of *Dioscorea hispida* at 1000x magnification scale. A. 20% treatment showed; 1. Irregular shape; B. and C. 30 and 40% treatment showed; 2. and 3. A round shape

Table 3. Plant height of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks after application

Treatment	Species of weeds				Average (cm)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	42.75	35.25	11.50	32.75	30.56±15.24
20%	46.25	32.50	13.00	31.25	30.75±14.69
30%	41.25	34.25	12.50	31.00	29.75±11.56
40%	38.00	15.25	12.25	29.25	23.68±18.94
Average (cm)	42.06±19.45c	29.31±9.65b	12.31±1.19a	31.06±4.53b	

Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Table 4. Root length (cm) of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks after application

Treatment	Species of weeds				Average (cm)
	T. alata		*P. amarus*	*C.mucunoides*	
Control	7.25	5.69±2.41ab	2.00	6.75	5.69ab
20%	8.25	6.19±2.83ab	1.75	7.25	6.19ab
30%	8.25	6.69±3.15b	2.00	7.75	6.69b
40%	6.50	5.19±2.94a	1.75	7.50	5.19a
Average (cm)	7.56±2.15b	7.00±1.67b	1.88±1.02a	7.31±1.30b	

Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Table 5. Morphological condition of weeds before and after treatment age 4 weeks

Weed Species	Figure		Phytotoxicity scoring result (%)
	Before treatment	After treatment	
T. alata			Toxicity level 15. Damage starts at week 3, showing abnormal leaf shape, color, and yellow spots.
C. pubescens			Toxicity level 15. Damage starts in week 3, showing abnormal leaf shape or color and many yellow spots on the leaves.
P. amarus			Toxicity level 20. Damage starts at week 3 with abnormal leaf shape or color, yellow spots, and shriveled leaves.
C. mucunoides			Toxicity level 15. Damage starts at week 3 with abnormal leaf shape or color and yellowish spots on the leaves.

Table 6. Phytotoxicity measurement of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks of age

Treatment	Species of weeds				Average (%)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	2.00	2.00	3.00	1.00	2.00±0.73
20%	2.00	2.00	3.00	1.00	2.00±0.73
30%	2.00	2.00	3.00	1.00	2.00±0.73
40%	2.00	2.00	2.75	1.00	1.94±0.68
Average (%)	$2.00±0.00^{b}$	$2.00±0.00^{b}$	$2.94±0.25^{c}$	$1.00±0.00^{a}$	

Note: The values were obtained from measurements using the scoring method for phytotoxicity levels from the Pesticide Commission and analyzed using ANOVA. Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Wet weight (g)

Table 7 shows that the wet weight of broadleaf weeds was affected significantly by the species of weed. Still, the interaction between the type of weed and bioherbicide concentration does not significantly affect the wet weight. Among the various broadleaf weeds in this study, the highest wet weight was found in *T. alata* at 7.97 g and the lowest in *P. amarus* at 1.74 g. Based on Table 7, the weed species affected the wet weight, but the interaction between the weed species and extract concentration did not significantly affect the wet weight. The results also showed that the wet weight *C. pubescens* and *T. alata* were not significantly different, whereas *C. mucunoides* and *P. amarus* were significantly different.

Dry weight (g)

The treatment of microencapsulated bioherbicide on various broadleaf weeds significantly affects the dry weight of broadleaf weeds. Table 8 presents the dry weight of broadleaf weeds treated with bioherbicide. The highest dry weight of broadleaf weeds treated with bioherbicides was obtained in *T. alata* (3.54 g), and the lowest was in *P. amarus* (0.29 g). It showed that the dry weight of *C. pubescens*, *T. alata*, *C. mucunoides*, and *P. amarus* differed significantly. However, the interaction between bioherbicide treatment and different weed species did not significantly affect dry weight.

Table 7. Wet weight of broadleaf weeds treated with microencapsulated bioherbicide *D. hispida* tuber extract at the age of 4 weeks.

Treatment	Species of weeds				Average (g)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	7.25	7.50	1.58	6.48	5.70±2.63
20%	9.00	7.63	1.20	6.43	6.06±3.23
30%	8.25	7.63	1.43	6.55	5.96±2.87
40%	7.38	6.15	2.75	5.05	5.33±2.64
Average (g)	7.97±1.53[c]	7.23±1.00[c]	1.74±1.44[a]	6.13±1.62[b]	

Table 8. Dry weight (gr) of broadleaf weeds treated with microencapsulated bioherbicide of *D. hispida* tuber extract at the age of 4 weeks after application.

Treatment	Types of weeds				Average (g)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	3.83	3.10	0.44	2.78	2.53±1.38
20%	3.52	3.18	0.27	2.47	2.36±1.34
30%	3.40	3.18	0.22	2.55	2.34±1.38
40%	3.43	2.45	0.21	2.35	2.11±1.42
Average (g)	3.54±0.60[d]	2.98±0.58[c]	0.29±0.12[a]	2.54±0.75[b]	

Discussion

Comparison of the morphological characteristics of Dioscorea *tubers*

The tubers of *Dioscorea* were used as a source of local food from generation to generation in Muna regency. Previous research conducted by Ariani and Geo (2019) showed that the Muna tribe utilizes *D. alata* and *D. esculenta* with the local names 'ghofa' and 'mafu' as the sources of carbohydrates by consuming their tubers. *D. hispida* is known as 'kolope' by local people and grows in the forest. People rarely use it as a food source because it has more sap and causes itching when it comes into contact with the skin.

Table 1 shows that one or more Dioscorea species could be found in each village. It indicated that *Dioscorea* tubers are abundant and commonly found in the forest. Still, the community has not utilized them much because they do not know the proper processing methods (Padhan and Panda 2020). *D. hispida* tubers are abundant in the area, so training is needed for the community to utilize *D. hispida* tubers as various processed products, such as chips, to reduce dioscorein content. The people in Muna usually boil and fry *Dioscorea* tubers and add salt to remove the sap from the tubers.

D. alata, *D. esculenta,* and *D. hispida* have different morphological characteristics. The morphology of *D. hispida* differs from the other 2 species; it has thick and stiff root hairs and brownish tuber skin. *D. alata* tubers are purplish and have sap or mucus (Lathifah et al. 2024). *Dioscorea* esculenta tubers have skin and flesh color variations from white to orange (Susila et al. 2020), and the color of the inner skin ranges from light brownish white to light yellowish (Pasireron et al. 2021). The tuber surface is rough, smooth, and slippery. The roots on the surface of the tuber have thorny characters on the surface of the tuber and cracks on the surface.

The tuber of *D. alata* and *D. hispida* was bigger than that of *D. esculenta* because the photosynthesis involved in tuber growth led to an increase in tuber size. Photosynthesis produces glucose, an energy source and a raw material for growth. Higher photosynthates in the tuber resulted in more extensive tuber growth. A study by Hamaoka et al. (2022) showed differences in the shape and size of tubers tend to be elongated (cylindrical), rounded, or oval, which is determined by the direction of photosynthesis translocation. Tubers with a larger diameter than the length indicate that the sink capacity is accumulated in the diametral direction and vice versa. Differences were also found in the flesh and skin colors of the tuber. The flesh of the tuber of *D. alata* and *D. hispida* is white-orange. However, the color of the inner skin of *D. hispida* is brown, and *D. alata* is white. Genetic factors and the influence of environmental factors such as temperature, humidity, and different soil conditions cause this variation. Plants grown in various ecological conditions tend to adapt to the surrounding environment. Plants' morphology varies because environmental factors are more dominant in influencing plants than genetic factors (Cao et al. 2021).

Morphology of microencapsulated tuber extract with various concentrations

Figure 5 shows the morphology of microencapsulated *D. hispida* tuber extracts at 20%, 30%, and 40% concentrations have different shapes. It relates to the encapsulation mechanism that attracts allelochemical compounds in the tuber extract. The microencapsulated extract has a ball shape but is irregularly round. The round shape of non-aggregate microencapsulation indicates that microencapsulation is physically stable (Misni et al. 2021). Microencapsulation consists of amphiphilic polymers containing hydrophobic compounds in the core and hydrophilic compounds surrounding the particles to prevent. Small spheres that are evenly distributed with a uniform particle distribution do not unite to form flocculation, indicating a polydispersion index. The smaller the polydispersion index value, the more uniform the distribution of particles in a component is in layer thickness and size. Meanwhile, the irregular shape of microencapsulation can be caused by the coating material (viscosity and solubility), the ratio of the core to the coating, and the inlet air temperature (Kori et al. 2022).

The morphology of microencapsulated extract indicates that a 40% concentration has an effective coating. The microencapsulated bioherbicide derived from *D. hispida* tubers demonstrated the most significant effect on broadleaf weed growth four weeks after application, with *T. alata* reaching 42.06 cm in height and *P. amarus* the smallest (12.31 cm). The dose of bioherbicide also affected the height. The negative control group had the highest height (30.56 cm), while the treatment group of 40% bioherbicide had the lowest plant height (23.68 cm). The findings of this study showed that the weed species and the bioherbicide concentration have different responses to the growth of broadleaf weeds. A study by Bashar et al. (2022) showed that the concentration of bioherbicides has various effects on the weed because the phytotoxic compounds contained in bioherbicides can reach target organisms through evaporation or decomposition with specific mechanisms depending on the organs and their chemical properties.

The effect of microencapsulation of tuber extract on various parameters

Applying different concentrations of microencapsulated *D.hispida* extract had a significant effect on root length. The weed species with the highest morphological damage was *P. amarus*. The research by Pranata et al. (2023) showed that the higher the dose of bioherbicide, the more bioactive herbicide is exposed, which affects the weed growth and further causes the weed to die. Bioherbicides mainly inhibit the function of essential enzymes (Table 3).

The interaction between the treatment of bioherbicide and weed species had the most effect on *P. amarus*, which has the shortest root. Previous studies showed that the average root length of *P. amarus* is 1-6 cm, *T. alata* is 6-9 cm, *C. pubescens* is 7-12 cm, and *C. mucunoides* is 7-12 cm (Stefanowska and Miroslawa 2022: Prasetyorini 2019; Rahman and Nur 2019; Feitoza et al. 2018), One factor that affects root elongation is the disruption of hormone activity that inhibits cell division. The phenolic compound in *Dioscorea* tubers disrupts cytokinin hormone activity, which results in inhibited cell growth and enlargement so that the formation of potential shoots (*plumules*) and radicles is inhibited (Amarasekara and Wickramarachchi, 2021). The findings of this study showed that the administration of bioherbicides affects root elongation by reducing the low rate of root cell growth (Zanfano et al. 2022) (Table 4).

Applying *D. hispida* tuber extract as a bioherbicide caused phytotoxicity in weed plants, which is indicated by plant abnormalities compared to negative control treatment., such as alteration in color and form, wilting, yellowish-brown lesions leaves, leading to the dropping of leaves (Kim et al. 2023). Table 6 showed that the phytotoxicity score for *T. alata*, *C. pubescens*, and *C. mucunoides* weeds was 15%, while *P. amarus* was 20%. This variation arises from different defense systems or resistance levels among these weed species, leading to varied symptomatic responses. A previous study by Strilbyska et al. (2022) indicated that allelochemicals decrease the permeability of cell membranes. Consequently, this can allow toxic substances to enter the plant, interfering with its defense mechanisms and metabolic functions. Dioscorin in *D.hispida* tuber extract causes plant death through physiological disorders. A study by Castillo et al. (2021) showed that disocorin in *D. hispida* tubers could reach 2000 mg/kg, and it can cause genetic changes. Applying bioherbicide also caused chlorosis symptoms, spots on the leaves, and bud death. A study by Bednarz et al. (2023) showed that allelochemicals can cause disease, necrosis, and chlorosis, which inhibit growth and suppress photosynthetic activity.

The impaired water absorption affects the growth of roots and inhibits photosynthesis, resulting in reduced plant absorption capacity, thus affecting wet weight. Islam et al. (2024) stated that toxic chemical compounds in plants can hinder growth and development and cause morphological damage. This is because the bioactive elements in the plants effectively disrupt water absorption and inhibit weed metabolism (Table 7). Several factors influence the outcomes of treatments on different weed species, one of which is the defense system unique to each weed type. According to Wang et al. (2024), it relates to physiological and morphological adaptations that help prevent or reduce the entry of harmful compounds into the plant when exposed to toxic substances. Each weed species has defense mechanisms, such as forming protective layers on the surface of leaves or roots, which can limit the absorption of harmful substances. Physiological changes resulting from treatment may lead to abnormal growth (dwarfism) and color changes in the leaves, stems, or roots. These changes signal disruptions in the plant's metabolic processes, impacting the weed's ability to absorb water and nutrients. Ultimately, these factors influence the wet and dry weight of the weeds and the root length. These findings are consistent with the research of Martinez et al. (2021), which suggests that growth decreases for each parameter observed, with the growth rate slowing down depending on the weed type and the concentration used.

The effect of microencapsulation of Dioscorea *tubers on weed growth*

The administration of various concentrations of microencapsulation of *D. hispida* tuber extract resulted in different growth of 4 species of weeds, namely *T. alata, C. pubescens, P. amarus*, and *C. mucunoides*. The differences in root length were found in the treatment of 40% concentration of bioherbicide. It could be caused by alkaloid compounds in the microencapsulation of *D. hispida* tuber extract. *D. hispida* tubers contain toxic dioscorin alkaloids (Hazrati et al. (2020). Dioscorin and diosgenin were derived from alkaloids and effectively inhibit weed growth, namely height, length, and biomass. The alkaloid content in *D. hispida* tubers is 31.4%, and *Dioscorea alata* L. is 12.52% (Indrawati et al. 2020). The growth rate that decreased the most was *P. amarus*. This is due to the different defense factors of each weed species (Motmainna et al. 2023). They state that the differences in inhibitory effects on several species of weeds occur due to the presence of morphological and physiological defense system factors against environmental stress. Morphological adaptation is based on the inhibition or prevention of the entry of harmful compounds into the plant, such as the presence of lignin. Lignin in the plant cell wall prevents the entry of allelochemical compounds into the membrane (Hasan et al. 2018).

In conclusion, there are 3 species of *Dioscorea* on Muna Island, i.e., *D. alata*, *D. esculenta*, and *D. hispida*. These three species have specific characteristics, namely the color of the tuber flesh. *D. alata* and *D. hispida* have orange-white tuber flesh, while *D. esculenta* has purple-white tuber flesh. The microencapsulated tuber extracts have different shapes at different concentrations of bioherbicide. The microencapsulated tuber extract at the concentration of 20% has irregularly shaped capsules, while the 30% and 40% concentrations show round shapes. The dark layer in microencapsulation has a larger area than the transparent layer, proving that the tuber extract is encapsulated in the encapsulation solution. The application of a 40% microencapsulated tuber inhibits weed growth with a toxicity level of 15%-20% out of 75% (ranging from abnormal to dead).), especially on *P. amarus*. It also inhibits root elongation. The potential of *D. hispida* as a bioherbicide needs further exploration. One of the species collected from Muna Island belongs to Dioscorea. The tubers can be consumed and used as medicinal ingredients. To promote the sustainability of this species, the author intends to investigate the use of *D. hispida* tubers in microencapsulation technology applications to manage weed growth and address related issues using natural herbicides.

ACKNOWLEDGEMENTS

The authors are grateful to Hasanuddin University, Makassar, Indonesia. They would like to thank the managers of the Botanical Laboratory of the Faculty of Mathematics and Natural Sciences and the Phytochemistry Laboratory of the Faculty of Pharmacy, Hasanuddin University. The landowners in Tampo Village, Napabalano District, Southeast Sulawesi, allowed their land to be used as a site for applying microencapsulated bioherbicides.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 185-190 DOI: 10.13057/asianjagric/g090119

Study of the contact effect of stearic acid on *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) in the laboratory

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Manuscript received: 13 November 2024. Revision accepted: 3 March 2025.

Abstract. *Sabit FA, Abdullah MA, Elhadeeti SAK. 2025. Study of the contact effect of stearic acid on* Trogoderma granarium *Everts, 1898 (Coleoptera: Dermestidae) in the laboratory. Asian J Agric 9: 185-190.* The hairy grain beetle, *Trogoderma granarium* Everts, 1898 is a hazardous pest that infests grains and causes great losses in the stores. Stearic acid is a suitable alternative to synthetic insecticides and a safe option to prevent infestation. The current experiment was conducted to investigate the effects of stearic acid on the adult, larval, pupal, and egg stages of *T. granarium*, through the direct treatment of stearic acid on beetle adults, larvae, pupal and eggs stages at exposure times of 5, 10, and 15 hours and concentrations of 20, 40, 60, and 80 ppm. Adult mortality rates varied, with the lowest value at 20 ppm concentration and exposure time of 5 hours being 46.66%, and the highest mortality (100.00%) was at 80 ppm and 15 hours. Larvae mortality rates varied between concentrations, with the highest rates at 80 ppm and 15 hours of exposure time, which reached 93.33%, and the lowest was 26.66% at 5 ppm and 5 hours. The lowest mortality rate of the pupal stage was 14.45% at 20 ppm concentration and 5 hours (exposure time), while the best results were 80.00% at 80 ppm concentration and 15 hours (exposure time). The finding showed that the optimal concentration for controlling *T. granarium*, the egg was 80 ppm stearic acid and an (exposure time) of 15 hours, resulting in the most significant egg damage of 61.54%. The study concluded that stearic acid has a positive lethal impact on *T. granarium* in different stages.

Keywords: Direct effect**,** stearic acid, *Trogoderma granarium*

INTRODUCTION

It is well acknowledged that grain crops like wheat, have high levels of carbohydrates, proteins, and fats. Therefore, people consider it as one of their staple food and tend to store or consume it in times of need (Gadimov et al. 2009). Insect infestations can damage or destroy products, resulting in losses of up to millions of dollars to producers and retailers annually (Demissie et al. 2008). Post-harvest insects migrate for mates, food, and shelter (Tyagi et al. 2019). Insect pests infest stored products from harvest through storage, processing, transportation, and retail and consumer, enabling researchers to identify potential weak points in storage that contribute to increased insect damage (Abdullah et al. 2023).

The hairy grain beetle *Trogoderma granarium* Everts, 1898 (khapra), which belongs to the Dermestidae family, Coleoptera order, and is among the most critical and dangerous insect pests that infest stored products, represents a major and continuous threat (Sabit and Ali 2020; Al-Saffar and Augul 2022; Hameed et al. 2023). Moreover, this species is considered the most crucial quarantine insect of stored products in various parts of the world in tropical and subtropical regions, such as Australia, Russia, and the USA (Kavallieratos et al. 2019; Zhao et al. 2021). It is one of the world's 100 most dangerous invasive species because its larvae can survive in severe conditions (Al-Saffar and Augul 2022). Like many storage pests, *T. granarium* has been transferred to other parts of the world in recent centuries through international trade, although adults do not fly. The khapra insect (*T. granarium*) is now distributed in more than 40 countries in Asia, the Middle East, Africa, and Europe. In Iraq, there are 31 species of stored product insects recorded, 16 genera, 8 families, and 2 orders of insects spread in most parts where grain is stored; the top rank is *T. granarium* (Khalique et al. 2018).

Larvae of *T. granarium* are considered much more harmful than adult beetles concerning control methods (Athanassiou et al. 2015). The larvae feed on whole stored products including cereal crops such as wheat, barley, rice, and other food products. Infestation can be heavy, with weight losses of 5-30%; in extreme infestations, it can be 73% globally (Singh et al. 2017). Damaged grains cause a significant loss in weight, germination, and value of damaged grains, mainly at severe infestation (Rajput et al. 2015).

The management of the khapra insect is based mainly on synthetic chemical pesticides (Abdullah et al. 2024; Haitham et al. 2022). The continuous use of insecticides to manage warehoused product insects has resulted in high environmental risks for humans and animals (Ali and Ali 2016; Almamoory and Al-Mayaly 2018). Hence, alternative management methods have been used for the control of *T. granarium*, such as the application of ozone, temperature, plant extracts, and fatty acids in its control (Sabeat 2017; Abdul Hadi and Al-Khazraji 2021; Hadi and Sabit 2023; Sabet and Sabr 2023).

Also, fatty acids have been applied lately to manage insect infestations. They have been extensively used, particularly oleic and linoleic acids, as they have been developed to have suitable pesticidal properties (Rahuman et al. 2008). *Polytrichastrum formosum* hexane extracts showed the highest lethal activity reaching 70.33% against *Sitophilus granarius* (C.Linnaeus, 1758). Contact toxicity effects using a single dose of palmitic, lauric, and myristic acids showed that the highest mortality rate (53.34%) was achieved using the myristic acid among the other tested pure fatty acids; lauric and palmitic acids were 4.32 and 17.75%, respectively (Abay et al. 2013). A study by Salinas-Sánchez et al. (2020) showed that ethyl acetate fraction of *Serjania schiedeana* Schltdl. (Sapindaceae) stems eradicated 78% at 10,000 ppm with an LC_{50} value of 6,008 ppm in 72 h against adults of *Melanaphis sacchari* (Zehntner, 1897). Commercial fatty acids and b-sitosterol have better aphicidal activity, with mean insecticidal concentrations ranging from 1,000 to 3,000 ppm in 72 h. The chemical analysis of the *S. schiedeana* extract showed that methyl palmitate is the main compound (38.6%). A study by Salinas-Sánchez et al. (2021) showed that a leaves fraction of *Ricinus communis* L eradicated 92% at 10,000 ppm, with an LC50 value of 1,427 ppm in 72 h against *Sipha flava* (Forbes, 1884) (Hemiptera: Aphididae). The chemical analysis revealed that linoleic acid is the primary compound in the active fraction (84.5%); in comparison, commercial linoleic acid at a concentration of 2,500 ppm had mortality and LC_{50} value of 85% and 1,181 ppm at 72 h after treatment, respectively. A study by Cantrell et al. (2011) showed that the fatty acids of *Jatropha curcas* L. seed oil have activity as mosquito repellent against *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) at a concentration of 20 nmol/cm^2.

In another study to estimate the effectiveness of fatty acids in the laboratory on the larvae of the *Earias insulana* (Boisduval, 1833) revealed that oleic acid and stearic acid affected more than linoleic acid at different concentrations the survival time of larvae and pupae reducing it to 18.93 and 10.19 days respectively compared to the other acids tested (Moustafa et al. 2018). A study by Aquilar-Marcelino et al. (2022) revealed that stearic and palmitic fatty acids were active against *M. sacchari* by fumigation of particles at rates of 74 and 63%, respectively, at 2500 ppm concentration and 72 hours exposure time. A recent study by Hadi and Sabit (2023) that evaluated the efficiency of fumigation using two fatty acids as a natural and safe alternative to synthetic pesticides in management eggs and pupa of *T. granarium*, the results showed that treating the insect eggs with fumes resulted in high rates of inhibition of egg hatching (Hadi and Sabit 2023). The effect of fatty acids on insect mortality involves physical and chemical disruption of the insect's protective barriers, leading to insect's death by suffocation, dehydration, or cell damage. It is often used alone or combined with other ingredients to enhance effectiveness (Bernklau et al. 2016). This study aimed to evaluate the effectiveness and efficiency of stearic acid by contact method as an accepted and harmless alternative to chemical pesticides in managing the larval, pupal, and adult stages of the hairy grain beetle *T. granarium*.

MATERIALS AND METHODS

Insect rearing

The beetle, *T. granarium* was obtained from infested grains in the warehouse insect laboratory – at the University of Baghdad. The beetles were placed in a containers made of sterilized plastic with a capacity of 500 mL. Insects were fed on wheat grains of the Ibaa 99 variety, and the new eggs deposited, adults, larvae, and pupae were separated carefully for further experiments. The insect rearing and experiments were carried out in the laboratory of the plant protection department, which is affiliated with Agricultural Engineering Sciences College/Baghdad University. New wheat grains were frozen at a temperature of -20°C for (14) days to ensure they were free from pests infestation, they were then placed in an incubator at 35 ± 1°C and 65 ± 5 (humidity). 3 g of KOH compound was placed in water (100 mL) in a tightly sealed glass container to stabilize the humidity.

Preparation of stearic acid concentrations

The stearic acid concentrations were prepared by putting 1 mL of 99% (ethanol) in a 1000 mL container and dissolving 20 microliters of stearic acid inside it using a microliter device and completed it to a volume of 1000 mL by adding distilled water in which fatty acid to the solvent ratio solution became (20) parts per million. Other concentrations (40, 60, and 80 ppm) were prepared, and the comparison treatments (ethanol and water) were prepared using a similar procedure.

Bioevaluation by contact with stearic acid on *T. granarium*

The different stages of *T. granarium* (eggs, larvae, pupae, and adults) were sprayed with a small hand sprayer at a capacity of 20 mL and at a distance of 15 cm from the dish at a rate of 1 mL/dish to ensure the homogeneity of the solution for all dishes (three replicates, 10 individuals from each stage for every replicate) for the different treatments and stages in addition to the control treatment (only treated with ethanol and water). All dishes were placed in the incubator under the indicated conditions (35 ± 1°C t and 65 ± 5 h). The mortality data were recorded every 5 hours and for 15 hours and corrected using (Abbott 1925) formula.

Statistical analysis

Experiments were designed according to Complete Randomized Design (CRD) (Shaaban and Al-Mallah 1991). Data were subjected to a one-way ANOVA of insect mortality, and statistical analysis was conducted using GenStat software at a significant level of $p>0.05$.

RESULTS AND DISCUSSION

Direct effect of stearic acid application on adults of *T. granarium*

Table 1 shows stearic acid's effect on *T. granarium* adults at concentrations of 20, 40, 60, and 80 ppm and exposure time of 5, 10, and 15h. The means of adult mortality percentages at 20, 40, 60, and 80 ppm concentration reached 58.88, 71.55, 79.00, and 91.11% respectively. The effect of exposure time (5, 10, and 15 h) had a mortality rate of 61.91, 79.17, and 84.33% respectively. The lowest mortality rate was 46.66% at 20 ppm and 5h, and the best results were 100.0% at 80 ppm and 15h.

Increasing concentration increases the mortality rate significantly in the adult stage of *T. granarium* (Table 1). However, the exposure time of 5 hours had a significantly lower mortality rate than that of 10 and 15 hours. The synergistic effect of the concentration and the exposure time had a more significant impact on the insect.

Direct effect of stearic acid on *T. granarium* larvae

Table 2 shows stearic acid's effect on larvae of *T. granarium* at concentration of 20, 40, 60, and 80 ppm and exposure time of 5, 10, and 15h. The larvae mortality percentages at concentration of 20, 40, 60, and 80 ppm were 36.66, 52.22, 67.78, and 76.66%, respectively. At the same time, three exposure times resulted in mortality rates of 45.83, 58.33, and 70.83%, respectively. The lowest mortality rate (26.66%) was obtained at the concentration of 20 ppm and the exposure time of 5h, and the highest mortality was 93.33% at the concentration of 80 ppm and at the exposure time of 15h. The lowest percentage of larvae mortality was obtained at a concentration of 20 ppm and exposure time 5h, which reached 26.66%, and the highest rate was 93.33% at 80 ppm and 15h exposure time.

The results showed that mortality percentage of 60 and 80 ppm concentration was not significantly different. However, the treatment of 20 and 40 ppm had a lower mortality percentage significantly than 60 and 80 ppm. The increasing exposure time increased significantly mortality percentage of the larvae. The synergistic effect of the concentration and exposure time treatments might had a greater impact on the larvae mortality rates.

Direct effect of stearic acid on *T. granarium* pupae

Table 3 shows the effect of stearic acid on the pupal stage of *T. granarium* at concentration of 20, 40, 60, and 80 ppm and exposure time of 5, 10, and 15h. The results showed that the highest mortality rate was 80.00% at concentration of 80 ppm and 15h as exposure time. The pupal mortality percentages at concentration of 20, 40, 60, and 80 ppm were 19.19, 29.08, 31.44, and 63.26% respectively, while their mortality rate at the three exposure time reached 25.75, 38.37, and 50.78% respectively. The lowest mortality was 14.45% at the concentration of 20 ppm and at the exposure time of 5h, and the highest mortality was 80.00% at the concentration of 80 ppm and at the exposure time of 15h.

The mortality percentage of 80 ppm was significantly higher than the other treatments in the pupae. However, the mortality rates for the 40 and 60 ppm treatments were not significantly different. Increasing exposure time increases the mortality percentage significantly. The inreaction of exposure time and concentration were all significant except for the interaction at the concentration of 40 ppm and exposure time of 10h, and the synergistic effect of the interaction treatments between concentration and exposure time was more influential on the mortality percentages.

Direct effect of stearic acid on *T. granarium* eggs

Table 4 shows the effect of stearic acid on the *T. granarium* egg stage at concentration of 20, 40, 60, and 80 ppm and exposure time of 5, 10, and 15h. The lowest rate of egg hatching inhibition was recorded at the concentration 20 ppm and exposure time 5h reaching 17.34%, while the highest rate was recorded at 80 ppm and exposure time 15h, namely 61.54%. The percentage inhibition means of the egg hatching at the four concentration reached 25.04, 33.7, 49.5, and 53.94% respectively, while the effect of exposure time at 5, 10, and 15 h on egg-hatching inhibition were 30.26, 40.30, and 51.07%, respectively.

The egg-hatching inhibition of the treatment of 60 and 80 ppm was not significantly different but significantly higher than that of 20 and 40 ppm. The egg hatching inhibition of exposure time of 5 and 10 hours was not significantly different but increasing exposure time of 15 hours increased egg-hatching inhibition significantly. The synergistic effect of the concentration and exposure time was more influential in the percentage of egg-hatching inhibition.

Table 1. Direct effect of stearic acid application on *T. granarium* adults

Concentration (ppm)	Mortality percentage			Mean
	Time (hour)			
	5	10	15	
20	46.66e	63.33d	66.66c	58.88d
40	60.00d	73.33c	81.33b	71.55c
60	67.66c	80.00b	89.33a	79.00b
80	73.33c	100.0a	100.0a	91.11a
Mean	61.91d	79.17b	84.33b	---
L.S.D (0.05)	Concentration 8.36 time 7.59			
	Concentration × time 14.07			

Table 2. Direct effect of stearic acid on *T. granarium* larvae

Concentration (ppm)	Mortality percentage			Mean
	Time (hour)			
	5	10	15	
20	26.66e	36.66e	46.66d	36.66
40	40.00e	53.33d	63.33c	52.22d
60	53.33d	70.00b	80.00b	67.78b
80	63.33c	73.33b	93.33a	76.66b
Mean	45.83e	58.33c	70.83c	---
L.S.D (0.05)	Concentration 9.41 time 7.95			
	Concentration × time 15.02			

Table 3. Direct effect of stearic acid on *T. granarium* pupae

Concentration (ppm)	Mortality percentage Time (hour) 5	10	15	Mean
20	14.45	20.00e	23.11e	19.19e
40	20.22e	28.13d	40.00d	29.08d
60	25.00d	38.89d	60.00b	31.44d
80	43.34c	66.44b	80.00a	63.26b
Mean	25.75e	38.37d	50.78c	---
L.S.D (0.05)	Concentration 9.66 time 8.38 Concentration × time 15.87			

Table 4. Direct effect of stearic acid on *T. granarium* eggs

Concentration (ppm)	Mortality percentage Time (hour) 5	10	15	Mean
20	17.34e	23.11e	34.67d	25.04e
40	20.22e	31.77d	49.11b	33.7c
60	37.55d	52.00b	58.94a	49.5b
80	45.94c	54. 33b	61.54a	53.94b
Mean	30.26c	40.30c	51.07b	---
L.S.D (0.05)	Concentration 7.92 time 6.86 Concentration × time 12.39			

Discussion

The lethal efficacy of fatty acids on some insects including *T. granarium,* has been studied previously (Abdullah and Muhammad 2005; Moustafa et al. 2018; Aquilar-Marcelino et al. 2022). This study evaluates the lethal effect of stearic acid against *T. granarium* stages in warehoused wheat grains.

The findings showed that stearic acid could control *T. granarium* at all its stages including adults, larvae, pupae, and eggs. All concentration of stearic acid were lethal and caused high rates of mortality for the adults at all exposure time. This result may be due to the penetration of stearic acid through the insect body wall, interfering with their metabolic processes and finally causing insect death (Peng et al. 2020; Kaczmarek et al. 2022). Fatty acids act as a deterrent for many insect species, making them less likely to feed or reproduce in areas where stearic acids are present. Additionally, stearic acids have been shown to disrupt the reproductive systems of certain insects, further impacting their population dynamics (Ferdous et al. 2021).

Stearic acid showed a high effect in causing the death of the larval and pupal stages of *T. granarium*, also it was effective at all of the concentration and exposure time. It destroys the exoskeleton of insects by covering the exoskeleton, which protects the insect externally and regulates humidity. Stearic acid can clog pores and disrupt normal physiological processes, including breathing through the spiracles, causing dehydration, suffocation, and ultimately, the insect's death (Kaczmarek and Bogus 2021).

Stearic acid showed high rates of egg-hatching inhibition at all its concentration and exposure time. It may be due to the ability of the fatty acid vapor to enter the egg and disturb its protoplasm, which has a deleterious effect on the respiratory and nervous parts (Hadi and Sabit 2023). After that, it affecting the growth and development of the embryo. It also can damage the embryo muscle tissue, resulting in loss the ability to breathe and, finally, embryo death. It can act as growth regulator that affects the progression of some biological processes for the embryo's growth and development, such as affecting the metabolism in the eggs, which thwarts the partial or total embryo formation (Mahgoub and El-Sisi 1997).

These findings agree with previous studies indicating the efficacy of fatty acids including stearic acid to control insects. Stearic acid is commonly used as an insecticide ingredient, effectively targeting many pests (Eldesouky et al. 2019). The mortality control rate of stearic acid is significant, as it is highly toxic to a wide range of insect species. Its action involves suffocating insects by enveloping their respiratory systems, ultimately resulting in death (Ibrahim 2020). In addition, stearic acid exhibits long-lasting effects, protecting against infestations by a range of insect pests (Ibrahim 2020). It is a valuable tool in both agricultural and domestic treatments. Its unrefined properties provide a reliable solution for managing insect-related problems with minimal environmental impacts (Aider et al. 2016; Aguilar-Marcelino et al. 2022). Stearic acid can affect metabolic rates and stress responses, impacting insect survival (Blomquist and Ginzel 2021; Refaey et al. 2022). It can have a toxic effect on insects by disrupting cell membranes, interfering with enzymes, and altering metabolic processes within the insect, leading to the collapse of critical cellular functions, which affects insect survival (Shah et al. 2023).

There is a positive relationship between the increase in concentration, exposure time and percentage rates mortality of adults, larvae and pupae and egg hatching inhibition (Prathiksh 2020; Siddiqui et al. 2022). Therefore, stearic acid can be considered as natural product promising potential source of safer alternative means with promising potential as a safer alternative to control *T. granarium* and different agricultural pests.

In conclusion, our results show that stearic acid is effective against all stages of *T. granarium* and can be considered an environmentally friendly alternative to the frequent use of synthetic traditional chemical insecticides within the concentrations and periods indicated in the integrated pest management plan. This study can inform decision-making processes regarding pest management strategies and implementing preventive measures in field and warehouse environments.

ACKNOWLEDGEMENTS

The author would like to thank all parties for the preparation and publication of the manuscript.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 191-200 DOI: 10.13057/asianjagric/g090120

Assessment of the water insecurity of rice and fish farmers in the coastal areas of Myanmar and Vietnam

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Manuscript received: 21 January 2024. Revision accepted: 26 March 2025.

Abstract. *Oo AT, Minh DD. 2025. Assessment of the water insecurity of rice and fish farmers in the coastal areas of Myanmar and Vietnam. Asian J Agric 9: 191-200.* Farm households along the coastlines of Myanmar and Vietnam are susceptible to climate-related hazards and the adverse effects of water insecurity. To understand the level of water insecurity of rice and fish farming households, this study examines water insecurity of households by using Guttman's Water Insecurity Indexing (WII) analysis. This study was conducted with 899 farm households from Myanmar and Vietnam. A total of 29 indicators were selected and constituted into four major components. According to a total aggregate score, rice-farming households are more susceptible to accessing water sources and services than fish-farming households in both countries. Compared to farm households in Myanmar, farm households in Vietnam are particularly vulnerable to access to water sources, with scores of 0.414 and 0.334. There is little variation in the water resource management score. However, Myanmar's farm households indicated more inadequate adaptation capacity (0.487) than Vietnam's (0.462). Moreover, an idealized Guttman's scale indicated that farm households in Myanmar had a lower level of intervention capability and adaptive capacity than farm households in Vietnam. The findings highlight the necessity of improving intervention capability and capacity and accessibility measures to improve the water security of farm households in both Myanmar and Vietnam.

Keywords: Adaptive capacity, climate-related hazards, farm households, Guttmann's analysis, water insecurity

INTRODUCTION

Water is essential for human life, socioeconomic development, poverty reduction, and other basic development objectives (MacAlister et al. 2023). However, multiple compounding effects of climate change have jeopardized the security of our water resources. Climate change has exacerbated the rise of sea levels, which impacts livelihoods in low-lying coastline areas. In coastal metropolitan regions in developing Asian countries, water security is under threat due to rapid urbanization, population growth, declining water quality, climate change, and ecological-hydrological changes (Krittasudthacheewa et al. 2019; Aboelnga et al. 2020; IPCC 2022). Farm households in the coastal areas of the Mekong Region are facing urgent water security crises, leading to significant water availability and quality disruptions for agriculture and domestic use (Krittasudthacheewa et al. 2019; MacAlister et al. 2023). One key factor contributing to water insecurity is the increased frequency and intensity of extreme weather events, and it has direct implications for farmers who rely on consistent access to water for irrigation, crop and fish production, and household needs (Krittasudthacheewa et al. 2019; IPCC 2022). Access to safe and secure water sources becomes a crucial concern during and after disasters, affecting not only agricultural production but also the overall health and livelihoods of the communities (Krittasudthacheewa et al. 2019; Lebel et al. 2023).

According to MacAlister et al. (2023), Vietnam and Myanmar fall into the category of water-insecure countries according to the national water security scores ranked for the Asia-Pacific. Myanmar has rich water resources but is plagued by poor infrastructure and increasing impacts of climate-related hazards (Oo et al. 2017; Oo 2018; MONREC 2019; Oo et al. 2023). On the other hand, in Vietnam, the Mekong Delta is heavily impacted by flood and climate change extremes. Even though Vietnam has made substantial progress in water resource management in recent years, it continues to face challenges in ensuring equitable access to safe water and safeguarding water quality (MONRE 2016; Tran et al. 2022; Lebel et al. 2023). By understanding the specific challenges these farming communities face, it is possible to enhance water security, build resilience, and implement targeted measures to meet the needs of each farming community in the face of increasing risks of water security.

There is an increasing number of studies on water insecurity assessment in different contexts and climate change's impact on water systems, management, and security (Jaramillo and Nazemi 2017; Danielaini et al. 2019; Kalogiannidis et al. 2023; Liu et al. 2024). As water security is a multi-dimensional construct in nature, several studies applied 'composite indices to their treatment in order to compare (normalization) and aggregate (weighting and aggregation)' for multiscale comparisons of complex concepts (Huynh and Stringer 2018; Vanham et al. 2018; Doeffinger and Hall 2021). Anthropologists have utilized Guttman's method to create ethnographically grounded scales that capture variation in a progressive, unidimensional concept across a set of indicators (Jepson and Vandewalle 2016; Danielaini et al. 2019; Aboelnga et al. 2020; Ding et al. 2024). In this study, we employ water insecurity indicators reported across various studies and local-specific dimensions of water insecurity. Similar to Danielaini et al. (2019), the indicators were selected and grouped into four dimensions of water insecurity, namely, insufficient accessibility, acceptability, capability, and adaptive capacity, and the grouping of dimensions was carried out given the definition of the indicators and related metrics of the indicators under broader dimension. Therefore, this study aims (i) to identify the key components of water insecurity in agriculture (rice) and aquaculture (fish) farming households; (ii) to evaluate the prevalent issues that shape the farm household's water insecurity; and (iii) to compare the difference in the water insecurity and vulnerability of both rice and fish farming households in Myanmar and Vietnam.

MATERIALS AND METHODS

Study area

In Myanmar, flooding and saltwater intrusion to farmlands were frequently recorded in Kyaiklat and Dedaye Townships. Kyaiklat Township includes 87 village tracts, while Dedaye Township includes 40 village tracts. Kyauktan Township is located in the SouthEast area of the Yangon Region and consists of 32 village tracts (MIP 2017; Oo 2018). This study covers the two coastal areas most afflicted by saltwater overflow and floods in Myanmar, where the Dedaye, Kyaiklat and Kyauktan Township were reported as the areas most affected by the climate-related extremes. The three townships that were chosen are all low-lying areas with elevations under 5 m (Oo et al. 2018). This empirical research study was carried out in 12 communities: of which 6 in Dedaye and Kyaiklatt Township and 6 in Kyauktan Township (Figure 1.A). By selecting 12 communities in these 3 townships, the impacts of saltwater intrusion and flooding at the individual farm level could be better understood. Six communities in Dedaye and Kyaiklatt Township in the Ayeyarwaddy Region: namely Ka Lar Su, Kyon Thin, Hmyaw Sin, Kunpa Laing, Kyee Chaung and Hpoe Shan Gyi villages, and 6 communities in Kyauktan Township in the Yangon Region: namely Kamarmat, Oke Pho, Thet Kel Kone, Yae Kyaw, Shwe Pyi Thit and Zwe Bar villages were selected.

In Vietnam, six communities spread across three Tam Giang – Cau Hai lagoon districts were chosen for this study. After consultations with commune-level officials, these villages were selected to ensure they met certain criteria, such as being part of the lagoon system and having both agriculture and aquaculture farming communities. There are 11 communes in the Quang Dien district, of which Quang Loi and Quang Thai were chosen. Of the 21 communes in the Phu Vang District, Vinh Xuan and Vinh Phu communes were selected. Additionally, Vinh Hung and Vinh Giang communes were chosen from among the 15 communes in the Phu Loc District (Figure 1.B). For the village profiles in the sampling areas, we developed gender and ethnicity related questions and sought to ensure that women and minority groups participated in the study.

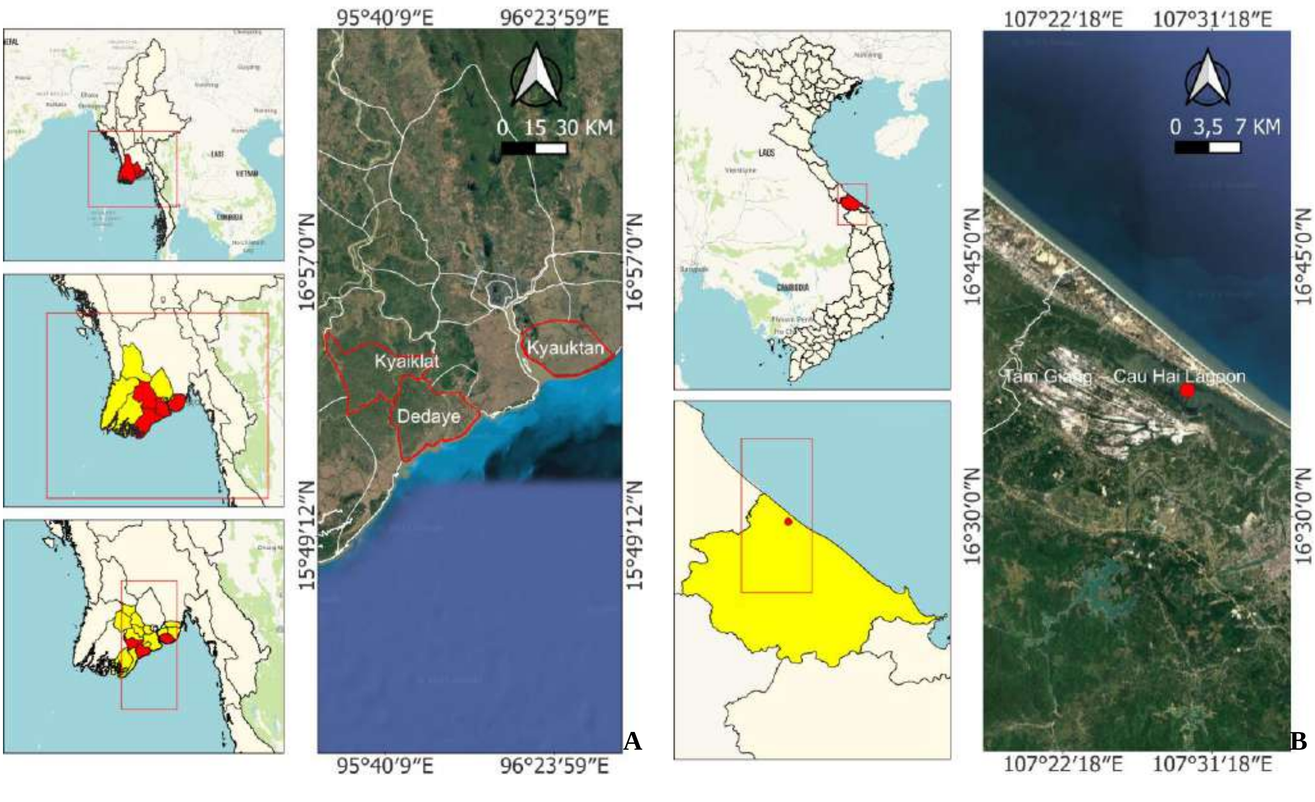

Figure 1. Map showing the study areas: A. Kyaiklat, Maubin City and Dedaye Township, Pharpon District in Ayeyarwaddy Region and Kyauktan Township, Yangon-S City in Yangon Region of Myanmar; B. Tam Giang – Cau Hai Lagoon, Thừa Thiên Huế, Vietnam

Considering the vast geographies and limited knowledge of the risks of water insecurity and climate change impacts, this study was conducted in two regions in Myanmar and one in Vietnam. The survey was carried out between September to October 2021 in Myanmar and from February to March 2022 in Vietnam. Given the complications and impacts of the COVID-19 pandemic during the surveys, a randomized sampling method was considered, where more samples were taken in low-travel restriction areas. For instance, Myanmar had fewer travel restrictions within communities at the time of the survey, while Vietnam had strict travel restrictions inside rural communities during the survey. Initially, villages were selected based on characteristics such as villages located near coastline areas and in which rice-farming and fish-farming farmers are presented. Data are collected in four steps: (i) field observation; (ii) selection of indicators with key stakeholders; (iii) final questionnaire preparation; and (iv) farm household surveys or interviews. A sample of 300 was considered from each sampling region. Regarding geographic disparities, this survey included 599 sample farmers in Myanmar and 300 respondents in Vietnam. A total of 899 samples were acquired for this research.

A total of 29 indicators were selected for this study and the selected indicators were grounded by an extensive literature review. During the survey, responses were assessed in dichotomous scale, one for positive responses and zero for negative responses. Farm households responses were then re-arranged in the data matrix and the experience-based indicators used in the scale development across dimensions. Guttman's scalograms, for example, assess individuals according to their frequency of responses, from highest to lowest. It also includes four dimensions: insufficient accessibility to water sources and related services, inadequate acceptable level of water risks, insufficient capability and adaptive capacity of water resource management (Marzi et al. 2018; Doeffinger and Hall 2021) (see detail in Table 1). Furthermore, indicators related to flooding and flood protection measures, as well as the impacts of climate and hydrological changes on farm households were considered and thus this study offers concrete findings of farm households and local communities and has direct implications for policymakers and development planners in Myanmar and Vietnam. A balanced indexing method was chosen to examine the difference in water insecurity level of farm households in two countries. The applicability, relevance, and uniqueness of the selected indicators were validated at the focus group discussions with key stakeholders, so that the selected indicators were able to capture the status of water insecurity in the specific regions and farming communities in Myanmar and Vietnam. Therefore, this study advances previous work on Marzi et al. (2018) and Doeffinger and Hall (2021) on experience-based scaling of water insecurity and uses an analytical framework including four dimensions or scales of household water insecurity at rice-farming and fish-farming in a coastal region of Myanmar and Vietnam.

During the assessment, all potential participants were requested to sign an ethical consent form; only those who agreed to participate were interviewed. Despite the COVID-19 pandemic in the region, the research team considered community participants norms, values, and ethics, such as following COVID-19 coping measures, keeping participant data anonymous, and practicing secure data management during the research assessment. Despite adhering to the suggested research aims, our study has certain limitations. Before the survey, the enumerators undertook testing and training utilizing several questionnaires. They might not have a solid grasp of the concepts around climate change and the different kinds of questionnaires. One questionnaire may also be used in this study to examine the unique characteristics of the two distinct contexts or nations. However, we conduct a daily data quality check to ensure our data are of the highest quality. Furthermore, this study only considers how climate change affects farm households; it ignores how it affects biodiversity, the ecosystem, cattle, and other animals.

Data analysis

According to MacAlister et al. (2023), global research approach to water security assessment highlights the inconsistency of data availability and the need for additional research to address disparities and knowledge gaps adequately. Therefore, countries should expand their data collection systems to fill the numerous gaps (Larsen et al. 2019; MacAlister et al. 2023; Anghileri et al. 2024). Guttmann's indexing and scaling analysis provides a structured methodology for capturing these complexities by incorporating indicators related to water availability, quality, accessibility, reliability, and governance (Marzi et al. 2018; Doeffinger and Hall 2021; Stoler et al. 2023). Guttmann's indexing and scaling approach also emphasizes the importance of considering not just the physical aspects of water availability but also the social, economic, and institutional dimensions that shape water security. Moreover, Guttmann's analysis goes beyond simple indicators by examining indicators such as water governance structures, community engagement, financial access to water services, and environmental sustainability (Doeffinger and Hall 2021; Veettil et al. 2022; Zhou et al. 2023). Therefore, applying Guttmann's water insecurity indexing analysis in the context of Myanmar and Vietnam offers a valuable framework for understanding the multifaceted nature of water security in these countries. This study considered indicators related to flooding and flood protection measures, as well as the impacts of climate and hydrological changes on farm households. Therefore, this study considers a comprehensive view and knowledge of the factors influencing water insecurity in Myanmar and Vietnam.

The open-ended questions gathered in the survey were carefully coded and categorized for analysis. The responses from the survey were evaluated using a dichotomous scale for each indicator, where a value of zero represented a negative response, and a value of one indicated a positive response. This method allowed for a clear and straightforward assessment of the participants perspectives. The survey comprised questions that addressed 29 indicators organized into four components. A composite indexing analysis using a balanced aggregate method was applied to convey the water insecurity level of rice and fish-farming households to flooding and saltwater intrusion in Myanmar and Vietnam. Each component comprises indicators standardized using a balanced average approach (Oo et al. 2018; Minh et al. 2019). The major component index of the rice and fish-farming households in both countries can be calculated after each index has been standardized following by equation (1):

$$M_h = \frac{\sum_{i=1}^{n} index\, S_h i}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, M_h denotes one of the four main components of agriculture or aquaculture farm households 'h', '$index\, S_h$' represents the subcomponent index or indicator denoted by 'i' of households 'h' and 'n'the number of indicators applied in each major component. This structured approach to data analysis not only facilitated a thorough understanding of the respondents views but also allowed for comparative insights between the two nations involved in the study. By using the Guttman scale, we were able to determine the levels of agreement or disagreement regarding each indicator, contributing to a more nuanced understanding of the issues. Overall, the methodology ensured systematic processing of qualitative data, providing valuable insights while maintaining a high level of reliability in the results obtained from both Myanmar and Vietnam.

RESULTS AND DISCUSSIONS

Accessibility to water sources and related services

Figure 2 displays climate-change extreme events in Vietnam and Myanmar between 1980 and 2020. Storms (49%) and floods (38%) were the most observed natural hazards in Vietnam, whilst floods (36%) and miscellaneous accidents (26%) were the most occurring natural disasters in Myanmar. Twenty-nine indicators were selected, validated, and evaluated to determine the differences in water insecurity levels of rice farming and fish farming households in Myanmar and Vietnam (Table 1). Of the 29 indicators chosen, 13 were included in the water accessibility component, 4 in the water acceptability component, 5 in the capability component, and 7 in the adaptive capacity component. The adaptive capacity component was considered to reflect the ability of both farming systems to the adverse effects of water-related problems and ecological-hydrological changes to mitigate or adverse the potential damages with these consequences. In the component of insufficient accessibility to water sources and related services, the mean index is 0.414, with rice-farming households experiencing a higher degree of insufficient access to water sources and water-related services at 0.412 and fish-farming households at 0.404. This study indicated that farm households in both countries have challenges with accessibility to water sources and related services. Although disputes with neighbors over water management occur, such instances constitute only slightly more than 20% of cases, with most families adopting their water treatment practices.

Nevertheless, households confront certain barriers in accessing public water sources and wastewater treatment, which is unsurprising, given the limited water-related services in most rural areas of the study. For instance, it is noted that Myanmar was among 26 countries with an estimated zero level of wastewater treatment (MacAlister et al. 2023). Furthermore, the drainage system is restricted, affecting not just farm households water use but also water utilization in production, particularly in water quality-sensitive sectors like fish farming. It is also noted that Myanmar and Vietnam were among the 20 lowest-scoring countries for water use efficiency in 2019 (MacAlister et al. 2023). The Vietnamese government has put considerable effort into implementing water supply and sanitation reforms and programs, but there is still a large rural and urban divide. Access to safe water and sanitation in Vietnam's rural areas was only around 45-51%, while it was about 82% in urban areas (Pham et al. 2023). In Myanmar, however, access to "improved water" is indicated as 83% (urban 93%, rural 78%) and to sanitation as 76% (urban 83%, rural 73%) (Global Water Partnership 2016). The findings also show that farm households generally experience issues with water resource management and accessibility, with the majority using their own water treatment practices.

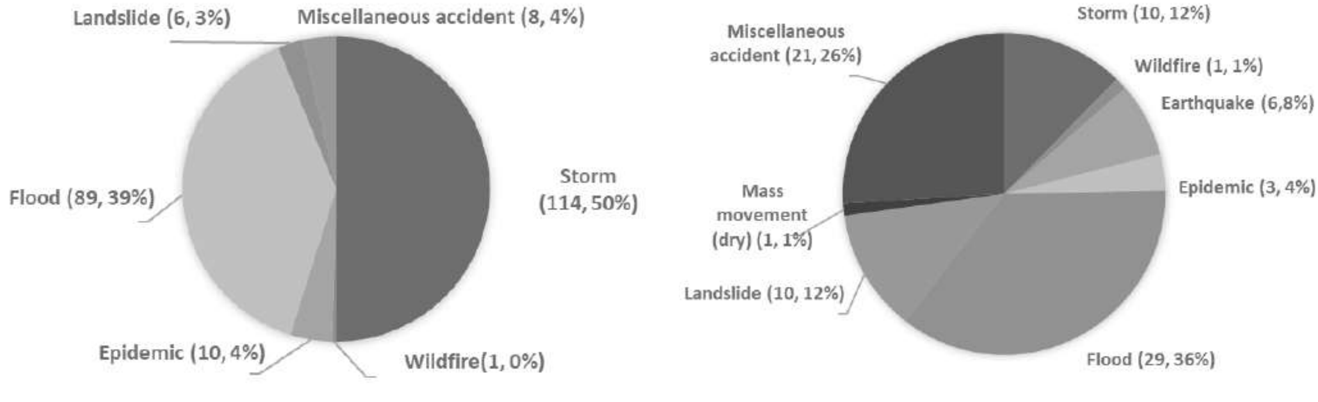

Figure 2. Occurrence of climate-change extreme events in Vietnam and Myanmar from 1980-2020 (Source: World bank climate change knowledge portal)

Table 1. Comparison of water insecurity indexing scores for two farming systems in Myanmar and Vietnam by weighting indicators

Indicators	Rice-farming households		Fish-farming households		Total cumulative scores	
	Vietnam Freq. (%)	Myanmar Freq. (%)	Vietnam Freq. (%)	Myanmar Freq. (%)	Vietnam Freq. (%)	Myanmar Freq. (%)
Idealised Guttman Scale for insufficient accessibility to water sources and related services						
Households report water conflict problems	88 (0.53)	51 (0.106)	58 (0.433)	6 (0.052)	146 (0.487)	57 (0.095)
Slept very few hours due to water collection duty	51 (0.31)	79 (0.164)	40 (0.298)	7 (0.061)	91 (0.303)	86 (0.144)
Dispute with neighbors/ tenants/ owner due to water	47 (0.28)	58 (0.12)	35 (0.261)	6 (0.052)	82 (0.273)	64 (0.107)
Reduced time for daily work/income-generative activities due to water collection	59 (0.355)	100 (0.208)	45 (0. 336)	12 (0.104)	104 (0.347)	112 (0.188)
Reduced time for studies or missed school due to water collection	47 (0.283)	48 (0.099)	38 (0. 294)	2 (0.017)	85 (0.283)	50 (0.084)
Collected water from an undesirable/ dirty source	39 (0.235)	89 (0.184)	35 (0.261)	12 (0.104)	74 (0.247)	101 (0.169)
The household does not have private sanitation facilities	46 (0.277)	368 (0.762)	32 (0.239)	99 (0.861)	78 (0.26)	467 (0.781)
Households do not have private water facilities	60 (0. 361)	311 (0.643)	48 (0.358)	39 (0.339)	108 (0.36)	350 (0.584)
Household members do not have access to improved non-drinking water source	65 (0.392)	105 (0.217)	54 (0.403)	14 (0.122)	119 (0.397)	119 (0.199)
The drainage system does not surround the household	121 (0.729)	70 (0.145)	97 (0.724)	22 (0.191)	218 (0.727)	92 (0.154)
Household members do not have access to improved drinking water sources	126 (0. 759)	98 (0.202)	98 (0.731)	15 (0.13)	224 (0.747)	113 (0.189)
Household members do not have access to improved sanitation facilities	37 (0.223)	431 (0.892)	27 (0.201)	108 (0.939)	64 (0.213)	539 (0.902)
Households do not have access to public drinking water system	123 (0.741)	392 (0.81)	97 (0.724)	51 (0.443)	220 (0.733)	443 (0.74)
Overall score	0.421	0.35	0.404	0.263	0.414	0.334
Idealised Guttman Scale for the inadequate acceptable level of water risks						
Households do not have access to public wastewater treatment	124 (0.747)	464 (0.961)	97 (0.724)	110 (0.957)	221 (0.737)	574 (0.96)
Having experienced an increase in flood event	32 (0.193)	153 (0.316)	34 (0.253)	17 (0.148)	66 (0.22)	170 (0.284)
Currently having difficulty/problem with flood/ flooding	96 (0.578)	117 (0.242)	77 (0.575)	9 (0.078)	173 (0.577)	126 (0.21)
Currently having difficulty/problem with water scarcity	71 (0.428)	267 (0.552)	63 (0.470)	32 (0.278)	134 (0.447)	299 (0.499)
Overall score	0.486	0.518	0.506	0.365	0.495	0.488
Idealised Guttman Scale for the insufficient capability of water resource management						
Having experienced a decrease in river water quality	47 (0.283)	262 (0.541)	44 (0.328	58 (0.504)	91 (0.303)	320 (0.534)
Currently not satisfied with flood protection	73 (0.439)	96 (0.198)	67 (0.5)	43 (0.035)	140 (0.467)	139 (0.232)
Currently not satisfied with healthy human settlement	53 (0.319)	7 (0.012)	41 (0.306)	1 (0.043)	94 (0.313)	8 (0.014)
Currently not satisfied with sufficient water availability	63 (0.379)	31 (0.064)	45 (0.336)	14 (0.122)	108 (0.36)	45 (0.075)
Currently not satisfied with water and sanitation infrastructures	77 (0.464)	60 (0.124)	55 (0.411)	7 (0.061)	132 (0.44)	67 (0.112)
Overall score	0.377	0.188	0.376	0.214	0.377	0.193
Idealised Guttman Scale for insufficient adaptive capacity in water resource management						
Currently not satisfied with healthy waterways	79 (0.476)	166 (0.344)	52 (0.388)	15 (0.13)	131 (0.437)	181 (0.303)
Disagree that it is important to use water much more efficient	71 (0.427)	44 (0.091)	53 (0.395)	4 (0.035)	124 (0.413)	48 (0.08)
Disagree that it is important to minimize water use consumption	67 (0.404)	52 (0.107)	55 (0.410)	5 (0.043)	122 (0.407)	57 (0.095)
Having the opinion that only the government is responsible for improving the eco-hydrological status	35 (0.211)	236 (0.488)	44 (0.328)	56 (0.487)	79 (0.263)	292 (0.487)
Having no solution to minimize the impacts of flooding events and salter instructions	33 (0.199)	268 (0.555)	40 (0. 298)	58 (0.509)	73 (0.243)	326 (0.546)
Climate and hydrological changes adversely affect household social and economy	127 (0.765)	472 (0.975)	100 (0.746)	113 (0.983)	227 (0.757)	585 (0.977)
Having concerns regarding the change in flooding events and saltwater intrusion into farms	119 (0.717)	439 (0.907)	95 (0.709)	114 (0.991)	214 (0.713)	553 (0.923)
Overall score	0.457	0.495	0.468	0.454	0.462	0.487

Acceptable level to water-related risks

Regarding the acceptability of risk associated with water resource management, farm households experience a relatively high risk level, as shown by index values of 0.495 in Vietnam and 0.48 in Myanmar. The fish-farming households exhibit a higher risk level, with an index value of 0.506, while the rice-farming households belong to a lower risk level, with an index value of 0.486 in Vietnam. The most challenging issue for farm households is not having access to the basic wastewater treatment system, with an index score of 0.737 in Vietnam and 0.96 in Myanmar. Notably, approximately fifty percent of farm households in this study area indicated a challenge and difficulty due to increased water shortages and freshwater supply. Thus, more concerted efforts are required to reduce those risks by improving the effectiveness and efficiency of water resource management policies and strategies in the study areas. Actions and measures that improve the accessibility and quality of water treatment systems may be beneficial in addressing this issue. Given that most farm households indicated a substantial challenge related to flood and saltwater intrusion, it is critical to have a comprehensive and integrated strategy for water resource management that addresses these issues in a coordinated and effective manner. This might involve establishing appropriate infrastructure such as dikes or embarkments, policies, and actions to improve water accessibility and quality and strengthen community resilience to flood and saltwater intrusion.

Incapability to manage water resources

Regarding the insufficient capability of farm households regarding water resource management, the indexing scores exhibit a relatively low level of capability, with an average score of 0.377 in Vietnam and 0.193 in Myanmar. This component has no statistical difference between the fish-farming and rice-farming households. About one-third of farm households in Vietnam reported they are not satisfied with flood protection, human settlement, water availability, and sanitation infrastructures. In contrast, a very small percentage of farm households reported this incapability with water resource management in Myanmar. Thus, this study highlights the need to prioritize measures that address the incapability of farm households related to flood protection, human settlement planning, water availability, and sanitation infrastructures in the study areas. Moreover, the study highlights the need for a comprehensive and integrated approach that considers human settlement, water supply, sanitary infrastructures, and resilience measures to reduce incapability and flood impacts on farming in the coastal areas of Myanmar and Vietnam.

Adaptation capacity in water resource management

The adaptive capacity component comprising seven criteria pointed out that the majority of farm households are still having insufficient capacities to cope with adverse changes and water insecurity in the region, with an average score of 0.462 in Vietnam and 0.487 in Myanmar. About 98% of farm households in Myanmar and 75% of farm households in Vietnam agreed that the worsening climate and hydrological changes are adversely affecting to social and economic conditions of their farm households. And, about 92 and 72% of farm households in Myanmar and Vietnam also agreed that they are experiencing the adverse effects of flood events and saltwater intrusion to farms. The findings suggest that farm households in both countries are extremely vulnerable to the adverse effects of climate and hydrologic changes, saltwater intrusion, and flooding. The results also indicate that the fish-farming households are slightly more vulnerable to climate and hydrological changes than the rice-farming households. In this study, about half of farm households in Myanmar and one-third of farm households in Vietnam are concerned over government responsibility for improving the current ecological-hydrological status in the study areas. Perhaps, the rest of the farm households perceived that it should be bilateral approaches while both communities and private and public organizations should conduct measures to improve the ecological-hydrological conditions in the region. Although the Vietnamese government has put considerable effort to meet most of the sustainable development goal-6 (water for all) by implementing mostly on water supply and sanitation over the past two decades, there is still need for increased capital, technology and expertise to upgrade infrastructure and water supply networks, mostly in the rural areas (World Bank Group 2023). In the case of Myanmar, the government spending on service was still low and amid the pollical crises, there are no or little concerns over to invest in national water sector by the government. Although the overall availability of water is abundant, there are distinct regional differences, such as lack of water or difficulty to access the water resources in dry zone, flooding in the delta, flash floods in the mountains and dry zone, salinization in the delta areas (Global Water Partnership 2016; World Bank Group 2023). Therefore, more investment to fulfill the needs for adequate availability of clean and sufficient water as well as empower the adaptive capacity of rural households in Myanmar is still largely needed. Therefore, both private and public organizations should make efforts to minimize the impacts of flooding, saltwater intrusion, and climate change impacts on water resources as well as less impacts on the social and economic conditions of farm households. Moreover, awareness campaigns should be organized to educate farm households about their role in improving water use efficiency and efficacy. These efforts will be crucial in minimizing the adverse effects of climate and hydrological changes on farm households livelihood activities and well-beings.

Idealized assessment of water insecurity by Guttman's dimensional scores

Following Guttman's scaling assessment, this study categorizes four dimensions of water insecurity, where we synthesized 13 criteria in the first dimension, four in the second dimension, 5 in the third dimension, and 7 in the fourth dimension (Table 2). The number of criteria in each dimension differs based on the compilation of previous research reference materials and information availability for each criterion. Sufficient and adequate data about rice- and fish-farming households can verify the different dimensions of water insecurity in the coastline areas of Myanmar and Vietnam. Figure 3 illustrates the significance of four dimensions among rice and fish-farming households in each country and a cumulative average percentage of farm households in each country. The results indicated that in terms of accessibility to water sources and services, most households belong to group A2-marginal water accessibility, accounting for over 65.7% in Vietnam and 85% in Myanmar. A relatively large proportion of households belong to group A3-low water accessibility, with an average of 28 and 11.7% for farm households in Vietnam and Myanmar, respectively. Despite the development of irrigation and machinery systems, fish-farming households usually encounter more difficulties in water accessibility than agricultural households in both countries.

Moreover, the results also indicate that the levels of acceptability to water-related risks are distributed across three main indices in Vietnam. But in Myanmar, the level of acceptability to water risks is mainly concentrated in category B1- high acceptability. The households that perceived the highest level of this dimension belong to group B1, with an average of 39.7% in Vietnam and 73.3% in Myanmar. It is interesting to note that both fish-farming and rice-farming households have similar levels of perceived acceptability. The second indicator (B2) consists of approximately 32.3% of households, where the rice-farming households have a higher percentage of perceived acceptability at 36.1%, while fish-farming households account for 27.6% of it.

Finally, a relatively low proportion of households (B3) agree that they have a high level of water risk acceptability at 22%. Only a very small percentage of households perceived a low level of damage in the criterion (B4) in Vietnam. In Myanmar, however, the rice-farming households perceived high acceptability to water risks at 69.6%, followed by a similar level of marginal (15.1%) and low acceptability (14.5%). However, the highest acceptability to water risks was observed by 88.7% of fish-farming households. In contrast, those farm households in Myanmar reported a small and equal contribution of marginal and low water acceptability. These findings suggest significant challenges posed by water-related risks such as saltwater intrusion and flooding in the study areas. Most farm households reported difficulties in accessing and utilizing water resources, and their livelihood and production processes are impacted by these water-related risks. The differences in levels of perceived damage between fish-farming and rice-farming households may reflect differences in the specific challenges posed by water-related risks. Thus, this finding highlights the need for targeted interventions to address the unique needs of different farming systems.

Regarding the insufficient capability of water sources, which is measured by five criteria, it is observed that farm households in both groups are quite satisfied with intervention capability dealing with issues related to water resource management in Vietnam. Most farming households belong to group C1, with an indexing score of 51.7%. However, there is not much difference between rice farming and fish farming households. The remaining levels are relatively evenly distributed for the indicator (C2) and (C3) - about 13% of households. Surprisingly, the proportion of households in the indicator C4 is quite high at 22.1%, and this ratio is not much different in both farm households. Compared to Vietnam, Myanmar is left behind in terms of insufficient capability of water resource management, with low incapability accounting for 45.7% and marginal incapability at 37.7%. Most rice-farming households account for C3-low capabilities (47.5%), while the fish-farming households belong to category C2 (marginal incapability with 40%). This could be due to Myanmar's limited infrastructure availability and water resource management capability compared to Vietnam. However, rice-farming and fish-farming households reported low and medium capabilities in Myanmar. Therefore, in terms of insufficient capability of water resource management, farm households in Vietnam are evaluated at high intervention capabilities, especially in fish-farming households. At the same time, this was accounted for in low and medium capabilities with farm households in Myanmar.

Moreover, this study employed seven indicators to assess the adaptive capacity of the farm households, such as satisfaction with healthy waterways, water utilization, and water consumption; only the government has responsibility for improving eco-hydrological status, and no solution to minimize the impacts of flooding events and salter instructions, climate and hydrological changes adversely affect household social and economy and concerns regarding the change in flooding events and saltwater instructions. We categorized farm households adaptive capacity level into four levels to measure farm households adaptive capacity level. The results show that households are evaluated at a high adaptive capacity of D1, 47%, whereas fish-farming households were reported at 48.5%, and rice-farming households were 45.8% in Vietnam. In Myanmar, however, the low adaptive capacity with ecological-hydrological changes was evaluated at D3, with the highest scores being 67.6%, where 65.2% of fish-farming households and 68.2% of rice-farming households were reported. This could be due to the lower literacy rate of Myanmar compared to Vietnam, as the education status of the farmers is directly related to the adaptive capacity of farmers in response to the impacts of extreme climate events and natural disasters (Oo et al. 2018; Oo 2018).

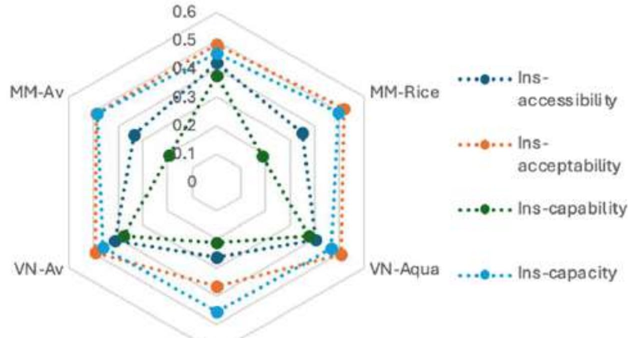

Figure 3. Dimensions of water-insecurity status for rice- and fish-farming households in Myanmar and Vietnam

Table 2. Comparison of water insecurity indexing scores for two farming systems in Myanmar and Vietnam

Indicators	Rice-farming households		Fish-farming households		Total cumulative scores	
	Vietnam Freq. (%)	Myanmar Freq. (%)	Vietnam Freq. (%)	Myanmar Freq. (%)	Vietnam Freq. (%)	Myanmar Freq. (%)
Idealised Guttman Scale for accessibility to water sources and related services (13 indicators)						
Adequate water-sanitation access – A1	3 (1.8)	4 (0.8)	3 (2.2)	1 (0.8)	6 (2)	5 (1.6)
Marginal water-sanitation access – A2	109 (65.7)	406 (83.9)	88 (65.7)	108 (94)	197 (65.7)	514 (85)
Low water-sanitation access – A3	45 (27.1)	65 (13.4)	39 (29.1)	5 (4.4)	84 (28)	70 (11.7)
Very low water-sanitation access – A4	9 (5.4)	9 (1.9)	4 (3)	1 (0.8)	13 (4.3)	10 (1.7)
Total	166	484	134	115	300	599
Idealised Guttman Scale for acceptability level to water risks (4 indicators)						
Highly likely water risks acceptability – B1	64 (38.6)	337 (69.6)	55 (41.1)	102 (88.7)	119 (39.7)	439 (73.3)
Marginal water risks acceptability – B2	60 (36.1)	73 (15.1)	37 (27.6)	6 (5.2)	97 (32.3)	79 (13.2)
Low water risks acceptability – B3	29 (17.5)	70 (14.5)	37 (27.6)	6 (5.2)	66 (22)	76 (11.8)
Very low water risks acceptability – B4	13 (7.8)	4 (0.8)	5 (3.7)	1 (0.9)	18 (6)	5 (1.7)
Total	166	484	134	115	300	599
Idealised Guttman Scale for insufficient capability for water resource management (5 indicators)						
High intervention capability for satisfying socioeconomic activities – C1	85 (51.2)	41 (8.5)	70 (52.2)	20 (17.4)	155 (51.7)	61 (10.2)
Marginal intervention capability for satisfying socio-eco activities – C2	19 (11.4)	180 (37.2)	20 (14.9)	46 (40)	39 (13)	226 (37.7)
Low intervention capability for satisfying socioeconomic activities – C3	26 (15.7)	230 (47.5)	13 (9.7)	44 (38.3)	39 (13)	274 (45.7)
Very low intervention capability for satisfying socio-eco activities – C4	36 (21.7)	33 (6.8)	31 (23.1)	5 (4.3)	67 (22.3)	38 (6.3)
Total	166	484	134	115	300	599
Idealised Guttman Scale for adaptive capacity for water resource management (7 indicators)						
High adaptive capacity to deal with eco-hydro changes – D1	76 (45.8)	1 (0.2)	65 (48.5)	1 (0.8)	141 (47)	2 (0.6)
Marginal adaptive capacity to deal with eco-hydro changes – D2	44 (26.5)	122 (25.2)	29 (21.6)	35 (30.5)	73 (24.3)	157 (26)
Low adaptive capacity to deal with eco-hydro changes – D3	31 (18.7)	330 (68.2)	24 (17.9)	75 (65.2)	55 (18.3)	405 (67.6)
Very low water risks acceptability – D4	15 (9)	31 (6.4)	16 (11.9)	4 (3.5)	31 (10.3)	35 (5.8)
Total	166	484	134	115	300	599

Note: Source = Household’s survey in 2021-2022

The second highest adaptive capacity group was evaluated at the medium adaptive capacity (D2), with scores of 26% in Myanmar and 24.3% in Vietnam. Only a small proportion of households reported high adaptive capacity scores (D1) being total scores of 0.6%, very low adaptive capacity scores (D4) being 5.6% in Myanmar, and low adaptive scores (D3) being total scores of 18.3% and very low adaptive capacity (D4) being total scores of 10.3% in the case of Vietnam. There are no significant differences between the two farm farming systems in Myanmar and Vietnam in these low and very low adaptive capacity categories. In this study, farm households in Myanmar are evaluated at low adaptive capacity, while the farm households in Vietnam belong to high adaptive capacity to deal with water resource management. Oo et al. (2018) observed that many social and economic conditions of the farm households influence the adaptive capacity. Thus, further studies are necessary to understand better the reasons or factors that influence the low and high adaptive capacity of farm households in Myanmar and Vietnam.

Regardless of the farming systems, rice-farming and fish-farming households in Myanmar and Vietnam are increasingly vulnerable to water security issues and susceptible to eco-hydrological changes and extreme climate events such as floods, water pollution, and saltwater intrusion. In terms of idealized Guttman's scaling analysis, the acceptability of water risks was evaluated as high scores, and the accessibility to water sources and related services in the region was recorded as medium in both Myanmar and Vietnam. In Myanmar, however, low intervention capability for satisfying socioeconomic activities and lower adaptive capacity to deal with ecological-hydrological changes were evaluated as the highest scores. In Vietnam, however, high adaptive capacity and high intervention capability were recorded. This study has highlighted the need to improve the intervention capability and adaptation capacity of farm households in the coastal areas of Myanmar. This study has pointed out the need for increased accessibility of farm households to water sources and water-related services in both countries. Therefore, policy interventions and community-based initiatives considering the different farming systems should play a vital role in developing adaptive strategies to mitigate water insecurity in the coastal areas. Strengthening flood protection measures, promoting awareness for improving water use efficiency, and supporting farming system resilience-building efforts are integral to addressing the long-term challenges posed by water insecurity in the rural areas of Myanmar and Vietnam.

Overall, the study highlights the importance of adopting a comprehensive approach that addresses the various dimensions of the water insecurity indices and considers the specific needs and challenges of farm households in ensuring the sustainable management and utilization of water resources in the coastal areas of Myanmar and Vietnam. By utilizing the localized indices and considering the various water user's groups in water insecurity evaluation, stakeholders can gain valuable insights into the complex ways that the water insecurity affects a diverse community. These insights can then used to inform targeted interventions, policy development, and resource allocation to improve water access, equity, and sustainability. Finally, we note that ongoing monitoring and evaluation are essential to track changes in water insecurity over time and assess the effectiveness of interventions aimed at addressing these challenges in both Myanmar and Vietnam. Further research is needed to better understand the reasons that influence the adaptive capacity and capability of farm households. Additional measurement considering the political, institutional, and environmental factors within the same income groups or farming systems can be conducted and compared within and between countries for mitigating adverse effects of natural disasters, and ecological-hydrological changes in the coastal areas of Myanmar and Vietnam.

ACKNOWLEDGEMENTS

Heartfelt thanks to the farmers from both Myanmar and Vietnam who were involved in the study, particularly during the COVID-19 pandemic in the region. The authors would like to express their sincere gratitude to the field-based enumerators, key farmers, and community-based organizations for their support of the field survey. The corresponding author greatly acknowledges the Stockholm Environmental Institute (SEI) Sustainable Mekong Research Network (SUMERNET) for the financial support to enhance the capacity of researchers in the Mekong Region.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 201-207

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090121

Unraveling key determinants of corn productivity: A correlation study of soil chemistry, biology, and climate in Sumedang, West Java, Indonesia

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Manuscript received: 22 January 2025. Revision accepted: 28 March 2025.

Abstract. *Sule MIS, Fitriatin BN, Siswanto SY, Nurbaity A. 2025. Unraveling key determinants of corn productivity: A correlation study of soil chemistry, biology, and climate in Sumedang, West Java, Indonesia. Asian J Agric 9: 201-207.* Corn productivity in areas with diverse agroecological conditions is influenced by several environmental factors such as soil chemistry, soil physics, soil biology, and climate conditions. The influence of these factors on corn productivity is significant and necessary to maintain or even increase corn plant productivity. This study aims to examine the conditions of soil chemistry, soil biology, and corn land climate and identify the correlation between environmental factors and corn yields, thereby determining the main factors of corn productivity in Sumedang, West Java, Indonesia. The study conducted using purposive sampling and an analytical approach at 18 observation locations, has practical implications for agricultural practices. By analysis was carried out by analyzing total phosphorus, cation exchange capacity, organic carbon, soil pH, phosphate-solubilizing bacteria, arbuscular mycorrhizal fungi, rainfall, temperature, and humidity; the study provides valuable insights for optimizing corn cultivation. The results of the correlation analysis show that each environmental factor has a different correlation strength with corn productivity. Soil chemistry factors have a weak-moderate correlation (0.26-0.43), soil biology is very weak (-0.36 to 0.02), and climate is weak-strong (-0.78 to 0.74). Environmental temperature conditions positively correlate ($r = 0.74$) to corn productivity. This is likely due to increased soil mineralization processes, germination, and faster plant development at optimal temperatures. Conversely, environmental humidity negatively correlates ($r = -0.78$) to corn productivity. This is likely because increased humidity can increase the risk of plant diseases (fungi), decrease photosynthesis efficiency, and limit soil aeration, thus affecting the health of corn plant roots. These findings underline the importance of maintaining the suitability of the corn cultivation environment amidst climate change, especially in tropical climates such as Indonesia.

Keywords: Agroecosystem, crop yield, microbial activity, soil fertility, sustainable agriculture

INTRODUCTION

Corn (*Zea mays* L.) is one of the leading food crops, and its existence is essential in Indonesia and the world. Corn has broad uses, such as animal feed, humans, and industrial raw materials (de Matos Nascimento et al. 2020). The average per capita corn consumption per week in Indonesia in 2024 will reach 0.04 kg (BPS 2024a). Corn in Indonesia is important in maintaining food and economic security, especially in villages. This is because cultivating corn in the village is one of the livelihoods of local farmers and a source of their income. Corn production in Indonesia in 2024 was predicted to reach 15 million tons and experience an increase of 2.93% compared to production in 2023 (BPS 2024b). However, achieving sustainable productivity is influenced by complex interactions between land characteristics (soil chemistry and biology) and the environment (climate conditions).

Soil health in the form of soil chemical and biological properties is one of the main factors that can influence corn plant productivity. Soil chemical characteristics are related to the availability of nutrients in the soil for corn plants, such as total phosphorus (P), Cation Exchange Capacity (CEC), organic Carbon (organic-C), and soil pH. P in the soil tends to be unavailable to plants because it is bound to aluminum (Al) and iron oxide (Fe), especially in acidic soils (Mabagala and Mng'ong'o 2022). Soil chemical properties can be influenced by cultivation practices carried out by farmers, such as the use of high doses of inorganic fertilizers. This condition causes competition between cations such as Mg^{2+} and Ca^{2+}, resulting in Mg deficiency in the soil. The ability of the soil to provide nutrients for plants is described by the CEC value (Yang et al. 2024). Apart from soil chemistry, factors that influence corn plants growth and productivity are the soil's biological conditions.

Soil biology in the form of soil microbiome structure and function affects soil fertility and plant health (Hartmann and Six 2023). Soil microbes play a role in decomposing organic matter, recycling nutrients, and increasing resistance to pathogens that cause plant diseases (Liu et al. 2022). The population and diversity of microbes such as Phosphate Solubilizing Bacteria (PSB) and soil Arbuscular Mycorrhizal Fungi (AMF) are the main factors that affect soil nutrient availability and plant productivity (Cavagnaro et al. 2015). AMF biomass is significantly positively correlated with corn productivity cultivated using manure in the long term (Jiang et al. 2020). The relationship between the microbial population and corn plant productivity is still unclear. Several recent studies have reported an unclear relationship between microbial diversity and plant productivity, with positive (Lankau et al. 2022; Shu et al. 2022) and negative (Ali et al. 2020; Fan et al. 2021) correlations. In addition to soil conditions, chemical and biological soil environmental factors such as climate conditions are also significant factors influencing the success of corn cultivation.

Climate conditions also significantly affect corn plant growth. At crucial stages such as flowering and grain filling, extreme environmental temperatures can disrupt pollination, seed formation, and grain filling, ultimately reducing yields (Gourdji et al. 2013). Climate change is characterized by an increase in temperature and changes in rainfall patterns, thereby threatening the success of corn production throughout the world in the future (Ocwa et al. 2023). The research results by Zhang and Yang (2019) stated that increasing days of extreme temperatures in 2050 could reduce corn yields by 27.3%. Changes in rainfall can affect the effectiveness of soil moisture and nutrients (Zheng et al. 2024). Changes in rainfall affect plant photosynthesis and microbial activity by changing the availability of water and soil nutrients and directly affecting the carbon exchange process in the plant-soil system (Zhu et al. 2021).

Previous studies on increasing corn productivity in Indonesia have been widely conducted. However, most of them only focused on one factor affecting corn productivity separately (biological, chemical, or climatic conditions). The research results on the effect of interaction between soil properties and climatic conditions on corn productivity that have been carried out comprehensively are still limited. Given these conditions, this study aims to examine the chemical conditions of soil, soil biology, and the climate of corn fields, as well as to identify the correlation between corn yields and environmental factors so that the main factors of productivity can be determined. This study was carried out in Sumedang, West Java, Indonesia, one of Indonesia's corn production centers.

MATERIALS AND METHODS

Sampling location

The study was conducted from September 2022 to March 2023 using a purposive sampling method to determine the sampling location. It was conducted with an analytical approach at 18 observation location points (Figure 1) with the same land use type: land planted with NKSumo 7283 corn variety. The characteristics of land management at the sampling location are that land processing uses the minimum tillage method with fertilizer input in the form of 3.5-7 tons/ha of manure, 200-350 kg of NPK fertilizer, and 200-350 kg of Urea fertilizer. The sampling location was in Cibugel District, Sumedang, West Java, Indonesia, with an altitude of around 500-1,000 m above sea level and a 10-30% slope. Soil samples from each sampling location were analyzed at the Soil Biology Laboratory and the Soil Chemistry and Plant Nutrition Laboratory of Universitas Padjadjaran.

Soil chemical analysis

Nitrogen total (%)

The nitrogen content analysis of the fertilizer was conducted using the Kjeldahl method, which involves several stages, including sample digestion, distillation, and titration (Gani et al. 2023). A completely homogenized sample of soil weighing precisely 250.0 mg was cautiously transferred into a Kjeldahl flask. Subsequently, 3 mL of H_2SO_4 and 0.25-0.50 g of selenium were added to the mixture, which was shaken until completely homogenized. The mixture was then allowed to stand for 2 to 3 hours. The digestion was performed incrementally at temperatures between 150 and 350°C until a clear solution was obtained. After the solution was cooled, a small volume of distilled water was added to inhibit crystallization.

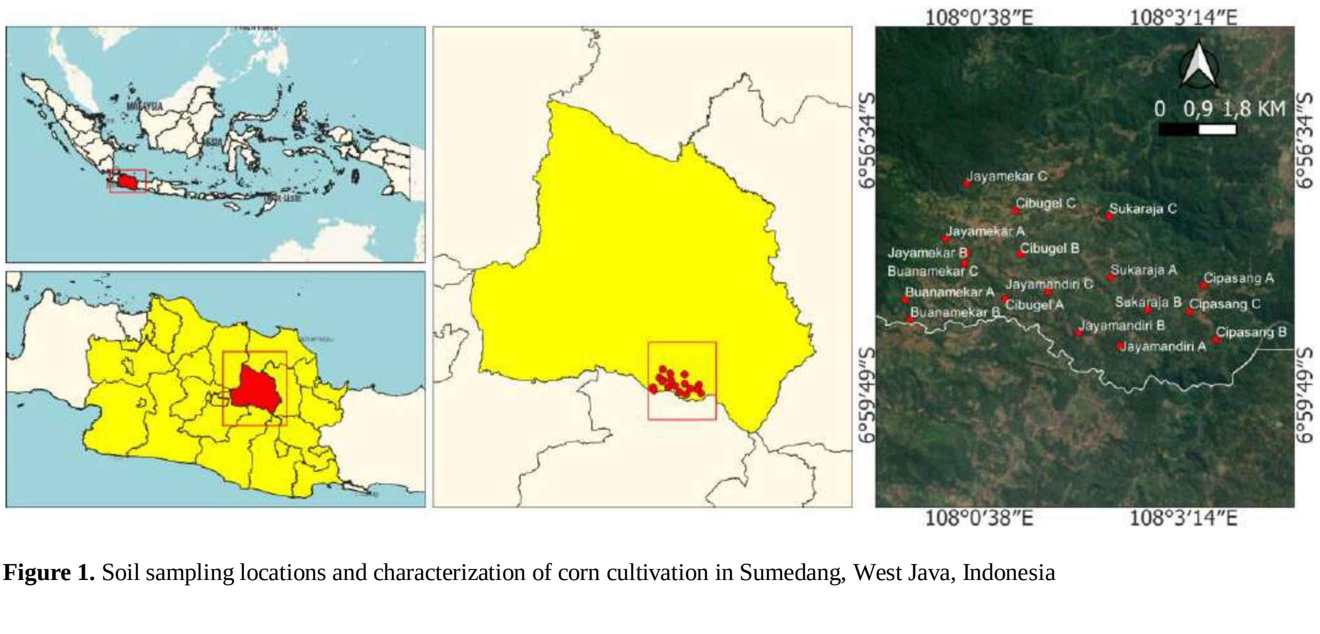

Figure 1. Soil sampling locations and characterization of corn cultivation in Sumedang, West Java, Indonesia

The distillate solution was poured into a boiling flask to a total volume of 250 mL, and deionized water was added until the flask reached the halfway mark. The distillate reservoir was set up in a 100 mL erlenmeyer flask, which contained 10 mL of 1% boric acid and three drops of Conway indicator. The distillation procedure involved the addition of 20 mL of 40% sodium hydroxide (NaOH). It was regarded as complete once the solution volume in the erlenmeyer flask reached 75. The resulting solution was then titrated with 0.05 N H_2SO_4 until a transition from green to pink occurred. The volume of H_2SO_4 used was measured, and the procedure was repeated to obtain the blank sample. The nitrogen content was calculated using the following formula:

$$\text{Nitrogen (\%)} = (Vc - Vb) \times N \times 14 \times fk$$

Where: Vc = Volume H_2SO_4 titration (mL); Vb = Volume H_2SO_4 blank (mL); N = Normality of raw solution H_2SO_4; 14 = Atomic weight of nitrogen; 100 = % conversion; fk = Moisture content correction factor.

Organic carbon (%)

Analysis of soil C-organic content was carried out using the method used by Gani et al. (2023). A total of 50-100 mg of soil sample was put into a 100 mL measuring flask, and 5 mL of 2N $K_2Cr_2O_7$ solution was added and then mixed until homogeneous. Furthermore, 7 mL of H_2SO_4 was added to the solution and left for 30 minutes. The 250 ppm standard C solution was prepared using 5 mL of 5000 ppm standard C solution added to a 100 mL measuring flask. Then, 5 mL of H_2SO_4 and 2N $K_2Cr_2O_7$ solution were added as in the 250 ppm standard C solution. Then, each solution was diluted to 100 mL and stirred until homogeneous. The absorbance value of each measurement was measured using a spectrophotometer with a wavelength of 651 nm. A standard curve was made to obtain a linear equation, which allows the determination of the x value of the solution. The C-organic content was calculated using the following formula.

$$\text{Organic-C (\%)} = \text{ppm curve} \times \text{mL} \times fk$$

Where: Ppm curve = the sample rate was derived from the regression curve showing the relationship between the standard series rate and the reading, adjusted by subtracting the blank value; fk = noisture correction factor = 100 / (100 - % moisture content).

Phosphate analysis (%)

The total P content of the soil was described using spectrophotometry as conducted by Irwandhi et al. (2025). Five hundred mg of soil samples from each location were weighed and put into a digestion flask. Then, 0.5 mL of $HClO_4$ and 5 mL of HNO_3 were added and stirred until homogeneous. Furthermore, the mixture of materials was heated in a reactor with an initial temperature of 100°C. During the process, food produced yellow vapor in the tube. When the yellow vapor disappeared, the temperature was set to 200°C, and digestion was considered complete when white vapor appeared with a remaining liquid of about 0.5 mL. The solution was then shaken and diluted using deionized water to a volume of 50 mL, then filtered using W-41 filter paper to obtain extract A. One mL of extract A was mixed with 9 mL of deionized water in a 20 mL test tube and stirred until homogeneous. The solution then evolved to produce a 10-fold dilution (extract B). The absorbance value was measured using a spectrophotometer with a wavelength of 693 nm. A standard phosphate curve was then created to produce a linear equation used to calculate concentration (ppm).

pH analysis

Soil pH was measured using 10 g of dried and ground soil samples. The soil sample was placed in a beaker, and 25 mL of deionized water (1:2.5) was added. The soil and deionized water mixture was stirred until homogeneous using a magnetic stirrer and was left for 30 minutes. After that, the soil pH value was determined using a calibrated pH meter.

Soil biological analysis

Isolation of phosphate-solubilizing bacteria was carried out using a serial dilution method up to the 10^{-6} level using Pikovskaya selective media (dextrose: 10 g L^{-1}, $Ca_3(PO_4)_2$: 5 g L^{-1}, $(NH_4)2SO_4$: 0.5 g L^{-1}, $MgSO_4.7H_2O$: 0.1 g L^{-1}, yeast extract: 0.5 g L^{-1}, $FeSO_4.7H_2O$: 0.0001 g L^{-1}, KCl: 0.2 g L^{-1}, $MnSO_4.7H_2O$: 0.0001 g L^{-1}, Agar: 15 g L^{-1}, pH 7). The cultures were then incubated at room temperature for 48 hours (Irwandhi et al. 2025).

Data analysis

Data analysis on each research variable was conducted using descriptive methods. Correlation analysis was conducted using Pearson correlation testing. Data visualization of the research findings was accomplished using Prism 9 software (Irwandhi et al. 2025).

RESULTS AND DISCUSSION

Chemical properties of corn cultivation

Soil chemical properties at a depth of 0-30 cm are analyzed, including total P, Cation Exchange Capacity (CEC), organic Carbon (C-organic), and soil pH (Figure 2). The figure shows that each research location point has different soil chemical properties. The total P content ranges from 6.26-17.39%, with location K showing the highest total P concentration at 17.39% (Figure 2.A). High total P content (location K) can increase the availability of P for maize plants. Phosphorus is an essential nutrient for plants, especially for root formation, plant growth, and seed formation (Malhotra et al. 2018). Sufficient P availability will support increasing maize productivity (Ademba et al. 2015).

The CEC value ranges from 9.01-16.40 cmol kg^{-1} (Figure 2.B), with the highest CEC value at location R (Jayamandiri 3), 16.40 cmol kg^{-1}. The soil CEC value is one of the important factors that can affect the availability of nutrients for plants. High CEC allows the soil to retain more cations so that the soil becomes rich in calcium

(Ca^{2+}), sodium (Na^+), magnesium (Mg^{2+}), and other cations. Previous studies have shown that increasing CEC affects the increase in chlorophyll content, nitrogen absorption, and plant biomass (Yang et al. 2024).

Organic-C levels vary around 1.12 to 2.27% at each location (Figure 2.C), with the highest organic-C content at location H (Cibugel 2), which is 2.27%. Soil organic-C content can indicate soil quality conditions such as soil structure, nutrient cycles, and soil biota (Hoffland et al. 2020). High organic-C can be used as an energy source for microorganisms in the soil (Creamer et al. 2022). Soil pH at each location has almost the same conditions (Figure 2.D), around 5.05-5.58, with location C (Cipasang 3) having the highest pH (5.58). Soil that is too acidic or alkaline can harm soil physical properties and reduce nutrient availability (Xia et al. 2024).

Biological properties of corn cultivation

Soil biology analysis showed that each location had soil biology conditions, such as Phosphate Solubilizing Bacteria (PSB) and Arbuscular Mycorrhizal Fungi (AMF) with different populations (Figure 3). These microbes play a role in providing P nutrients for corn plants. The PSB population at each location varied from 1.05 to 21.00 × 10^6 CFU g^{-1}, with location I (Cibugel 3) having the highest PSB population, 21.00 × 10^6 CFU g^{-1}. The AMF population varied from 130-426 spores g^{-1}, with location O (Buanamekar 3) having the highest AMF population (426 spores g^{-1}). A high AMF population in corn cultivation land will increase P availability by up to 102.90% for corn plants (Kazadi et al. 2021). PSB can increase P availability for plants by changing bound P, while AMF can increase plant P absorption through root tissue (Billah et al. 2019). A high population of phosphate-solubilizing microbes can increase the efficiency of P fertilization (Elhaissoufi et al. 2021). In addition, phosphate-solubilizing microbes can also produce phytohormones such as Indole-3-Acetic Acid (IAA), cytokinins, and gibberellins, which can be used to support plant growth (Sodiq et al. 2019).

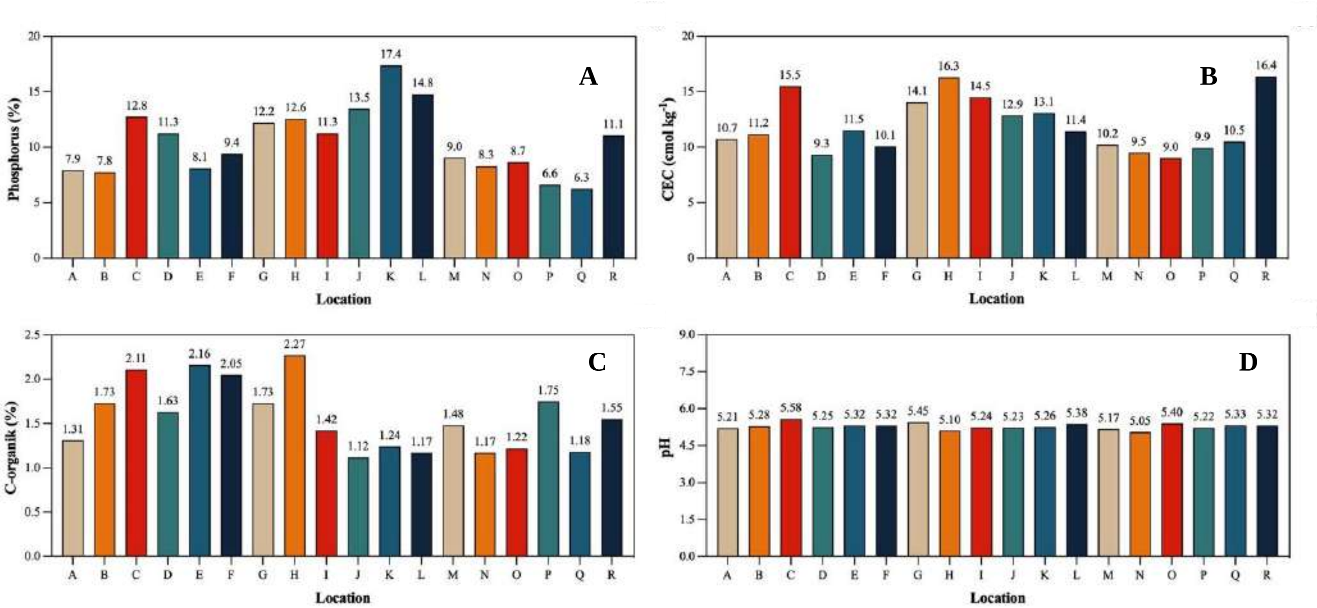

Figure 2. Soil chemical conditions at corn cultivation locations. A. Phosphorus; B. Cation exchange capacity; C. Organic carbon; D. pH

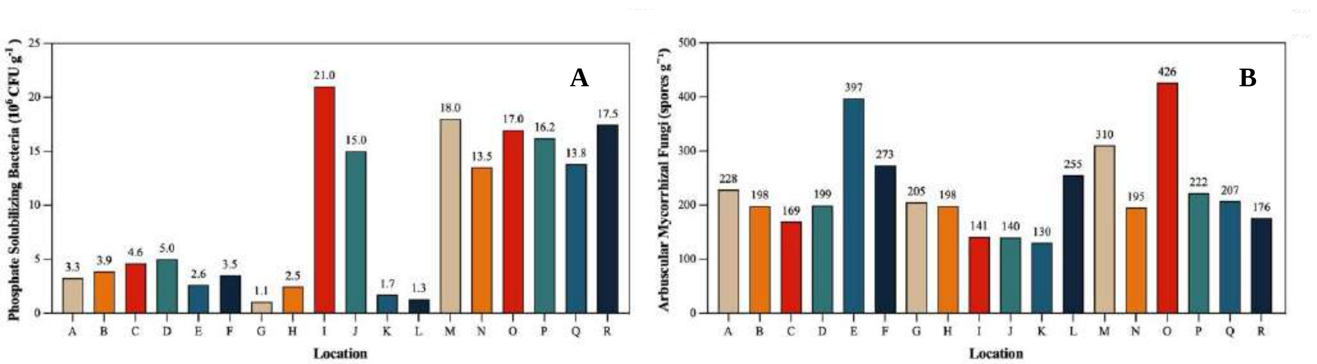

Figure 3. Soil biological conditions at corn cultivation locations. A. Phosphate solubilizing bacteria; B. Arbuscular mycorrhizal fungi

Climate condition of corn cultivation

Average climate conditions such as rainfall, temperature, and humidity during corn cultivation can be observed in Figure 4. The average rainfall (Figure 4.A) was around 315 to 380 mm annually, with the highest rainfall at location F (Sukaraja 3). The appropriate rainfall to support optimal corn growth was around 300-500 mm during cultivation activities (Wang et al. 2020). The average temperature (Figure 4.B) and humidity (Figure 4.C) at each location were around 22.4-23.7°C and 78.5-80.2%. These conditions are still suitable for corn plant growth because the optimal temperature is around 20.0-30.0°C (Beegum et al. 2023). Climatic conditions will also indirectly impact soil microorganisms, so they can affect biogeochemical processes that affect the availability of nutrients and plant pathogens (Ughamba et al. 2025).

Corn productivity

The results of the corn productivity analysis (Figure 5) show a variation in yield among locations of around 6.1 to 10.4 tons ha^{-1}. The locations with the highest productivity were R (Jayamandiri 3), Q (Jayamandiri 2), and D (Sukaraja 1), with productivity of 10.4, 10.1, and 10.0 tons ha^{-1}, respectively. This condition has a fairly high difference, around 63.9-70.9% higher compared to location J (Jayamekar 1), with a productivity of 6.1 tons ha^{-1}.

Pearson's correlation analysis of growth traits in maize

Correlation analysis was conducted to assess the relationship between various environmental factors on corn plant productivity in the forms of phosphate solubilizing bacteria (PSB), arbuscular mycorrhizal fungi (AMF), total phosphate (TP), cation exchange capacity (CEC), organic carbon (OC), pH (pH), rainfall (RF), temperature (T), and humidity (HD). The results of the Pearson correlation analysis in Figure 5 indicate that each corn productivity has a different level of relationship with environmental factors. The strength of the correlation between variables can be assessed using the absolute value of r, very strong (0.90-1.00), strong (0.70-0.89), moderate (0.40-0.69), weak (0.10-0.39), and negligible (0.00-0.10) (Pengphorm et al. 2024). The results of the correlation analysis showed that biotic factors in the forms of PSB and AMF each had a very weak or negligible correlation (0.02 and -0.11) on corn productivity. This may be due to the influence of soil chemical properties such as pH and limited availability of organic matter. This aligns with previous research that AMF colonization is influenced by soil and climate conditions (Li et al. 2025).

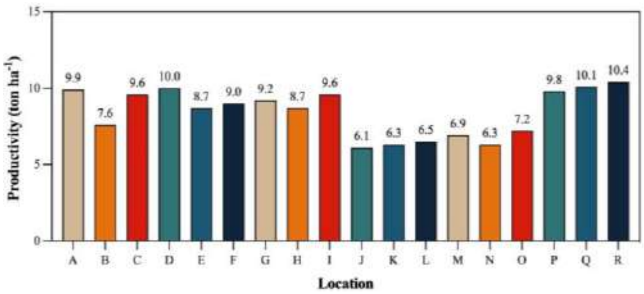

Figure 5. Corn productivity in corn cultivation in Sumedang, West Java, Indonesia

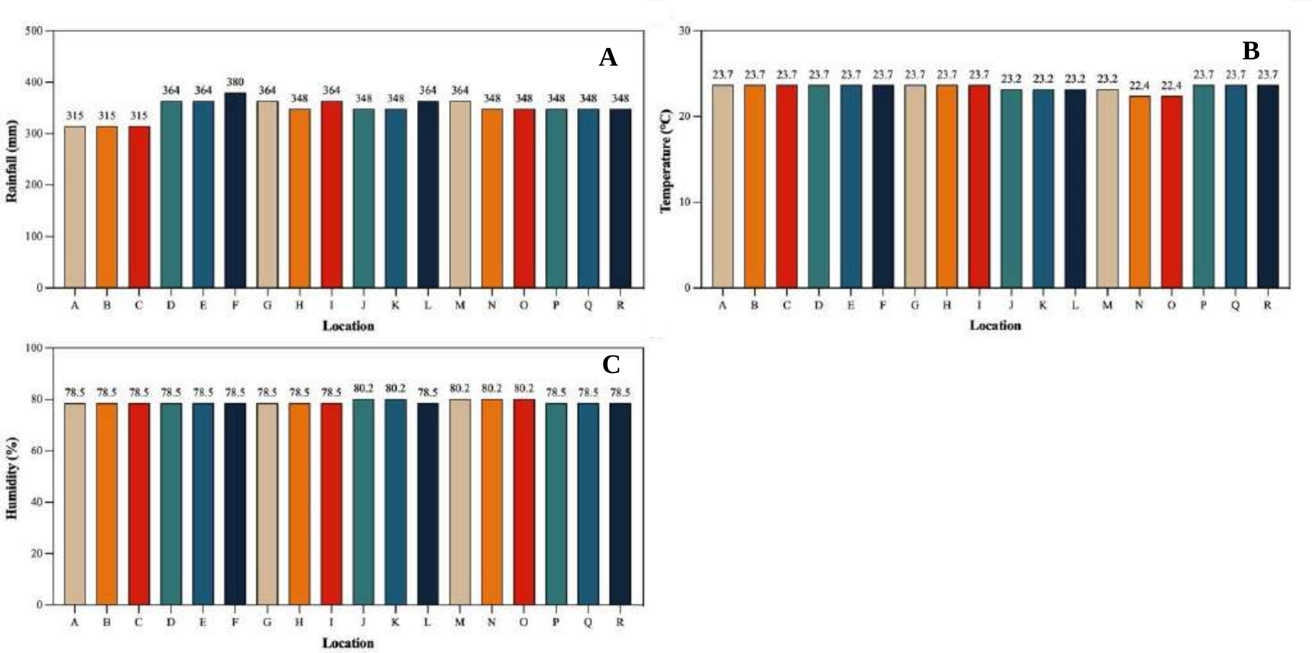

Figure 4. Climate conditions at corn cultivation locations. A. Rainfall; B. Temperature; C. Humidity

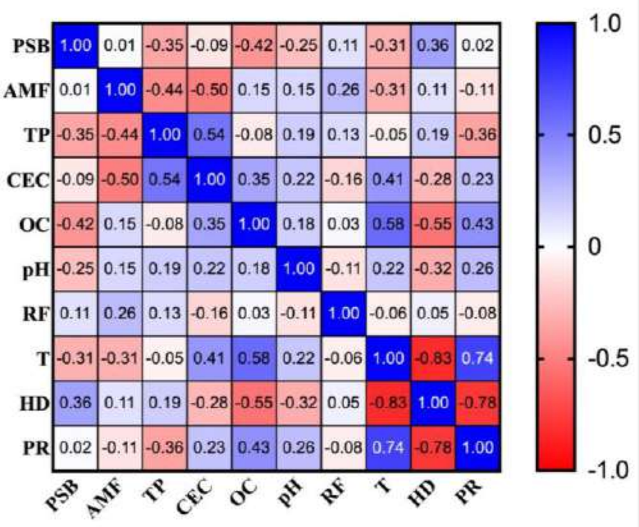

Figure 6. The correlation between maize productivity and biotic-abiotic environmental factors was analyzed. The environmental factors considered include Phosphate Solubilizing Bacteria (PSB), Arbuscular Mycorrhizal Fungi (AMF), Total Phosphate (TP), Cation Exchange Capacity (CEC), Organic Carbon (OC), pH (pH), Rainfall (RF), Temperature (T), Humidity (HD), and Productivity (PR)

Figure 6 shows that soil chemical factors such as TP, CEC, organic-C, and pH have weak to moderate correlations with r values of -0.36, 0.23, 0.43, and 0.26, respectively. The weak negative correlation between TP and productivity indicates that high TP content is not always directly proportional to the P that plants can absorb. In acidic soils, P is not available to plants because Al^{2+} and Fe^{2+} bind it, so plants cannot absorb it (Basílio et al. 2022). Corn productivity has a very low correlation with rainfall (-0.08), indicating that rainfall does not significantly impact productivity. Climatic factors such as temperature have a strong positive correlation (0.74), while humidity has a strong negative correlation. This shows that higher environmental temperatures within the optimal range can increase productivity, while high environmental humidity can decrease productivity. In environments with high humidity, it can increase the presence of plant pests and diseases and inhibit nutrient absorption.

In conclusion, soil chemical factors such as TP, CEC, organic-C, pH, PSB, and AMF analysis at each location had different conditions, with the highest results at respective locations K (17.39%), R (16.40 cmol kg^{-1}), H (2.27%), C (5.58), I (21 × 10^6 CFU g^{-1}), and O (426 spores g^{-1}). The average rainfall, temperature, and humidity conditions at each location were respectively around 315-380 mm, 22.4-23.7°C, and 78.5-80.2%. The results of the correlation analysis show that each environmental factor has a different correlation strength with corn productivity. Soil chemistry factors have a weak-moderate correlation (0.26-0.43), soil biology is very weak (-0.36 to 0.02), and climate is weak-strong (-0.78 to 0.74). Temperature conditions have a positive correlation with a value of 0.74, while humidity has a negative correlation of -0.78 on corn plant productivity. Climate factors (temperature and humidity) are determining factors and greatly influence the success of corn cultivation.

ACKNOWLEDGEMENTS

The author would like to express his appreciation to the Universitas Padjadjaran Academic Leadership Grant (ALG) for their valuable support and funding of the research and publication of this article.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 208-214
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090122

The impacts of crop diversity and sustainable farming on food security, climate, and biodiversity in Dumanjug, Cebu, Philippines

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Manuscript received: 13 December 2024. Revision accepted: 26 March 2025.

Abstract. *Lillo EP, Alcazar SMT, Malaki ABB, Chavez MLM, Cañarijo III DM, Redoblado BR, Margate MA, Diaz JL, Mago JE, Belanizo J, Beceril R, Revillas MJ, Abrigana M, Davirao C, Obando ME, Diaz GGG, Gonzaga CF, Cagara BN. 2025. The impacts of crop diversity and sustainable farming on food security, climate, and biodiversity in Dumanjug, Cebu, Philippines. Asian J Agric 9: 208-214.* The Philippines, an archipelagic Southeast Asian country, faces a complex and interconnected set of challenges involving food security, biodiversity conservation, and sustainable agricultural practices. The impacts of climate change, the loss of crop diversity, and the reduction in agricultural output brought on by unsustainable farming practices are some of the country's complex issues. This study examines crop diversity and sustainable farming practices in Dumanjug, Cebu, Philippines, and their implications for food security, climate change adaptation, and biodiversity conservation. The research evaluates the farmer's diverse cropping systems and sustainable agricultural techniques by employing participatory observation and a survey questionnaire. Results highlight the reliance on local seeds, adoption of sustainable practices such as intercropping and crop rotation, and the cultivation of indigenous food crops like *Cocos nucifera* L., *Moringa oleifera* Lam., and *Musa acuminata × balbisiana (ABB Group)*. Despite challenges such as typhoons, droughts, and limited resource access, these practices contribute to food security, economic stability, and ecosystem resilience. The study underscores the importance of promoting local seed systems, climate-resilient farming techniques, and supportive policies to address systemic vulnerabilities and enhance agricultural sustainability. The implementation of these recommendations is expected to bring about benefits such as increased crop diversity, improved food security, and enhanced resilience to climate change. Findings provide evidence-based recommendations for policymakers, agricultural extension workers, and local communities, contributing to regional and national efforts to achieve sustainable development goals.

Keywords: Agricultural resilience, Cebu, climate change adaptation, Dumanjug, local seeds, sustainable farming practices

INTRODUCTION

The Philippines is an archipelagic country in Southeast Asia facing significant problems with food security, biodiversity preservation, and sustainable agricultural practices. Among the critical concerns are the declining agrarian productivity caused by unsustainable farming methods, climate change impacts, and the erosion of crop diversity (Ortega-Espaldon and Medina 2024). As a nation deeply rooted in agriculture, these challenges are particularly felt in rural communities that depend on farming for their livelihoods, and the interconnected targets of both food security and environmental sustainability must be prioritized in these contexts. Addressing both food security and environmental sustainability is very important.

Through exploiting traditional knowledge and modern innovations, the Philippines can enhance food security, resilience to climate change, and biodiversity conservation. Recognizing that traditional knowledge and modern agricultural technologies are not mutually exclusive is essential. Traditional practices often reflect centuries of adaptation to local environments and can offer insights into sustainable farming methods. By integrating these practices with modern technology, farmers can create a synergistic approach that enhances productivity while respecting cultural heritage and local biodiversity. Engaging with local communities and understanding their knowledge can lead to innovative solutions that increase crop yields without disregarding the wisdom of traditional techniques. The combination of traditional and modern agricultural approaches has been proven effective and serves as a blueprint for other regions. These efforts will drive the country towards sustainable development in its agricultural communities, promoting a more resilient and self-sufficient future. Dumanjug in Cebu province is an important case where crop diversity meets sustainable farming practices. Dumanjug demonstrates how diverse cropping systems can enhance farming resilience by integrating traditional and modern agricultural practices. Crop diversity is essential for agricultural biodiversity, protecting against pests, diseases, and climate change that threaten global food production (Chittapur and Umesh 2017; Altieri 2018). Research shows that diverse cropping systems help stabilize ecosystems, improve soil health, and increase nutrient availability, improving crop yields and long-term sustainability (Beillouin et al. 2021).

The approach towards eco-friendly activities such as organic farming, intercropping, crop rotation, and agroforestry has received attention as an efficient means of building soils, improving water quality, and decreasing reliance on chemicals (Edwards 2020; FAO 2020). In the Philippine context, these approaches have shown the potential to enhance biodiversity and ensure long-term food security (Parreño-de Guzman et al. 2015). Unfortunately, smallholder farmers continue to be hesitant to widely adopt these beneficial practices due to economic and technical barriers (e.g., limited access to resources and insufficient technical support) (Heckelman 2019; Touch et al. 2024). Environmental shocks, like abnormal trends in weather variability and pest outbreaks, also persist in damaging rural farming systems (Romeh 2019; Ortega-Espaldon and Medina 2024).

In order to tackle these challenges and establish resilient agricultural systems, it is crucial to comprehend the significance of crop diversity and sustainable farming practices. Studies show that diversifying crops enhances local food security and reinforces farming communities ability to adapt to climate change and other pressures (Vernooy 2022). In addition, diverse farming systems contribute to the preservation of local biodiversity, aligning with global strategies for accomplishing Sustainable Development Goals (SDGs), especially SDG 2 (Zero Hunger), SDG 13 (Climate Action), and SDG 15 (Life on Land) (United Nations 2015; Lee at al. 2016). This study assesses crop diversity and sustainable farming practices in Dumanjug, Cebu, while identifying farmers common challenges. Through field observations and interviews, it seeks to evaluate the contributions of diverse cropping systems and sustainable agricultural techniques to food security, climate resilience, and biodiversity conservation. These findings aim to inform policymakers, agricultural extension workers, and local communities, promoting informed decision-making and sustainable development in agricultural areas. Policymakers can develop supportive frameworks to provide economic incentives and technical help to smallholder farmers, encouraging the adopting of sustainable practices. Agricultural extension workers can spread the knowledge and skills necessary for eco-friendly farming, bridging the gap between research and real-world application. Local communities can also play a vital role in preserving agricultural biodiversity by adopting the knowledge acquired about diverse cropping systems and participating in sustainable initiatives. Additionally, this research will contribute to regional and national discussions on enhancing food sovereignty to achieve sustainable development in agricultural communities across the Philippines.

MATERIALS AND METHODS

Study area

The study was conducted in the Municipality of Dumanjug, a third-class municipality situated in the southwestern part of Cebu Province, Philippines (Figure 1). Dumanjug is composed of 37 barangays with Barangay Tubod-Duguan as the biggest (556.69 hectares) and Barangay Sima as the smallest (85.16 hectares). The municipality has eight coastal barangays namely: Kanyuko, Bitoon, Tangil, Tapon, Poblacion, Looc, Calaboon and Camboang. A total population of 57,823, as recorded in the 2020 census. The municipality features a diverse landscape, encompassing both upland and lowland agricultural zones. This geographical and ecological diversity provides an ideal setting to explore and assess crop diversity and the sustainability of farming practices across varied environmental conditions.

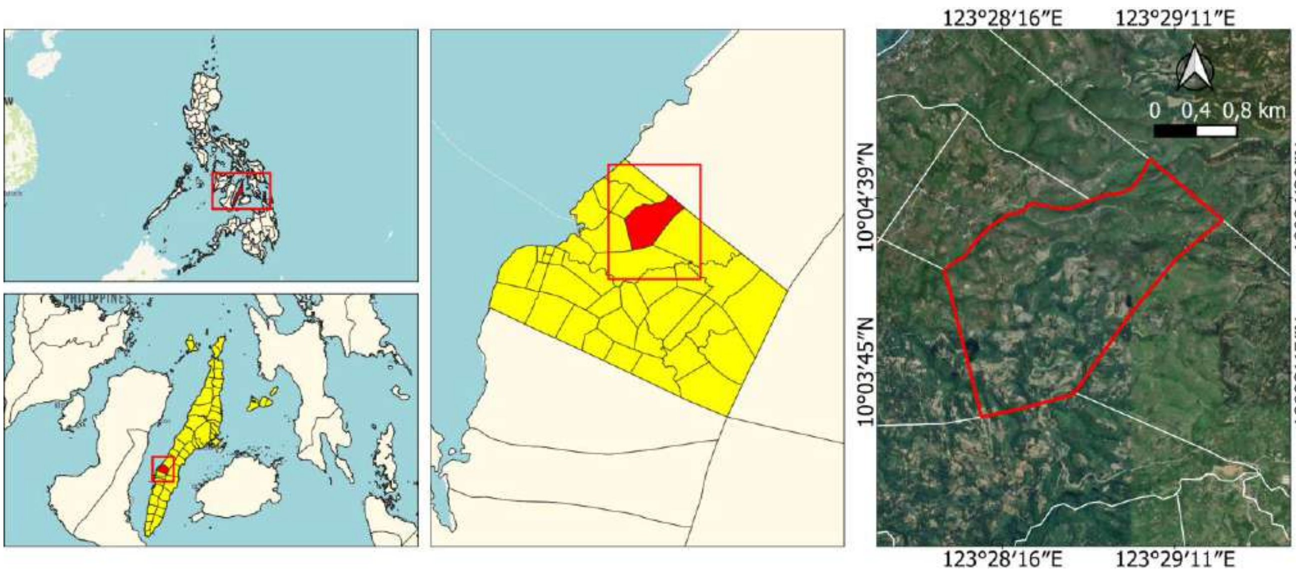

Figure 1. Map of Kanghumaod, Dumanjug, Cebu Province, Philippines

Barangay Kanghumaod, in particular, was selected as the focal area due to the active participation of the KAACWWA (Kanghumaod Association of Active Community Workers and Agriculturalists), a local People's Organization (PO) (Figure 1). The majority of farmers affiliated with KAACWWA manage landholdings of less than a hectare, with many operating as "sa-op" (tenant farmers). Under this traditional tenancy arrangement, farmers cultivate the land and share a portion of the harvest with the landowner. The prevalence of small-scale and tenant farming in the area underscores the importance of studying how agricultural practices in such settings can be optimized for sustainability and productivity.

Data collection

The study was conducted in the months of March-May, 2024 including the analysis of data. It conducted a courtesy call to the municipality of Dumanjug and asked for an available list of POs for farmers. From the list, POs whose goal is to produce food crops were selected, disregarding those POs producing timber, handicrafts, etc. Hence, the PO of KAACWWA was selected as the subject for the study. The goal is to conduct a 100% sampling from a total of 150 PO members, but unfortunately, only 28 farmers were readily available and accessible, and the rest were hesitant and other had worked in the city and other region.

This study utilized a mixed-methods approach consisting of participatory observation and survey questionnaires to collect qualitative and quantitative data. Kawulich (2005) describes that participatory observation is important during field visits to selected farms to observe farming practices first-hand and the context around agricultural activities. Also, this helped validate the data collected through the questionnaires, ensuring accuracy and a thorough understanding of the farming systems. A modified version of the survey questionnaire is used to fit the study's objectives in obtaining information on the types of crops planted, farming practices adopted, and challenges faced by the farmers.

Data analysis

The gathered data was recorded and arranged using Microsoft Excel. Data analysis was performed utilizing statistical tools in Excel, focusing on descriptive and inferential statistics to derive meaningful insights. The Shannon Diversity Index (*H*') was calculated to evaluate crop diversity on the farms, as this index effectively measures species richness and evenness (Shannon 2001; Magurran 2013):

$$H' = -\sum(pi \cdot lnpi)$$

Where:
pi: Represents the proportion of each crop species

Descriptive statistics, such as means, percentages, and frequency distributions, were used to summarize farming practices and challenges data. Furthermore, qualitative data from observations were thematically analyzed to identify recurring patterns and contextual insights, following Braun and Clarke's (2006) thematic analysis framework.

RESULTS AND DISCUSSION

Source of seeds/planting stocks

The findings of this study emphasize the farmers' preference towards local sources as well as the sources of planting materials as opposed to commercial sources (Figure 2). It is not surprising in all cases since many of these people could identify benefits from local sources of seed use. Firstly, local seeds tend to better adapt to the local climate conditions and soil, which enhances crop resilience and yield (Liu et al. 2018). It becomes imperative in these climate-changing times where unpredictable weather conditions continue to bring about crop problems under the negative influence of soil degradation. Hence, by using local sources, farmers can reduce reliance on outside sources of inputs while adapting to varying environmental conditions. Secondly, local seeds are usually cheaper or even free an excellent bonus, especially for small-scale farmers with limited funds (Ahmed and Sarma 2022). Commercial seeds become too expensive and often become a barrier for many farmers. In terms of food security, this is a serious problem because small-scale farmers contribute significantly to the food production of localities. Thirdly, local seeds mirror traditional agricultural practices inherited through generations (Sharma et al. 2020). They have been used in many farmers lives and may appear natural or sustainable compared to commercial, generally associated with large-scale, industrialized agriculture. Local seeds are important for biodiversity conservation because they are often attached to specific crop varieties and agroecological practices that support ecosystem services. Lastly, in some rural areas, limited access to commercial seeds exists. However, local seeds, as demonstrated by Montalvo-Romero et al. (2023), are more practical in such cases. This reliability is particularly valuable in the face of infrastructural challenges or the absence of nearby suppliers. Therefore, local seeds are the best choice for farmers living in remote locations, providing a secure solution to their seed needs. This reliability is especially important in the context of climate change, where farmers may need to adapt to changing environmental conditions, and the availability of commercial seeds may not be guaranteed.

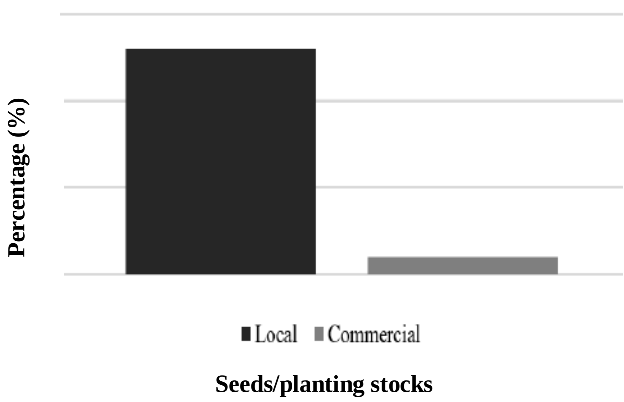

Figure 2. Source of seeds/planting stocks

This paper illustrated the importance of using local seeds among farmers in food security, biodiversity conservation, and climate change. Reviving local seeds encourages local seed production, which manages small farmers, advocates traditional agricultural practices, and increases the resiliency of crops and yields. Furthermore, using local seeds can help conserve biodiversity and promote ecosystem services, which are important and critical for maintaining ecosystem health and resilience. While hybrid seeds may offer higher productivity in certain contexts, local seeds are often better adapted to specific climatic and soil conditions, leading to resilience against pests and diseases. It is important to value the role of local seeds in promoting food sovereignty and biodiversity. Additionally, there can be long-term benefits associated with local seeds regarding sustainability and lower input costs. Educating farmers about both options advantages and potential trade-offs can empower them to make informed decisions that align with their unique circumstances.

Crop diversity and its role in food security, economic stability, and ecosystem resilience

The findings from the study highlight the relevance of indigenous food crops, namely *Cocos nucifera* L., *Moringa oleifera* Lam. and *Musa acuminata* × *balbisiana* (ABB Group), which is rooted deep in the local agriculture of the locality (Figure 3). These crops provide essential nutrition at a community level, but their impact goes beyond this to generate economic opportunities for farmers. *C. nucifera*, also known as the "tree of life," has a wide range of food and medicine uses and industrial applications, thus being a valuable asset to the community (Henrietta et al. 2022). *Moringa oleifera*, the so-called "superfood," has been characterized by its vitamin, mineral, and antioxidant content that, among others, offers numerous health benefits for humans and livestock (Henrietta et al. 2022). Additionally, *M. acuminata* × *balbisiana* is a crop that stimulates household nutrition and market demand. It forms a significant part of the local food system.

The integration of perennial crops like *C. nucifera* and *Theobroma cacao* L. into agroforestry systems further enhances biodiversity conservation, climate change mitigation and ecosystem services (Nair et al. 2021). Through sustainable farming practices, trees and shrubs associated with traditional crops are introduced to improve resilience and pursue environmental health (IPBES 2019). The variety among the crops, including staple crops such as *C. nucifera*, *M. oleifera*, *Musa acuminata* × *balbisiana*, *Zea mays* L., and *Ipomoea batatas* (L.) Poir. ensures food security. These crops have carbohydrates and nutrients needed by communities that are particularly vulnerable to food insecurity (FAO 2017). For farmers, continuous income from such crops motivates them to keep investing in their agricultural practices and their livelihoods (FAO 2017).

The cultivation of indigenous food plants also has broader ecological implications. The wide variety of crops planted, including underutilized species such as *Capsicum frutescens* L., *Cucurbita maxima* Duchesne, *Spondias purpurea* L., *Carica papaya* L., *Colocasia esculenta* (L.) Schott, *Abelmoschus esculentus* (L.) Moench, and *T. cacao* enrich the genetic diversity of farming systems. This genetic diversity reduces the risk of crop failures caused by pests, diseases, or climate variability, ensuring greater agricultural sustainability (IPBES 2019).

Agricultural operations can enhance the provision of specific ecosystem services and biodiversity (Bethwell et al. 2021), however, poor management often leads to biodiversity loss, degradation of ecosystem conditions, and deterioration of services (Huang et al. 2015). These crops support ecological resilience by fostering the adaptability of farming systems to climate change (IPBES 2019).

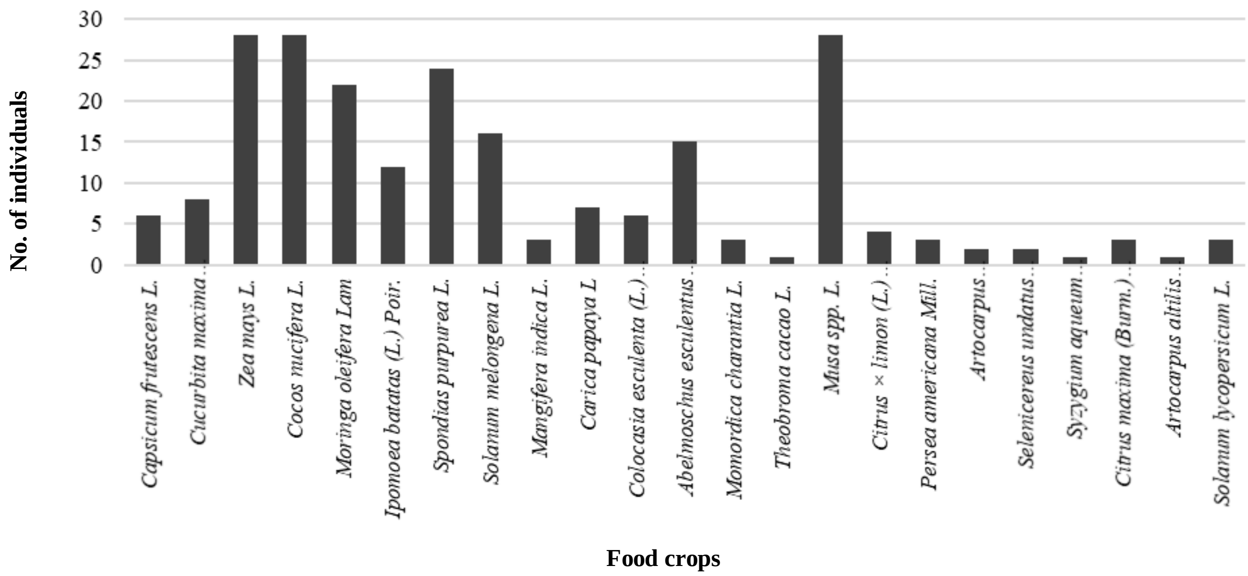

Figure 3. Food crops planted

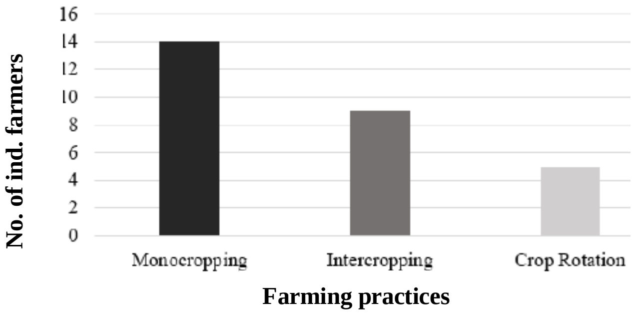

Figure 4. Farming techniques utilized by farmers

Farmers cultivating a diverse range of crops not only ensure food security and economic stability but also strengthen ecosystem resilience and biodiversity. This will encourage and promote the use of indigenous food plants as an advocacy measure against climate change and to improve farmers livelihoods, biodiversity, and long-term environmental sustainability.

A Shannon Diversity Index of 2.69 would indicate a relatively high level of species diversity within the community. The greater the value of the Shannon Diversity Index, the greater the diversity of species there. This index points towards the community's good combination of species, which supports a healthy and balanced ecosystem.

Although diverse cropping systems can present challenges in terms of logistics and management, they also offer significant advantages, such as improved soil health, pest control, and resilience to climate variability (Davis et al. 2012; Hufnagel et al. 2020). It is crucial to educate farmers about the long-term benefits of diversity, including increased ecosystem stability and reduced risk of total crop failure. Simplifying farming logistics through proper planning, education, and access to resources can help mitigate these challenges, allowing farmers to reap the rewards of diverse cropping systems while improving overall efficiency.

Farming techniques employed

In this study, the dominant farming practice is monocropping, which is characterized by the cultivation of a single crop species on a large scale (Figure 4). This approach is often favored due to its simplicity and short-term profitability (Jhariya et al. 2021). However, monocropping has several drawbacks, including soil degradation and depletion of specific nutrients, which can lead to increased reliance on fertilizers and pesticides (Tilman et al. 2002). This can have negative impacts on biodiversity, as well as contribute to climate change through the release of greenhouse gases. Despite these challenges, the potential for change is within our reach, offering hope for a more sustainable and resilient agricultural future.

Moreover, some farmers in the region are adopting more sustainable practices such as intercropping and crop rotation. Intercropping, combining different crops, has improved soil quality, increased biodiversity, and enhanced host plant resistance to pest attack (Perfecto and Vandermeer 2016). Also, such diverse crops would indirectly act as biological pest control as their life cycles are disrupted or they are attracted to beneficial insects (Altieri 2018). The fact that nine farmers in the area have intercropped indicates a growing awareness of the advantages of crop diversity and the willingness to adopt more sustainable practices.

Crop rotation, a method where various crops are planted in succession on the same plot of land, is a sustainable practice that enhances soil health while minimizing the accumulation of pests and diseases (Liu et al. 2022). This strategy can also interfere with the life cycles of pests and diseases, as those that harm one type of crop are less likely to persist when a different crop is sown in the next season (Mahato et al. 2021). While only 5 farmers in the area are currently practicing crop rotation, this method has the potential to improve long-term agricultural sustainability and resilience.

Disturbances and challenges faced by farmers

In this study the farmers in Dumanjug, Cebu, face various disturbances and challenges, impacting their livelihoods and agricultural productivity (Figure 5). Typhoons emerge as the most significant disturbance, with a notable proportion of respondents reporting varying levels of impact. Of the total, 10 respondents were extremely affected, seven were moderately affected, six were rarely affected, and five were unaffected. Typhoons are recurrent events in the Philippines, particularly in the Visayas Region, due to the country's geographical location in the typhoon belt. These extreme weather events can destroy crops, infrastructure, and homes, disrupting agricultural cycles and leading to economic losses (Yumul et al. 2011). The result of the study implied that farmers need disaster-resilient farming practices, such as the use of storm-resistant crops and sustainable soil management techniques, alongside government support through insurance and rapid response systems.

Drought is identified as another critical disturbance, with eight respondents extremely affected, 14 moderately affected, and six rarely affected. Unlike typhoons, no respondent reported being completely unaffected by drought. Prolonged dry spells can severely affect rain-fed agriculture, a common practice among smallholder farmers. Reduced water availability for irrigation leads to crop failure, food insecurity, and income loss (Quibria 2024). Addressing drought impacts requires long-term solutions such as implementing water-saving technologies, the promotion of drought-tolerant crop varieties, and investing in community-based water management systems.

While less impactful overall, landslides, flooding, earthquakes, fire, and pests/diseases affect specific groups within the community. Four respondents in each category reported being extremely affected, while the majority (24 respondents) reported no impact. These events, though localized, can disrupt farming activities, damage agricultural lands, and pose safety risks. For instance, landslides caused by heavy rains in sloped agricultural areas can lead to soil erosion and loss of arable land (Panda 2021). Pests and diseases, though not widespread, have the potential to escalate if not addressed promptly, emphasizing the urgent need for Integrated Pest Management (IPM) systems and proactive monitoring.

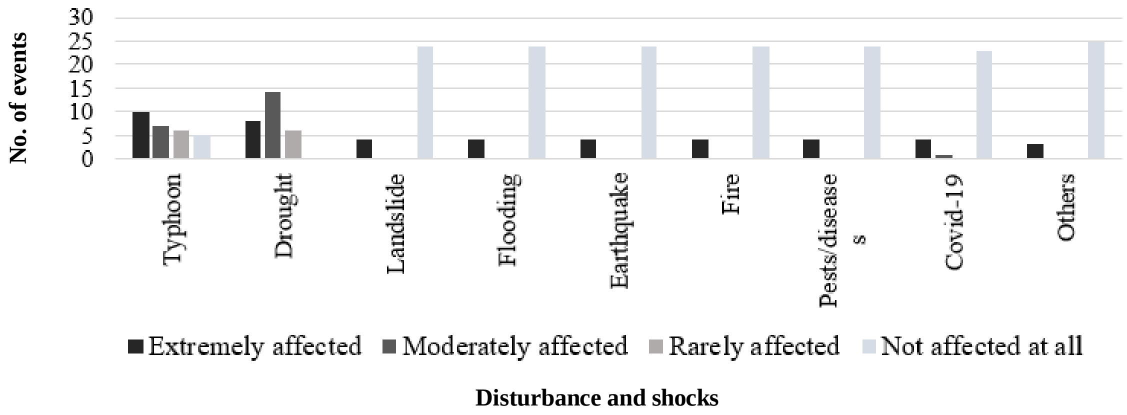

Figure 5. The disturbances and shocks encountered by farmers

The direct impact of the COVID-19 pandemic on the farming community would be minimal, with only four respondents saying that they were greatly affected and one moderately affected. However, there is a high likelihood of the indirect effects of the pandemic, especially the disrupted supply chains, limited access to markets, and fluctuating input prices, which worsened existing vulnerabilities (Somashekhar and Basavaraja 2021). For farmers, these disruptions highlight the need for diversification strategies and underscore the potential of digital tools for accessing markets, and policies that support local food systems to enhance resilience during future global disruptions.

Challenges classified under the "other" category consist of insufficient financial resources, small plots of land, lack of farming ordinances, and changes in market prices. These systemic problems arise from chronic structural inequities that restrict farmers from adapting to external shocks. The lack of affordable credit restricts investment into better farming practices. Small areas further restrict productivity and income generation (Quibria 2024). Additionally, the lack of binding regulations on sustainable farming methods leads to inconsistency in land use and agricultural policies. Therefore, this points to the need for stronger local governance. It will also be detrimental to farmers economic stability. Thus, cooperative marketing strategies and value chain improvements are needed.

The research emphasizes the importance of a cohesive and localized method for enhancing resilience among farmers in Dumanjug, Cebu. These interventions are designed through participatory methods and supported by evidence-based policies aimed at protecting farmers livelihoods while ensuring sustainable agricultural practices that are environmentally friendly.

Conclusion, this study highlights the vital role of crop diversity and sustainable farming practices in ensuring food security and ecological resilience in Dumanjug, Cebu. By prioritizing local seed systems, intercropping, and crop rotation, farmers can strengthen agricultural sustainability while preserving biodiversity. The study is relevant as it emphasizes the need for a two-pronged strategy, including self-consumption and income generation, in addressing food security. The impacts of typhoons and droughts highlight the need for targeted interventions to build community resilience. However, long-term success will require collaborative efforts involving policymakers, local organizations, and community leaders. Targeted interventions supporting smallholder farmers, improving disaster resilience, and promoting sustainable agriculture are essential for safeguarding the livelihoods and food security of local communities in Dumanjug.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the individuals and organizations that have contributed to the success of this research. We are particularly grateful to the Municipality of Dumanjug for their unwavering support and cooperation, which enabled us to conduct the study in their area of responsibility. We also extend our appreciation to the Barangay Kanghumaod for providing the necessary resources and security support. We would like to thank the PO farmer members of KAACWWA for sharing their valuable insights, experiences, and knowledge, which greatly helped us achieve the objectives of this study. We are also grateful to the CTU Argao Campus for their academic support and for providing a conducive environment for research. We would like to acknowledge the hard work and dedication of our project staff and science research assistants, who played a crucial role in data collection, analysis, and support. Their efforts were instrumental in the completion of this project. We would also like to express our heartfelt gratitude to the Department of Science and Technology (DOST) and the Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD) for providing the necessary support for the successful implementation of this study. Finally, we would like to acknowledge the dedication and hard work of our team's families, who have provided strength, support, and understanding throughout this journey.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 215-225

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090123

Morphological diversity, ethnobotany, and economic value of banana plants (*Musa* spp.) in the Keerom lowland area, Papua Province, Indonesia

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Manuscript received: 18 January 2025. Revision accepted: 12 April 2025.

Abstract. *Suharno, Suparno A, Yuliana, Tanjung RHR, Zebua LI, Prabawardani S. 2025. Morphological diversity, ethnobotany, and economic value of banana plants (*Musa *spp.) in the Keerom lowland area, Papua Province, Indonesia. Asian J Agric 9: 215-225.* Bananas (*Musa* spp.) are an agricultural crop of significant economic value, highly sought after as a popular fruit for consumption. The global diversity of bananas is substantial and continues to grow with the emergence of new varieties, including in Papua, Indonesia. This study aims to determine the diversity of bananas based on local knowledge, ethnobotany, and the economic value of bananas in the lowland area of Keerom, Papua. Data were collected from a survey in eight villages representing the Arso, West Arso, and Skanto Districts. The diversity was determined based on morphological characters referring to descriptors for bananas, with 67 characters. Based on the local community's knowledge, 13 varieties of bananas were found. The findings also show that the population and distribution of bananas in Keerom vary. *Kepok boy, kepok abu-abu, raja, barangan*, and *nona* varieties are widely distributed, while *ambon, jarum, goroho, raja nangka, susu,* and *merah* varieties are not as widely distributed. Based on the morphological characters, supported by the results of PCA analysis and Eigenvalue tests, the banana varieties can be divided into four groups. Regarding utilization, the fruit is mostly for consumption, the leaves are for food wrappers, and the blossoms are processed as vegetables. Other parts of bananas have not been utilized optimally. The price of bananas at the consumer level averages IDR 8,500 per bunch (USD 0.5). This selling value has increased by 144.17% from the price of bananas at the farmer level. The findings also show that the banana varieties in Keerom are relatively diverse, yet most of them have not been optimally utilized. However, Indonesia has significant potential for developing food products using bananas as a primary ingredient, offering opportunities to maximize utilization and contribute to national food security.

Keywords: Bananas, food source, Keerom, *Musa*, traditional knowledge

INTRODUCTION

The diversity of natural resources utilized by Indonesian communities is remarkably high (Anggraeni et al. 2017), including bananas (*Musa* spp.) (FAO 2023), which can be consumed in various forms, either directly as fresh fruit or as processed products (Schönhart et al. 2009; Dwivany et al. 2020). Bananas are one of the staple food sources for all groups. Historically, however, bananas were considered a special and luxurious food reserved for leaders in certain parts of the world (Schönhart et al. 2009). Aside from being a food source, bananas serve as a commercial crop and play a significant role in supporting national economies (Subrahmanyeswari and Gantait 2022).

Bananas are classified as herbaceous plants that resemble trees in appearance. The plant features a pseudostem, which is formed by the concentric arrangement of leaf sheaths. Banana leaves are produced sequentially, culminating in the emergence of an inflorescence from the terminal part of the plant (Beaton et al. 2023). Banana plants are highly adaptable to various environmental conditions, particularly drought, making them invaluable for food security programs (Hapsari and Lestari 2016). Several factors, including climate, planting media, and altitude, influence the cultivation and distribution of banana plants (Nugraha et al. 2024).

Carl Linnaeus derived the genus *Musa*, while the name *Musa* was derived from the Arabic word *mauz* by Antonius Musa (Emperor Linnaeus's physician) (Subrahmanyeswari and Gantait 2023). The name banana comes from the Arabic word *banan*, which means finger. Each *banan* resembles a finger. Hence, a bunch of them resemble a hand (Häkkinen et al. 2012; Subrahmanyeswari and Gantait 2023). Bananas are the oldest fruit plants known to humans (Subrahmanyeswari and Gantait 2023), which have been cultivated since the agricultural culture system (Denham et al. 2003). The domestication of the genus *Musa* originated from Southeast Asia, as inferred based on chloroplast and cell nucleus genes (Li et al. 2013), producing various varieties of bananas, including the *raja* variety (which means king in the Indonesian language). Indonesia is considered the center of origin of diverse banana varieties, encompassing rich genetic resources of wild and cultivated bananas (Daniells et al. 2001). There are at least 325 *Musa* cultivars recorded in Indonesia (Valmayor et al. 2002), distributed across Bali, Java, Kalimantan, Papua, Sulawesi, Sumatra, Maluku, and Nusa Tenggara (Meitha et al. 2020).

As a perennial crop, bananas offer food security for many developing countries (Mertens et al. 2021; Beaton et al. 2023). With a production of more than 144 million tons annually, bananas are considered one of the most important fruit crops in the world (Meitha et al. 2020). The widespread distribution of bananas makes this plant a promising local food source to meet regional fruit needs. Exploring banana diversity is crucial, as it is significant based on the traditional knowledge of local communities (Cenci et al. 2021; Ardiyani et al. 2023). Several conservation efforts have also been made to preserve wild banana germplasm ex-situ in the form of seeds, in vitro, cryopreservation, or as living plants. Most of the available germplasm is stored in the form of in vitro cultures or frozen meristems at the International Musa Germplasm Transit Centre (ITC) in Belgium (Panis et al. 2005).

In Papua, the banana plants are substantially diverse (Zebua et al. 2023), yet the exploration in the areas is limited. Therefore, this study aims to determine the diversity of banana plants, their utilization, and economic value for the community in the Keerom lowlands, Papua. This research benefits the community by contributing to the development of bananas as a source of food and functional food, as well as their potential to increase community income.

MATERIALS AND METHODS

Study area

This study was conducted in three districts, namely Arso District (Arso II-Track 5, Poros Street, and PTPN II Village), West Arso (Yowang, Young, Tuwi, and Sanggaria Village), and Arso Skanto (Arso 7 Village), Keerom District, Papua Province (Figure 1). The Keerom area is partly a lowland area with an altitude of 40-60 masl. The average temperature during observation ranged from 24 to 34°C (Table 1). The land in this area is mostly used for agriculture and oil palm plantations, with some areas being forests. According to data from Statistics Indonesia (BPS - *Badan Pusat Statistik*) of Keerom District (2024)), the average temperature in this area is between 27.1 and 28.7°C, with humidity falling between 74.8 and 76.80% each month throughout the year, and average rainfall being 128.15 mm/year.

Data collection

Data were collected using a survey method in three districts, namely Arso District (consisting of three villages: Arso II-Track 5, Poros Street, and PTPN II), West Arso District (consisting of four villages: Yowang, Young, Tuwi, and Sanggaria), and Arso Skanto (consisting of one village: Arso 7). These districts are located in Keerom lowland, which the government earmarked as a transmigration destination for farmers.

The diversity of banana varieties was determined by describing the morphological characteristics, as specified in the Descriptors for Banana (*Musa* spp.) book published by the joint CIRAD-Inibap-IPGRI. There are 67 characteristics to be observed, including the habitus and the plant organs, such as roots, stems, leaves, fruits, blossoms, and seeds. The similarities in the characteristics form the basis for determining kinship relationships in phylogenetic trees, which was carried out using the NTSYs software. Then, to see the dominant characteristics that set one group of kinship apart from another, the Principal Component Analysis (PCA) method was used through the calculation of Eigenvalues.

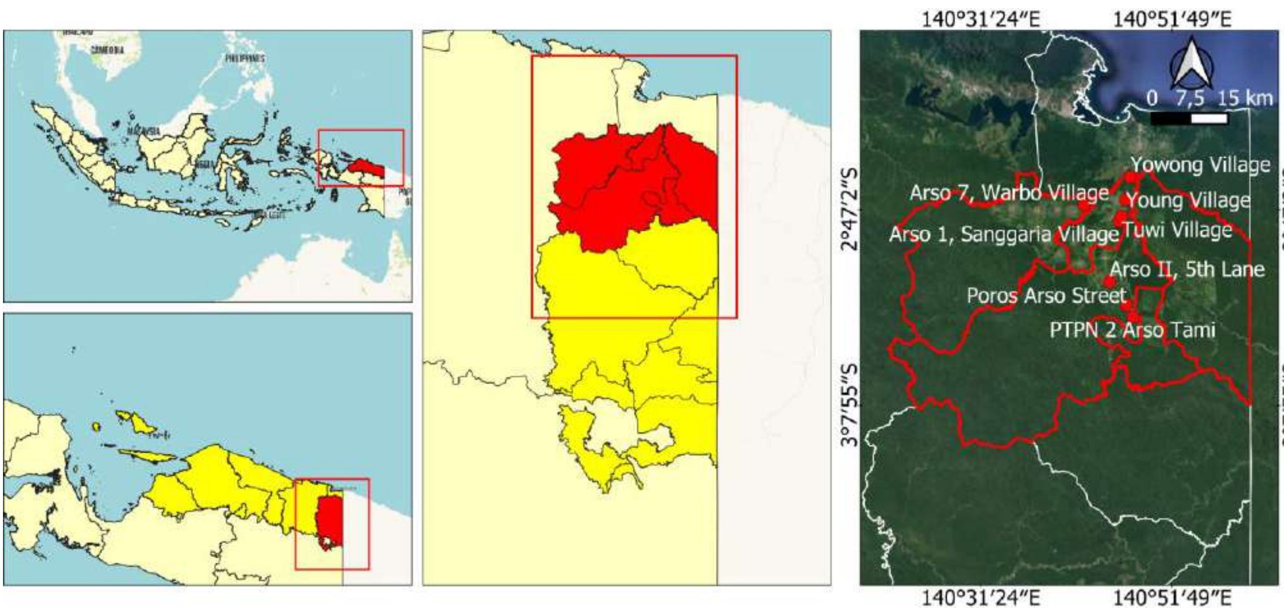

Figure 1. Research locations in the lowland areas in Keerom, Papua, Indonesia

Table 1. Locations and environmental conditions in the Arso area, Keerom, Papua, Indonesia

District	Village	Coordinate points	Location altitude (masl)	Temperature (°C)
West Arso	Young	140,78191°; -2,76645°	40	30 - 34
West Arso	Tuwi	140,77414°; -2,79637°	40	27 - 30
West Arso	Yowong	140,79013°; -2,72569°	60	26 - 30
West Arso	Sanggaria	140,76985°; -2,80423°	49	24 - 31
Skanto	Warbo	140,70773°; -2,77589°	46	25 - 32
Arso	PTPN2 Tami	140,79475°; -2,97447°	54	27 - 32
Arso	Arso II, Track 5	140,75324°; -2,91197°	48	25 - 32
Arso	Poros Street	140,78315°; -2,95297°	42	26 - 33

PCA can find which characteristics have high contribution values to their variations, both positively and negatively. Giuliani and Vici (2024) claimed that PCA can identify kinship relationships and determine characteristics with high contribution values. The Principal Components (PC) are determined based on Eigenvalues, with values <1 excluded from the calculation of the number of principal components.

The banana ethnobotanical survey was conducted using a mixture of qualitative and quantitative methods (Martin 2004; Iskandar et al. 2018). Techniques adopted from various disciplines have been combined to form a collaborative approach called Participatory Rural Appraisal (PRA) (Martin 2004). Some of the techniques used are observation, participant observation, semi-structured interviews, and economic value of banana fruit. The interviews involved 62 respondents spread across the observation points in Keerom District. Meanwhile, the economic values were observed in Keerom District and several markets in Jayapura City since the initial survey results showed that bananas from Keerom were also marketed in Jayapura City. The study also explored how bananas were utilized by the community in Keerom, focusing on the parts of the plant used and their economic value. Most bananas served as a source of family income, with their selling prices surveyed at the farmer, middleman, and consumer levels.

Data analysis

The observation data were analyzed descriptively and qualitatively. The soil physicochemical characteristics were examined to see the level of soil fertility. The samples were sent to the Integrated Laboratory Unit of the Bogor Agricultural Institute (IPB - Institut Pertanian Bogor). The parameters measured included the degree of acidity (pH), organic carbon content, N, P, cation exchange capacity (CEC), K, Ca, Na, and Mg (Table 2). The results were presented in the form of tables and figures.

RESULTS AND DISCUSSIONS

The results show that land in the study area is mostly used for agriculture and oil palm plantations, and is highly suitable for agricultural activities (Tabel 2). According to Suharyanto et al. (2022), entisols and inceptisols are the most suitable soil types for agricultural use in the Arso Keerom area. Inceptisols, in particular, are prevalent across nearly all districts in Keerom and can be developed extensively for agricultural purposes. Environmental conditions substantially affect the adaptation of banana plant varieties. Local bananas are usually more adaptive than imported bananas, which, according to Fettig et al. (2023), includes the adaptation to local agroecological conditions. Local banana varieties are used for various purposes, including traditional medicine and cultural events.

Diversity of banana varieties

Based on the local knowledge, there are 13 varieties of bananas, namely: *Kepok boy, kepok abu-abu, raja, raja nangka, barangan, nona, susu, ambon, merah, hutan, tanduk, jarum,* and *goroh*o (Figure 2). The population and distribution of banana varieties in Keerom vary. *Kepok boy, kepok abu-abu, raja, barangan, and nona* are widely distributed, while *ambon, jarum, goroho, raja nangka, susu* and *merah* are not as widely distributed (Table 3). The populations of certain varieties, such as *kepok boy*, *kepok abu-abu,* and *raja* are relatively high, while other varieties, such as *goroho, jarum, susu, hutan, merah,* and *raja nangka* are scarce.

Morphologically, the habitus of banana plants in Keerom is diverse (Figure 2), with some plants heights reaching >3 m. Likewise, *barangan, ambon*, and *kepok* have the longest leaf blades among other varieties. The leaf stalk channels (petiole) also vary (Figure 3), including overlapping margins (Figure 3.A), curved inward margins (Figure 3.B), open with spreading margins (Figure 3.C), and wide with erect margins (Figure 3.D-F).

The characteristics of each variety's fruit also vary, especially the number of hands on each bunch and the number of fingers on each hand (Figure 4). *Tanduk* bananas have fewer hands and fingers compared to other varieties, such as *kepok boy* and *kepok abu-abu.* The features of the fruit (including the length and width) and the characteristics of the ovules also vary (Figure 5). Most varieties have different shapes, sizes, and blossom colors. *Hutan* bananas are distinctive with their green fruit and blossoms, while red bananas have red fruit and blossoms. However, most banana blossoms are red, even though the fruit is green, such as in *kepok, susu, raja,* and *jarum* bananas. According to Hapsari and Lestari (2016) and Hastuti et al. (2019), the characteristics of banana fruit and blossoms are key variables in banana diversity.

The shapes of the banana blossom (male bud shape) (Figure 6) are either like a top (Figure 6.A and 6.F), lanceolate (Figure 6.E), intermediate (Figure 6.B), and ovoid (Figure 6.D). The shape of the male bud also affects the shape of the bract base (medium and large shoulders) and bract apex (pointed, slightly pointed, intermediate, and obtuse). The analysis of morphological characteristics using NTSYs shows that the varieties of bananas in Keerom can be grouped into four clusters. The first cluster shows the closeness between *goroho* and *jarum* bananas. The second cluster shows a kinship between *ambon* bananas, *kepok abu-abu*, and *barangan*. The third cluster shows a kinship between *tanduk*, *raja*, *susu, and kepok asam*. The fourth cluster shows a kinship between *raja nangka, nona, merah,* and *hutan* (Figure 7).

The PCA results show that three main components can be used as the basis for grouping banana diversity in Keerom. The morphological diversity of bananas in the PCA1, PCA2, and PCA3 components reached values of 14.27, 13.05, and 11.45%, respectively. The PCA2 score of the main component shows that bananas from Arso are divided into four groups (Figure 8). Group I consists of three varieties of bananas, namely, *ambon*, *kepok abu-abu*, and *barangan*. Group II consists of two varieties of bananas, namely *goroho* and *jarum*. Group III consists of four varieties of bananas, namely *tanduk*, *raja*, *susu*, and *kepok asam*. Group IV consists of four varieties of bananas, namely *raja nangka*, *merah*, *nona*, and *hutan*.

Table 2. Results of soil fertility analysis in the Keerom lowland area, Papua, Indonesia

Physio-chemical parameters	Unit	Location (Sample)		
		Tuwi (AR-12)	PTPN2 Tami (AR-T.1)	Arso II, Track 5 (AR-7)
pH (H_2O) (1:1)	-	6.99	7.79	7.41
pH ($CaCl_2$)	-	6.28	6.63	6.33
C-organic	% w/w	2.99	7.05	2.10
Total nitrogen	% w/w	0.36	0.46	0.34
C/N Ratio	-	8.31	15.33	6.18
P Bray as P_2O_5	mg/kg	<9.88	135.73	<9.88
Cation exchange capacity (dry base)	cmol/kg	26.31	25.16	39.14
Cation exchangeable (dry base)	cmol/kg	7.36	3.27	0.78
Base saturation	%	27.98	13.00	1.98
Potassium (K)	cmol/kg	<0.05	2.06	<0.05
Sodium (Na)	cmol/kg	<0.09	<0.09	<0.09
Calcium (Ca)	cmol/kg	1.67	<36.00	0.78
Magnesium (Mg)	cmol/kg	5.69	1.21	<0.08
Aluminum exchangeable (dry base)	me/100g	<0.02	<0.02	<0.02
Acid exchangeable (dry base)	me/100g	0.44	0.45	0.27

Note: Sample analysis was conducted at the Integrated Laboratory Unit of IPB, Bogor

Table 3. Banana diversity in the Arso lowland area in Keerom, Papua, Indonesia

Banana's local name	Observation location							
	Young	Tami	Tuwi	Arso II	Yowong	Sanggaria	Warbo	Poros Arso
Kepok boy	+++	+++	+++	+++	+++	+++	+++	+++
Kepok abu-abu	++	++	++	++	+++	++	++	++
Raja	++	+	++	++	++	++	++	++
Raja nangka	-	-	+	-	-	-	-	-
Barangan	++	-	+	++	++	++	+	+
Nona	+	+	-	-	++	++	+	+
Susu	-	-	-	+	-	-	-	-
Ambon	-	-	-	+	-	-	-	-
Merah	-	-	-	-	-	+	-	-
Hutan	+	-	-	-	+	-	-	-
Tanduk	+	+	+	+	-	+	-	-
Jarum	-	+	-	-	-	-	-	-
Goroho	+	-	-	-	-	-	-	-

Note: - not found; + found in limited quantities; ++ many; +++ abundant

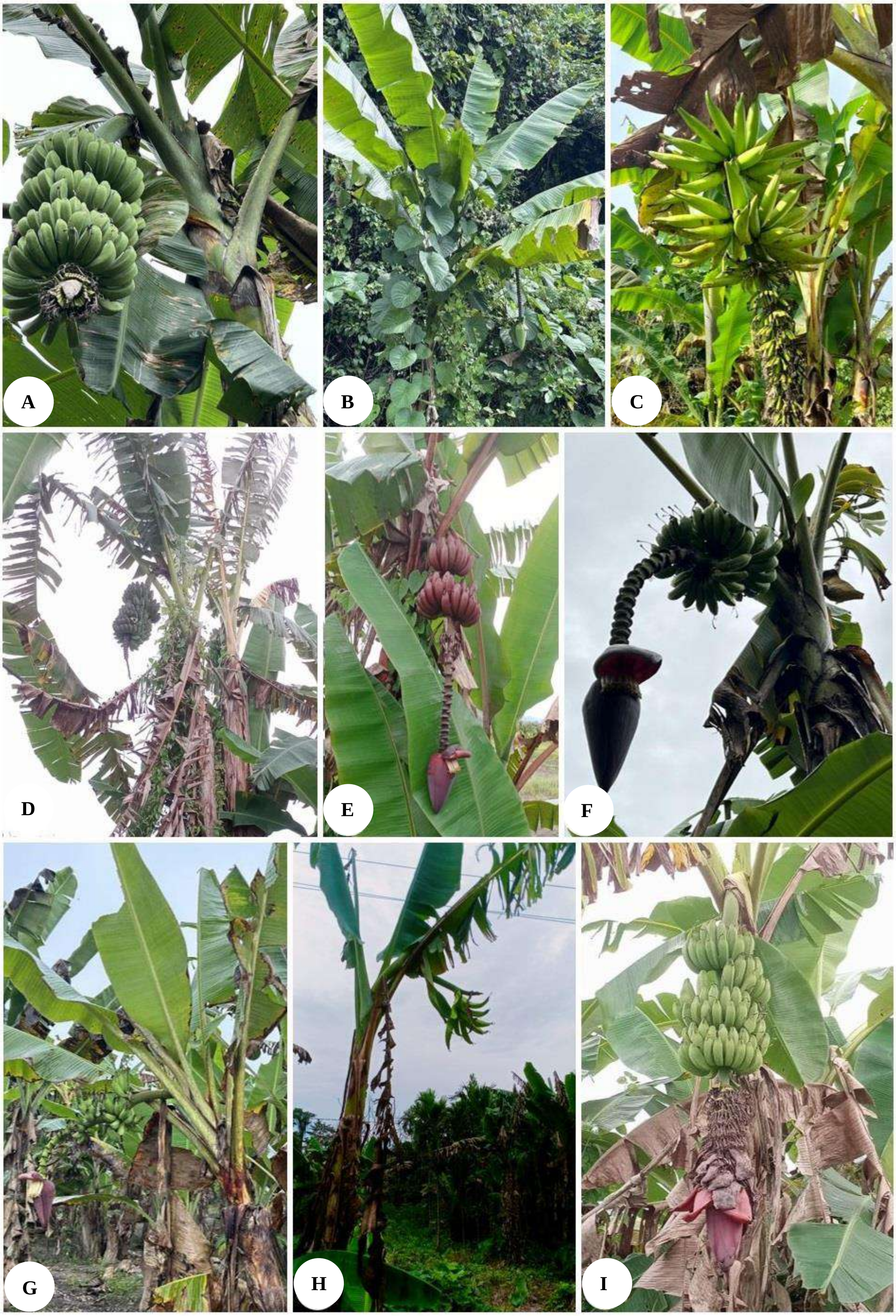

Figure 2. Diversity of banana habitus in the Arso lowland, Keerom, Papua, Indonesia: A. *Barangan*; B. *Hutan*; C. *Jarum*; D. *Kepok boy*; E. *Merah*; F. *Nona*; G. *Susu*; H. *Tanduk*; I. *Raja*

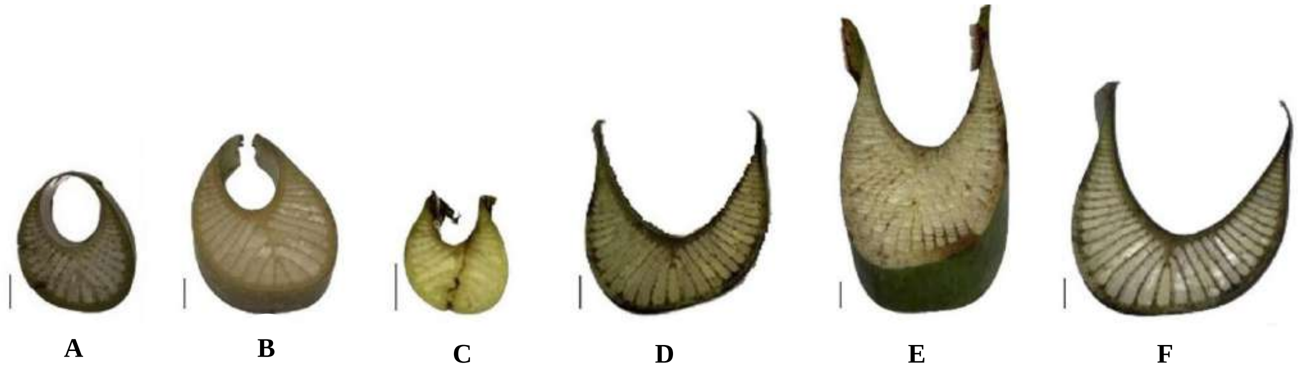

Figure 3. Diversity in cross-sectional morphology of leaf stalks (petioles) in several banana varieties found in Keerom, Papua, Indonesia: A. *Tanduk*; B. *Kepok asam*; C. *Nona*; D. *Barangan*; E. *Jarum*; F. *Kepok abu-abu* (scale bar: 1 cm)

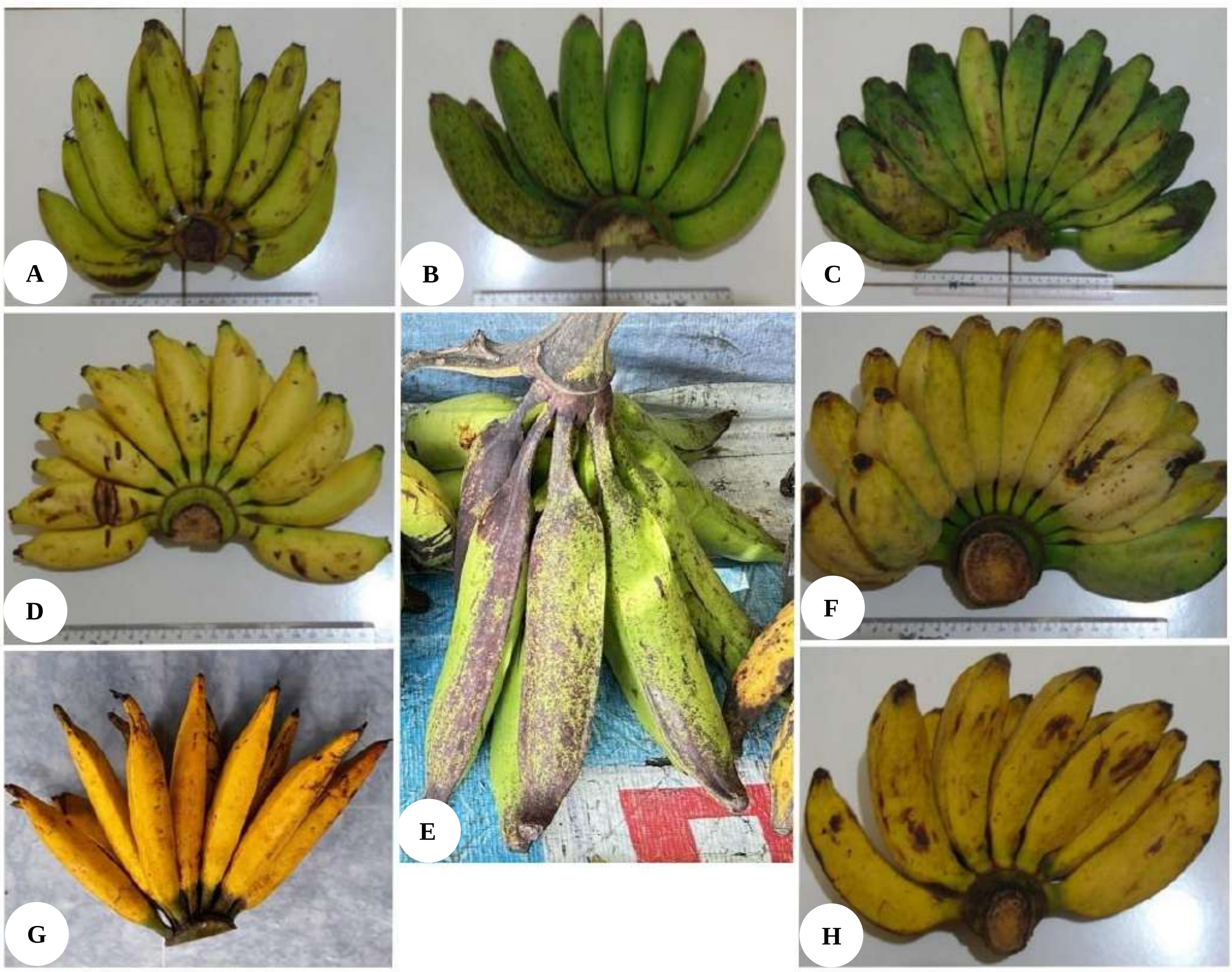

Figure 4. Banana varieties found in the lowland area of Keerom, Papua, Indonesia: A. *Ambon*; B. *Barangan*; C. *Kepok boy*; D. *Nona*; E. *Tanduk*; F. *Kepok abu-abu*; G. *Jarum*; and H. *Raja*

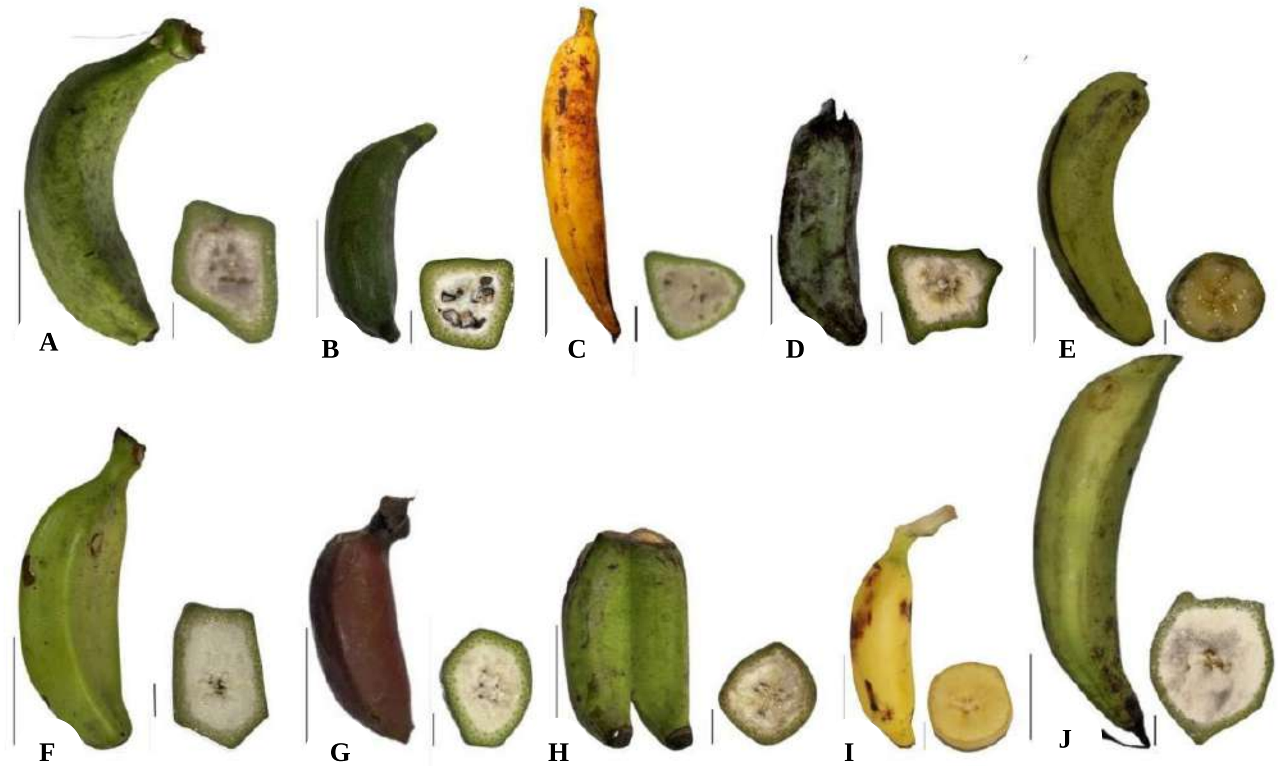

Figure 5. Varieties of banana fruit (scale bar: 5 cm) and cross-section of the fruit with various ovule positions (scale bar: 1 cm): A. *Barangan*; B. *Hutan*; C. *Jarum*; D. *Kepok abu-abu*; E. *Ambon*; F. *Kepok boy*; G. *Merah*; H. *Susu*; I. *Nona*; J. *Tanduk*

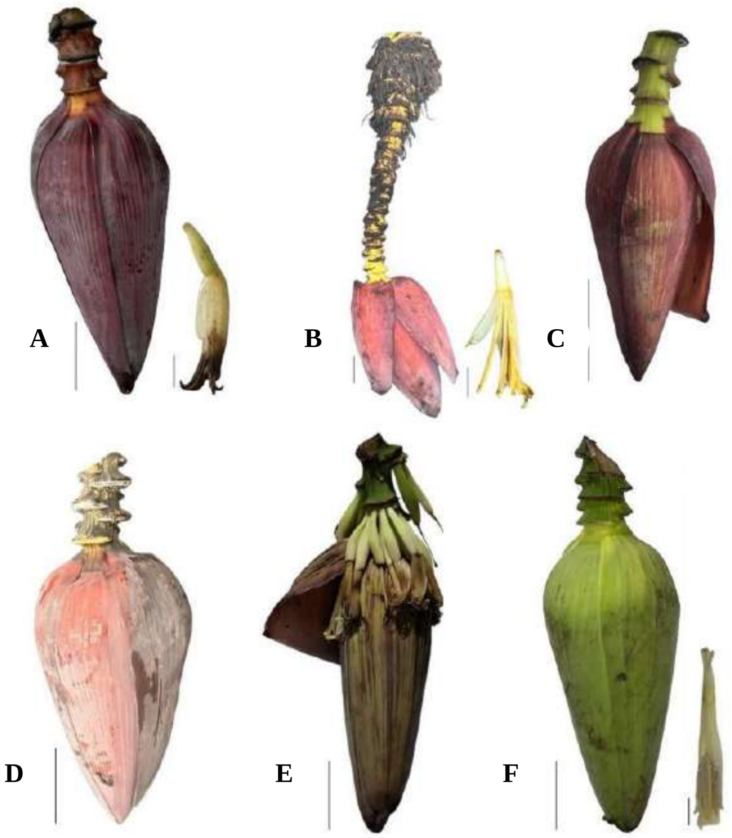

Figure 6. Varieties of banana blossom (male bud): A. *Susu*; B. *Raja*; C. *Nona*; D. *Merah*; E. *Jarum*; and F. *Hutan* (scale bar: 5 cm) and banana flowers (scale bar: 1 cm)

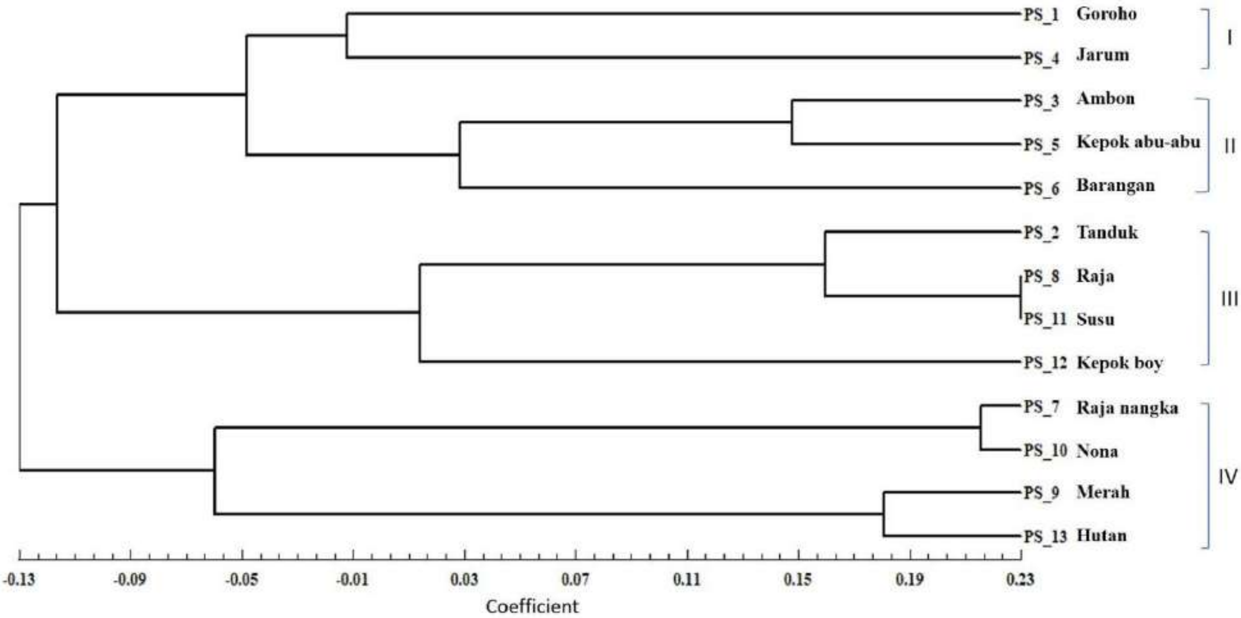

Figure 7. Banana kinship relationships are based on morphological characters, as observed in Keerom, Papua, Indonesia

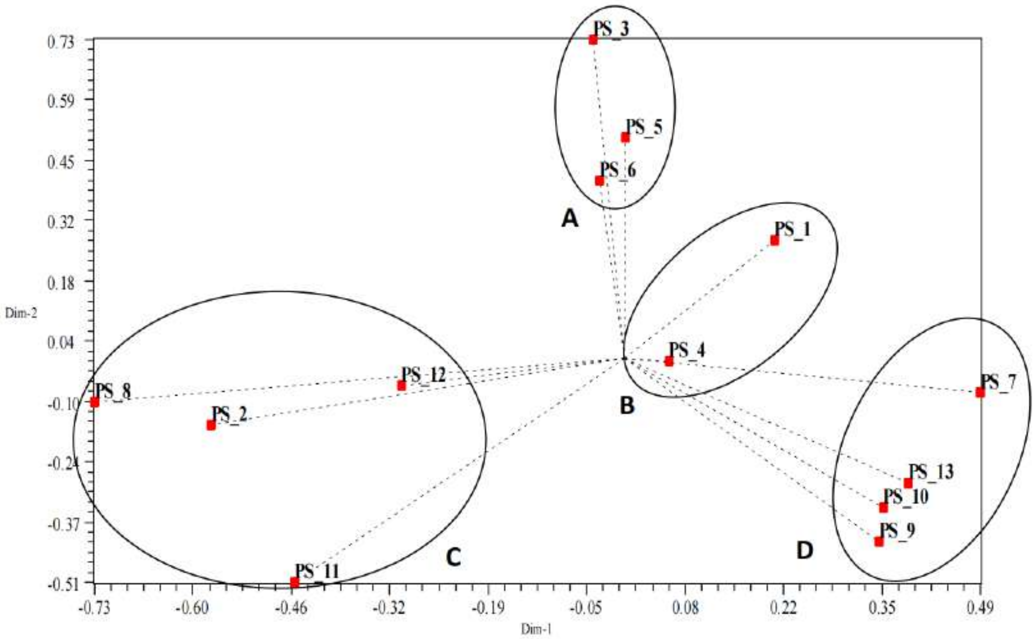

Figure 8. Plot score of two main banana components based on the 67 morphological characters of banana varieties in Keerom, Papua, Indonesia: PS_1. *Goroho*; PS_2. *Tanduk*; PS_3. *Ambon*; PS_4. *Jarum*; PS_5. *Kepok abu-abu*; PS_6. *Barangan*; PS_7. *Raja nangka*; PS_8. *Raja*; PS_9. *Merah*; PS_10. *Nona*; PS_11. *Susu*; PS_12. *Kepok boy*, and PS_13. *Hutan*

The three PCAs contribute to the principal component analysis based on the morphological characters. The results show that principal component 1 (PCA1) contributed 58.20% of the variation, PCA2 47.76%, and PCA3 38.80%. The results of the Eigenvalue test showed that nine main banana characters contributed most substantially to the grouping, namely: 1) leaf blade length (K22), 2) leaf vein lines (K33), 3) the color of cigar leaf dorsal surface (K36), 4) rachis appearance (50), 5) male bud type (K51), 6) ripe fruit flesh color (K60), 7) heart color (K65), 8) length (K66), and 9) heart diameter (K67). Based on their morphological characteristics, the fruit and banana blossom exhibit significant differences among the varieties of bananas observed.

Understanding the diversity of bananas involves studying native wild species, their relatives, and both primitive diploid forms and more advanced triploid varieties (Perrier et al. 2009). Therefore, molecular techniques are essential for accurately uncovering the diversity of banana varieties (Sardos et al. 2016; Rahmah et al. 2023).

Ethnobotany of banana plants: The utilization by the community

Observations reveal that the community widely utilizes banana plants as a local food source (Table 4). Most banana varieties are primarily used as a fruit source and sold to generate income for the local community. Other parts of the banana plant are also utilized: the leaves are used as wrapping materials for goods or food, and the banana blossom is consumed as a vegetable. A small portion of the community uses the stem as a component in plant fertilizers.

On various occasions, bananas are cultivated alongside other plants such as sugar cane, sweet potatoes, cassava, and various species of tubers in the agricultural system of indigenous people in Papua who still practice shifting cultivation. People in the mountains typically use sweet potatoes (batatas) and other species of tubers as their staple foods (Kogopa 2024). Meanwhile, in the lowlands, sago serves as a staple food source for the community (Kadir et al. 2022; Kadir et al. 2024).

Some Papuan people use bananas as part of traditional ceremonies and events (Zebua et al. 2023). In several other regions, bananas are used in a wide variety of ways, including as desserts such as fresh ripe bananas, boiled ripe bananas (*pisang rebus*), fried ripe bananas (*pisang goreng*), and unripe bananas made into *rujak bebek*, mixed with cassava or sweet potatoes and chili sauce. Other uses include *banana kolek* (bananas mixed with sugar and coconut flour), banana chips (*kripik pisang*), and offerings for traditional ceremonies (Iskandar et al. 2018). Several varieties of bananas can also be used as raw material for making "banana flour," which can be developed into a source for various types of food products (Keiluhu et al. 2024).

Banana-based food sources are highly important and offer significant nutritional value, making them beneficial for various forms of treatment (Ranjha et al. 2022). According to Palma et al. (2022), The potassium content, along with other elements such as calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), and rubidium (Rb), in the banana blossom samples studied was found to be higher than previously reported in the literature. This suggests that banana blossoms, as utilized by the community, are highly nutritious and serve as a valuable alternative for meeting the community's nutritional needs. Ferreira et al. (2023) also stated that banana stem flour is rich in phenolic compounds and exhibits strong antioxidant properties. In vivo studies indicate that supplementing a hypercaloric diet with banana stem flour helps prevent pathological damage by reducing total cholesterol and glucose levels, suggesting a potential hepatoprotective effect. Therefore, incorporating banana stem flour as a supplement can enhance the intake of fiber, antioxidants, and bioactive compounds.

The economic value of bananas

The observation results indicate that farmers sell bananas in bunches. When calculated, the selling price of the *tanduk* banana per hand is the highest, reaching IDR 8,000, followed by *ambon* bananas at IDR 3,750 and *raja* bananas at IDR 3,000 per hand. Other varieties of bananas are sold at various prices ranging from IDR 1,600 to IDR 2,500 per hand, with the lowest price being for *susu* bananas. Overall, at the farmer level, the average selling price of bananas is IDR 3,481.25 per hand (Table 5). Farmers can sell bananas directly to the market or through middlemen (collectors). At the collector level, the average selling price reaches IDR 5,125 (an increase of 47.22%). The selling price of bananas in the market (at the consumer level) reaches an average of IDR 8,500 per hand, which has increased by 144.16% compared to the selling price at the farmer level.

Based on the observation results, the *kepok boy* and *kepok abu-abu* varieties are commonly found in the market. This is understandable given their wide distribution and abundant availability (Table 3), which results in lower selling prices despite their popularity among consumers. Interview results revealed that the *tanduk* bananas command a higher price due to their popularity and durability (it does not spoil easily), similar to the *raja* bananas. On the other hand, several other banana varieties, such as the *nona* bananas, are often sold at lower prices, even though they are in demand by many consumers.

Table 4. Utilization of bananas by variety in the Keerom lowland, Papua, Indonesia

Variety	Utilization
Kepok boy	The fruit is consumed as food, while the leaves are used to wrap various food and beverage ingredients. Additionally, banana blossoms are utilized as vegetables
Kepok abu-abu	The fruit is consumed as food, while the leaves are used to wrap various food and beverage ingredients. Additionally, banana blossoms are utilized as vegetables
Raja	The fruit is consumed, and the leaves are used for wrapping
Raja nangka	The fruit is consumed, and the leaves are used for wrapping
Barangan	The fruit is consumed
Nona	The fruit is consumed
Susu	The fruit is consumed
Ambon	The fruit is consumed, and the leaves are used for wrapping
Merah	The fruit is eaten; however, this variety of banana is not widely known due to its limited population and the community's lack of awareness about it
Hutan	Birds primarily consume the fruit, and it remains unutilized by the community
Tanduk	The fruit is consumed
Jarum	The fruits are consumed, primarily prepared through baking
Goroho	The fruit is consumed, typically fried

Table 5. Banana price values in Keerom, Papua, Indonesia and surrounding areas

Variety	Price at the farmer level (per bunch) (IDR)	Selling price (per hand) (IDR)		
		Farmer	Middlemen/collectors	Market (consumer)
Kepok boy	15,000-20,000/bunch (7-8 hands)	2,500	3,000	5,000
Goroho	15,000-20,000/bunch (7-8 hands)	2,500	3,000	5,000
Kepok abu-abu	15,000-20,000/bunch (7-8 hands)	2,500	3,500	5,000
Raja	20,000-25,000/bunch (8-10 hands)	3,000	5,000	10,000
Raja nangka	For self-consumption	-	-	-
Barangan	20,000/bunch (8-9 hands)	2,200	3,500	5,000
Nona	10,000-15,000/bunch (6-8 hands)	1,800	5,000	8,000
Susu	10,000-12,000/bunch (6-8 hands)	1,600	3,000	5,000
Ambon	25,000-30,000/bunch (7-8 hands)	3,750	5,000	10,000
Merah	-	-	-	-
Hutan	-	-	-	-
Tanduk	20,000-25,000/bunch (2-3 hands)	8,000	10,000	15,000
Jarum	-	-	-	-
Average		3,094.44	4,555.56	7,555.56
Price difference at the farm level		0	1,461.12	4,461.12
Price increase		0	47.22%	144.17%

Note: -: not yet known/not yet economically valuable. Price increases are calculated based on prices from farmers. 1 USD = 17,000 IDR

The identification results also indicate that not all varieties of bananas are sold in Keerom. Certain varieties, such as *kepok abu-abu*, *kepok boy*, *raja*, and *barangan* bananas, are frequently sent to other cities, such as Jayapura City, due to the high demand for bananas there. Most bananas are sold in local markets, with none being marketed in supermarkets. This suggests that post-harvest management for farmers in Keerom has not been fully optimized. According to Saha et al. (2021) and Sah et al. (2023), post-harvest handling of bananas directly impacts the quality of the fruit, which in turn can increase its selling price and, consequently, the income of the community. Fettig et al. (2023) and Wijayanto (2024) added that this handling includes the availability of planting materials, development of post-harvest and processing technology, marketing, investment, and consumer education.

Although banana cultivation in Keerom is not yet intensively managed by the community, it is still sufficient to contribute to the income of local farmers. However, farmer assistance is essential to improve the management system and increase their income. Additionally, underutilized banana varieties can be optimized through ongoing, sustainable support. According to Eyland et al. (2020), the diversity of bananas in New Guinea (including Indonesia) is exceptionally high, warranting more in-depth research to understand and utilize their potential fully. Castañeda-Álvarez et al. (2016) and Mertens et al. (2020) emphasize the importance of conducting further studies on the diversity of plant species, including bananas and their relatives, to prevent extinction. This is crucial in light of the growing abiotic and biotic pressures associated with climate change, particularly those driven by anthropogenic factors such as the increasing global population, habitat loss and fragmentation, improper land use, and inadequate conservation strategies.

In general, it can be concluded that 13 banana species were found based on local knowledge, grouped into four clusters. The *kepok boy* and *kepok abu* bananas have a wider population and distribution, while *merah*, *jarum*, *raja nangka*, and *goroho* bananas are very limited. Banana parts that are commonly utilized by the community are the fruit, leaves, and heart of the banana. The selling price of bananas at the farm level averaged IDR 3,094.44 (USD 0.2) and IDR 7,555.56 (USD 0.45) per hand at the consumer level, an increase of 144.17%. Increasing the value-added utilization of banana plants in Keerom requires the assistance of farmers and the utilization of appropriate technology.

ACKNOWLEDGEMENTS

We would like to thank the Head of West Arso, Skanto, and Arso Sub-district for permitting this research. To Annisa Ruhani, Diaz Toruan, Zet Banundi, Andro Siahaan, Wetty Queenisa Kabes, Gilbert Marani, Irma Rahayu, and Yuliana, thank you for helping the authors collect research data in the field.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 226-232
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090124

Socio-demographic profile of food farmers in Carcar City, Cebu, Philippines amid challenges and opportunities for agricultural development

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Manuscript received: 30 January 2025. Revision accepted: 21 April 2025.

Abstract. *Lillo EP, Diaz GGG, Diaz JLB, Mago JE, Davirao CR. 2025. Socio-demographic profile of food farmers in Carcar City, Cebu, Philippines amid challenges and opportunities for agricultural development. Asian J Agric 9: 226-232.* This research specifically examines the socio-demographic profile of food farmers residing in Carcar City, Cebu, focusing on their age, sex, educational attainment, income, household size, land ownership, and farming experience. The study employed a descriptive quantitative research design, which involved conducting structured interviews and administering surveys. The results showed that most farmers are middle-aged or older, implying that those aged 36-55 years comprise 46%, while those aged 56 years and older comprise 40%. The youngest age group is the young adults, comprising 14% of individuals aged 18-35 years old. Most of the women engaged in farming activities are middle-aged, which correlates with their household responsibilities. The economics of return could limit opportunities due to low monthly earnings, ranging from PHP 5,000 to 15,000 (89 to 267 USD), and insecure land tenure, which discourages sustainable agricultural practices. Farmers educational attainment is limited and restrictive in terms of modern technological acceptability, as more than 70% of them received only primary education. Strategic interventions are thus needed to address these issues, including enhancing youth engagement in agriculture, increasing training programs, and developing mechanisms to improve financial support and secure land tenure. Therefore, working together with government agencies, stakeholders, and community organizations is crucial in reviving the agricultural sector, promoting food security, and achieving sustainable rural development. The results consistently indicated a clear need for comprehensive policies that would enhance farmer resilience and economic viability.

Keywords: Agriculture, Carcar City, farming practices, socio-demographics, sustainable development

INTRODUCTION

Depending on the sharpness of view and the laws of one society's economy, sociology, politics, and other relevant areas, agriculture has been a long-term cornerstone in the foundation of human society. It is a food producer and a supplier of raw materials-based industries, and it also plays a vital role in the livelihoods of people worldwide. Farmers are the backbone of agriculture, producing food and commodities that sustain human life and support economic development. Farmers today face numerous challenges, including climate change, environmental degradation, socioeconomic disparities, and resource depletion, all of which threaten the stability of food systems and sustainable development. These have significantly hindered farmers ability to maintain productivity and resilience, especially in developing countries (FAO 2021). By 2050, the world population is expected to reach 9.7 billion, as predicted by the United Nations (2022). In addition to the increasing number of people, agriculture will face serious challenges. Age, gender, educational background, and access to resources also increase the challenge in terms of the politics of resilience and productivity among those reliant on agriculture (World Bank 2020). Thus, beyond restricting solution spaces, these constraints and barriers will require multi-regional considerations and a thorough understanding of farmers capabilities and opportunities.

In the Philippines, agriculture is the country's most important economic sector, accounting for nearly a quarter of the entire workforce (Philippine Statistics Authority 2022). The agriculture sector is vital as it contributes 20% of the gross national domestic product and 37% to the national labor force. Within this agricultural sector, there are approximately 4.5 million farms that directly support 25 million Filipino farmers and their families (Oakeshott et al. 2016). Although it is significant, this sector has continually encountered challenges, including a growing aging farmer population, limited access to formal education, capital, and technology, as well as a decreasing interest among the youth. These challenges are exacerbated by unpredictable changing climatic conditions, which cause crop failures and considerably reduce agricultural yields (Rola et al. 2016). The capability of farmers to adjust to and cope with the impacts of climate change is also strongly related to their socioeconomic setup aspects, including income, social security, and assets.

There is a point at which a city like Carcar, situated in southern Cebu's agricultural belt, reflects the issues and opportunities of the agricultural sector within the Philippines. A site renowned for its rich, ancient soils and agricultural heritage, it continues to play a significant role in the region's food production. Above all, farming communities in Carcar are subject to increasing urbanization and industrial pressures alongside the encroaching horizons of new economic trends. These are indeed worrying trends regarding the sustainability of traditional farming practices and the survival prospects of their beneficiaries.

Yet, these few observations on Carcar as an agricultural city fail to fulfill the set promise of data regarding the socio-demography of Carcar farmers. Such understanding is relevant because it defines how farmers access resources as well as how they internalize and respond to external pressures. As Serrat (2017) notes, certain socio-demographic factors, such as age, education, and land ownership, appear to significantly influence their decisions and ability to adopt modern technologies into their farming practices. Furthermore, it has been observed that many rural development programs have failed because their planners did not adequately consider the socio-demographic contexts of their target populations (Pingali et al. 2019).

The socio-demographic assessment that food farmers are undergoing in Carcar City aims to fill this void by conducting an all-encompassing study. This includes the distribution of age, gender, education level, income, household number, terms of land ownership, and farming experiences. The identified patterns and associative nature can be used to generate insights for subsequent policy and program designs that support the agriculture sector in Carcar and similar locations. This inquiry builds upon existing literature and aims to provide locally relevant contexts that are feasible and practical. The research aims to study the barriers and opportunities in rural agricultural communities through a closer understanding of the socio-demographic profiles of food producers in Carcar City. This initiative aims to provide policymakers, stakeholders in agriculture, and local government units with evidence of human-intervention-based effects, thereby enhancing the efficiency, resilience, and sustainability of agriculture in Carcar and beyond.

MATERIALS AND METHODS

Study area

The conducted study encompassed four barangays (Napo, Buenavista, Guadalupe, Ocaña) in Carcar City, Cebu, located in southern Cebu, Philippines (Figure 1). In the Philippine administrative structure, a barangay is the smallest unit, comparable to a village, district, or neighborhood. Carcar City is endowed with a rich agricultural tradition, and agriculture remains one of the primary pursuits for many of its inhabitants. The tropical monsoon climate features a wet season from June to October, characterized by rainfall, and a dry season from January to May, which facilitates the planting of crops such as corn, rice, vegetables, and root crops. The second major contributor to the local economy, after agriculture, is livestock farming. The morphology of the land varies from flat, low-lying areas to high, rolling hills, generally in contrast to the types of crops cultivated and the different farming practices employed. Adaption of farmers would depend on the distance of the farm from Cebu City and the availability of urban markets for the sale of their produce. Nevertheless, agriculture sustainability is challenged by threats from urban expansion, land-use changes, and minimal infrastructure development. According to the Philippine Statistics Authority's 2020 report, Carcar City has a total population of 136,453, a significant number of whom are engaged in agricultural activities, primarily focused on crop and livestock production. The area benefits from fertile soil conditions and access to local markets, but it also faces challenges such as land conversion, resource limitations, and climate variability that impact agricultural productivity.

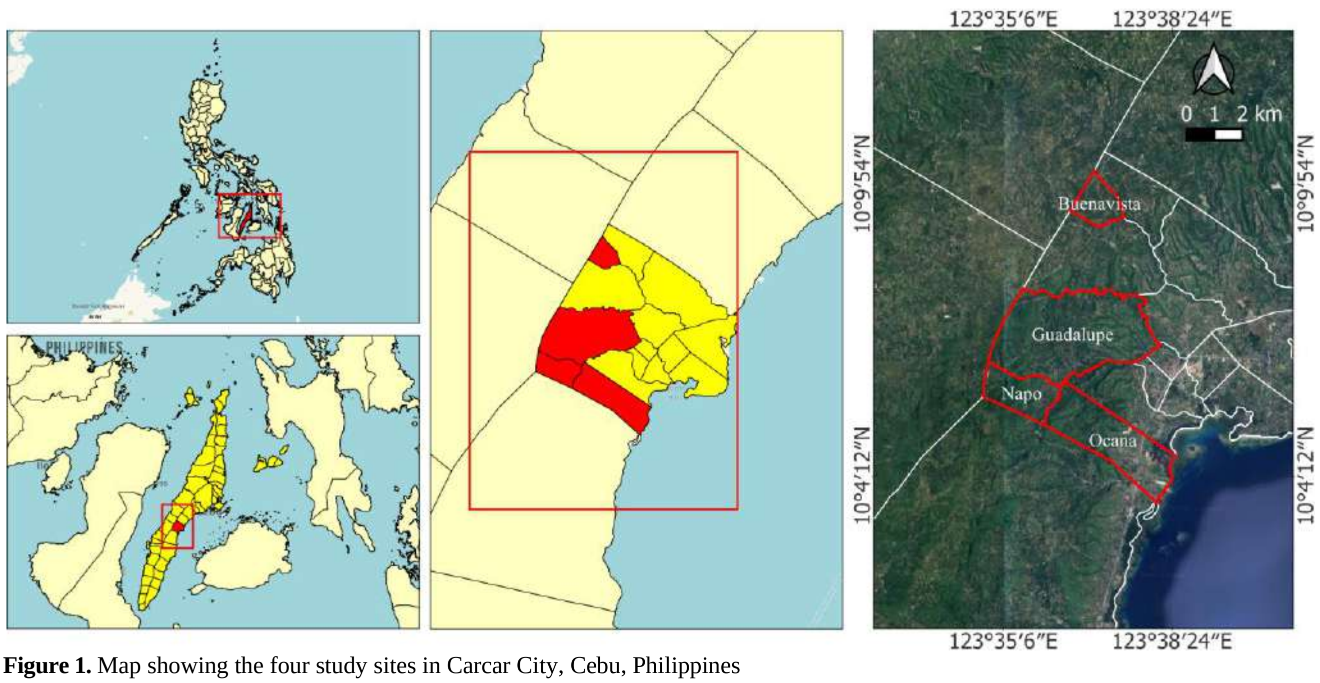

Figure 1. Map showing the four study sites in Carcar City, Cebu, Philippines

Data collection

A quantitative design was employed to systematically analyze the socio-demographic characteristics of farmers in Carcar City. This approach is commonly used in social science research to identify patterns and relationships within population groups (Creswell 2014). Data were gathered using a structured questionnaire specifically designed to meet the study's objectives. The questionnaire was divided into three sections: i. Demographic profile, which covered age, gender, education, and household size; ii. Economic information, which included questions on income, farming experience, and land ownership; and iii. Access to resources, focusing on agricultural inputs, training, and support programs. The instrument was adapted from previously validated questionnaires used in similar agricultural studies (Israel 1992; FAO 2016) and customized to align with the local context. The surveys were administered in the local dialect (Cebuano) by trained enumerators over one month to facilitate comprehension and engagement. Both closed-ended and open-ended questions were included. Close-ended questions, such as multiple-choice and Likert-scale items, were used for quantitative analysis (Bryman 2016), while open-ended questions allowed for the collection of additional qualitative insights. Before full deployment, the questionnaire underwent pre-testing with five farmers to ensure clarity and relevance, and necessary adjustments were made to refine ambiguous questions. Ethical considerations were observed, with participants providing informed consent and responses kept confidential, following standard research ethics guidelines (Babbie 2020). Trained enumerators conducted interviews to minimize bias, and data were double-checked for inconsistencies before analysis to ensure accuracy and validity (Groves et al. 2009).

Data analysis

The collected data were encoded and analyzed using descriptive statistics, including frequency distributions and measures of central tendency, which summarized key socio-demographic characteristics. Cross-tabulations examined relationships between variables such as age, gender, education, income, and land ownership. Additionally, comparative analyses were conducted to identify disparities within the farming community. Results were visualized using figures (Figures 2-8) to enhance interpretation, ensuring a comprehensive understanding of the socio-demographic factors influencing agricultural practices in Carcar City.

RESULTS AND DISCUSSION

Based on socio-demographic considerations, the gathering of information is imperative as it significantly contributes to the analysis and interpretation of data. The 50 agricultural producers participating in this study were categorized based on their demographic profiles, economic status, and access to resources. The demographic characteristics of the respondents are detailed in Table 1.

Table 1. Socio-demographic characteristics of the informants per barangay

Socio-demographic profiles	Categories	Buen avista	%	Guadalupe	%	Napo	%	Ocaña	%
Sex	Male	1	5.88	5	62.50	4	44.44	15	93.75
	Female	16	94.12	3	37.50	5	55.56	1	6.25
Age	18-35	2	11.76	2	25.00	0	0.00	3	18.75
	36-55	11	64.71	2	25.00	5	55.56	5	31.25
	56 and above	4	23.53	4	50.00	4	44.44	8	50.00
Educational attainment	Elementary level	8	47.06	3	37.50	4	44.44	8	50.00
	Secondary level	7	41.18	3	37.50	1	11.11	8	50.00
	College level	2	11.76	2	25.00	4	44.44	0	0.00
Role in the family	Husband	4	23.53	5	62.50	4	44.44	14	87.50
	Wife	13	76.47	3	37.50	5	55.56	2	12.50
Household size	1-3 Members	3	17.65	4	50.00	1	11.11	8	50.00
	4-6 Members	7	41.18	2	25.00	6	66.67	5	31.25
	7-9 Members	4	23.53	2	25.00	2	22.22	2	12.50
	10+ Members	3	17.65	0	0.00	0	0.00	1	6.25
Farming experience	1-5 Years	0	0.00	3	37.50	3	33.33	8	50.00
	6-10 Years	7	41.18	2	25.00	0	0.00	1	6.25
	11-20 Years	1	5.88	2	25.00	6	66.67	6	37.50
	21-30 Years	1	5.88	1	12.50	0	0.00	1	6.25
	31+ Years	8	47.06	0	0.00	0	0.00	0	0.00
Income range (PHP)	< 5,000	3	17.65	0	0.00	0	0.00	2	12.50
	5,000-10,000	6	35.29	2	25.00	4	44.44	8	50.00
	10,000-15,000	5	29.41	3	37.50	2	22.22	5	31.25
	15,000-20,000	3	17.65	2	25.00	2	22.22	0	0.00
	> 20,000	0	0.00	1	12.50	1	11.11	1	6.25
Land tenure	Own land	10	58.82	5	62.50	3	33.33	12	75.00
	Tenant	4	23.53	2	25.00	6	66.67	3	18.75
	Sharecropper	3	17.65	1	12.50	0	0.00	1	6.25
Resource/training type	Financial support (Loans)	7	41.18	3	37.50	1	11.11	6	37.50
	Agricultural training	5	29.41	1	12.50	4	44.44	5	31.25
	Access to modern equipment	3	17.65	2	25.00	2	22.22	3	18.75
	Government subsidies	2	11.76	2	25.00	2	22.22	2	12.50

Aging workforce and declining youth engagement

As shown in Figure 2, farmers aged 36-55 form the largest segment, comprising 46% of the respondents, while those aged 56 and above account for 40%. The youngest cohort, aged 18-35, represents only 14% of the population, indicating a significant disengagement among young people. Gender distribution among Carcar's farming population is balanced, with men and women each representing 50% (Figure 2). However, gender roles vary across different age groups. Among middle-aged farmers (aged 36-55), women predominate at 34%, while men account for only 12%.

Educational attainment and its impact on agricultural practices

Figure 3 illustrates the educational attainment among farmers in Carcar City, highlighting a significant gap in formal education. The majority, 46% (23 farmers), are at the elementary level, followed by 38% (19 farmers) at the secondary level, while only 16% (8 farmers) have reached the college level.

Economic constraints and financial vulnerability

Most farming households in Carcar City earn between PHP 5,000 (89 USD) and PHP 15,000 (267 USD) per month, reflecting their significant economic vulnerability (Figure 4). This income range is insufficient to meet the financial demands of larger households, particularly those with seven or more members, who experience heightened financial strain due to increased living expenses (Figure 5). Conversely, smaller households with one to three members often struggle with labor shortages, which can limit their farming capacity and productivity. Mid-sized households, consisting of four to six members, tend to achieve a more favorable balance between income generation and labor availability.

Land tenure insecurity and its consequences

Land ownership and tenure play a crucial role in determining the number of years farmers till the land and their ability to sustain productive agricultural practices in Carcar City. The results were as follows: 60% of farmers are landowners of the land they cultivate; further, the remaining share is distributed among 30% of farmers who do not own land, and the remaining 10% are sharecroppers (Figure 6). The farmer is, therefore, said to enjoy favorable longevity, as ownership seals long-term investment in sustainable practices that can include crop diversification, soil management, and even infrastructure improvements. The stability also promotes the iteration of generations in the fields, and the farming knowledge passed down with it is passed on to heirs. Thus, landowners comprise the larger experience categories, with the majority having 11-20 years (30%) and 31+ years (16%) (Figure 7), indicating a strong relationship between ownership and years spent farming. In contrast, tenants and sharecroppers face significant challenges related to tenure insecurity, which limits their ability to remain on the same land for extended periods. Tenants, comprising 30% of farmers, are likely concentrated in the 1-10 years of experience range (48%), as short-term agreements and the risk of eviction discourage long-term investments. Sharecroppers, who comprise 10% of the population, are among the most vulnerable due to the unstable nature of their agreements and the obligation to share a portion of their harvest with landowners.

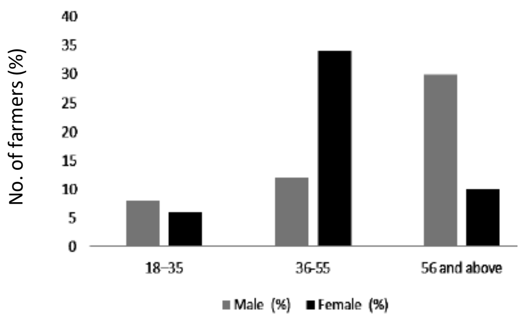

Figure 2. Age and gender distribution of farmers

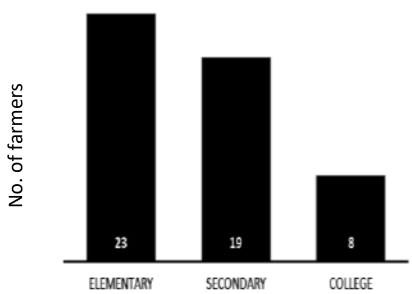

Figure 3. Educational attainment of farmers

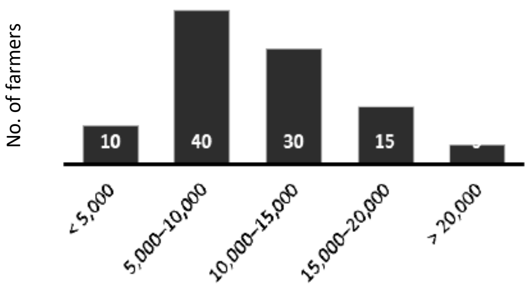

Figure 4. Monthly income range of farmers (in PHP)

Figure 5. Household size of farmers

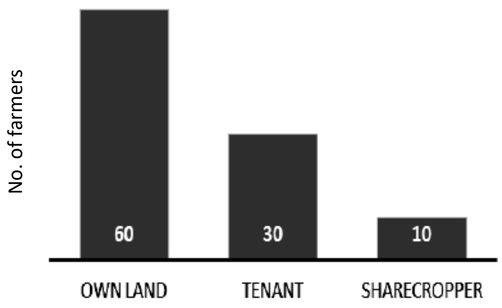

Figure 6. Land ownership and tenure status

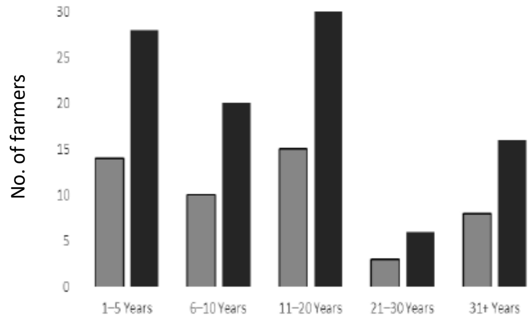

Figure 7. Farming experience (years)

Figure 8. Access to resources

Access to resources

The result highlights limited access to resources as a significant barrier to productivity among Carcar's farmers. Approximately 34% of respondents reported limited financial resources, and only 30% had received agricultural training, as shown in Figure 8. These constraints reduce productivity and resilience, especially in the face of climate variability.

Discussion

The demographic trend of an aging farmer population in Carcar City presents a significant challenge to the long-term sustainability of agricultural practices. The predominance of older farmers in this sector heightens the risk of stagnation due to their potential resistance to adopting advanced technologies and contemporary agricultural methodologies. Palis (2020) found that the average age of Filipino rice farmers is 53 years, and a majority prefer their children to pursue non-agricultural careers, citing the physical demands and economic uncertainties of farming as deterrents. As shown in Figure 2, only 14% of farmers fall within the age range of 18-35, underscoring the limited participation of young people in agricultural endeavors. This limited involvement of young individuals in farming is likely a consequence of the prevailing perceptions that depict agriculture as a physically demanding, financially unstable profession with minimal opportunities for career advancement (Chipfupa and Tagwi 2021). The phenomenon of an aging farmer demographic is increasingly recognized as a global concern. As highlighted by Heide-Ottosen (2014), a discernible pattern exists of rising percentages of older farmers, coupled with a decline in the numbers of younger farmers in rural areas worldwide.

The younger cohorts are progressively inclined toward urban employment, which engenders a labor deficit within the agricultural domain. In contemporary society, younger individuals exhibit considerably greater mobility than their predecessors, resulting in enhanced opportunities for education and skill acquisition. This contributes to a decreased willingness to return to rural settings for agricultural pursuits after completing their educational endeavors (Farrell et al. 2021). Tackling this challenge necessitates the implementation of agricultural curricula in educational institutions, the provision of financial incentives for aspiring young farmers, and increased access to contemporary farming technologies (Gaithuma et al. 2025). The process of modernization possesses the capacity to captivate the interest of younger demographics (Guerra 2018). The integration of innovative practices, such as the use of robotics, drones, farm management software, and more sustainable agricultural techniques, has the potential to motivate the younger generation to take control of agricultural enterprises and enhance their viability (Farrell et al. 2021).

Conversely, the agricultural community in Carcar is undergoing a transformation in labor dynamics, particularly in terms of gender roles. Women are increasingly attaining prominence within middle-aged farmer collectives, thereby demonstrating their dual responsibilities in both agricultural and domestic spheres (Kristjanson 2022). Nevertheless, men continue to play a crucial role in many physically demanding agricultural tasks, particularly among the older demographic. Such disparities in gender roles may necessitate targeted interventions aimed at women farmers to facilitate access to resources, training, and financial support, thereby enhancing their productivity and economic autonomy.

Furthermore, the adoption of mechanization and labor-sharing arrangements could contribute to the equitable distribution of gender roles within agricultural activities. Educational attainment plays a crucial role in shaping agricultural practices, influencing productivity, sustainability, and the adoption of modern techniques. Asfaw and Admassie (2004) found that elevated educational attainment among agricultural producers is correlated with a greater propensity for adopting advanced agricultural technologies, which subsequently leads to enhanced crop yields and economic resilience. Conversely, Paltasingh and Goyari (2018) found that farmers with limited educational backgrounds tend to exhibit diminished productivity due to their reliance on traditional farming practices and inadequate financial management skills. The data from Figure 3 indicates a significant challenge in adopting modern agricultural practices, which can directly affect productivity, efficiency, and sustainability. With 46% of farmers at the elementary level and only 16% reaching college level, the limited formal education suggests several key implications for agriculture. Farmers with lower educational levels may lack the awareness or technical skills necessary to implement modern farming methods, such as precision agriculture, climate-resilient crops, and mechanization. Traditional knowledge persists among many farmers and remains a valuable resource. Yet, when combined with some technical information, it may not adequately respond to the immediate concerns of agriculture. Farmer education through field schools, workshops, and extension services ought to be a priority if farm productivity is to be enhanced and sustained (Dawe 2018).

Such programs would, therefore, be based on relevant on-the-job training to make them accessible and credible to less formally educated farmers. Any new vision for agricultural extension must bridge the existing educational gaps between the relatively small number of formally educated farmers and traditionalists who are often distracted. Economic constraints and financial vulnerability pose significant challenges to farmers, particularly those with lower educational attainment and income levels. Farms managed by less-educated farmers may experience lower yields and reduced profitability, which can contribute to economic instability in rural areas.

The data from Figure 4 indicates that most individuals earn a subsistence income, ranging from PHP 5,000 to PHP 15,000 (89 to 267 USD), with very few exceeding PHP 20,000 (348.59 USD). This trend has a direct impact on agricultural sustainability, investment capacity, and overall livelihood security among low-income individuals. Farmers with low incomes struggle to afford essential agricultural inputs, including seeds, fertilizers, and equipment. Odhiambo et al. (2004) concluded that farmers with larger families allocate a greater proportion of their income to household needs rather than reinvest in farm productivity, leading to stagnation in agricultural development. The larger households contend with heavy financial burdens, while their smaller counterparts confront dwindling farm capacities. Financial assistance from the government, cooperative farming programs, and market access enhancements are necessary steps to balance these imbalances and establish a floor for household incomes (Villanueva 2019). Access to microfinance programs and cooperatives would enable farmers to pool resources, procure modern equipment, and enter more lucrative markets. Many farmers will continue to remain poor, unable to make any investments in farming, and therefore, likely to experience even reduced productivity without outside help. Land tenure insecurity presents significant challenges to agricultural productivity, limiting farmers' ability to make long-term investments and adopt sustainable practices. Farmers who own their land are more likely to invest in infrastructure, soil conservation, and mechanized farming, ensuring long-term productivity and sustainability. In contrast, tenants and sharecroppers face uncertainties that discourage improvements and the adoption of technology (Sumalde and Francisco 2021). Without secure tenure, farmers may hesitate to implement sustainable techniques such as crop rotation, soil conservation, and mechanized activities, as they lack assurance over the long-term benefits of their investments (de Janvry and Sadoulet 2022). Strengthening land tenure policies-whether through more secure lease agreements or land ownership programs for tenant farmers-can serve as a strong incentive for sustainable agriculture. Additionally, increasing access to financial resources, improving infrastructure such as irrigation and farm-to-market roads and fostering cooperative farming models can significantly enhance agricultural resilience. Investments in rural infrastructure not only lower production costs but also improve market competitiveness, ultimately empowering farmers to achieve greater economic stability and sustainability (Pingali et al. 2019).

In conclusion, the aging farming population in Carcar City presents significant obstacles to agricultural sustainability. The challenges associated with this demographic trend include limited youth participation, educational gaps, economic constraints, gender disparities, and insecurity of land tenure. Addressing these issues requires a multi-faceted approach involving modernization, targeted education programs, financial assistance, and enhanced infrastructure. Encouraging youth involvement through technology-driven initiatives and financial incentives is crucial for ensuring the long-term sustainability of the agricultural sector. Moreover, improving land tenure security and providing targeted support for women farmers will contribute to a more resilient and equitable agricultural system. By implementing these strategies, Carcar City can foster a more sustainable and productive agricultural future.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Department of Budget and Management (DBM) for considering and approving our proposal and allocating the necessary budget for this study. We extend our appreciation to Cebu Technological University-Argao for its unwavering support in facilitating this research and for granting the team the opportunity to conduct the study. Our heartfelt thanks go to Carcar City Mayor Mario Patricio P. Barcenas and the barangay captains for their assistance in coordinating the research activities within the study area. Most importantly, we are deeply grateful to the People's Organization (PO) and the farmers who generously shared their time and insights, making this study possible.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 234-243

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090125

Influence of fertilization and defoliation interval on forage yield and chemical composition of *Cenchrus ciliaris* under sandy soil

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Manuscript received: 23 February 2025. Revision accepted: 30 April 2025.

Abstract. *Lugundi HS, Maleko DD, Kizima JB, Nziku ZC, French P, Urassa V, Mangesho W, Gooddluck S, Lugeye S, Mgonja H, Mngulu S, Selemani IS. 2025. Influence of fertilization and defoliation interval on forage yield and chemical composition of* Cenchrus ciliaris *under sandy soil. Asian J Agric 9: 234-243.* Improper defoliation intervals and nutrient-deficient soil limit the yield and quality of forages, resulting in poor dairy production. Using inorganic fertilizers to improve soil nutrition is expensive and pollutes the environment. The study was conducted to assess the influence of organic fertilization and defoliation intervals on the yield and chemical composition of *Cenchrus ciliaris* L. during the rainy season. The study was designed as a 3 × 4 factorial arrangement in a Completely Randomized Design (CRD), a method that allows for a comprehensive analysis of multiple factors and their interactions. It included three cutting intervals (9, 16, and 23 days) and three fertilizer types (compost cow manure, green processed bioorganic fertilizer, and liquid processed bioorganic fertilizer), plus a control with four replications. The study revealed that all fertilizer types had a significant influence on biomass yield, metabolizable energy, and crude protein percentages. In addition, fertilizer applications significantly decreased NDF, ADF, and ADL. On the other hand, the results show that the longer the cutting interval, the higher the biomass yields, but lower protein content and metabolizable energy. *Cenchrus ciliaris* fertilized compost cow manure and cut at a 23-day interval had a higher biomass yield and reasonable nutritive value. The study suggests the importance of considering fertilization and a cutting interval as appropriate management practices for *C. ciliaris* in related soil types and climates.

Keywords: *Cenchrus ciliaris*, crude protein, defoliation interval, fertilization, forage biomass yield

Abbreviations: GDP: Gross Domestic Product, TALIRI: Tanzania Livestock Research Institute, TARI: Tanzania Agriculture Research Institute, SUA: Sokoine University of Agriculture

INTRODUCTION

The agricultural sector employs a large number of people in Tanzania. It has a high economic livelihood contribution of about 66.3% to the population of Tanzania (Country profile United Republic of Tanzania 2015). However, poverty still exists mostly in rural areas. Hence, the transformation of the sector is important. Through the revolution of the agriculture sector, including the Livestock subsector, the livelihood of most poor people, predominantly in small-scale farming, will be improved (Kinyondo and Magashi 2017). Generally, livestock is mostly important, particularly in developing countries. It contributes to the livelihood of rural areas by about 60% in developing countries (FAO-RULIS 2019). In Tanzania, the contribution of livestock is very little compared to the number of animals in the country (Engida et al. 2015).

Tanzania is the second nation after Ethiopia, with the largest livestock in Africa, having a population of 37.9 million cattle, 27.6 million goats, and 9.4 million sheep (Budget speech MLF 2024/2025). The livestock's significant gain in the country is more in the number of livestock rather than increased productivity (Waziri and Uliwa 2020). In the economy of Tanzania, livestock contributes only 7.1% of the Gross Domestic Product (GDP). The low contributions of livestock are associated with a number of factors, including low livestock productivity (Engida et al. 2015). The scarcity of forage is among the factors that affect livestock productivity (Maleko et al. 2019). The availability and quality of grazing pastures, which serve as a feed source for animals, are continuously declining, particularly during the dry season. The increased competition from other land uses, including crop production, decreases grazing land (Mengistu 2018). Additionally, an increase in livestock numbers, an increase in population, and climatic changes contribute to conflicts among land users (Mwamfupe 2015).

The scarcity of quality forages limits dairy production in Tanga. Most smallholder farmers use natural pastures, mainly collected from the roadside, and crop residues as the main forage resources for their dairy cattle. The natural pastures are normally low in quality and quantity, and hence, there is no assurance of forage availability throughout the year for sustainable milk production. To reduce the shortage of forage and improve its quality, high-yielding, and quality forages that adapt to climate change need to be recommended. *Cenchrus ciliaris* L. is among the promising forages for improvement of dairy production; it has high productivity performance, resistance to grazing, fire, and resilience to climatic change (Patidar and Mathur 2017; Maeresera 2020; Ruvuga et al. 2022). *Cenchrus ciliaris* is high temperature-resistant, surviving in an area up to 50°C (Siller-Clavel et al. 2022). In large distributed area of practiced pasture production, *C. Ciliaris* can potentially give economic benefits to farmers. (Lutatenekwa et al. 2021). Rotation grazing is one of the important systems practiced by a few farmers with established *C. ciliaris*. Yet, the proper interval of time for grazing and resting the forage was not clearly known, which might result in feeding the animal low-quality forages. Therefore, the study to document the proper defoliation interval for better yield and quality of forages by simulation grazing was inevitable.

On the other hand, soil nutrient deficiency was identified as one of the limiting factors on forage productivity and quality (Hassan et al. 2015). Kizima et al. (2015) reported the high yield performance of *C. ciliaris* when using urea in Tanzania. Nonetheless, in addition to their high cost, prolonged applications of inorganic fertilizers may lead to environmental pollution, mineral imbalances, and ecological issues (Rashmi et al. 2020). Consequently, the application of eco-friendly organic fertilizer may fulfill its intended purpose (Wu 2017).

Information on improving the yield and quality of *C. ciliaris* through a combination of organic soil fertilization and harvest management in Tanzania is lacking. It was anticipated that the application of organic fertilizers would increase the yield and quality of *C. ciliaris* in Tanga Municipal. The objective of the current study was to improve forage yields and quality through appropriate cutting intervals and the use of environmentally friendly fertilizers during the rainy season. Specifically, the study assessed the growth attributes, chemical compositions, and yields of *C. ciliaris* in response to varying levels of organic fertilizer and simulated defoliation.

MATERIALS AND METHODS

Study area

The study was conducted on the station during the rainy season, between February and May 2024, at the Tanzania Livestock Research Institute (TALIRI)-Tanga Centre, found in the Tanga Region, eastern zone of Tanzania. The institute is located at a latitude of 5°05'19.24"S and a longitude of 39°03'51.15"E, at an altitude of 60 meters above sea level, in a tropical wet and dry season with yearly temperatures varying from 26 to 33°C, with the hottest month temperatures in January and February, and receives bimodal rainfall of 1230 to 1400 mm annually (Habimana et al. 2024). An experimental site is indicated in Figure 1, where the trend of temperature and rainfall during the period of the experiment is shown in Figure 2.

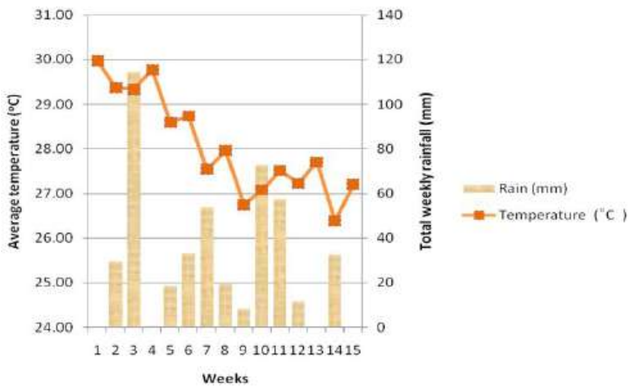

Figure 2. Trend of mean temperature and rainfall during the experimental period recorded from the HOBO data logging weather station installed at TALIRI, Tanga

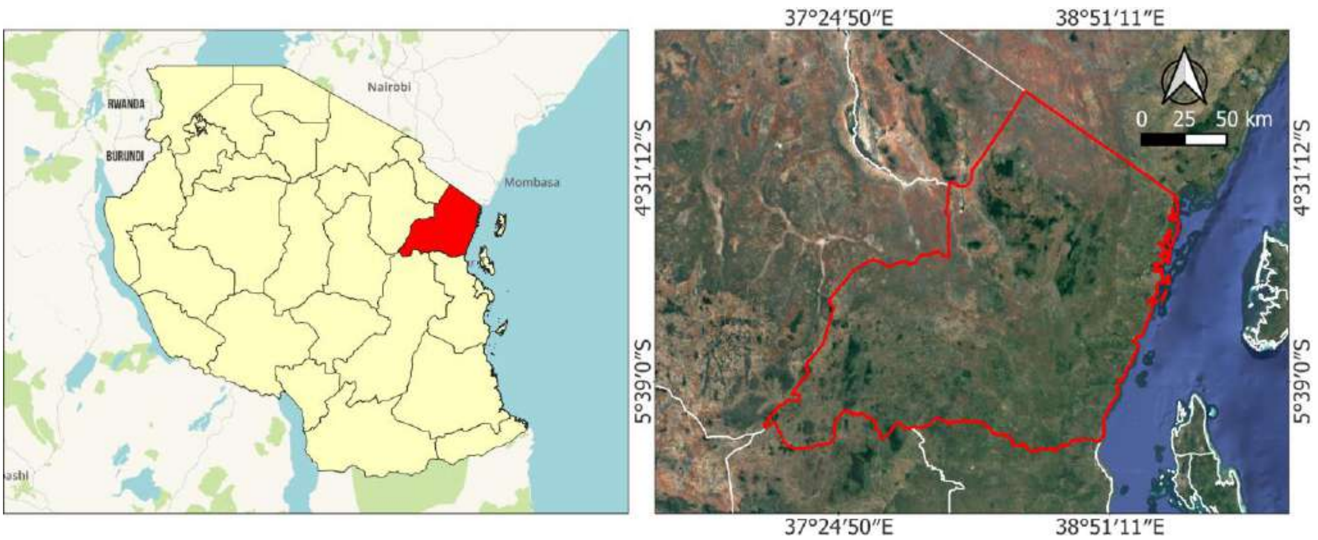

Figure 1. A study area in Tanga Municipality at TALIRI, Tanzania, indicating an experimental site

Procedures

Fertilizer collection and soil testing

The types of fertilizer used were processed bio-organic fertilizer (green and liquid fertilizers) and compost cow manure. A green fertilizer with 0.13, 0.04, 5.74, and 13.14% total nitrogen, potassium, phosphorus, and phosphate, respectively, was purchased from Afrint Bio Solution Ltd. This company supplies commercial organic products for organic farming in Tanzania. The liquid fertilizer with respectively 0.112, 0.046, and 0.0043% total nitrogen, potassium, and phosphorus was a plant source type of organic fertilizer collected from Lukwangule Organic Product Producer from Morogoro, Tanzania, which produces and supplies organic fertilizer for agricultural production.

Compost cow manure was collected and heaped for at least one year to allow decomposition. The part of the heaped manure was collected based on the amount needed for plot application and thoroughly mixed to create heaped composite manure. Subsequently, samples were obtained from five parts of the new heap and thoroughly mixed to obtain a composite sample for laboratory tests, where total nitrogen after laboratory testing was 1.55%. The source of compost cow manure was from the TALIRI cattle herds that were grazing near the station campus. The soil and compost-cow manure samples testing were done at the Tanzania Agricultural Research Institute (TARI) - Mlingano. The physical and chemical properties of the soil are shown in Table 1.

Experimental design and layout

The study was done in a 3 × 4 factorial arrangement in a Complete Randomized Design (CRD). There were two factors (the cutting interval and fertilizer type). Due to the fast re-growth of *C. ciliaris* in the coastal region during the rainy season, the data were collected in cutting intervals of three levels (9, 16, and 23 days re-growth from vegetation standardization by a homogenous cut). The fertilizer types had four levels (No Fertilizer (NF) as a control, compost cow manure (F1), green fertilizer (F2), and liquid fertilizer (F3)). These were set in four replicates to make a total of 48 subplots. Each plot had an area of 15 m² (5 × 3 m) with a spacing of 0.33 m inter-row and a 1 m path between each experimental plot. This experiment was done in two harvesting schedules in the rainy season, at early-mid rain and mid-late rain.

Site preparation and application of fertilizer

The standing sward of *C. ciliaris* established in May 2023 was used in the experiment. Pegs, manila string, and 100-meter tape were used for the plot's demarcation. The grass in all demarcated plots was cut uniformly using a grass cutter (shaving machine) at 5 cm above the ground. The full charged and sharp machine was used in uniform cut, where the blade height of the machine was adjusted to 5 cm above the ground. The grass shaving was started at one end of the plot and moved straight in overlapping rows. The consistent cutting height was highly considered to ensure the uniformity in all plots; however, any noted deviation in uneven terrain was repeated and made correct. The starting cutting time was recorded using a stopwatch, where the average cutting time used to complete all plots was about 11/2 hours, which was done in a single day. All harvested grasses were finally removed from the plots, and the plots were left clean. Throughout the time of uniform cut, no rainfall was recorded. A rate of 12 t/ha of compost cow manure (F1) was applied by broadcasting based on the nutrient deficiency of the soil on the following day after the uniform cut. This was done once throughout the experimental period. The rates of 7 kg ha^{-1} and 8 L ha^{-1} of green (F2) and liquid fertilizers (F3), respectively, were applied by spraying on the same day. Due to the leaching effect of sandy soil, three application times of the processed bio-organic (green and liquid) fertilizers with the same rate, respectively, were done in each harvesting schedule, with a seven-day interval skipped. A 7 kg and 8 L ha^{-1} of green and liquid fertilizers, respectively, were diluted in 1,400 and 4,000 L of water prior to application at each application time.

Data collection

Growth and yield

The data on growth and yield attributes for the first cut were collected based on the following parameters: plant height, tiller number, tiller diameter, biomass fresh yield, and dry matter yield. Following 9 days, subsequently data collection was repeated at 16 days and 23 days.

The data were collected in a sample size of one meter in length along a single row at the center of the plots. This was done to avoid edge effects. Plant heights were measured using a tape measure, whereby three plants (tallest, medium, and short) were measured, and an average height per plant in centimeters was calculated. Then, to obtain tiller numbers, three plants within the selected row were randomly selected and manually counted to get an average number of tillers per plant. By using a digital veneer caliper, the tiller diameter was measured from three randomly selected tillers from the randomly selected plants to obtain an average diameter per tiller per plant.

Table 1. Physical and chemical properties of the soil collected at 0-15 cm before fertilizer application on the experimental site

Particle size (%)	%textural composition	Remarks
Sand	62	
C. silt	16	
F. silt	12	
Clay	10	
Physical/chemical characteristics		
pH	6.0	Medium acid
Total nitrogen (%)	0.1	Very low
Organic carbon (%)	1.20	Low
Carbon to nitrogen ratio (C: N)	12	Good
Exchangeable bases		
Sodium (meq 100g^{-1})	0.15	High
Potassium (meq 100g^{-1})	1.41	Very high
Phosphorus (mg Kg^{-1})	6.62	Low
Calcium (meq/100g)	2.40	Medium
Magnesium (meq 100g^{-1})	0.32	Low
Cation Exchange Capacity (CEC) (meq 100g^{-1})	5.60	Low

The fresh grass sample was collected and weighed using the spring balance at 5 cm above the ground level from the sampling unit, which was one meter long along the row of the plot. The sample was then sent to the laboratory and put into an oven at 65°C for 48 hours to dry. After drying, a spring balance was used to measure the dry weight, and the yields were finally converted to t/ha. The same procedures were used at all times of data collection.

Chemical composition

The collected and dried samples were ground, sieved, and packed, ready for proximate analysis. Proximate analysis was done at the laboratory of Sokoine University of Agriculture (SUA). Total dry matter was obtained by drying the ground sample in an oven set to 105°C overnight. Ash and organic matter contents were determined after burning samples using a muffle furnace at 550°C for 3 hours, following the AOAC procedure (1990). Total Nitrogen (N) was determined using the Kjeldahl method and multiplied by 6.25 to obtain Crude Protein (CP). The ether extract (crude fat) was determined using the Tecator Soxtec extraction method. Nitrogen-free extract (%NFE) was calculated using %NFE = 100% - (%CP + %CF + %EE + %Ash) (National Research Council 2001), where CP, CF, and EE are crude protein, crude fiber, and ether extract, respectively. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) were determined using the procedure of Van Soest et al. (1991) by the Ankom fiber analyzer DELTA at the laboratory of TALIRI Tanga. Metabolizable Energy (ME) was calculated using the formula of ME = 1.549 + 0.0102 CP + 0.0275 EE + 0.0148 NFE - 0.0034 CF (Ellis 1981), where CP is crude protein, EE is ether extract, NFE is nitrogen-free extract, and CF is crude fiber.

Data analysis

Data were analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS 2000), and the following model was used:

$$Yijk = \mu + A_i + B_j + (AB)ij + E_{ijk}$$

Where: Yijk: Observation (growth, biomass yield, and chemical composition), μ: Overall mean, A_i: effect due to i^{th} fertilizer types, B_j: effect due to j^{th} defoliation interval, (Ab_{ij}): effect due to interaction of fertilizer type and defoliation interval, and E_{ijk}: Sampling error.

The Duncan Multiple Range Test was used to compare the different means for all observations at a 0.05 level of significance.

RESULTS AND DISCUSSION

Growth and yield

Table 2 shows the effects of fertilizer types and cutting intervals on growth and yield parameters, as well as their interactions with each other, as shown in Table 3. All types of fertilizer were found to significantly influence plant height, tiller number, and tiller diameter of *C. ciliaris* ($P < 0.05$) compared to unfertilized plots. The compost cow manure influenced the highest plant height (58.9 cm) and tiller diameter (2.65 mm), followed by green and liquid fertilizers, respectively. However, there was no significant difference among the 3 types of fertilizers in the effect on tiller numbers per plant. Cutting intervals significantly influenced the plant height, tiller number, and tiller diameter ($P < 0.05$), where the mean values were increasing with long cutting intervals of 9, 16, and 23 days, respectively.

Higher dry matter yields (1.89 t DM ha^{-1}, 1.74 t DM ha^{-1}, and 1.69 t DM ha^{-1}) for *C. ciliaris* were recorded in the plots that received compost cow manure, green manure, and liquid fertilizer, respectively. The application of fertilizers provides essential nutrients for plant growth (plant height, tiller number, and diameter), resulting in a higher biomass yield. The same results were reported by Karydogianni et al. (2022); Alhammad et al. (2023), that the increase in yield is attributed to a considerable correlation with N fertilization.

Table 2. Effect of types of fertilizer and cutting interval on plant height, tiller number, tiller diameter, and biomass yield of *C. ciliaris*

Factors	Levels	Variables			
		Plant height (cm)	Tiller number	Tiller diameter (mm)	DM yield (t DMha^{-1})
Types of fertilizer	NF	40.50^c	24^b	1.90^c	1.12^b
	F1	58.89^a	33^a	2.65^a	1.89^a
	F2	55.43^{ba}	32^a	2.48^b	1.74^a
	F3	52.68^b	33^a	2.42^b	1.69^a
	p-value	<0.0001	<0.0001	<0.0001	<0.0006
	SEM±	1.4	2	0.06	0.13
Cutting intervals	9 days	37.26^c	27^c	1.86^c	0.83^c
	16 days	51.63^b	30^b	2.50^b	1.44^b
	23 days	66.73^a	35^a	2.72^a	2.56^a
	p-Value	<0.0001	<0.0001	<0.0001	<0.0001
	SEM±	1.2	1	0.05	0.12

Note: Means with different superscripts within the column of the same factor are significantly different at P<0.05, SEM: Standard Error of the Mean, NF: No Fertilizer (control), F1: Compost cow manure, F2: Green processed bio-organic fertilizer, F3: Liquid processed bio-organic fertilizer

Table 3. Effect of fertilizer type and cutting interval on plant height, tiller number, tiller diameter, and dry matter yield of *C. ciliaris* and their interaction

Types of fertilizer	Cutting intervals	Variables			
		Plant height (cm)	Tiller number	Tiller diameter (mm)	DM yield (t DM ha^{-1})
NF	9 days	32.24^{b}	21	1.53^{b}	0.71^{b}
	16 days	41.13^{a}	22	2.00^{a}	1.05^{ab}
	23 days	48.12^{a}	28	2.17^{a}	1.59^{a}
F1	9 days	41.04^{c}	29	2.12^{c}	0.92^{b}
	16 days	53.51^{b}	33	2.75^{b}	1.47^{b}
	23 days	82.12^{a}	38	3.09^{a}	3.27^{a}
F2	9 days	39.16^{c}	26	1.88^{b}	0.83^{c}
	16 days	55.41^{b}	33	2.73^{a}	1.67^{b}
	23 days	71.71^{a}	37	2.82^{a}	2.72^{a}
F3	9 days	36.63^{c}	30	1.93^{c}	0.86^{c}
	16 days	56.45^{b}	33	2.52^{b}	1.57^{b}
	23 days	64.96^{a}	38	2.83^{a}	2.65^{a}
	p-value	<0.0001	0.9789	0.3789	0.0564
	SEM±	2.43	3	0.1	0.23
	Interaction	**			

Note: Means with the different superscript within the column of the same factor s are significantly different at P<0.05, SEM: Standard Error of the Mean; NF: No Fertilizer (control), F1: Compost cow manure, F2: Green processed bi-organic fertilizer, F3: Liquid processed bio-organic fertilizer, **: Significant interaction at p<0.05

In comparing the performance between fertilizer types, the composite cow manure performed relatively better in yield than processed bioorganic fertilizers (both green and liquid). The higher performance of composite cow manure could be due to a lower leaching effect on nutrients compared to processed bioorganic fertilizer that was in liquid form. Kang et al. (2011) reported greater leaching of nutrients when using organic liquid fertilizer compared to solid organic fertilizer, supporting this claim. Similarly, Prado et al. (2023) reported the decreased leaching of nutrients to soil through the use of manure-based fertilizer. Tan et al. (2011) also reported a high yield of corn using the solid manure organic fertilizer, more than the liquid cattle organic fertilizer.

In considering the influence of cutting interval on the grass yield, the means of growth and yield parameters were observed to differ significantly ($P < 0.05$) on all cutting intervals. The highest performance was recorded at 23-day cutting intervals, and the lowest was at 9-day cutting intervals. The highest performance at 23 days could be due to enough resting time for the plant before harvest, which influenced more leaves, tiller numbers, and diameter, resulting in a higher biomass yield. These results are comparable to the results of Gilo et al. (2022), who reported low yields at most harvest frequencies.

There were no significant interactions between fertilizer types and cutting intervals on the tiller number, tiller diameter, and biomass yield; however, a significant interaction ($P < 0.05$) was observed on plant height, where the highest mean value (82.12 cm) was recorded from the plots fertilized with compost cow manure harvested at a 23-day interval. Plots fertilized with green and liquid fertilizers, as well as unfertilized plots at the same cutting interval, performed differently in terms of plant height. This might contribute highly to biomass yield, where the highest value, 3.27 t DM ha^{-1}, was recorded from plots fertilized with compost cow manure harvested at a 23-day interval. López-Chuken (2012) reported that nitrogen fertilization and a long cutting interval yielded the highest yield, which these results agreed with.

Apart from the effects of defoliation interval and fertilization, the result reported in this study could also be influenced by the properties of sandy soil under which the study was conducted. The response of the plant to defoliation interval and fertilization can change depending on the soil properties, including water retention capacity, retention of nutrients, and microbial biomass status (Brady and Weil 2016). Therefore, the performance of *C. ciliaris* under different soil types, such as sandy soil, may respond differently to defoliation interval and fertilization, to limit the generalizability of this study. The defoliation interval and fertilization are key management practices for *C. ciliaris* discussed in this study; however, spacing, mulching, rotation grazing, and water harvest are also important to maintain soil moisture and nutrients (Ojo et al. 2020; Fenetahun et al. 2021; Mangani et al. 2022). Therefore, this management practice can be combined and implemented to enhance productivity in different climatic conditions.

Chemical composition

Percentage Crude protein (%CP)

The effect of fertilizer type and cutting interval on crude protein percentage is shown in Table 4, and their interaction is indicated in Table 5. The percentage of crude protein was significantly ($P < 0.05$) affected by cutting interval, fertilization, and their interactions. All types of fertilizer used in this study significantly influenced higher %Cp than unfertilized plots ($P < 0.05$). The highest %CP (16.73%) of *C. ciliaris* was recorded at composite cow manure, followed by liquid and green processed fertilizer, respectively. The lowest %CP (13.47%) was recorded from unfertilized plots. This indicates that the %CP of the plant increased with fertilization. This effect might be due to N applied from types of fertilizer to the soil that could be quickly absorbed and transformed into nitrate-nitrogen that is further included in the organic material of the plant (Maeresera 2020). In contrast, López-Chuken (2012) reported no significant increase in the crude protein content of *C. ciliaris* by N fertilization. However, a significant increase in crude protein content with an increase in fertilization in the current findings is agreed upon by the results reported by Kizima et al. (2015) on the same species.

The cutting interval significantly affected the crude protein content (P < 0.05). It was recorded higher (18.40% Cp) at a 9-day interval of cutting and lower at a 23-day cutting interval. This indicates that the percentage of crude protein decreases as plant maturity increases. The shorter cutting interval gives higher protein content than a long cutting interval. This might be due to the decrease in leaf-to-stem ratio and increase in structural carbohydrate and lignin content as the plant matures (Yigzaw 2019). The results of the present study also agree with those reported by Kisambo et al. (2023) that an increase in harvesting interval results in a decrease in crude protein content of the plant due to grass maturation.

The interaction of fertilization and cutting interval on crude protein is shown in Table 5. The crude protein was significantly affected by the interaction. The highest mean value (19.24%) for crude protein was recorded from the plots fertilized with liquid processed bio-organic fertilizer, followed by green processed organic fertilizer, compost cow manure, and control, respectively. All these higher records were at a 9-day cutting interval. From this interaction, the crude protein content was improved from 10.62% at 23 days of harvest to 19.24% at 9 days of harvesting intervals. These results are much higher than those of López-Chuken (2012), who reported 18% CP of *C. ciliaris* at a 21-day harvest.

Table 4. Effect of types of fertilizer and cutting interval on chemical composition (proximate) of *C. ciliaris*

Factors	Levels	Variables					
		DM (%)	ASH (%)	OM (%)	CP (%)	EE (%)	NFE (%)
Types of fertilizer	NF	94.91^{c}	13.86^{d}	81.04^{b}	13.47^{d}	2.04^{d}	36.04^{a}
	F1	95.73^{ba}	15.12^{a}	80.61^{c}	16.73^{a}	2.06^{c}	33.48^{c}
	F2	95.63^{b}	14.02^{c}	81.61^{a}	15.74^{c}	2.08^{b}	34.70^{b}
	F3	95.91^{ab}	14.13^{b}	81.79^{a}	15.88^{b}	2.09^{a}	34.71^{b}
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SEM±	0.08	0.02	0.08	0.02	0.002	0.09
Cutting intervals	9 days	94.98^{b}	15.39^{a}	79.59^{c}	18.40^{a}	2.12^{a}	31.61^{c}
	16 days	95.06^{b}	14.61^{b}	80.45^{b}	15.02^{b}	2.07^{b}	34.97^{b}
	23 days	96.59^{a}	12.85^{c}	83.74^{a}	12.95^{c}	2.01^{c}	37.62^{a}
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001
	SEM±	0.07	0.02	0.07	0.02	0.002	0.08

Note: Means with the different superscript within the column of the same factor are significantly different at P<0.05, SEM: Standard error of the mean, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract and NFE: Nitrogen free extract, NF: No fertilizer (control), F1: Compost cow manure, F2: Green processed bio-organic fertilizer, F3: Liquid processed bio-organic fertilizer

Table 5. Effect of fertilizer type and cutting interval on chemical composition (proximate analysis) of *C. ciliaris* and their interaction

Types of fertilizer	Cutting interval	Variables					
		DM (%)	ASH (%)	OM (%)	CP (%)	EE (%)	NFE (%)
NF	9 days	94.51^{b}	16.10^{a}	78.41^{c}	16.63^{a}	2.09^{a}	32.44^{c}
	16 days	94.08^{c}	13.94^{b}	80.14^{b}	13.14^{b}	2.03^{b}	36.03^{b}
	23 days	96.13^{a}	11.55^{c}	84.58^{a}	10.63^{c}	2.01^{c}	39.66^{a}
F1	9 days	95.56^{b}	16.04^{a}	79.52^{b}	18.78^{a}	2.12^{a}	30.90^{c}
	16 days	94.88^{c}	15.26^{b}	79.62^{b}	16.07^{b}	2.05^{b}	33.96^{b}
	23 days	96.73^{a}	14.05^{c}	82.69^{a}	15.35^{c}	2.00^{c}	35.59^{a}
F2	9 days	94.43^{c}	14.66^{a}	79.77^{c}	18.95^{a}	2.13^{a}	30.89^{a}
	16 days	96.04^{b}	14.33^{b}	81.71^{b}	15.16^{b}	2.09^{b}	35.77^{b}
	23 days	96.41^{a}	13.06^{c}	83.36^{a}	13.12^{c}	2.01^{c}	37.45^{c}
F3	9 days	95.40^{b}	14.75^{b}	80.65^{b}	19.24^{a}	2.14^{a}	32.23^{a}
	16 days	95.25^{b}	14.90^{a}	80.35^{b}	15.71^{b}	2.11^{b}	34.11^{b}
	23 days	97.09^{a}	12.74^{c}	84.36^{a}	12.68^{c}	2.02^{c}	37.79^{c}
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SEM±	0.13	0.04	0.14	0.04	0.004	0.16
	Interaction	**	**	**	**	**	**

Note: Means with the different superscript within the column of the same factor are significantly different at P<0.05. SEM: Standard Error of the Mean, DM: Dry Matter, OM: Organic Matter, CP= Crude Protein, EE= Ether Extract and NFE= Nitrogen Free Extract, NF: No Fertilizer (control), F1: Compost Cow Manure, F2: Green processed bio-organic fertiliser1, F3: Liquid processed bio-organic fertilizer, **: Significant interaction at p<0.05

This difference might be due to the significant interaction effect of both fertilizer type and cutting interval reported in the current study. The interaction of compost cow manure and cutting interval at 16 days and 23 days was recorded (16.07 and 15.35%), respectively, more than other types of fertilizer used in the current study. This indicates that compost cow manure gives better crude protein on *C. ciliaris* when harvested at these two mentioned cutting intervals.

Percentage Ether Extract (%EE)

Table 4 indicates the effect of fertilization and cutting interval on the percentage of ether extract, and Table 5 indicates the interaction of the two. All types of fertilizer significantly ($P < 0.05$) affected the percentage of ether extract (%EE) compared to unfertilized plots. The highest mean value (2.09%) of ether extract was recorded from plots fertilized with liquid bio-organic fertilizer, and unfertilized plots were recorded with the lowest value (2.04%) of ether extract. This indicates that the percentage of ether extract increases with fertilization. The current results agreed with those of Culicov et al. (2024), who used organic fertilizer on three grass species (*Agrostis capillaris* L., *Festuca rubra* L., and *Nardus stricta* L.) and reported an increase in %EE with fertilization. Furthermore, the significant increase in %EE with fertilization on rhodes grass, which is similar to the present study, was reported by Christopher (2020).

Cutting the interval significantly ($P < 0.05$) affected the percentage of ether extract. The percentage of ether extract was recorded as the highest (2.12%) at 9 days of harvest, and the lowest mean value (2.01%) of ether extract was recorded at 23 days of harvest. This indicates that the content of ether extract decreases with the increase of plant age. This might be due to plant maturation. These results are supported by Aiyesa et al. (2019), who reported a significant decrease in ether extract with plant maturity on *Cenchrus purpureus* (Schumach.) Morrone. (Mendoza-Pedroza et al. 2022) also reported a significant decrease in the content of ether extract with an increase in the harvesting age of *Pennisetum purpureum*. The interaction of types of fertilizer and cutting interval significantly ($P < 0.05$) recorded higher (2.14%) EE on liquid processed bio-organic fertilizer at 9 days of harvest, and the lowest value was recorded in 23 days of harvest on the plots harvested with compost cow manure.

Percentage nitrogen-free extract (%NFE)

The effect of fertilization types and cutting interval (Table 4) and their interaction (Table 5) significantly ($P < 0.05$) affected the percentage of nitrogen-free extract (%NFE). The highest free nitrogen extract (36.04%) on fertilization was significantly ($P < 0.05$) recorded from unfertilized plots, followed by liquid processed bio-organic fertilizer, green processed bio-organic fertilizer, and compost cow manure, respectively. This indicates that the %NFE decreases with fertilization. Mrázková et al. (2020) found a decrease in %NFE from 48.2-47.85% in the grasses by application of organic fertilizer, which supports the current finding.

The long harvest interval of 23 days was significantly ($P < 0.05$) recorded as the highest mean value (37.62%) NFE, while the harvest at 9 days recorded the lowest value (31.61%). This shows that %NFE increases with the increase in harvesting age. This might be due to the maturation effect on the nutrients of the plant. In contrast, Jagadeesh et al. (2017) found a significant decrease in %NFE of *P. purpureum* among stages of plant growth. Differences in plant species might cause this difference. However, the current finding is also supported by Jagadeesh et al. (2017), who reported the increases in %NFE of *P. purpureum* in another stage of growth.

Fiber fraction and metabolizable energy

The effect of fertilizer types and cutting intervals on the percentage of Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), and Metabolizable Energy (ME) is shown in Table 6. The % CF, % NDF, % ADF, ADL, and I were significantly influenced by fertilizer type and cutting interval ($P < 0.05$). The application of fertilizer significantly decreased the fiber content ($P < 0.05$). The lowest 27.66, 55.92, 31.42, and 3.17% of crude fiber, neutral detergent fiber, acid detergent fiber, and acid detergent lignin, respectively, were recorded from plots fertilized with compost cow manure. The highest %CF, %NDF, %ADF, and %ADL were recorded from unfertilized plots. Hassan et al. (2015) also reported a decrease in fiber content by fertilization. Fertilizer tends to influence plant growth by increasing new leaves and shoots, which results in reduced lignification of the plant. The fiber contents were significantly increased with increasing cutting intervals ($P < 0.05$). The lowest %CF, %NDF, %ADF, and %ADL were recorded from a 9-day cutting interval, while the highest records were from the 23-day interval of cut. The increase of fiber content with increasing intervals of cut was due to the decrease in leaves-to-stem ratio, advances in structural carbohydrates, and cell wall content as the plant matures (Kefyalew and Alemu 2020). The current findings agree with Yigzaw (2019).

The application of fertilizer significantly increased the metabolizable energy of *C. ciliaris* ($P < 0.05$). The highest 5.0 MJ/kg DM Metabolizable Energy (ME) was recorded from compost cow manure, followed by green and liquid fertilizer, respectively. The lowest metabolizable energy was recorded from non-fertilized plots. This result agreed with the results of Hazary et al. (2015), who reported an increase in ME of jumbo grass with increasing N fertilization. The Metabolizable Energy (ME) was significantly decreased with increasing cutting interval ($p < 0.05$). The highest, 5.33 MJ $kgDM^{-1}$, was recorded from a 9-day interval of cutting, and the lowest was recorded from a 23-day cutting interval. Similarly, Yigzaw (2019) reported a decrease in ME with an increase in the intervals of cut. This could result from increasing the cell wall composition, which lowers plant digestibility and reflects the low ME of the grass.

This study reveals that extending cutting intervals increases forage yield while reducing nutritive values such as crude protein, ME, and ether extract. With extended cutting intervals, the fibre content increases. This indicates poor digestibility and palatability of the plant (Naseri et al. 2017; Fayos-Febrer et al. 2023), which can negatively affect livestock intake and performance. Therefore, this study suggests the importance of balancing both forage yield and quality to ensure palatability of the forage for better livestock performance.

The findings of this study reveal that the organic fertilizer improves both biomass yield and quality, particularly the use composted cow manure is used in sandy soil. Further, Reganold and Wachter (2016) reported long-term benefits on the use of organic fertilizer through enhanced soil health, decreased environmental degradation, and improved system resilience as crucial for sustainable productivity in shifting climatic patterns. Despite all these advantages of organic farming, some critics may argue that dependence on organic farming practices may fall short in satisfying the increasing demand for dairy production in a dynamically changing climate. Therefore, suggested innovative approaches, integrating organic practices with scientific advancements such as improved genetically diverse forage cultivars, exactitude nutrient management, and rotation grazing can help meet productivity demand while ensuring adaptability in the face of climate challenges without compromising environmental stewardship (Sekhar et al. 2024).

Economic analysis on the cost of fertilizers used for forage yield

The cost and benefit analysis to determine the return from forage production is shown in Table 7. The composted manure had a higher labor charge application compared to green and liquid bio-organic fertilizer; this might be due to the high volume, extra time, and physical effort required in the application of composted manure, which could reflect a high labor charge. The returns obtained from plots fertilized with composted cow manure and liquid fertilizer were higher compared to unfertilized plots. However, the application of composted cow manure had the highest return among all types of fertilizer. The application of green fertilizer yielded a low return compared to the control; this might be due to the high price of this type of fertilizer. Despite the lowest return of green fertilizer in a single aspect of forage yield, yet, the nutritive value of forage obtained from plots fertilized with green fertilizer, which was not economically analysed, was higher than that obtained from unfertilized plots. This reason explains the substantial benefit of this type of fertilizer over the non-use of fertilizer.

Compared to unfertilized plots in this study, all fertilized plots had higher nutritional value, especially crude protein, ether extract, and metabolizable energy, which are very essential to improving livestock productivity. Apart from improving the nutritional value of the forage, the ecological benefits of organic fertilizer to the soil have not been economically evaluated. Organic fertilization, especially composted cow manure, may contribute to maintaining soil structure by enhancing microbial activities and organic matter that resulting in long-term soil health and fertility (Adugna 2016).

This study reveals that the use of composted cow manure has economic advantages over other types of fertilizer. This might be the reason for the less costly and high-yielding effect in the use of compost cow manure observed in this study. Composted cow manure has a long-term effect, leading to improved productivity and resilience for sustainable farming. It is locally available with less cost and does not need frequent application as compared to commercial organic fertilizers. Commercial fertilizers need frequent application and are obtained at a high price, and with price fluctuation, which makes them economically impractical to many smallholder farmers. Since the availability of organic fertilizer varies depending on the area, the farmers have to be equipped with knowledge on how to utilize the locally available organic fertilizer for economical and sustainable farming.

Table 6. Fiber fraction and metabolizable energy of *C. ciliaris* as influenced by types of fertilizer and cutting intervals

Factors	Levels	Variables				
		CF (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ Kg DM^{-1})
Types of fertilizer	NF	29.50^{a}	62.26^{a}	34.80^{a}	3.58^{a}	4.42^{c}
	F1	27.66^{d}	55.92^{c}	31.42^{c}	3.17^{b}	5.0^{a}
	F2	28.49^{c}	58.82^{b}	32.70^{b}	3.20^{b}	4.88^{b}
	F3	28.76^{b}	58.89^{b}	32.72^{b}	3.24^{b}	4.82^{b}
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SEM±	0.06	0.23	0.3	0.06	0.02
Cutting intervals	9 days	26.24c	55.06c	30.06c	2.41c	5.33a
	16 days	28.41b	58.04b	33.50b	3.21b	4.70b
	23 days	31.17a	63.82a	35.17a	4.26a	4.32c
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SEM±	0.05	0.2	0.26	0.05	0.02

Note: Means with the different superscript within the column of the same factor are significantly different at P<0.05, SEM: Standard error of the mean, CF: Crude Fiber, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin and ME: Metabolisable Energy, NF: No Fertilizer (control), F1: Compost cow manure, F2: Green processed bio-organic fertilizer, F3: Liquid processed bio-organic fertilizer

Table 7. Return estimated from the total forage yield of three cuts in difference fertilizer treatments

Income and expenditure	Cutting intervals	Types of fertilizer			
		NF	F1	F2	F3
Biomass yield (t DM Ha^{-1})	9 days	0.71	0.92	0.83	0.86
	16 days	1.05	1.47	1.67	1.57
	23 days	1.59	3.27	2.72	2.65
Total biomass yield (t DM Ha^{-1})		3.35	5.66	5.22	5.08
Income Sales (USD)		319	539	497	484
Cost of fertilizer per hectare (USD)		0	83	222	129
Fertilizer application labor charge per Ha (USD)		0	25	18	18
Expenditure (USD)		0	108	240	147
Returns (USD)		319	431	257	337

In conclusion, the current study reveals that the fertilization of *C. ciliaris* by using organic fertilizer improves its quantity and quality when harvested at the proper stage. Despite the fact that all types of organic fertilizer improved the quality and quantity at all harvesting stages compared to unfertilized plots (control), the compost cow manure became superior in many parts. At a cutting interval of 23 days, plots fertilized with compost cow manure produced a 3.27 t DM ha^{-1} biomass yield and 15.35% crude protein, which are reasonable for dairy cattle production. It may accommodate many cows and supply enough protein content. The %CP produced at this stage was beyond the threshold levels: 7.5% required for rumen function, 10.6% needed for tropical grass species, as well as 15% minimum needed for animal growth and lactation, as reported by Hassan et al. (2015). Therefore, under sand soil tropical sub-humid conditions, the use of compost cow manure and grazing/cutting intervals of 23 days could be recommended for Buffel grass; this will not need to supplement crude protein-based feeds for dairy cattle. However, further study to explore the influence of defoliation and fertilization on different types of soil and agroecological zones is recommended.

ACKNOWLEDGEMENTS

The authors are grateful to the Tanzania Livestock Research Institute (TALIRI), in collaboration with the Agriculture and Food Development Authority of Ireland, TAEGASC-Ireland, through the Maziwa Faida Project, for funding this study. We extend our acknowledgment to all staff members of TALIRI Tanga for their excellent cooperation during the data collection for the current study.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 245-254

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090126

Diversity analysis of soil microbial communities in Inpari 32 rice fields following indigenous endophytic bacteria applications

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Manuscript received: 31 January 2025. Revision accepted: 12 May 2025.

Abstract. *Ridwansyah R, Prihatiningsih N, Lestari P, Kharisun, Djatmiko HA, Irwandhi. 2025. Diversity analysis of soil microbial communities in Inpari 32 rice fields following indigenous endophytic bacteria applications. Asian J Agric 9: 245-254.* The challenge of rice farming in Indonesia is exacerbated by land degradation resulting from the use of chemical fertilizers and pesticides, which is further intensified by climate change. The use of endophytic bacteria in rice farming is an environmentally friendly and sustainable approach that increases microbial diversity and soil productivity. This study investigates the effects of indigenous endophytic bacterial applications on soil microbial diversity in Inpari 32 rice (*Oryza sativa* L.) fields. The study involved the application of indigenous endophytic bacteria to rice plants, the isolation and characterization of soil bacteria following application, and the evaluation of microbial diversity using the Shannon-Wiener diversity index. A hypersensitivity test was conducted to assess the safety and compatibility of the plant with bacteria. The study applied the endophytic bacterial formula evenly to the lower and upper surfaces of rice leaves using a sprayer, with treatments administered five times at 56, 62, 67, 72, and 77 days after planting. The results demonstrated that the treatment enhanced the diversity of soil microbial, including genera such as *Paenibacillus*, *Micrococcus*, *Methylobacterium*, *Streptomyces*, *Actinomyces*, *Acremonium*, and *Penicillium*. The diversity index indicated that treatments from: (i) Petanahan Kebumen; (ii) Karangwangkal Banyumas; (iii) Sumbang Banyumas, and the consortium; (iv) fell into the moderate diversity category. The hypersensitivity test revealed that the isolate coded C104 1 U2 exhibited necrosis symptoms, indicating pathogenicity, while the other isolates showed no necrosis symptoms, suggesting they were beneficial non-pathogenic microbial. Necrosis symptoms indicate the presence of pathogenic microbial, whereas their absence confirms the presence of beneficial microbial. This study provides a scientific foundation for developing innovative agricultural practices to enhance microbial diversity and ecosystem resilience in rice cultivation systems.

Keywords: Characterization, endophytic bacteria, hypersensitivity, soil microbial, soil microbial diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop that is essential to the world's population, serving as a primary food source (FAO 2021). In Indonesia, rice is a staple food for nearly 95% of the population, and its demand is expected to increase in line with population growth (Ani et al. 2024). Sustainable rice production activities are critical. Because the current agricultural system faces threats from climate change, soil degradation, and declining biodiversity (Nguyen et al. 2023). This condition is exacerbated by the reliance on chemical fertilizers and pesticides, which leads to environmental degradation and finally impacts on the soil microbial communities (Jin et al. 2022). Innovative and environmentally friendly strategies are urgently needed to enhance soil health and improve rice plant productivity in the face of environmental changes. Rhizomicrobiome engineering using local endophytic bacteria is a sustainable approach that can be applied (Irwandhi et al. 2024).

Endophytic bacteria are a group of microorganisms that inhabit plant tissues without harming their host plants (Afzal et al. 2019). These bacteria play a crucial role in synthesizing phytohormones, enhancing nutrient absorption in plants, and mitigating damage caused by pathogens by producing secondary metabolites, lytic enzymes, and hormone activators (Chaudhary et al. 2022). Indigenous endophytic bacteria from local ecosystems uniquely adapt to specific environmental conditions and host plants. This ability makes them the ideal candidates for biofertilizer development (Kuźniar 2025). Endophyte applications have been shown to stimulate beneficial soil microorganisms, thereby increasing biodiversity and soil resilience. In rice ecosystems, these bacteria can enhance nutrient cycling, suppress pathogenic organisms, and improve plant health, thereby contributing to sustainable crop production (Prihatiningsih et al. 2019, 2020).

Plants play an important role in determining microbial diversity in the rhizosphere. Plant roots cause physical and chemical changes in the rhizosphere that affect microbial diversity in and around the rhizosphere. Microbial populations in the rhizosphere are generally more numerous and diverse than in non-rhizosphere soils. Exudates produced by plant roots influence the activity of rhizosphere microbial. Some rhizosphere microbial play a crucial role in nutrient cycling and soil formation processes, influencing plant growth and microbial activity and acting as biological control agents against root pathogens (Sun et al. 2021).

Soil microbial, such as bacteria and fungi, demonstrate their ability to socialize and interact with other colonies by establishing intercellular communication through a mechanism called Quorum Sensing (QS). Gram-positive and Gram-negative bacteria apply quorum sensing for communication, but they produce different autoinducers. Gram-positive bacteria utilize modified oligopeptides, known as Autoinducer Peptides (AIPs). In comparison, Gram-negative bacteria mainly rely on N-acyl homoserine lactone (AHL) molecules (autoinducer-1, AI-1), while fungi rely on Volatile Aroma Compounds (VOCs) often used in fungal communication between fungi (Verbeke et al. 2017; Heffernan et al. 2023).

Developing and cultivating the Inpari 32 rice variety in Indonesia aims to obtain high rice yields and resistance to biotic stresses (Sitaresmi et al. 2023). Although its application has contributed to increased rice production, maintaining soil health in the Inpari 32 cultivation system remains challenging. The effect of local endophytic bacteria on soil microbial diversity in Inpari 32 land has not been thoroughly studied. Soil microbial diversity plays a crucial role in maintaining the functions of agricultural ecosystems, including nutrient recycling, organic matter decomposition, and pathogen suppression (Gupta et al. 2022). A diverse microbial community will be more resilient to environmental disturbances and provide various ecosystem services important for sustainable agriculture (Pedrinho et al. 2024). The Shannon-Wiener diversity index is a widely used metric for assessing microbial diversity, as it considers both species richness and evenness (Magurran 2013). Additionally, hypersensitivity testing is crucial to ensure that bacterial inoculants do not cause adverse effects on plants or disrupt native microbial communities (Swandi et al. 2019). This study investigated the impact of inoculating native endophytic bacteria on soil microbial diversity in Inpari 32 rice fields. It focused on the application of these bacteria, their effects on soil microbial communities, and their implications for sustainable rice production.

MATERIALS AND METHODS

Location

The research was conducted in Linggasari Village, Kembaran Sub-district, Banyumas District, Central Java, Indonesia, from July to November 2024. Soil analysis was conducted in the Soil Science Laboratory and Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, Indonesia. The endophytic bacterial isolates used in this study were obtained from various locations. Isolates A5 and A6 were from Petanahan, Kebumen; KR4 and KR7 were from Karangwangkal, Banyumas; and SB3 were from Sumbang, Banyumas. Below are the original locations of the endophytic bacterial isolates seen in Figure 1.

Isolation and identification of endophytic bacteria

The process of isolation and identification of endophytic bacteria begins with plant root sampling using purposive random sampling method. Isolation was carried out by selecting two healthy rice plants, taking the roots, cleaning them with running water, and weighing 100 g each. The endophytic bacteria isolation process was carried out by sterilizing the root surface using sodium hypochlorite solution, rinsed three times using sterile water, then macerated using a sterile porcelain mortar, and added as much as 100 mL of sterile water. The 1 mL suspension obtained was mixed with 9 mL of sterile water, then incubated at 80°C for 30 minutes to isolate *Bacillus* sp. (Singh et al. 2016). Serial dilutions up to five times were carried out and the results of the fourth and fifth dilutions were grown on Nutrient Agar (NA) media using the spread plate method. Colonies that appeared were observed based on their macroscopic and microscopic morphological characteristics.

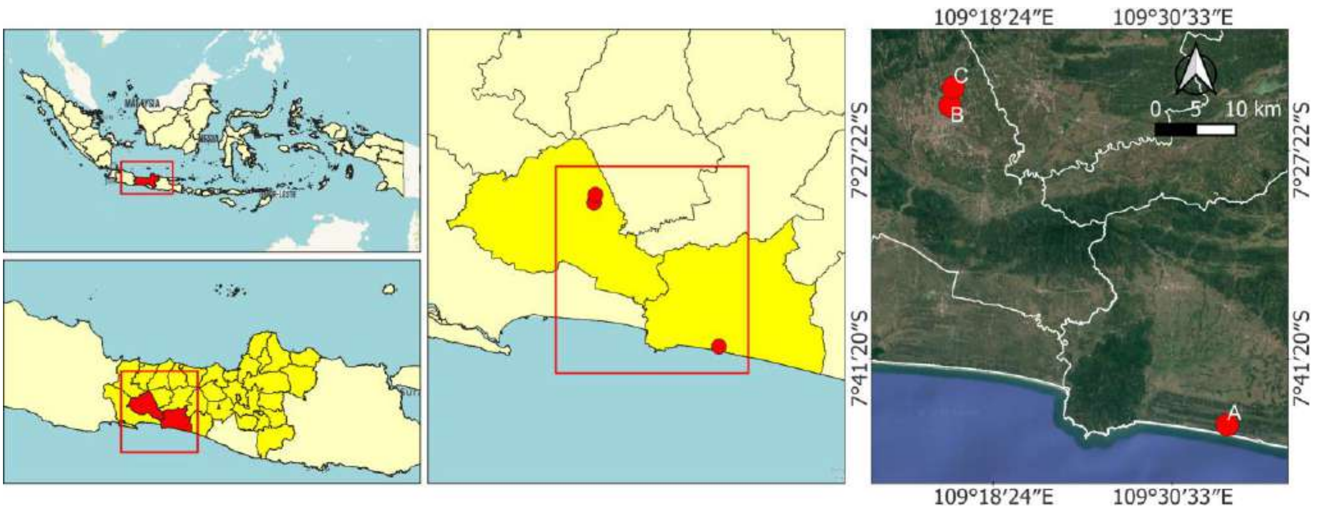

Figure 1. Locations of sources of endophytic bacterial isolates: A. Petanahan, Kebumen; B. Karangwangkal, Banyumas; C. Sumbang, Banyumas in Central Java, Indonesia

Application of endophytic bacteria

This research was conducted using a Randomized Group Design (RGD) consisting of 5 treatments, namely K, A, B, C, and D. Each treatment was repeated 5 times, so there were 25 treatment plots. The treatments were control (K), treatment of endophytic bacteria isolates A5, A6 (A), treatment of endophytic bacteria isolates KR4, KR7 (B), treatment of endophytic bacteria isolate SB3 (C), and consortium of endophytic bacteria isolates A5, A6, KR4, KR7, SB3 (D). Endophytic bacteria were applied by dissolving 250 mL of the endophytic bacteria formula into 5 L of water (0.5%) to create a ready-to-spray solution. The application was made by spraying evenly on the lower and upper surfaces of the leaves five times at the ages of 56, 62, 67, 72, and 77 days after planting using a sprayer. Soil microbial isolation was carried out during the vegetative phase of rice, following one application of endophytic bacteria, and in the generative phase of rice after five applications.

Isolation of soil microbial

A total of 1 g of soil sample was dissolved in 9 mL of distilled water to form a stock solution. The sample solution was subjected to a series of dilutions, ranging from 10^{-1} to 10^{-4}. The 10^{-3} and 10^{-4} solutions were spread onto a petri dish containing Potato Dextrose Agar (PDA) medium, each at a volume of 50 µL. Pure bacterial cultures were grown on Nutrient Agar (NA) medium and incubated at room temperature for 24 hours. Pure fungal cultures were grown on a PDA medium and incubated at room temperature for three days.

Characterization of soil microbial

Bacterial characterization was performed on pure isolates exhibiting good colony growth in the culture medium. Characterization of bacterial isolates was carried out macroscopically and microscopically. A macroscopic characterization of bacterial isolates was conducted based on the 7th edition of Bergey's Manual of Determinative Bacteriology. It records the colony shape characteristics (circular, irregular, spindle, filamentous, rhizoid), optical characteristics (transparent, translucent, opaque), colony margin (entire, lobate, undulate, serrate, filamentous, curled), elevation (flat, raised, convex, umbonate, crateriform), colony texture (smooth, dry, mucoid), colony color (white, yellow), and colony size (pinpoint, small, moderate, large) (Breed et al. 1957). Microscopic characterization of the bacterial isolates was performed using Gram staining, catalase testing, endospore testing, and observation of cell morphology under a microscope. The macroscopic characterization of fungal isolates was conducted according to the 2nd edition of Pictorial Atlas of Soil and Seed Fungi, by recording the colony color and shape characteristics. The microscopic characterization of fungal isolates was done by observing hyphae, conidia, and conidiophores (Watanabe 2002).

Diversity index (H')

The diversity of soil microbial will affect the biodiversity of a soil ecosystem. Diverse soil microbial will balance the food chain of soil microorganisms. It is the most widely known and widely used concept of diversity. The Shannon-Wiener index is calculated using the following formula (Shannon 1948):

$$H' = -\sum_{i=1}^{S} (pi)(In\ pi)$$

$$pi = \sum \mathrm{ni}/\mathrm{N}$$

Where: H' = Diversity index Shannon-Wiener; pi = Number of individuals of a species/total number of all species; ni = Number of individuals of the i-th species; N = Total number of individuals.

The criteria for the value of the Shannon-Wiener diversity index (H') are as follows: $H' \leq 1$ = Low diversity; $1 < H' < 3$ = Moderate diversity; $H' \geq 3$ = High diversity.

Hypersensitivity test of soil bacteria

The hypersensitivity test aims to obtain non-pathogenic soil bacteria. It is conducted by growing bacteria in 5 mL of Nutrient Broth (NB) medium and shaking the culture for 24 hours. A 0.3 mL bacterial suspension grown in a Nutrient Broth medium is injected into a jade plant (*Crassula ovata* (Mill.) Druce) leaf. After 5 days, the symptoms are observed; if necrosis occurs, the bacteria are pathogens in plants. If it does not exhibit necrosis symptoms, it may be caused by antagonistic bacteria.

RESULTS AND DISCUSSION

Character of soil microbial in the vegetative phase of rice

The isolation of soil microbial during the vegetative phase of rice revealed the presence of six bacterial isolates and three fungal isolates, highlighting the diversity of the microbial community present in the experimental plot. The bacterial isolates were predominantly Gram-positive, with most exhibiting rod-shaped morphology (bacilli). This finding aligns with previous studies showing that Gram-positive bacteria, especially bacilli, are well-suited to the soil environment due to their robust cell wall structure, which enhances survival under various environmental conditions (Madigan and Martinko 2015).

A total of 9 soil microbial isolates were isolated from all treatment plots (K, A, B, C, and D), indicating the important role of environmental and management factors in shaping soil microbial composition. The differences in soil microbial composition indicate differences in nutrient availability, microbial competition, and the influence of indigenous endophytic bacteria. The treated plots consistently showed a higher number of microbial isolates compared to the control. It is influenced by the ability of endophytic bacteria to produce bioactive compounds, increase nutrient accessibility, and create profitable niches for beneficial microbial (Santoyo et al. 2016; Trivedi et al. 2020). Indigenous endophytic bacteria can also support soil ecosystem resilience through microbial interactions and suppress pathogen populations in the soil (Compant et al. 2016).

Morphological and microscopic analyses of the isolates, illustrated the structural characteristics of the soil microbial community (Figure 2). Macroscopic observation showed that different colony morphologies among isolates, including color, texture, and edge shape variations, indicate functional and genetic diversity within the microbial community. Further microscopic analysis confirmed the dominance of bacilli, consistent with their well-documented role as Plant Growth-Promoting Rhizobacteria (PGPR) and their ability to produce bioactive compounds, including phytohormones and antimicrobial agents (Ni et al. 2024). These findings are consistent with previous studies, which have shown the potential of indigenous microbial applications to enhance soil microbial diversity and ecosystem resilience (Tan et al. 2022). The dominance of Gram-positive bacteria in the treated fields suggests that the applied endophytic bacteria may have created conditions that favor the proliferation of these organisms. This can be attributed to the production of secondary metabolites by endophytic bacteria, which suppress pathogenic microbial while promoting the growth of beneficial species (Narayanan and Glick 2022).

The morphological characteristics of soil bacteria colonies are presented in Table 1 (macroscopically) and Table 2 (microscopically). The six soil bacteria isolates macroscopically showed small colonies. The colonies were bright white and round, with flat elevations and intact edges. Microscopically, it exhibited biochemical characteristics, including Gram-positive staining, catalase positivity, and the formation of endospores. Details of the morphological characteristics of fungi are presented in Table 3. Based on morphological observations during the vegetative phase of rice, six soil bacteria isolates and three soil fungal isolates were identified. The bacterial isolates belonged to genus of *Paenibacillus*, while the fungal isolates belonged to genus of *Acremonium*. The variation in the presence of soil microbial is influenced by plant cultivation, fertilizer application, and the role of the microbes given (Senevirathne et al. 2024).

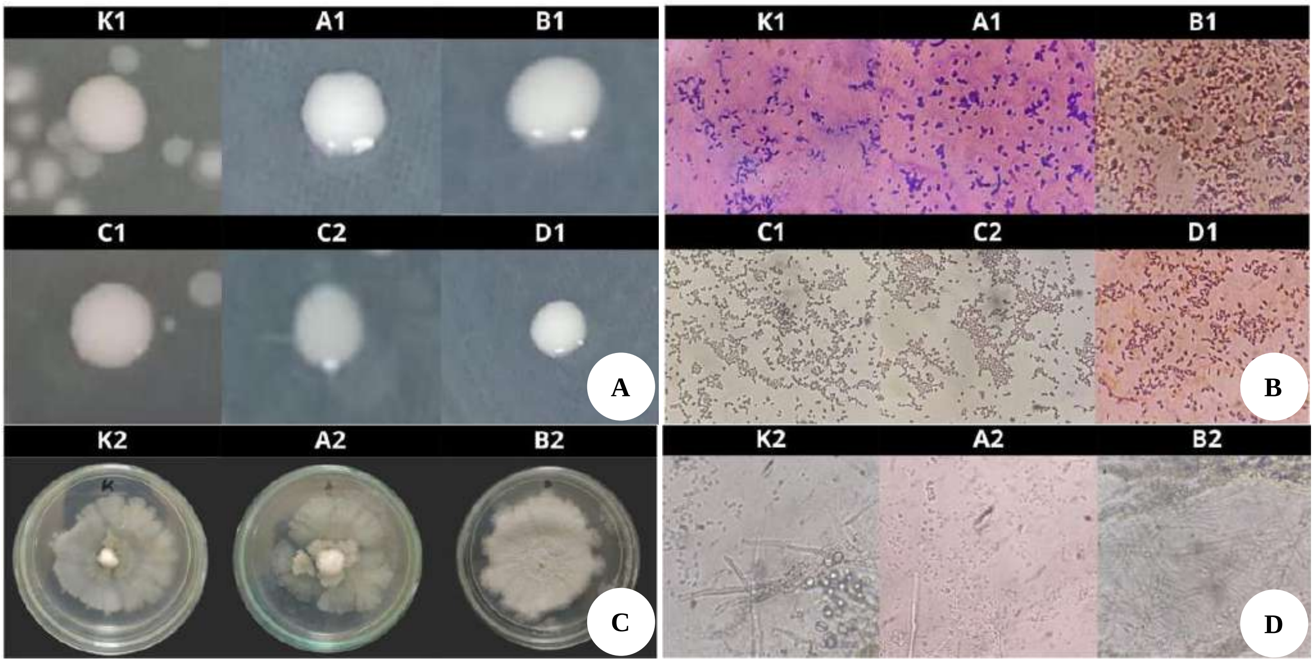

Figure 2. Soil microbial isolation in the vegetative phase of rice: A. Macroscopic soil bacteria; B. Microscopic soil bacteria; C. Macroscopic soil fungi; D. Microscopic soil fungi

Table 1. Macroscopic morphological characteristics of soil bacteria in the vegetative phase of rice

Isolate code	Size	Pigmentation	Optical characteristics	Shape	Elevation	Surface	Margin	Genera
K1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
A1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
B1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
C1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
C2	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
D1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*

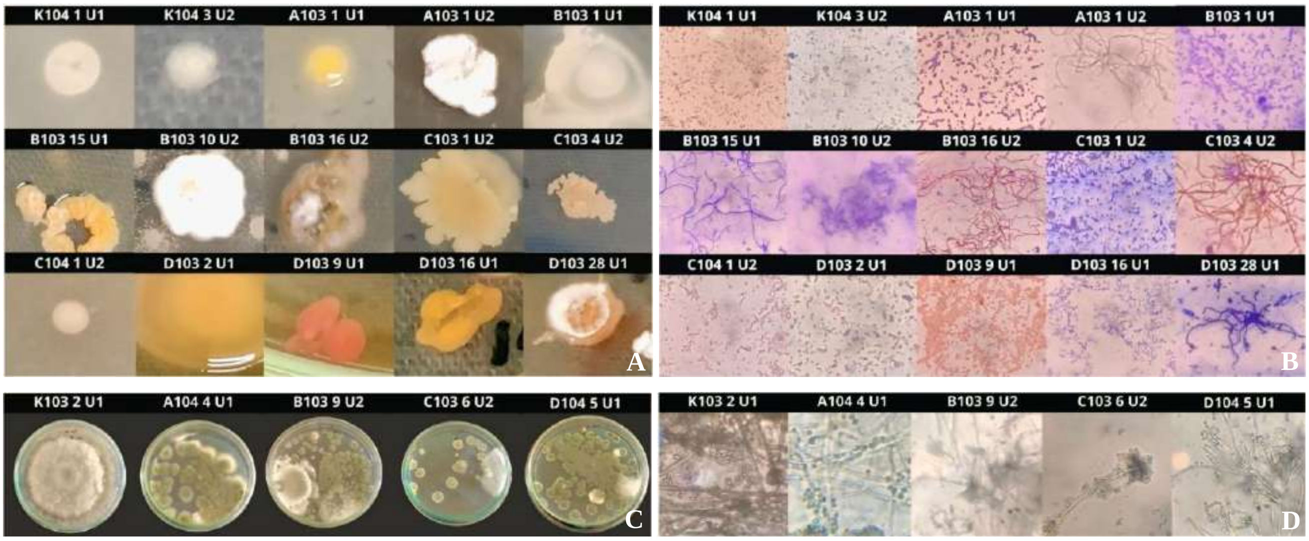

Figure 3. Soil microbial isolation in the generative phase of rice: A. Macroscopic soil bacteria; B. Microscopic soil bacteria; C. Macroscopic soil fungi; D. Microscopic soil fungi

Table 2. Biochemical and microscopic morphological characteristics of soil bacteria in the vegetative phase of rice

Isolate code	Catalase	Gram staining	Endospores	Cell shape	Genera
K1	+	+	+	Bacilli	*Paenibacillus*
A1	+	+	+	Bacilli	*Paenibacillus*
B1	+	+	+	Bacilli	*Paenibacillus*
C1	+	+	+	Bacilli	*Paenibacillus*
C2	+	+	+	Bacilli	*Paenibacillus*
D1	+	+	+	Bacilli	*Paenibacillus*

Table 3. Morphological characteristics of soil fungi in the vegetative phase of rice

Isolate code	Color	Colony shape	Hyphae	Conidium	Conidiophores	Genera
K2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium
A2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium
B2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium

Character of soil microbial in the generative phase of rice

Soil microbial in the generative phase of rice after indigenous endophytic bacterial treatment were successfully isolated from as many as 15 bacterial isolates and 5 fungal isolates. The results of macroscopic and microscopic soil microbial isolation are shown in Figure 3. Based on several tests carried out (Tables 4, 5, 6), five soil bacteria genera namely, *Paenibacillus*, *Micrococcus*, *Methylobacterium*, *Streptomyces*, and *Actinomyces*, and two soil fungal genera namely, *Acremonium* and *Penicillium* were identified.

Characteristics of the soil bacteria genus

Genus Paenibacillus *Ash et al., 1994*

Based on the results of macroscopic observations, isolates in the vegetative phase showed similar morphological characteristics. Meanwhile, isolates after treatment with codes K104 1 U1, K104 3 U2, B103 1 U1, and C104 1 U2 had round, regular (circular) and irregular (irregular) colonies, even edges (entire) and grooved (lobate), smooth surfaces, and milky white to yellowish white. Microscopic observations revealed rod-shaped, Gram-positive, catalase-positive cells with endospores. The results of the observations are based on the identification of bacteria in 7th edition of Bergey's Manual of Determinative Bacteriology (Breed 1957), where this genus is described as Gram-positive, rod-shaped, catalase-positive, and possessing endospores. This genus is widespread and can be found in the soil. Based on research by Grady et al. (2016), *Paenibacillus* sp. can be found in the soil or around the roots of plants that act as Plant Growth-Promoting Rhizobacteria (PGPR) by binding nitrogen and phosphate solvents, producing Indole Acetic Acid (IAA), and siderophores, and as inducers of systemic resistance (induced systemic resistance). According to Grady et al. (2016), more than 20 *Paenibacillus* sp. can bind free nitrogen.

Table 4. Macroscopic morphological characteristics of soil bacteria in the generative phase of rice

Isolate code	Size	Pigmentation	Optical characteristics	Shape	Elevation	Surface	Margin	Genera
K104 1 U1	Small	Clear white	Opaque	Circular	Flat	Smooth	Entire	*Paenibacillus*
K104 3 U2	Small	Clear White	Opaque	Circular	Flat	Smooth	Entire	*Paenibacillus*
A103 1 U1	Small	Yellow	Translucent	Circular	Raised	Smooth	Entire	*Micrococcus*
A103 1 U2	Small	Clear white	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
B103 1 U1	Large	Clear white	Translucent	Irregular	Flat	Smooth	Lobate	*Paenibacillus*
B103 15 U1	Small	Yellow with clear white in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
B103 10 U2	Moderate	Clear white	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Actinomyces*
B103 16 U2	Small	Green with clear white in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
C103 1 U2	Moderate	White yellowish	Opaque	Irregular	Flat	Smooth	Lobate	*Micrococcus*
C103 4 U2	Small	White	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Actinomyces*
C104 1 U2	Small	White	Translucent	Circular	Raised	Smooth	Entire	*Paenibacillus*
D103 2 U1	Moderate	White yellowish	Opaque	Circular	Raised	Smooth	Entire	*Micrococcus*
D103 9 U1	Small	Pink	Opaque	Circular	Raised	Smooth	Entire	*Methylobacterium*
D103 16 U1	Small	Yellow	Opaque	Circular	Raised	Smooth	Entire	*Micrococcus*
D103 28 U1	Pinpoint	White with brown in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*

Table 5. Biochemical and microscopic morphological characteristics of soil bacteria in the generative phase of rice

Isolate code	Catalase	Gram staining	Endospores	Cell shape	Genera
K104 1 U1	+	+	+	Bacilli	*Paenibacillus*
K104 3 U2	+	+	+	Coccobacilli	*Paenibacillus*
A103 1 U1	+	+	+	Coccobacilli	*Micrococcus*
A103 1 U2	+	+	-	Filamentous	*Streptomyces*
B103 1 U1	+	+	+	Bacilli	*Paenibacillus*
B103 15 U1	+	+	-	Filamentous	*Streptomyces*
B103 10 U2	-	+	-	Filamentous	*Actinomyces*
B103 16 U2	+	+	-	Filamentous	*Streptomyces*
C103 1 U2	+	+	+	Bacilli	*Micrococcus*
C103 4 U2	-	+	-	Filamentous	*Actinomyces*
C104 1 U2	+	+	+	Bacilli	*Paenibacillus*
D103 2 U1	+	+	-	Bacilli	*Micrococcus*
D103 9 U1	+	-	-	Bacilli	*Methylobacterium*
D103 16 U1	+	+	-	Coccobacilli	*Micrococcus*
D103 28 U1	+	+	-	Filamentous	*Streptomyces*

Table 6. Morphological characteristics of soil fungi in the generative phase of rice

Isolate code	Color	Colony shape	Hyphae	Conidium	Conidiophores	Genera
K103 2 U1	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	*Acremonium*
A104 4 U1	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
B103 9 U2	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
C103 6 U2	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
D104 5 U1	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*

Genus Micrococcus *Anon.*

Based on the results of macroscopic observations of the treatment isolates in the generative phase of codes A103 1 U1, D103 2 U1, C103 1 U2, and D103 16 U1, the colonies were round, regular (circular), and irregular (irregular) with flat edges (entire) and lobed, smooth surfaces, and yellow. The microscopic observations revealed that the isolates were cocci, Gram-positive, and catalase-positive but did not possess endospores. The results of the observations are based on the identification of bacteria in 7th edition of Bergey's Manual of Determinative Bacteriology, where this genus is described as Gram-positive and round-celled.

Most are found in tetrads or irregular groups, catalase positive, and do not have endospores. This genus is widespread in various terrestrial and aquatic ecosystems. According to research by Patel et al. (2021), *Micrococcus* is a Plant Growth-Promoting Bacteria (PGPB) and biological control agent against soil-borne pathogens. *Micrococcus* can produce Indole Acetic Acid (IAA), ammonia, potassium solubilizers, and phosphate solubilizers, which directly enhance plant growth.

Genus Methylobacterium *Patt et al., 1976*

Based on the results of macroscopic observations of isolate code D103 9 U1, the colony is circular with flat edges (intact), a smooth surface, and pink. The microscopic observations show that the cells are rod-shaped, Gram-negative, catalase-positive, and do not have endospores. The results of the observations are based on the identification of bacteria in the Breed et al. 1957 (7th edition of Bergey's Manual of Determinative Bacteriology). The genus is Gram-negative, rod-shaped, catalase-positive, and does not form endospores. This genus can be found on the surfaces of leaves, soil, dust, and freshwater. According to research by Dourado et al. (2015), *Methylobacterium* strains are reported to produce phytohormones, including cytokinins and auxins, which stimulate cell division and elongation, respectively. *Methylobacterium* can produce IAA, indicating that its inoculation can increase the concentration of IAA in plants and encourage increased plant growth.

Genus Streptomyces *Waksman & Henrici, 1943*

Based on the results of macroscopic observations of isolates in the generative phase of plants with isolate codes A103 1 U2, B103 15 U1, B103 16 U2, and D103 28 U1, the colonies are irregularly shaped with wavy edges, wrinkled surfaces, and are white, yellow, green, and brown. The microscopic observations revealed that the isolates are filamentous, Gram-positive, and catalase-positive and do not form endospores. The results of the observations are based on identifying bacteria in the 7th edition of Bergey's Manual of Determinative Bacteriology (Breed et al. 1957), where this genus is characterized as Gram-positive, filamentous, catalase-positive, and does not form endospores. This genus inhabits the soil surrounding plant roots, known as the rhizosphere, as well as niches within and between root cells, referred to as endophytic compartments. According to research by Vurukonda et al. (2018), *Streptomyces* endophytes contribute to the growth of their host plants as plant growth promoters, thereby increasing the ability of their hosts to survive in stressful environmental conditions. The interaction between IAA-producing bacteria and host plants is important in plant diversity.

Genus Actinomyces *Harz, 1877*

Based on the results of macroscopic observations of isolates treated in the generative phase with isolate codes B103 10 U2 and C103 4 U2, the colonies formed were irregular in shape with wavy edges, wrinkled surfaces, and white. The microscopic observations revealed that the isolates were filamentous, Gram-positive, catalase-negative, and lacked endospores. The results of these observations are based on the identification of bacteria in the 7th edition of Bergey's Manual of Determinative Bacteriology, where this genus is described as Gram-positive, rod-shaped, filamentous, with colonies in the form of branched hyphal tissue, catalase-negative, and lacking endospores. This genus is widespread and can be found in the soil. According to research by AbdElgawad et al. (2020), *Actinomyces* plays a role in the decomposition of organic matter and nitrogen fixation (approximately 15%), as well as serving as a phosphate solvent. *Actinomyces* is known to produce various antibiotics, biological control agents, and plant growth-promoting chemicals.

Characteristics of the soil fungi genus obtained

Genus Acremonium *Link, 1809*

The results of macroscopic observations of isolates in the generative phase in the isolated code K103 2 U1 showed that the colonies were flat, protruding in the middle, and had a cotton-like texture. Meanwhile, microscopic observations revealed that the isolate had septate, upright hyaline hyphae, cylindrical conidia, single-celled hyaline spores, and upright conidiophores. These were identified by consulting the Watanabe 2002 (2nd edition of Pictorial Atlas of Soil and Seed Fungi), which describes this genus as having upright, septate, hyaline conidiophores (phialids). Phialosporous conidia are clavate, cylindrical or elliptical, hyaline, single-celled, and usually biguttulate. The hyphae formed are often crustaceans. According to research by Han et al. (2020), members of the genus *Acremonium* fungi are the dominant endophytes that can be isolated. This fungus increases antifungal activity in host plants. Members of the genus *Acremonium* are generally found in corn and rice.

Genus Penicillium *Link, 1809*

Macroscopic observations of isolates in the generative phase in isolate codes A104 4 U1, B103 9 U2, C103 6 U2, and D104 5 U1 showed that the colonies were flat, fibrous, and velvety textured. Meanwhile, microscopic observations revealed that the isolates possess long, branched, multinucleated, septate fibrous hyphae, conidia arranged in chains, and conidiophores that are upright and branched, with round, hyaline conidia. The results of the observations are by the identification of fungi in the Watanabe 2002 (2nd edition of Pictorial Atlas of Soil and Seed Fungi), where this genus has hyaline, upright, branched conidiophores like penicillate at the top with 2-3 setulae, vertical phialides on each metal, and conidial heads that are somewhat aggregated, compact, and composed of conidia bound to each phialide. Phialids taper gradually or are cylindrical with pointed tips. Phylosporous conidia have a hyaline or yellowish-brown mass, elliptical or oval in shape, single-celled, and rough or slightly serrated on the surface. According to research by Han et al. (2020), *Penicillium* is a rhizosphere fungus that effectively suppresses various damping-off and fusarium wilt diseases. Additionally, these fungi can stimulate plant growth.

Diversity index

The results of the calculation of soil microbial diversity using the Shannon-Wiener index in each treatment showed that the diversity was low and moderate. The low H' index value of the vegetative phase of rice was found in the Control, A, B, C, and D treatments with H' index values of 0.08, 1.10, 1.13, 0.00, and 0.00, respectively. The moderate H' index value of the generative phase of rice was found in the control, A, B, C, and D treatments with H' index values of 1.33, 1.36, 1.24, 1.00, and 1.56, respectively. The results of the soil microbial diversity calculation for the vegetative and generative phases of rice are presented in Table 7.

Application of endophytic bacteria in treatment A increased the H' index value from 0.10 to 1.36, treatment B from 0.13 to 1.36, treatment C from 0 to 1.00, and treatment D from 0 to 1.56. This was caused by one of the environmental factors, namely soil. Soil quality significantly impacts the biological conditions of the soil, particularly the presence of microbial in the plant rhizosphere. The treatment of endophytic bacterial consortium showed the highest increase in bacterial diversity among the others. According to Prayudyaningsih et al. (2015), the rhizosphere is a part of the soil around the plant roots. The microbial population in the rhizosphere is generally more numerous and diverse than in non-rhizosphere soil. The abundance of rhizosphere bacteria can be increased by applying endophytes, especially in the form of consortia. The endophyte application aims to stimulate the stability of microbial abundance in the soil. Endophytes can degrade several organic compounds in the soil, thereby enhancing the availability of food sources for various soil microbial, which maximizes the function of endophytes.

Hypersensitivity test of soil bacteria

A hypersensitivity test using *C. ovata* (Figure 4; Tables 8 and 9) against 22 bacterial isolates revealed that two bacterial isolates, with isolate codes *Xanthomonas oryzae* (Xoo) as a control and C104 1 U2, were pathogenic. This was indicated by necrosis on *C. ovata* leaves after 5 days of incubation. Hypersensitivity reactions are characterized by rapid and localized cell death following bacterial inoculation. This reaction occurs in infected plants during the introduction of pathogens and is also an effort to inhibit pathogen growth (Balint-Kurti 2019).

Hypersensitivity test using *C. ovata*, according to Umesha et al. (2008) plants of the Crassulaceae family are succulent plants, have thick enough leaves that store water and bacterial cells can be easily infiltrated. The microclimate in the mesophyll of the leaves of the *C. ovata* plant offers suitable conditions for bacterial proliferation resulting in the expression of hypersensitivity reactions.

Table 7. Soil microbial diversity index in the vegetative and generative phases of rice

Treatment	Vegetative phase		Generative phase	
	Soil microbial diversity (H')	Criteria	Soil microbial diversity (H')	Criteria
Control	0.08	Low	1.33	Moderate
A	0.10	Low	1.36	Moderate
B	0.13	Low	1.24	Moderate
C	0	Low	1.00	Moderate
D	0	Low	1.56	Moderate

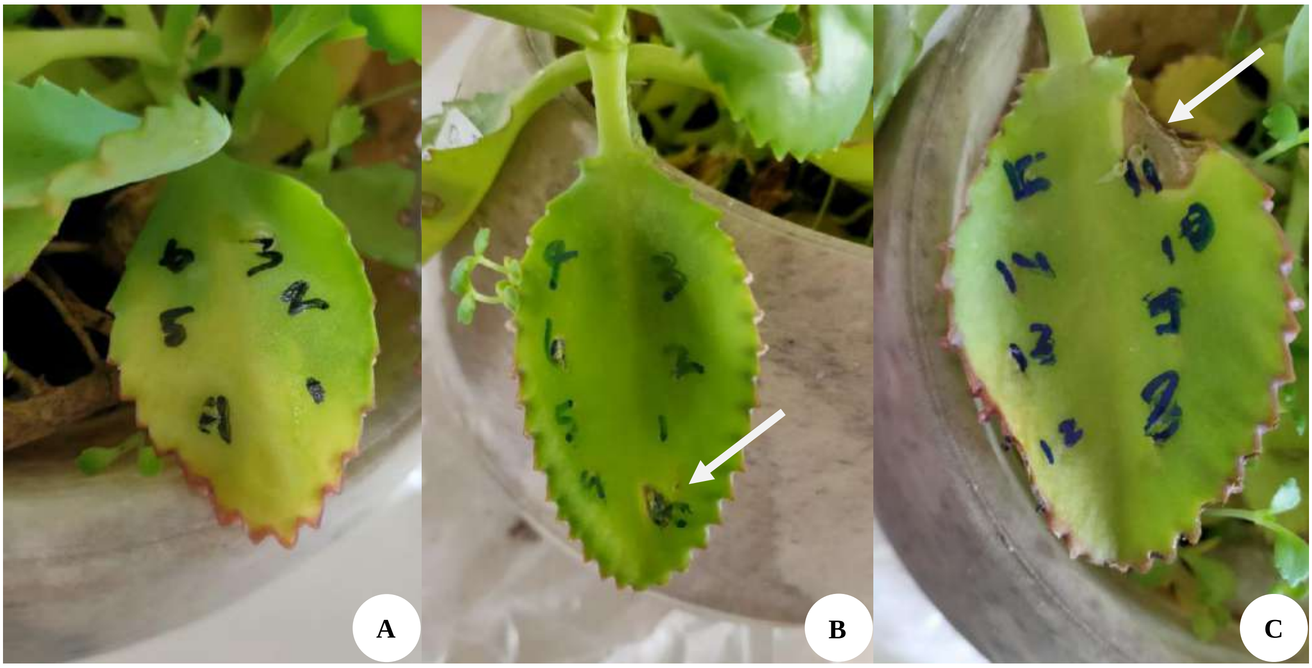

Figure 4. Hypersensitivity test results: A. Soil bacterial isolates in the vegetative phase of rice; B. Soil bacterial isolates in the generative phase of rice (codes 1 to 7); C. Soil bacterial isolates in the generative phase of rice (codes 8 to 15). The arrow on the hypersensitivity test results indicates that the isolate is a pathogen

Table 8. Results of hypersensitivity tests of soil bacterial isolates found in the vegetative phase of rice

Test code	Isolate code	Hypersensitivity tests
1	K	-
2	A	-
3	B	-
4	C	-
5	C2	-
6	D	-

Table 9. Results of hypersensitivity tests of soil bacterial isolates found in the generative phase of rice

Test code	Isolate code	Hypersensitivity tests
K (control)	**Xoo**	+
1	K104 1 U1	-
2	K104 3 U2	-
3	A103 1 U1	-
4	A103 1 U2	-
5	B103 1 U1	-
6	B103 15 U1	-
7	B103 10 U2	-
8	B103 16 U2	-
9	C103 1 U2	-
10	C103 4 U2	-
11	**C104 1 U2**	+
12	D103 2 U1	-
13	D103 9 U1	-
14	D103 16 U1	-
15	D103 28 U1	-

In conclusion, the isolation of soil microbial during the vegetative and generative phases of rice plants revealed an increase in the diversity of soil microbial, comprising the genera *Paenibacillus*, *Micrococcus*, *Methylobacterium*, *Streptomyces*, *Actinomyces*, *Acremonium*, and *Penicillium*. The diversity index indicated that treatments A, B, C, and D fell into the moderate category. The highest increase in soil microbial diversity was observed in the treatment with the endophytic bacterial consortium. The results of the hypersensitivity test showed symptoms of necrosis in isolate C104 1 U2, while the other isolates did not exhibit these symptoms. This indicates that there is only one pathogenic soil microbe, and other microbes have the potential to develop as antagonistic agents.

ACKNOWLEDGEMENTS

Thanks to *DRTPM Kemendikbudristek* for supporting this research in "*Terapan Scheme*" with contract number: 20.81/UN23.35.5/PT.01.00/VI/2024, and to the farmer and students for their teamwork in this research.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 255-263 DOI: 10.13057/asianjagric/g090127

Morphological and genetic variation of cassava (*Manihot esculenta*) based on DNA markers barcoding *rbcL* and ITS

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Manuscript received: 22 November 2024. Revision accepted: 14 May 2025.

Abstract. *Afifah N, Susilowati A, Purwanto E. 2025. Morphological and genetic variation of cassava (*Manihot esculenta*) based on DNA markers barcoding* rbcL *and ITS. Asian J Agric 9: 255-263.* The cassava crop holds significant economic value as both a food source and a commercial crop. This study aims to characterize cassava varieties using morphological and molecular methods. Five cassava varieties were identified in Boyolali District, Central Java, Indonesia: M, U, KP, HM, and MH. Morphological observations following the guidelines in the book Selected Morphological and Agronomic Descriptors for the Characterization of Cassava. Molecular observations were conducted using a DNA barcoding technique, with the *rbcL* and ITS as DNA markers. The research involved several stages, beginning with the isolation of genomic DNA using the Genaid plant DNA extraction kit, then the visualization of the genomic DNA by using gel electrophoresis and amplification of gene targets—subsequently, bidirectional Sanger sequencing. The DNA sequences of each sample were subjected to BLAST analysis on the NCBI website for species identification. The NTSYSpc was utilized to examine relationships based on morphological characteristics. For molecular characteristics, MEGA11 was used for nucleotide composition analysis, genetic distance calculation, and phylogenetic tree construction. The morphological analysis revealed substantial variations in the colors of leaf petioles, the distance between stem nodes, and the texture of tuber bark. All five cassava varieties exhibited a high degree of genetic similarity to *Manihot esculenta* Crantz, demonstrated by their high query coverage and E-values.

Keywords: Cassava, DNA barcoding, genetic diversity, *Manihot esculenta*, morphological characteristics

INTRODUCTION

Cassava, scientifically known as *Manihot esculenta* Crantz, is a member of the Euphorbiaceae family. Although primarily classified as a tropical plant, cassava also adapts well to subtropical regions. This plant exhibits distinctive morphological traits, including branching and enlarging taproots, finger-like leaves, and stems with numerous branches (Ha et al. 2016). Cassava is widely cultivated because it thrives in a variety of soil conditions. In Indonesia, cassava is grown in both dry and wet climates, with only a small percentage being cultivated in rice fields (Aristin et al. 2022). As an annual crop, cassava can survive for several years and produces thickened or swollen roots known as tubers, which are rich in starch and commonly used as a staple food. The yield of sweet-tasting tubers depends on factors such as soil fertility, soil structure, climate, and the harvesting date. Tubers with a sweet flavor typically have lower levels of hydrogen cyanide (HCN) and are safe for consumption. In contrast, tubers with a bitter taste contain higher HCN levels, making them less suitable for direct consumption, though they can be used primarily in flour production (Nur 2019).

The diversity of cassava is evident in its morphological characteristics, which include variations in leaves, stems, and tubers. Both genetic and environmental factors influence these variations. Understanding these traits is essential for identifying phenotypic germplasm, which can be valuable for plant breeding programs (Karim et al. 2020). With advancements in technology, distinctions between cassava types can now be examined not only through morphological but also through molecular characteristics. Molecular markers have become useful tools to supplement morphological assessments due to their high accuracy, rapid processing, and specific results (Perwitasari et al. 2020). By comparing and analyzing the morphological characteristics of different varieties within a single species, researchers can better understand the relationships among cassava varieties. Moreover, the management and conservation of germplasm heavily rely on the analysis of genetic diversity.

The ribulose-1,5-bisphosphate carboxylase large subunit gene (*rbcL*) is part of a chloroplast gene-coding region and demonstrates a high amplification success rate across various gymnosperm and cryptogamae plant species. The Consortium for the Barcode of Life (CBOL) recommends the use of the *rbcL* gene as a standard gene encoder in plants due to its high PCR amplification success rate (Li et al. 2021; Trujillo-Argueta et al. 2022). The *rbcL* gene exhibits superior success during the PCR amplification process compared to other DNA barcoding markers, further confirming its accuracy (Moura et al. 2019). The Internal Transcribed Spacer (ITS), which is found within nuclear ribosomal DNA, is also recommended as a DNA barcode for flowering plants. The nuclear ribosomal DNA consists of three ribosomal subunits—18S, 5.8S, and 26S—separated by ITS1 and ITS2. Typically, ITS sequences range from 700 to 900 base pairs (Hapsari et al. 2018;

Acharya et al. 2022). Plant varieties can be distinguished by their ITS sequences, which also aid in constructing phylogenetic trees. Variation between species can arise from mutations in the ITS region, where even closely related species exhibit a high degree of variability (Abozaid and Fattah 2024). The ITS genes have a high success rate in PCR amplification and provide excellent species discrimination, making them reliable markers in the study of new species (Hariri et al. 2021). Therefore, this study aimed to assess the diversity and relationships among cassava varieties using both morphological characters and DNA barcoding with the *rbcL* gene marker and ITS sequences.

MATERIALS AND METHODS

Sample collection

The study was conducted in the gardens of cassava farmers in Boyolali District, Central Java, Indonesia, from January to March 2024. The tools used for this study included a ruler, a digital camera, and stationery. The materials comprised five varieties of cassava: *M. esculenta* (M) with red-colored leaf petioles, *M. esculenta* (U) with purple-colored leaf petioles, *M. esculenta* (KP) with green-colored leaf petioles, *M. esculenta* (MH) with reddish-greenish petioles, and *M. esculenta* (HM) with reddish-green petioles. A survey method with purposive sampling was employed in various community gardens across several sub-districts in the Boyolali District. The selected cassava plants were between 4 to 8 months old and had reached adulthood, exhibiting different morphological characteristics.

Morphological characterization

Morphological characterization was conducted following the referenced guidelines outlined in the "Cassava Plant Selected Morphological and Agronomic Descriptors for the Characterization of Cassava" by IITA (Sikteubun et al. 2022). The leaves were measured for both length and width and observed for the number of lobes, leaf margins, and leaf pulse color. Leaf stems were measured for length, and leaf stem color was observed. The stem was observed for the distance between nodes, protrusion of nodes, and stem color. The tuber was observed for shape, texture of the outer skin, and tuber flesh color. Following the morphological analysis, the samples were molecularly identified in the UNS Molecular Biology Laboratory.

Genomic DNA extraction

Isolating DNA from cells and cellular components is known as DNA extraction. To remove impurities and contaminants, the detached leaf samples were thoroughly cleaned and allowed to air dry before extraction. The first step in DNA extraction involves crushing and grinding the leaf samples into a fine paste or powder using a mortar and pestle. This process mechanically breaks down the cell walls. Next, the DNA was extracted using the Geneaid Genomic DNA Mini Kit (Plant) according to the kit's manual. This procedure purposes to obtain pure DNA from all five varieties of samples, which will be used for further analysis.

Visualization of DNA

The visualization of genomic DNA is the initial step before performing Polymerase Chain Reaction (PCR). This process determines whether the obtained DNA is pure or contaminated and assesses its quality. DNA visualization was carried out using five components: a DNA sample, GelRed, 1X TAE buffer, a DNA ladder, and a 0.8% agarose gel, along with loading dye. The loading dye serves two purposes: it facilitates the loading of DNA into the wells, thereby minimizing cross-contamination between samples, and it acts as a tracking dye. The dye imparts color to the transparent DNA, allowing for monitoring the migration of the sample in the gel, thus preventing it from traveling too far and being lost in the buffer (Suryandari 2024).

Electrophoresis is a technique used to separate DNA molecules based on their size. The bands formed during this process are analyzed by examining the position of the DNA after migration (Amiteye 2021). The basic principle of electrophoresis involves the movement of charged molecules or ions through a semisolid medium under the influence of an electric field. Negatively charged DNA molecules migrate through the agarose gel when an electric current is applied, moving from the negative pole toward the positive pole (Hall 2020; Zheng and Wang 2023). The quality of the DNA can be evaluated based on the electrophoretic analysis. The brightness of the resulting bands can vary due to several factors, including the quality of DNA isolation, the presence of contaminants, and the conditions during the electrophoresis process. Degraded DNA typically forms fuzzier or fragmented bands, while intact DNA generates clear and well-defined bands (Tanzil and Fanata 2024).

PCR amplification with *rbcL* and ITS primers

Good-quality genomic DNA was used for polymerase chain reaction (PCR) amplification with the selected primers, as detailed in Table 1 (Perwitasari et al. 2020; Heylen et al. 2021; Thongkhao et al. 2022). The amplification was conducted in a PCR tube using an Applied Biosystems Veriti Thermal Cycler. Each PCR reaction had a total volume of 50 µL per tube, which included 5 µL (100 ng) of DNA sample, 16 µL of deionized distilled water (ddH2O), 25 µL of MyTaq HS Red Mix (Bioline), and 2 µL (10 pmol) of each primer (forward and reverse) (Omonhinmin et al. 2022). The PCR process for amplifying the *rbcL* gene involved an initial denaturation step at 95°C for 3 minutes. This was followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 53°C, and elongation at 72°C for 1 minute. After these cycles, there was a final extension at 72°C for 40 seconds. For amplifying the ITS regions, the PCR conditions included an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C, and elongation at 72°C for 1 minute. This was also followed by a final extension at 72°C for 40 seconds. The amplified PCR products were then subjected to electrophoresis on a 0.8% agarose gel stained with GelRed for 25 minutes. The bands were compared with a 1Kb DNA ladder. The number of bands observed indicates the quantity of PCR product in the sample. When a single band is observed, it signifies the amplification of a specific DNA target. Conversely, the formation of two or more bands indicates the amplification of nonspecific band products, which occurs when primers anneal in multiple locations. After electrophoresis, the PCR product is sent to a sequencing service provider using the same primer for sequencing.

Sanger Bi-directional sequencing for *rbcL* and ITS

The PCR products were sent for sequencing using the same primers by PT. Genetika Science Indonesia. The sequencing method employed was Sanger sequencing.

Data analysis

Morphological characteristic data were analyzed using the Cluster Analysis model of the NTSYSpc program, version 2.02i (Suhasini and Yuvaraja 2023). A higher similarity coefficient value indicates lower morphological diversity. The clustering analysis utilized the SAHN function with the UPGMA method. This analysis aimed to calculate the similarity coefficient value to assess the diversity among the five cassava varieties. For molecular characterization, the sequences were aligned for similarity searching with a database in GenBank at NCBI (National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool). Nucleotide composition and Pairwise genetic distance were calculated using the Molecular Evolutionary Genetic Analysis (MEGA)11 program (Tamura et al. 2021). The construction of a phylogenetic tree was performed using the Neighbor-Joining (NJ) method with the Tamura 3-parameter (T92) for the *rbcL* sequence and Tamura 3-parameter + Gamma (T92+G) for the ITS sequence, with a bootstrap value of 1000 (Saleky et al. 2022).

RESULTS AND DISCUSSION

Morphological characteristics

Leave morphology

The morphological characteristics of the five cassava varieties showed variations in the color of leaf stalks, young leaves, stems, and tubers. Cassava plants observed in various regions of the Boyolali District exhibited leaves with incomplete stems, lacking leaf sheaths (or vaginal structures), and consisting only of leaf stalks (*petioles*) and leaf blades (*lamina*). The leaf stalks of cassava displayed a range of colors, including red, purple, green, reddish-green, and greenish-red. The petiole has an elongated round shape with one end tapering and thickening at the base, with lengths ranging from 12 to 30 cm. The leaf blades were characterized by a round (*orbicular*) shape at their broadest part and tapering to a point at the tip (*apex folii*), creating a narrower appearance. The edges of the leaves were smooth, and they meet and attach at the base. Each leaf blade featured a prominent midrib (*costa*) running through the center, with all midribs originating from the tip of the leaf stalk. Lateral veins (*nervus lateralis*) extended close to the leaf edges, bending upwards to connect with the midrib above. The leaf veins were clearly visible, branching from the lateral veins, and exhibit various colors—green, red, and reddish-green—extending less than halfway along each lobe (Figure 1). Cassava leaves had a palmate vein pattern (*palminervis*) with an odd number of lobes, typically ranging from 5 to 9. The largest and longest lobe was positioned in the center, while the side lobes were smaller in size. The edges of cassava leave featured finger-like indentations (*palmatipartitus*), with notches extending more than halfway down the leaf from each side, resembling finger-like projections. The leaf flesh was thin and soft (*intervenium*), and the leaves exhibited a dark green color on the upper side and a lighter green on the underside, with a smooth (*laevis*) and dull (*opacus*) surface texture. Juvenile leaves exhibited a variety of colors, including red, light green, and reddish-green. Cassava leaves were considered simple leaves (*folium simplex*) each stalk typically beared only one leaf with deep notches.

Figure 1. Morphological characteristics (leaves) of cassava varieties: A. *Manihot esculenta* (M); B. *M. esculenta* (U); C. *M. esculenta* (KP); D. *M. esculenta* (HM); E. *M. esculenta* (MH)

Table 1. PCR primers for the *rbcL* and ITS markers. Forward (F) and reverse (R) primers with their 5'-3' sequences were used for amplifying DNA sequences

Loci	Primer	Sequence	Ta (°C)
rbcL	aF	5'ATGTCACCACAAACAGAGACTAAAGC3'	53
rbcL	R23	5'TTTTAGTAAAAGATTGGGCCG3'	53
ITS	F	5'AGAAGTCCACTGAACCTTATC3'	57
ITS	R	5'CGCTTCTCCAGACTACAATTC3'	57

Note: Perwitasari et al. (2020); Heylen et al. (2021); Thongkhao et al. (2022)

Stem morphology

The cassava stem had a round shape, was woody, segmented, and elongated, with a height ranging from 40 to 110 cm. The color of the stem varied depending on the outer bark; younger stems were typically green, while older stems were orange or light brown. The inner pith of the stem was white, soft in texture, and had a smooth structure similar to cork. The outer colors of the stem included yellowish-green, green, and reddish-green, while the cortex was either light green or dark green (Figure 2). The growth of the stem was straight, and it exhibited a dichotomous branching pattern. Each stem featured a protrusion at the stem node, which is the remnant of the leaf stalk's base. There were two types of stem node protrusions: semi-prominent and prominent. In the semi-prominent model, the protrusion was not very noticeable, and the remains of the leaf stalk base appeared flat against the stem surface. Conversely, in the prominent model, the protrusion was clearly visible, giving the stem a serrated appearance. The distance between stem nodes, also known as internodes, was relatively short, measuring less than 8 cm.

Tuber morphology

The tubers of cassava were in various shapes, including cylindrical, cone-cylindrical, and mixed forms. These tubers are specialized roots that change shape and function primarily as storage organs for food reserves. Morphologically, the tuber section can be divided into three parts: stalks, tubers, and tails. The skin of the tuber can have either a rough or smooth texture. Rough-skinned tubers are generally easy to peel, while smooth-skinned varieties can be more challenging to remove. The outer skin color varied, typically appearing in dark or light brown shades (Figure 3), and the tuber cortex was either purple or pink. The flesh of the tuber was commonly white or cream. There were also two types of tuber stalk extensions: *sessile* and *pedunculate*. In the *sessile* model, the tuber stalk is longer, allowing the tuber to hang away from the stem's base. In contrast, the *pedunculate* model features a very short or absent stalk, causing the tuber to attach directly to the base of the stem.

Dendrogram of cassava varieties in Boyolali

A dendrogram is a branching diagram in the form of a tree representing the degree of similarity in morphology or other observable traits regardless of their evolutionary relationship. This study used dendrograms to describe relationships based on morphological characters. The dendrogram illustrates the separation of five varieties of *M. esculenta* into two distinct groups (Figure 4). The first group includes *M. esculenta* (KP), *M. esculenta* (M), and *M. esculenta* (U), while the second group comprises *M. esculenta* (HM) and *M. esculenta* (MH) (Figure 4).

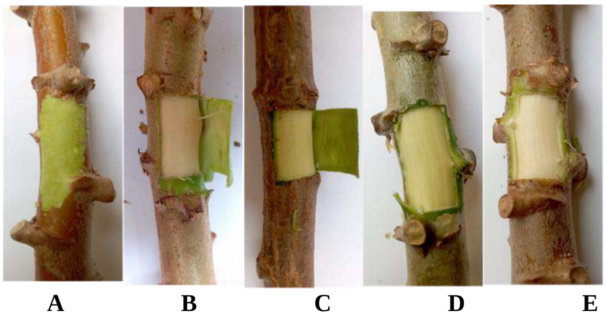

Figure 2. Morphological characteristics (stem) of cassava (*Manihot esculenta*) varieties: A. *M. esculenta* (M); B. *M. esculenta* (U); C. *M. esculenta* (KP); D. *M. esculenta* (HM); E. *M. esculenta* (MH)

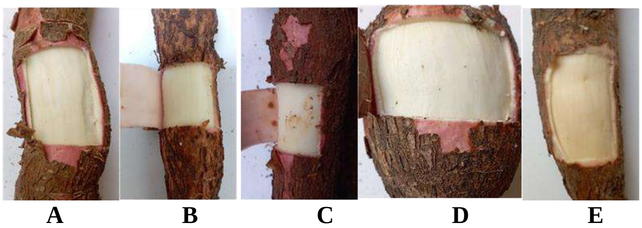

Figure 3. Morphological characteristics (tuber) of cassava (*Manihot esculenta*)varieties: A. *M. esculenta* (M); B. *M. esculenta* (U); C. *M. esculenta* (KP); D. *M. esculenta* (HM); E. *M. esculenta* (MH)

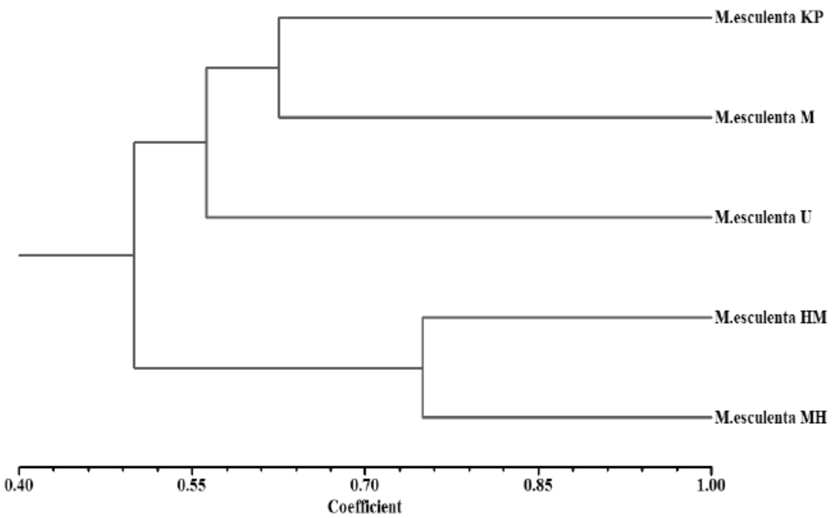

Figure 4. The dendrogram of morphological characteristics of five cassava (*Manihot esculenta*) varieties in Boyolali District, Central Java, Indonesia

The varieties MH and HM exhibit the closest relationship, with a similarity coefficient of 0.76. These two varieties shared several characteristics, including a lack of branching patterns on the stem, similarities in stem node protrusions, young leaf color, tuber skin color, and tuber surface texture. In contrast, KP, M, and U, exhibited the most distant relationship with a similarity coefficient of 0.57. The three varieties in this group were characterized by a branching model on the stem, a rough outer skin texture, and ease of peeling the cortex. Additionally, the similarity coefficient between KP and M is 0.63.

Morphological characterization is essential for gathering comprehensive data and information about this plant. Morphological traits are essential for characterizing and classifying plants in the taxonomy field (Hassemer and Baldini 2020; Zahara 2020). Morphological identification is essential for identification and classification despite being regarded as a traditional taxonomy method. This method helps investigate the similarities and origins of plants by exposing their growth patterns, forms, and external structures. Key indicators for recognizing, characterizing, and categorizing plants are morphological traits. These traits can be quantitatively measured, such as petiole length, or qualitatively described and observed, such as *petiole* color. Figure 4 shows that *M. esculenta* (KP), *M. esculenta* (M), and *M. esculenta* (U) have a closer kinship than *M. esculenta* (HM) and *M. esculenta* (MH). Even so, the five varieties of *M. esculenta* are still in the same ancestral line.

Molecular characteristics: *rbcL* and ITS amplicon

The visualization of genomic DNA isolation revealed a comparatively thin band along with some smears. Amplification of five DNA samples from the *M. esculenta* variety using the *rbcL* primer produced a distinct, thick, and fluorescent DNA band that was clearly visible. The amplicon indicated that all five varieties amplified the target region of the ribulose-1,5-bisphosphate carboxylase large subunit, specifically at 1500 bp (Figure 5.A). DNA amplification was performed on five samples of different varieties of *M. esculenta* using the Internal Transcribed Spacer (ITS). The results revealed a thick, single, and clearly visible DNA band. This indicates that the five samples of *M. esculenta* were successfully amplified within the target ITS region, which sizes ranged between 750 and 1000 base pairs (Figure 5.B).

The PCR product band indicates that both the *rbcL* and ITS primers were amplified effectively, resulting in a robust amplicon of the expected target size. The success of the amplification process, as well as the thickness of the DNA band, can be influenced by several factors, including template DNA concentration, appropriate PCR components, the quality of genomic DNA extraction for use as the DNA template, the specificity of the primers, and proper optimization of the annealing process (Hoose et al. 2023). The band produced for the *rbcL* marker gene exhibits a length of approximately 1500 bp. According to the CBOL (2009) data, the *rbcL* gene typically has a length range of 1430 bp. The R23 primer amplifies a region slightly beyond the *rbcL* gene, resulting in an amplicon product of 1500 bp, thus capturing the complete *rbcL* gene sequence. There is a minor overlap in the gene following the *rbcL* region. On the other hand, a study by Sagala and Sogandi (2022) reported that the *rbcL* gene fragment length varies from 500 to 1400 bp. The PCR product of the target gene obtained is dependent on the specific primers used. The bands produced for the ITS marker show a length range of 750 to 1000 bp. This aligns with findings from Rani et al. (2024), which indicated that the ITS1-ITS2 primers flank the 5.8S RNA region, resulting in sequence lengths of 500 to 700 bp and making them common for DNA barcoding studies. Additionally, research by Hariri et al. (2021) noted that the ITS has advantageous characteristics with a typical length of around 700 bp and is present in multiple copies within the core genome. This ITS demonstrates high species discrimination, making it suitable for research on new species.

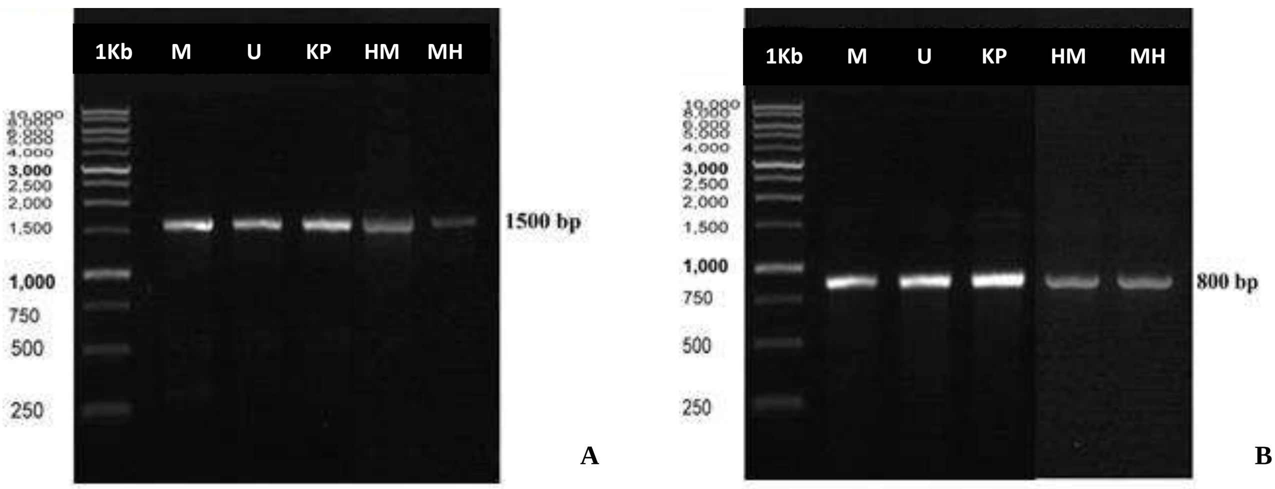

Figure 5. Visualization of PCR products of *Manihot esculenta* varieties. Amplicon genes marker: A. *rbcL*; B. ITS

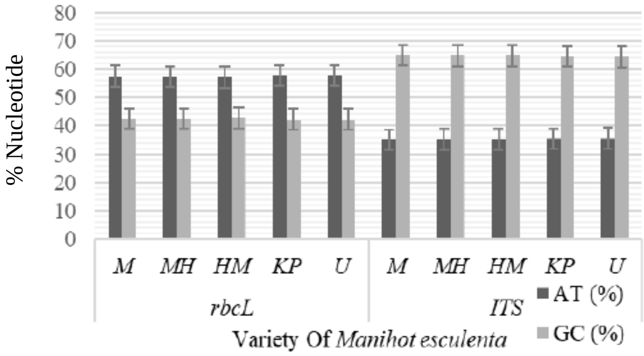

Figure 6. Nucleotide composition in amplicons of *Manihot esculenta* variety using *rbcL* and ITS

Table 2. Pairwise genetic distance among *Manihot esculenta* varieties based on *rbcL* and ITS markers

Markers	Minimum	Maximum	Means	Standard error
rbcL	0.0073	0.0191	0.0105	0.0013
ITS	0.0036	0.1628	0.0311	0.0192

Sequence (nucleotide composition)

DNA sequencing of both *rbcL* and ITS amplicons yielded high-quality sequences. The nucleotide composition of the *M. esculenta* variety's amplicons exhibited variability in AT and GC content. Overall, the *rbcL* amplicons showed a higher AT nucleotide composition (57.4%) than GC content, while the ITS sequence had a higher GC nucleotide component (64.6%) compared to that of AT (Figure 6).

The average nucleotide composition of adenine-thymine (AT) content in the *rbcL* gene from various *M. esculenta* variety was found to be 57.4%, a finding that has significant implications for our understanding of the species as it is higher than the Guanine-Cytosine (GC) content of 42.5%. In contrast, the Internal Transcribed Spacer (ITS) region has a lower AT content of 35.3% compared to a GC content of 64.6%. This is in line with the statement by Kolter and Gemeinholzer (2021) that the ITS gene contains more G and C content. Genes with a higher proportion of G and C nucleotides tend to maintain a lower mutation rate (Niu et al. 2017).

The composition of these nucleotides is related to the hydrogen bonds formed. Guanine (G) and Cytosine (C) form three hydrogen bonds, while Adenine (A) and Thymine (T) form only two. This results in different DNA denaturation temperature used, because the GC pair has more hydrogen bonds so it requires more heat energy to break them than the AT pair (Farikh et al. 2023). The higher AT content compared to GC is attributed to the greater variability in nucleotide composition and higher nucleotide substitution rates in the amplified genes (Madduna et al. 2020). The GC percentage is typically unique to each species. Generally, it is expected to fall within the range of 40-60% to be considered conserved.

Pairwise genetic distance

Pairwise genetic distance refers to the level of genetic variation between species, quantified as a numeric value. In this analysis, the pairwise genetic distance substitution model was employed using the MEGA application to assess pairwise genetic distance. Additionally, DNA sequence alignment was analyzed within the MEGA application; a smaller pairwise genetic distance value between two organisms indicates a closer evolutionary relationship (Sahaba et al. 2021). The aligned sequences from the five cassava varieties revealed a low pairwise genetic distance of 0.0073 for the *rbcL* gene. In contrast, the sequence for *Cnidoscolus aconitifolius* (Mill.) I.M.Johnst. (AB267937.1) showed the highest pairwise genetic distance among the analyzed sequences at 0.0191 (Table 2). *C. aconitifolius* was chosen as an outgroup since it has similar stem and leaf morphology, but it does not produce tubers and is in the same family. *C. aconitifolius* is commonly known as Japanese papaya or cassava with thorns. For the ITS sequences, the five varieties showed a shallow pairwise genetic distance of 0.0036, while the *C. aconitifolius* sequence (MH978980.1) displayed the highest pairwise genetic distance of 0.1628 among the other sequences. The value of genetic distance ranges from 0 to 1. The value of 0 indicates that individuals have a fairly close kinship relationship, while a value of 1 indicates that individuals have a fairly distant kinship relationship.

Pairwise genetic distance is a measure used to determine the relationships between organisms based on nucleotide differences, which reflect the level of genetic diversity. A low pairwise genetic distance value indicates a close relationship, while a high pairwise genetic distance value suggests a more distant relationship (A'yun et al. 2024).

Phylogenetic relationship

The phylogenetic tree analysis of *rbcL* sequences reveals the relationships among five varieties of *M. esculenta*. Notably, *M. esculenta* (M) and *M. esculenta* (KP) are situated on different branches compared to *M. esculenta* (U); however, they share the same ancestral line with a bootstrap value of 99%, indicating a close genetic relationship. The bootstrap value provides a measure of confidence in the branches, with values above 90% considered reliable. In addition, the varieties *M. esculenta* (MH) and *M. esculenta* (HM) also exhibit a close relationship, even though they occupy different branches, supported by a bootstrap value of 99%. Using the Neighbor-Joining (NJ) method with the T92 model, the analysis successfully distinguished the sequences of *Manihot carthagenensis* (Jacq.) Müll.Arg., *Manihot grahamii* Hook., and *C. aconitifolius*. These three sequences were categorized as outgroups since they belong to the same family, Euphorbiaceae but not esculenta. The *rbcL* sequence from *M. esculenta* in the UK (LT576833.1) exhibits a closer relationship to *M. esculenta* (MH) (Figure 7).

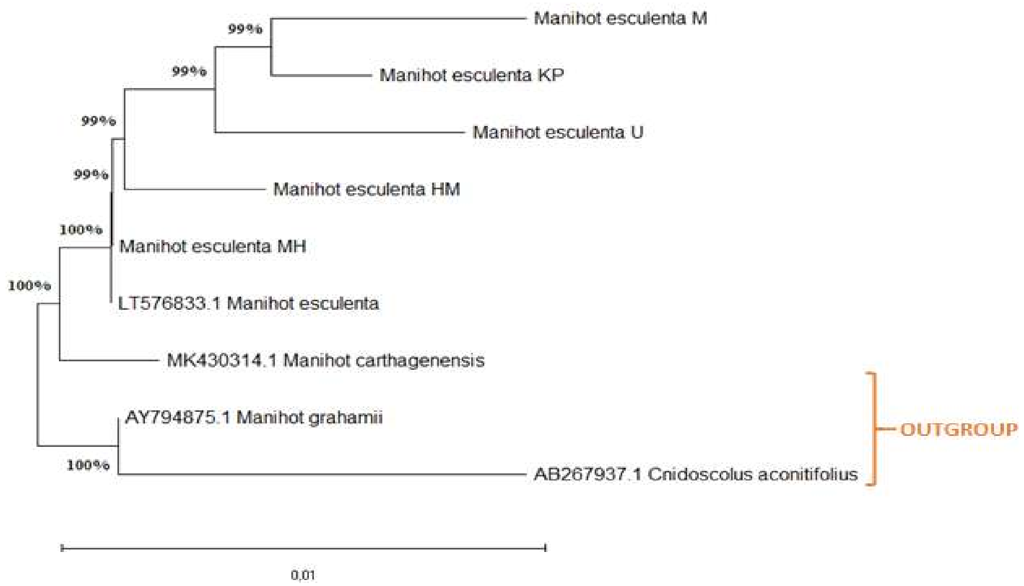

Figure 7. The phylogenetic relationships among different varieties of *Manihot esculenta*, as determined by the *rbcL* genetic marker

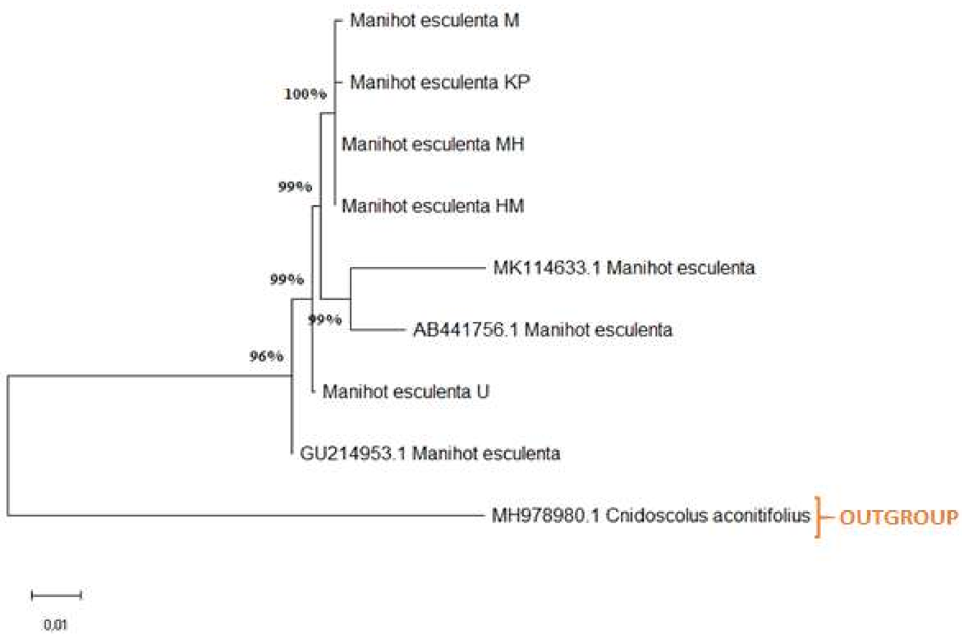

Figure 8. The phylogenetic relationships among varieties of *Manihot esculenta* are differentiated based on the ITS genetic marker

The tree construction process included a bootstrap analysis with 1000 repetitions (Sulistyaningsih et al. 2018). In this context, bootstrap values are categorized as strong/supportive (>85%), moderate (70-85%), weak (50-69%), and poor (<50%) for the branches formed. The construction indicates that the five varieties of *M. esculenta* form a group with a bootstrap value of 99%. The NJ method was chosen since it effectively produces branch lengths from trees, even when the topology changes, by simulating the variations in evolutionary changes. Additionally, the T92 model helps determine the positioning and calculate the Pairwise genetic distances among the five varieties of *M. esculenta* and other species.

Based on the data from the observation of the ITS sequence in Figure 8, it is separated between *M. esculenta* species and *C. aconitifolius*. However, these two species have morphological similarities. *C. aconitifolius* does not produce tubers and is in a different genus. Based on the phylogenetic tree, *M. esculenta* and *C. aconitifolius* have a fairly distant relationship but are still in the same line of ancestors.

The analysis of the phylogenetic tree constructed from the ITS sequence reveals the relationships among five varieties of *M. esculenta*. The varieties—*M. esculenta* (M), *M. esculenta* (KP), *M. esculenta* (MH), and *M. esculenta* (HM)—all cluster on the same branch, exhibiting a bootstrap value of 100%. Two other varieties, *M. esculenta* (AB441756.1), originating from Bandung, and *M. esculenta* (MK114633.1), from Sri Lanka, occupied different branches but remained within the same ancestral lineage with a bootstrap value of 99%. The variety *M. esculenta* (U) had significant differences, with the most extensive branching compared to the other four varieties, and it had a bootstrap value of 99%. Additionally, *M. esculenta* (GU214953.1), from the USA, shows the furthest branching among all varieties, with a bootstrap value of 96%. According to Subari et al. (2021) and Vertiana et al. (2023), branch confidence is reliable if the bootstrap value exceeds 90%, while a value below 25% suggests the branch is not trustworthy. These findings indicate a very close relationship among the five tested varieties.

The Neighbor-Joining (NJ) method, utilizing the T92 + G model, effectively identifies *C. aconitifolius* as an outgroup. Analyzing the phylogenetic tree constructed from ITS sequences reveals groupings based on taxonomic relationships. Generally, these groupings can be categorized into three types: monophyletic, paraphyletic, and polyphyletic. In this case, the phylogenetic tree of the five *M. esculenta* varieties is classified as monophyletic, which indicates that all members of this group share a common ancestor. This suggests they have similar genetic traits or patterns, with only minor variations. In agricultural applications, phylogenetic tree analysis aids in selecting superior varieties based on their genetic relationships. The identification of superior varieties is determined by examining the relationship patterns in the phylogenetic tree, alongside factors such as genetic diversity and proximity, which can enhance resilience and productivity. Plant breeding typically involves combining phylogenetically more distantly related varieties to create superior offspring. This approach facilitates a more effective combination of genes that benefits both the plant and the environment.

Additionally, molecular identification of organisms using DNA barcoding can enhance accuracy in recognizing species with high morphological similarity, thus reducing identification errors based on morphology alone. While morphological identification is a crucial first step in understanding and classifying plants, it is generally more time-consuming than molecular methods. Therefore, conducting both morphological and molecular identifications simultaneously is recommended to achieve more precise and accurate results.

In conclusion, the research conducted in Boyolali District revealed variations in the physical traits of cassava plants. Five different varieties were studied for differences in young leaf color, stem characteristics, and tuber skin texture. Kinship analysis using NTSYSpc indicated that the KP and HM varieties share a 100% similarity, while both these varieties show a notable difference of 34% from the U variety. A phylogenetic tree constructed using MEGA 11 software demonstrated a stable structure and high genetic similarities among the varieties. These findings are valuable for future agricultural development efforts, particularly in creating improved cassava varieties and documenting plant genetic resources.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the people of Boyolali District, especially the cassava farmers who have shared information about cassava plants. We would also like to thank those who helped during the field visit.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 264-275 DOI: 10.13057/asianjagric/g090128

Rice field soil quality assessment through various environmental diversity sources in Ende District, East Nusa Tenggara, Indonesia

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Manuscript received: 30 January 2025. Revision accepted: 26 May 2025.

Abstract. *Mutiara C, Bolly YY, Hutubessy JIB, Aggrey H, Dahiba S, Palelet EY, Romadhon MR, Irmawati V, Hasanah K. 2025. Rice field soil quality assessment through various environmental diversity sources in Ende District, East Nusa Tenggara, Indonesia. Asian J Agric 9: 264-275.* Ende District, located in East Nusa Tenggara, Indonesia, is an important agricultural area known for its diverse food commodities, particularly rice. Despite ongoing rice cultivation, local production satisfies less than 50% of the community's needs, necessitating substantial imports from outside the region. Wewaria Sub-district, with 1,526.4 hectares of rice fields, is the largest rice-producing area in the district and the sole source of premium-quality rice. However, rice productivity in Ende remains low due to constraints such as suboptimal seed quality, inadequate infrastructure, and poor agronomic management. This study highlights the crucial influence of soil quality on rice yields and investigates both the physical and chemical characteristics of soil in Wewaria. A descriptive exploratory survey was conducted, focusing on environmental variability in relation to drainage patterns, altitude, and slope gradients. Land mapping units were delineated using thematic maps, Geographic Information System (GIS) tools, and soil sampling to determine representative locations for analysis. Preliminary assessments revealed key limitations, including nutrient deficiencies, low soil pH, and limited availability of potassium and nitrogen—factors that significantly restrict productivity. The findings emphasize the necessity of targeted soil quality assessments to guide sustainable agricultural practices, improve rice yields, and address critical challenges faced by local farmers. This research seeks to provide a comprehensive analysis of soil conditions in Wewaria to inform the development of effective strategies for enhancing rice production and strengthening regional food security.

Keywords: Agricultural sustainability, food security, soil fertility, soil health, Wewaria Sub-district

INTRODUCTION

Ende District, located in East Nusa Tenggara, Indonesia, is known for its diversity of food commodities, with rice being one of the most widely consumed staples among the local population. Despite being a rice-producing area, local production in Ende satisfies less than 50% of the community's needs, with the remainder sourced from outside the region (Indonesian Central Agency of Statistics 2021). The district comprises a total of 4,264.4 hectares of rice fields, including 3,268 hectares of irrigated fields and 996.4 hectares of rainfed fields. Among these, Wewaria Sub-district accounts for 1,526.4 hectares—approximately 35.79% of the total area (Ende District Agricultural Service 2021). It not only has the most extensive rice field coverage in the district but also serves as the sole source of premium-quality rice. In view of this, the local government has proposed to designate Wewaria as a central food production zone.

From 2018 to 2020, rice productivity in Ende District ranged between 3 and 4 tons per hectare (Indonesian Central Agency of Statistics 2021). Interviews with agricultural extension workers and local farmers in Wewaria indicate that achieving yields close to 4 tons per hectare is difficult and generally considered suboptimal. According to productivity classifications, yields below 5 tons per hectare are categorized as low, 5-6 tons as medium, and above 6 tons as high (Indonesian Central Agency of Statistics 2007). Numerous factors affect both rice availability and field productivity. In response, the district government undertook a preliminary study in 2021 to assess the rice sector in Wewaria, identifying issues related to seed pricing, availability of quality seeds, inadequate infrastructure, and farmer group management (Fatima et al. 2022). However, soil conditions—an essential factor in rice productivity—remain insufficiently analyzed.

Soil characteristics significantly influence the quantity and quality of crop production (Mujiyo et al. 2022). Variations in the physical and chemical properties of soil can directly impact rice yield outcomes (Pratama et al. 2024). Moreover, soil attributes are intrinsically linked to agricultural performance (Herawati et al. 2024). In Wewaria, rice cultivation typically lacks organic inputs, with inorganic fertilizers applied at rates of 250-300 kg per hectare. Theoretically, such application rates should support yields exceeding 5 tons per hectare (Hasnain et al. 2020). However, persistent low productivity indicates underlying constraints associated with soil quality.

Assessing soil quality provides a pathway to enhance productivity by identifying critical limitations (Surendran et al. 2021). Dossou-Yovo et al. (2020) reported that potassium and total nitrogen availability are key constraints in rice cultivation. Similarly, Michael (2021) identified common issues such as nutrient deficiencies, low soil pH, and phosphorus fixation. Targeted soil quality research enables the formulation of site-specific strategies to address such constraints. For example, the combined application of nitrogen fertilizer and rice straw can improve both yield and soil health (Zhang et al. 2021). Moreover, conservation practices such as no-tillage and organic farming systems have demonstrated superior outcomes in rice productivity and soil quality compared to conventional methods (Denardin et al. 2020; Romadhon et al. 2024).

Despite these insights, no comprehensive studies have been conducted in Wewaria to evaluate the physical, chemical, and biological aspects of rice field soils. As a result, productivity improvement efforts remain fragmented and ineffective. Therefore, this study aims to assess the soil quality of rice fields in Wewaria Sub-district through a combination of field surveys, soil sampling, and laboratory analysis, thereby providing evidence-based strategies to improve rice productivity in the region.

MATERIALS AND METHODS

Research time and location

This study was conducted in Wewaria Sub-district, located within Ende District, East Nusa Tenggara Province, Indonesia (Figure 1). Geographically, Wewaria lies between 8°39′49.8″-8°28′18.3″ S and 121°47′46.7″-121°37′07.9″ E. As the most extensive rice-producing area in Ende, Wewaria holds considerable agricultural significance, particularly as a source of premium rice. The region encompasses approximately 1,526.4 hectares of rice fields, which are key to understanding local agricultural practices and production challenges. The research specifically focuses on evaluating soil and climatic conditions that influence rice productivity, with emphasis on pH levels, nutrient availability, and overall soil quality.

Research materials

Field activities were conducted in rice fields managed by Universitas Flores, which supports the local farming community in Wewaria. The primary environmental variables considered for analysis were drainage systems, altitude, and land slope. Two main types of rice field drainage systems—irrigated and rainfed—were identified (Figure 2). To delineate Land Mapping Units (LMUs), researchers overlaid the Rupa Bumi Indonesia base map with thematic maps representing environmental diversity across the study area. These included drainage type, elevation (Figure 3), and slope characteristics (Figure 4). The resulting land classifications (Table 1) informed the selection of sampling points, which were then georeferenced on the working map (Figure 5). Climatic data for the region were obtained from the Weather-Spark website (2024), which aggregates long-term climate data globally.

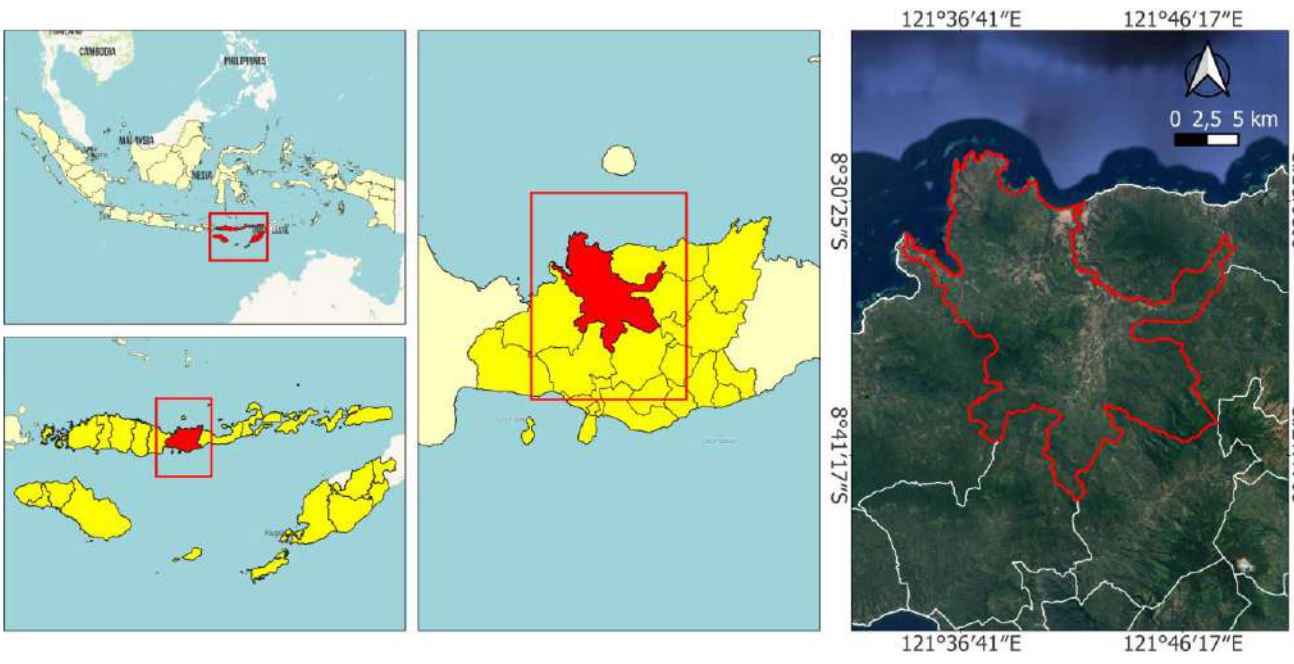

Figure 1. Map of the study site in Wewaria Sub-district, Ende District, East Nusa Tenggara Province, Indonesia

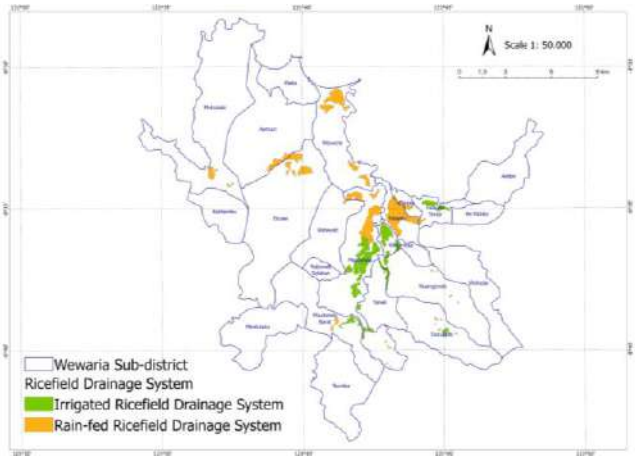

Figure 2. Map showing irrigated (green) and rain-fed (orange) ricefield drainage systems in Wewaria Sub-district, Lio, Indonesia

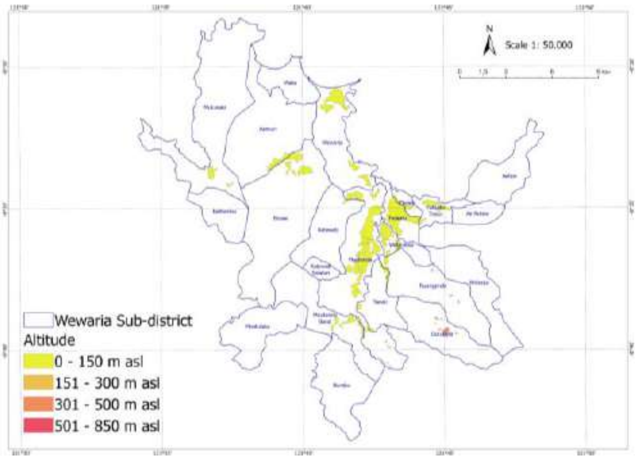

Figure 3. Map illustrating altitudinal variation (masl) across landscape in Wewaria Sub-district, Lio, Indonesia

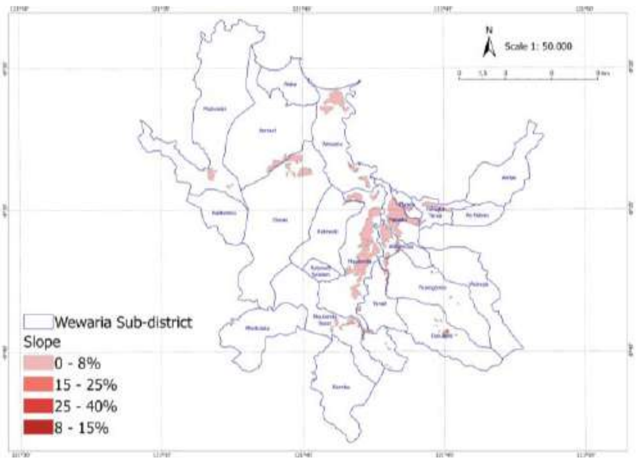

Figure 4. Map showing slope gradients across the landscape in Wewaria Sub-district, Lio, Indonesia

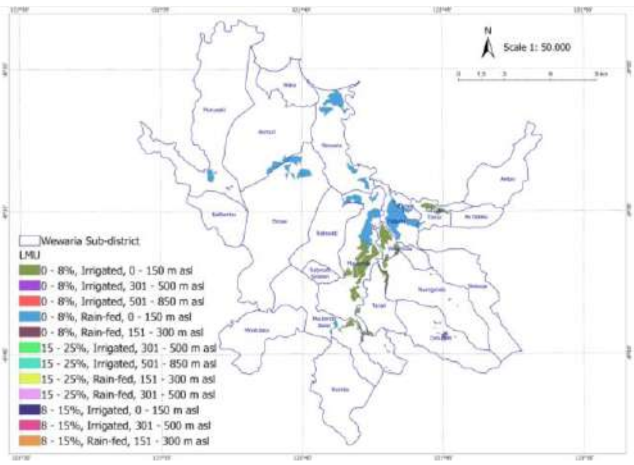

Figure 5. Map showing Land Mapping Unit (LMU) locations classified by slope, altitude, and ricefield drainage systems in Wewaria Sub-district, Lio, East Nusa Tenggara, Indonesia

Table 1. The Land Mapping Units (LMU) based on environmental diversity

LMU	Ricefield Drainage System	Altitude (masl)	Slope (%)
1	Irrigated	0-150	0-8
2	Irrigated	301-500	0-8
3	Irrigated	501-850	0-8
4	Rain-fed	0-150	0-8
5	Rain-fed	151-300	0-8
6	Irrigated	301-500	15-25
7	Irrigated	501-850	15-25
8	Rain-fed	151-300	15-25
9	Rain-fed	301-500	15-25
10	Irrigated	0-150	8-15
11	Irrigated	301-500	8-15
12	Rain-fed	151-300	8-15

Note: A total of 12 LMU types were each sampled three times (n=3), resulting in 36 soil samples for soil quality index observation

Research methods

A descriptive and exploratory research design was adopted to evaluate soil quality across various landscape units. The methodology involved both field-based observations and systematic soil sampling. Soil quality assessment was conducted using a scoring approach that integrated environmental parameters, morphological data, and laboratory analysis results (Table 2). A total of 12 LMUs were identified, with three replicate samples collected from each, resulting in 36 sampling points for Soil Quality Index (SQI) evaluation.

Research analysis on Soil Quality Index (SQI)

The Soil Quality Index (SQI) was determined using a combination of Principal Component Analysis (PCA) and the Minimum Data Set (MDS) approach, following the classification framework proposed by Cantú et al. (2007). PCA was utilized to statistically evaluate the laboratory-derived soil parameters (Table 2), allowing for dimensionality reduction by identifying the most influential variables—a method supported by prior studies (Nehrani et al. 2020; Zhan et al. 2020). Based on the PCA results, the MDS approach was then employed to select key indicators from each Principal Component (PC), ensuring minimal redundancy while preserving the dataset's integrity. The primary objective of this analysis was to uncover correlations among soil quality indicators that significantly contribute to SQI estimation. The distribution of the accumulated results of the SQI weighting that has been calculated is then classified based on the distribution of the total weight as an SQI class criteria presented in Table 3. Correlation analyses were conducted to assess the relationships between individual soil quality parameters and the resulting SQI values, as shown in Figure 13.

$$\text{SQI} = \sum_{i=1}^{n} 1 = Wi \times Si^{n}$$

Where: SQI = Soil quality index; Wi = Weighting index; Si = Score index; n = Number of soil quality indicators.

Data analysis

The soil quality parameters obtained from different cultivation systems were subjected to Analysis of Variance (ANOVA) to assess the impact of varying paddy field cultivation practices on the Soil Quality Index (SQI). When significant differences were detected, a Duncan's Multiple Range Test (DMRT) was conducted to determine pairwise differences in SQI values across cultivation systems. Furthermore, Pearson correlation analysis was employed to evaluate the relationships between SQI and various environmental factors, thereby enabling a clearer understanding of the associations between individual parameters and the overall index. This statistical analysis provided a foundation for identifying key interrelationships and determining the principal factors influencing soil quality under different agricultural management practices.

RESULTS AND DISCUSSION

Analysis of SQI

The results of the Principal Component Analysis (PCA) are presented, showing the contribution of eight principal components (PC1 to PC8) to the total variance (Table 4). The first principal component (PC1) accounted for 15.3% of the total variance and is most strongly associated with Base Saturation (BS), active pH (pH-act), and Bulk Density (BD). The second Principal Component (PC2) explained 14.8% of the variance and included aluminum (Al), iron (Fe), and potassium (K). The third component (PC3) contributed 11.7% to the total variance, incorporating phosphorus (P), electrical conductivity (EC), and water content (WC). The fourth component (PC4) explained 9.5% of the variance and was most strongly associated with penetration resistance (PRdx). The fifth component (PC5) accounted for 8.1% of the variance and comprised manganese (Mn) and Particle Density (PD). The sixth component (PC6), contributing 7.7% of the variance, included the carbon-to-nitrogen ratio (C/N) and Cation Exchange Capacity (CEC). The seventh principal component (PC7) accounted for 7.1% of the variance and consisted of carbon biomass (CMB). The eighth principal component (PC8) explained 5.3% of the variance and included Psty, potentiometric pH (pH-pot), nitrogen (N), and carbon (C).

Table 3. SQI class criteria

Value	Class criteria
0.80 - 1.00	Very high
0.60 - 0.79	High
0.40 - 0.59	Medium
0.20 - 0.39	Low
0.00 - 0.19	Very low

Source: Cantú et al. (2007)

Table 2. Soil Quality Index (SQI) scoring criteria: Limiting factors and corresponding relative scores (1-5) for physical, chemical, and biological soil indicators used to assess land suitability

Indicators		Limiting factors and relative scores				
		1	2	3	4	5
Physical	Particle density (g/cm^3)	>1.6	1.5-1.6	1.4-1.5	1.3-1.4	<1.3
	Bulk density (g/cm^3)	>1.5	1.4-1.5	1.3-1.4	1.2-1.3	<1.2
	Porosity (%)	>20	18-20	15-18	10-15	<10
	Water content (%)	>30	<2	2-8	9-20	20-30
	Electrical conductivity (dS/m)	<3	3-5	5-7	7-10	>10
Chemical	pH level	<5; >8.2	5.0-5.4; 7.8-8.2	5.4-5.8; 7.4-7.8	5.8-6.0; 7.0-7.4	6.0-7.0
	Total-N (%)	<0.1	0.1-0.2	0.21-0.5	0.51-0.75	>0.75
	Available-P (ppm)	<10	10-15	16-25	16-35	>35
	Available-K (mg/100g)	<0.1	0.1-0.2	0.3-0.5	0.6-1	>1
	Organic-C (%)	<0.5	0.5-1	1-3	3-5	5-10
	C/N ratio	<5	>25	16-25	5-10	11-15
	Cation Exchange Capacity (me/100g)	<5	5-16	17-24	25-40	>40
	Base saturation (%)	<20	21-30	31-50	51-70	>70
	Potential redox (mV)	0-100	101-200	201-300	301-400	>400; <(-100)
	Al (ppm)	>50	40-50	35-40	20-35	<20
	Fe (ppm)	>53	53-19	19-5	5-3	<3
	Mn (ppm)	>20	15-20	10-15	5-10	<5
Biological	C-Microbes Biomass (µg/g)	>25	20-25	10-20	5-10	<5

Source: Indonesian Center for Research and Development of Agricultural Land Resources (2024)

Table 4. Principal Component Analysis (PCA) results for each Soil Quality Indicator (SQI). The table presents eigenvalues, proportions of explained variance, cumulative variance, and the loadings of each SQI variable on the first eight principal components (PC1-PC8). Values marked with an asterisk (*) indicate the highest loading for each variable, contributing to the Minimum Data Set (MDS) selection

Eigenvalue	2,9109	2,8137	2,2222	1,7975	1,5347	1,4554	1,3401	1,0005
Proportion	0,153	0,148	0,117	0,095	0,081	0,077	0,071	0,053
Cumulative	0,153	0,301	0,418	0,513	0,594	0,670	0,741	0,793
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
PD	0,199	0,076	0,243	0,095	***0,461**	0,112	0,004	-0,414
BD	***0,244**	0,124	-0,179	-0,110	-0,223	-0,380	-0,211	-0,029
Psty	0,009	0,173	-0,380	0,158	-0,239	0,200	-0,035	***0,454**
WC	-0,105	-0,146	***0,301**	-0,286	-0,412	-0,036	0,178	-0,077
EC	0,274	-0,093	***0,355**	-0,017	-0,161	0,348	0,045	0,180
pH-act	***0,409**	-0,048	-0,021	0,053	0,084	0,274	-0,280	0,034
pH-pot	0,280	-0,160	-0,016	0,238	-0,037	0,232	-0,019	***0,302**
N	-0,145	0,215	0,389	-0,060	0,164	-0,293	-0,187	***0,459**
P	0,089	-0,187	***0,447**	-0,212	-0,131	0,192	0,076	0,093
K	0,073	***0,466**	0,092	-0,173	0,176	0,037	0,217	-0,052
C	-0,254	0,091	-0,046	-0,491	0,282	0,128	-0,115	***0,334**
C/N	-0,087	-0,115	-0,381	-0,393	0,080	***0,420**	0,055	-0,196
CEC	-0,395	0,051	0,098	0,186	-0,266	***0,298**	-0,096	-0,133
BS	***0,468**	-0,102	-0,083	-0,092	0,032	-0,246	0,197	0,070
PRdx	-0,198	-0,067	0,129	***0,416**	0,188	0,059	-0,363	-0,019
Al	0,082	***0,494**	0,050	-0,024	-0,118	0,128	-0,148	-0,117
Fe	0,152	***0,495**	0,040	0,009	-0,046	0,241	0,072	0,035
Mn	-0,084	-0,164	-0,058	0,105	***0,424**	0,079	0,397	0,284
CMB	-0,105	0,194	0,011	0,331	-0,113	-0,032	***0,610**	0,009

Note: PD: Particle Density; BD: Bulk Density; Psty: Porosity; WC: Water Content; EC: Electrical Conductivity; pH-act: Actual pH; pH-pot: Potential pH; N: Total Nitrogen; P: Available Phosphorus; K: Available Potassium; C: Organic Carbon; C/N: Carbon/Nitrogen Ratio; CEC: Cation Exchange Capacity; BS: Base Saturation; PRdx: Potential Redox; Al, Fe, Mn: Aluminum, Iron, and Manganese concentrations, respectively; CMB: Carbon Microbial Biomass

The influence of these parameters on the SQI is illustrated in Figure 6. These indicators displayed considerable weights and positive intercorrelations, indicating their mutual relevance to soil quality. Despite these correlations, the selection of a single indicator from each principal component ensures that the indicators used in MDS are independent within the PCA grouping framework. Consequently, all 19 indicators were retained for MDS construction. Each MDS indicator was assigned a weight index (Wi), calculated by dividing the variance explained by its respective principal component by the cumulative variance of all selected components. The SQI was subsequently derived by multiplying each indicator's Wi value by its normalized MDS score. The final computed SQI values are depicted in Figure 6.

The spatial analysis of the Soil Quality Index (SQI) revealed two distinct soil quality classes—low and medium—across the Land Mapping Units (LMUs) of the study area. The low SQI class dominates LMUs 1-19 and 23-25, particularly concentrated in the northern and eastern regions of the Wewaria Sub-district. In contrast, medium SQI values are primarily observed in LMUs 20-22 and 26-36, which are situated in the northern sector of the region (Figure 7). Overall, the findings suggest that soil quality across the study area is predominantly low, with limited zones of moderate quality. This uneven distribution highlights a critical need for targeted interventions to improve soil health, especially in low-SQI regions.

The primary objective of the study was to determine the underlying factors influencing the variability in SQI across different LMUs. Utilizing statistical techniques, particularly Analysis of Variance (ANOVA), the study identified several significant contributors to the observed differences in soil quality. Among these, the type and efficiency of rice field drainage systems emerged as a critical determinant. Inadequate drainage can lead to excess soil moisture, reduced aeration, salt accumulation, and degradation of soil structure—conditions that severely impair root function and nutrient uptake (Adelana et al. 2022). Additionally, topographic slope was found to significantly influence SQI variation. Steeper slopes accelerate runoff and erosion, increasing the risk of nutrient loss and further reducing soil productivity (Rosskopf et al. 2020).

The interplay between natural factors (such as slope and rainfall) and anthropogenic influences (notably land management practices) plays a defining role in shaping soil quality. The differentiation between low and medium SQI classes reflects this interaction, emphasizing the importance of site-specific soil conservation strategies (Tian et al. 2022). The robustness of the ANOVA framework enabled the validation of these relationships (Table 5), supporting the conclusion that integrated soil management approaches—including improved drainage infrastructure and erosion control—are essential to elevate soil quality in vulnerable regions (Martín-Sanz et al. 2022).

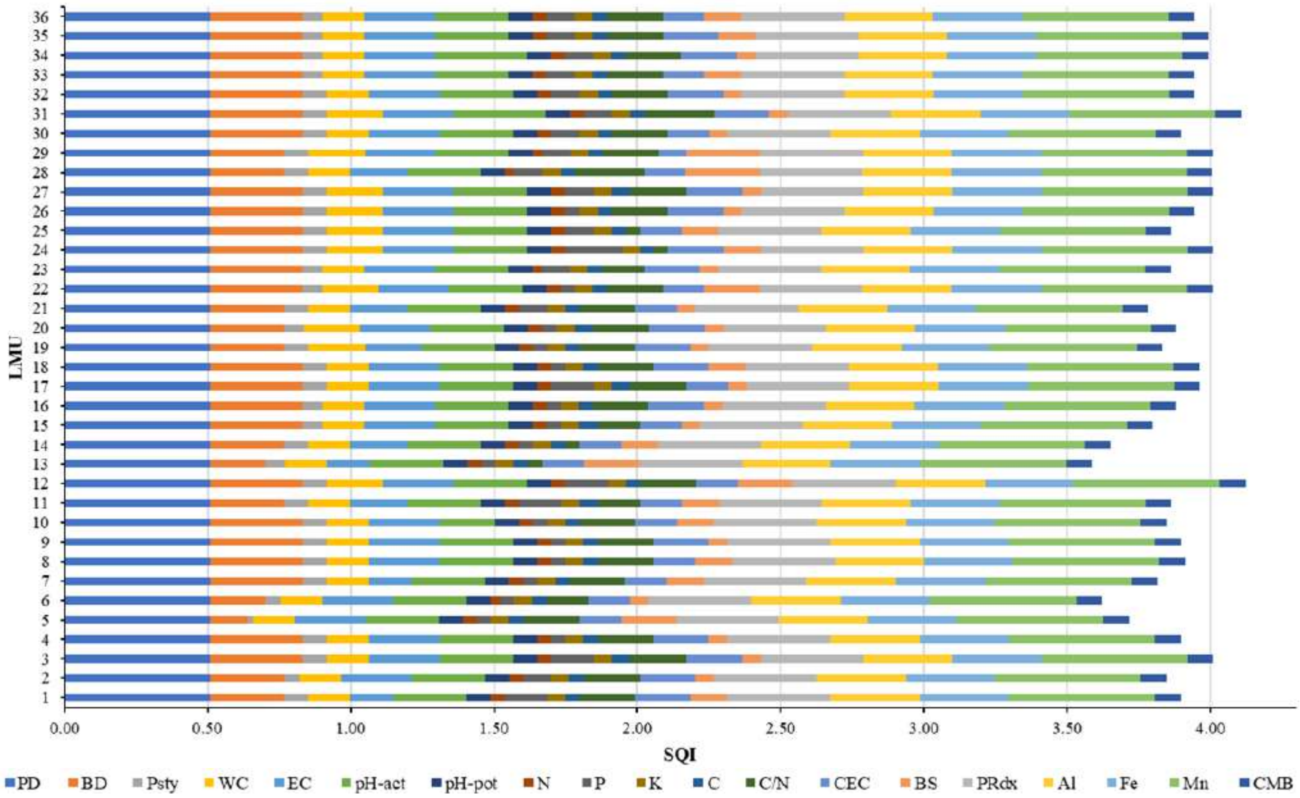

Figure 6. Stacked bar chart of Soil Quality Index (SQI) across Land Mapping Units (LMUs), showing contributions of individual indicators

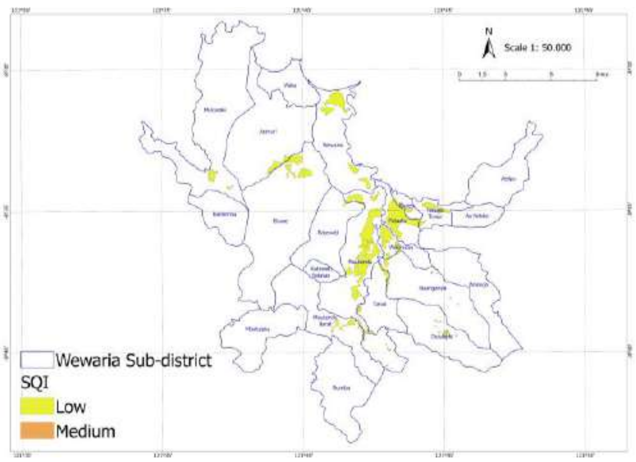

Figure 7. Spatial distribution of Soil Quality Index (SQI) classes (low and medium) across Wewaria Sub-district

Table 5. The ANOVA result of environmental diversity on SQI

Environmental diversity	Ricefield drainage systems	Altitude	Slope
SQI	0.000**	0.472ns	0.007**

Note: ns: non significant; *: significant; **: very significant (ns>0.05; *<0.05; **<0.01)

Furthermore, the study highlights the contrasting challenges faced by drainage-based and rainfed rice cultivation systems. In drainage-based systems, persistent waterlogging and salinization constrain oxygen availability and limit nutrient uptake, leading to poor plant performance. As reported by Devkota et al. (2022), ineffective drainage infrastructure intensifies salinity stress, ultimately reducing soil fertility and rice yields. On the other hand, rainfed rice systems are highly susceptible to intermittent moisture stress, which promotes soil erosion and nutrient depletion. According to Majumdar et al. (2023), fluctuations in soil moisture negatively impact soil microbial dynamics and structure, further diminishing its capacity to support crop growth.

These findings reinforce the necessity for tailored soil management strategies for both cultivation systems. Addressing the site-specific drivers of degradation is crucial to maintaining long-term soil functionality and agricultural sustainability in rice-based agroecosystems. The slope of a paddy field exerts a critical influence on soil fertility and overall quality. Steeper slopes are typically associated with increased erosion, reduced water retention, nutrient leaching, and compaction—factors that collectively impair soil fertility (Parvez et al. 2024). In contrast, flatter terrains offer more favorable conditions for rice cultivation, including improved moisture retention, enhanced nutrient stability, and higher accumulation of organic matter. To address the adverse effects of slope gradients, site-specific management interventions such as erosion control, water regulation, and the application of soil amendments are essential for maintaining long-term soil health and productivity (Dewi et al. 2022).

Results from the Duncan's Multiple Range Test (DMRT) comparing irrigation systems in relation to Soil Quality Index (SQI) reveal that rainfed rice fields exhibit significantly higher SQI values than other systems (Figure 8). The lowest SQI values were recorded in irrigated rice fields, particularly those utilizing the Irrigation Cultivation Technique (IKT). Across all cultivation methods, statistically significant differences in SQI were observed, with the rainfed system outperforming the irrigation-based system by 2.63%. Al Viandari et al. (2022) reported that rainfed rice fields maintain sufficient water availability to support optimal nutrient cycling, a condition further enhanced by the sustained application of appropriate agro-technologies. Furthermore, Ray and Chakraborty (2021) observed a markedly higher diversity of soil organisms in rainfed fields compared to irrigated systems, suggesting enhanced biological activity under natural rainfall regimes.

Rainfed rice cultivation supports greater microbial diversity than irrigated systems, largely due to ecologically dynamic soil environments (Liu et al. 2022). Natural rainfall induces periodic fluctuations in soil moisture, which foster microbial richness by supporting diverse physiological groups (Jiang et al. 2021). Additionally, rainfall introduces external organic inputs, enriching substrates available to soil microorganisms. Temperature and aeration conditions in rainfed systems also vary more frequently, creating favorable conditions for aerobic microbial communities. In contrast, the prolonged waterlogged conditions typical of irrigated rice fields often create anaerobic environments, thereby suppressing microbial diversity (Majumdar et al. 2021). Moreover, the intensive use of chemical inputs in irrigated systems can diminish soil microbial populations, further limiting community complexity. Therefore, differences in water management practices, moisture variability, and organic matter dynamics play a key role in shaping microbial community structures across rice cultivation systems (Muhammad et al. 2022).

Slope-related processes also significantly impact soil fertility and quality, as illustrated in Figure 9. Steep slopes experience more severe soil erosion, leading to the loss of topsoil and associated nutrients and organic matter, which directly reduces soil fertility (Arunrat et al. 2022). Rapid surface runoff on such slopes also limits water infiltration, compromising moisture availability for crop uptake. In contrast, gentler slopes facilitate higher infiltration rates, promoting soil moisture retention and the accumulation of surface organic matter, both of which enhance soil fertility. Additionally, better drainage on flatter terrains improves aeration, thereby stimulating microbial activity crucial for organic matter decomposition and nutrient cycling (Ahmad et al. 2020). Soils on steep slopes are often more degraded, coarser in texture, and less capable of retaining water and nutrients. Consequently, steeper landscapes pose greater challenges for maintaining soil fertility, whereas flatter areas offer more favorable conditions for sustainable soil and crop management (Derakhshan-Babaei 2021).

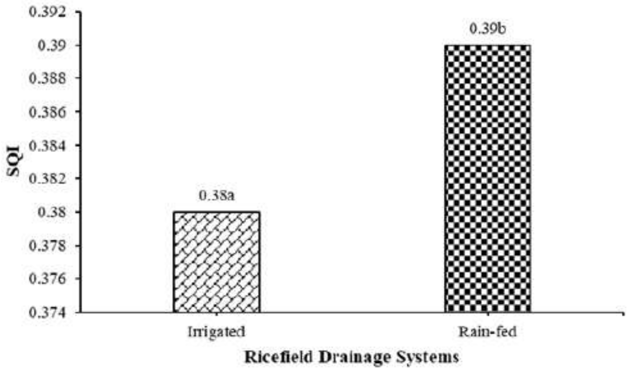

Figure 8. Effect of ricefield drainage systems on SQI based on DMRT. Different letters indicate significant differences ($p < 0.05$)

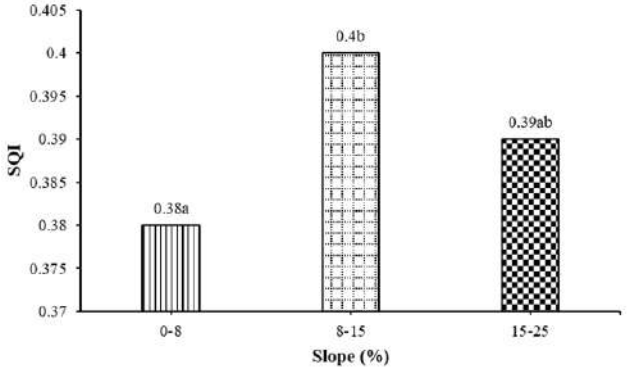

Figure 9. Effect of slope (%) on SQI based on DMRT. Different letters denote significant differences ($p < 0.05$)

Examination of climate conditions and their connection to SQI

Ende, located in East Nusa Tenggara, is characterized by a tropical savanna climate (Aw) based on the Köppen climate classification (Figure 10). This region displays distinct seasonal patterns, with a marked wet season extending from November to April and a dry season from May to October. Temperatures remain relatively stable throughout the year, with daily averages ranging from 23 to 33°C. Rainfall is moderate, peaking between December and March. These climatic conditions are shaped by the region's geographic and topographic characteristics, which contribute to microclimatic variations that may influence Soil Quality Index (SQI) dynamics.

Rainfall in Ende District, East Nusa Tenggara, exhibits marked seasonal and interannual variability. Over the past five years (2020-2024) (Figure 11), the rainy season typically spans approximately five months, beginning around November 16 and extending to about April 17, during which the probability of rainfall exceeds 29%. February is generally the wettest month, with an average of 15 rainy days. The district receives an average annual rainfall of 1,129 mm, with the majority occurring between November and April. Notably, historical data indicate significant fluctuations in annual precipitation, with a high of 1,439 mm recorded in 2017 and a low of 568.51 mm in 2022.

Temperature variation in the region is minimal throughout the year. The diurnal temperature amplitude is approximately 6.0°C, with maximum daytime temperatures reaching 33°C and nighttime lows averaging 23°C. However, rainfall distribution across Ende District may vary locally due to geographic and topographic heterogeneity, which can influence microclimatic conditions and, in turn, affect soil moisture and quality.

Monthly air humidity data for Ende District over the past five years (2020-2024) reveal considerable temporal variability. Minimum, maximum, and average humidity levels for Ende City have been consistently recorded and are accessible through official sources such as the Indonesian Central Agency of Statistics (2022) and Weather-Spark (2024). These humidity patterns are influenced by a range of geographic, topographic, and seasonal factors, which contribute to localized microclimatic conditions. Given the complexity and variability of these factors, researchers are encouraged to reference the most recent datasets and perform detailed analyses to gain an accurate and comprehensive understanding of the region's climatic profile (Figure 12).

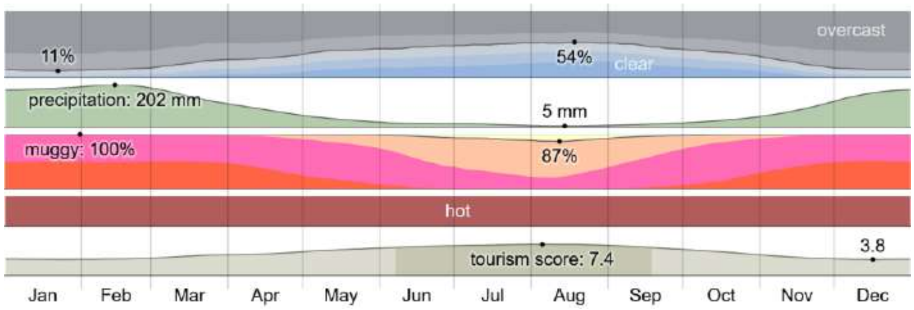

Figure 10. Monthly climate trends in Ende from 2020 to 2024, showing variation in cloud cover, precipitation, humidity, temperature, and tourism score

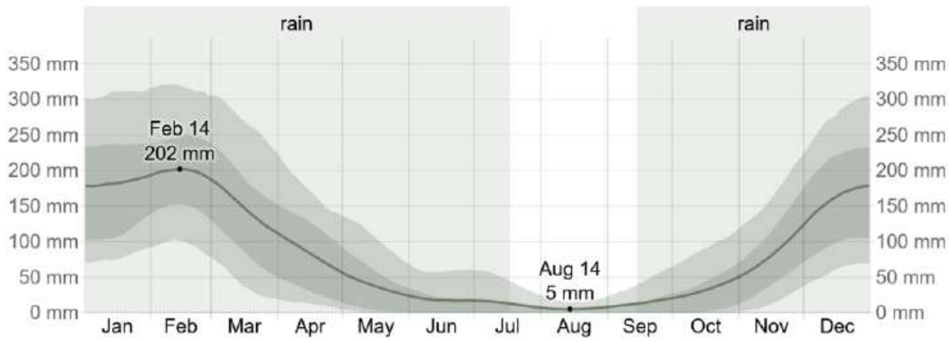

Figure 11. Average monthly rainfall in Ende in the past 5 years (2020-2024)

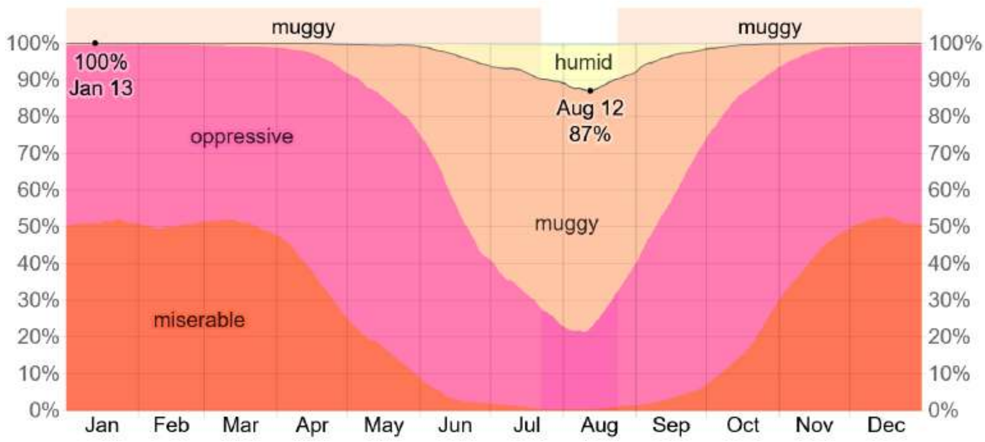

Figure 12. Average monthly humidity comfort levels in Ende in the past 5 years (2020-2024)

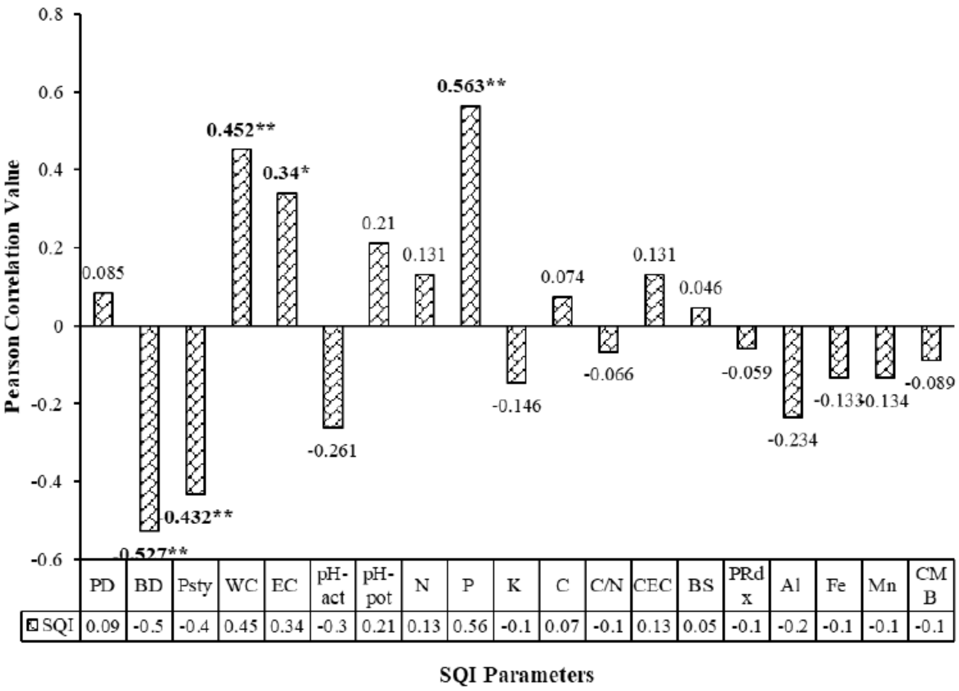

Figure 13. Pearson correlation analysis of various soil parameters with the Soil Quality Index (SQI). Note: PD: Particle Density; BD: Bulk Density; Psty: Porosity; WC: Water Content; EC: Electrical Conductivity; pH-act: actual pH level; pH pot: potential pH level; N: Total-N; P: Available-P; K: Available-K; C: Organic-C; C/N: C/N ratio; CEC: Cation Exchange Capacity; BS: Base Saturation; PRdx: Potential Redox; CMB: C-Microbes Biomass; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

The SQI determining factors analysis

A correlation analysis was conducted to determine the relationships between various soil physicochemical parameters and the Soil Quality Index (SQI), a key indicator for evaluating overall soil health (Figure 13). The analysis identified five key parameters that significantly influence SQI: bulk density, porosity, water content, Electrical Conductivity (EC), and available phosphorus (available-P).

Among these, water content ($r = 0.452$, $p<0.01$), electrical conductivity ($r = 0.340$, $p<0.05$), and available-P ($r = 0.563$, $p<0.01$) exhibited significant positive correlations with SQI. Adequate water content enhances microbial activity and nutrient mobility, while moderate EC levels reflect a favorable ionic balance that supports nutrient availability and plant health (Alkharabsheh et al. 2021). Available phosphorus, an essential macronutrient, directly contributes to plant growth and soil biological function, and its higher concentration was associated with better soil quality (Singh et al. 2020).

In contrast, bulk density ($r = -0.527$, $p < 0.01$) and porosity ($r = -0.432$, $p<0.01$) showed significant negative correlations with SQI. High bulk density typically indicates soil compaction, which restricts air and water movement, root penetration, and microbial activity—key elements of soil health (Hu et al. 2021; Samaei et al. 2022). Similarly, low soil porosity is associated with poor soil structure and reduced capacity for water and nutrient retention (Hafeez et al. 2022; Mondal and Chakraborty 2022).

These findings emphasize the importance of maintaining balanced soil physical and chemical properties for sustainable soil management. Ensuring optimal moisture levels, salinity, nutrient availability, and soil structure can improve SQI and enhance agricultural productivity. The identification of these five key determinants—bulk density, porosity, water content, EC, and available-P—offers valuable insights for targeted interventions to maintain or restore soil health in tropical savanna agroecosystems (Maharjan et al. 2020; Selim 2020).

Discussion

Improving soil health is a multifaceted challenge that requires addressing both direct soil quality indicators and broader agronomic constraints that can adversely affect crop productivity. This study identifies low phosphorus (P) availability as a significant limiting factor in the paddy fields of Wewaria Sub-district, where phosphorus levels range from low to deficient. Given phosphorus's essential role in plant development, its deficiency can significantly hinder rice growth unless corrected through targeted fertilization strategies (Kurniawan et al. 2023).

To address phosphorus limitations, integrating organic fertilization methods is recommended. Organic matter enhances P availability by competing for adsorption sites and facilitating the mineralization of organic phosphorus into plant-accessible forms. Rice husk biochar, in particular, has shown promise in augmenting soil P levels and improving the availability of other macronutrients like potassium (Pogorzelski et al. 2020). The synergistic use of biochar and organic fertilizers is thus considered a best management practice for optimizing nutrient availability.

Additionally, natural rock phosphate can serve as a long-term phosphorus source through gradual weathering and mineralization. In cases of acute phosphorus deficiency, the application of inorganic phosphorus fertilizers may be warranted, though care must be taken to apply them within agronomic recommendations to prevent nutrient imbalances (Amar et al. 2022).

Combining organic and inorganic fertilizers has been shown to yield superior outcomes in both productivity and soil health. Roba (2018) and Qaswar et al. (2020) found that balanced fertilization regimes significantly improve soil fertility and crop yields. Similar findings by Mahmood et al. (2017) and Mi et al. (2018) indicated that the co-application of NPK and compost not only enhances rice yields but also improves key soil parameters such as pH and phosphorus content. A study by Murnita and Taher (2021) recommends an optimal ratio of 25% NPK and 75% organic fertilizer, which yielded 8.05 tons/ha of rice and improved soil chemical properties, including available phosphorus (86.56 ppm), organic carbon (3.33%), and total nitrogen (0.21%).

In addition to soil amendments, incorporating biological agents is a promising approach. Organic inputs fuel microbial activity by supplying carbon, thereby enhancing soil biological processes (Cahyani et al. 2023). In particular, Arbuscular Mycorrhizal Fungi (AMF) significantly improve phosphorus uptake by extending root access to otherwise unavailable nutrient pools and releasing organic acids that mobilize P (Raymond et al. 2021). AMF also improve soil aggregation and water retention by producing glomalin, a glycoprotein that stabilizes soil structure (Holátko et al. 2021).

The organic farming system emerged as the most effective cultivation practice for improving soil health in this study. Compared to conventional and semi-organic systems, organic agriculture enhances soil aggregation, boosts microbial activity, and elevates soil organic carbon levels—contributing to better nutrient cycling and overall soil fertility (Yang et al. 2020; Yadav et al. 2021). These improvements create a more favorable environment for plant growth, ultimately supporting higher yields and long-term agricultural sustainability.

Climate also plays a critical role in rice farming systems in Ende, which is characterized by a tropical savanna climate (Aw). The distinct wet (November-April) and dry (May-October) seasons influence both irrigated and rain-fed rice cultivation practices. Adequate rainfall during the wet season supports rice growth, while the dry season necessitates efficient water management, especially in irrigated systems (Bose et al. 2024). In rain-fed systems, the timing and reliability of rainfall are crucial for crop success, underscoring the importance of climate-adaptive planning (Bell et al. 2022).

Irrigated rice systems benefit from consistent water availability during the wet season, with stable temperatures (23 to 33°C) and high humidity fostering optimal growth. However, evapotranspiration increases during the dry season, requiring effective irrigation strategies to mitigate water loss (Pizarro et al. 2022). Rain-fed systems, in contrast, rely heavily on seasonal rainfall patterns, with an average annual precipitation of 1,129 mm—peaking between December and March. Strategic planting schedules aligned with these patterns are vital for ensuring adequate water availability and avoiding crop failure (Mugi-Ngenga et al. 2023; Yan et al. 2023).

In conclusion, this study reveals that soil nutrient deficiencies, particularly phosphorus, potassium, and nitrogen, along with low soil pH, are major constraints to rice productivity in Wewaria Sub-district. Soil quality assessment based on the Soil Quality Index (SQI) classified the region into low and medium SQI categories, with variations attributed to slope and drainage conditions. Five critical SQI parameters were identified, highlighting the need for a comprehensive soil management strategy. To address these challenges, a holistic and integrated approach is required, which includes, combining organic and inorganic fertilization for balanced nutrient supply, leveraging biochar and rock phosphate for long-term soil fertility, incorporating biological agents like mycorrhizal fungi to enhance nutrient uptake, transitioning towards organic farming systems for sustainable soil and crop productivity, and aligning agronomic practices with climatic conditions to optimize water use and planting cycles.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Directorate of Research, Technology, and Community Service, Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, for the research grant support in 2024 (Grant No. SP DIPA - 023.01.2.723023/2024). Appreciation is also extended to the *Lembaga Penelitian dan Pengabdian Masyarakat*, Universitas Flores, the Ende District Government, and the Wewaria Sub-district authorities for their invaluable support and cooperation during the implementation of this research.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 276-287 DOI: 10.13057/asianjagric/g090129

The effect of fermented cacao pod husk using *Phanerochaete chrysosporium* on nutritive value, digestibility, fermentation parameters, and methane emissions

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Manuscript received: 1 March 2025. Revision accepted: 26 May 2025.

Abstract. *Pratama SM, Wajizah S, Wahyudi I, Ilham, Zulfahrizal, Munawar AA, Samadi. 2025. The effect of fermented cacao pod husk using* Phanerochaete chrysosporium *on nutritive value, digestibility, fermentation parameters, and methane emissions. Asian J Agric 9: 276-287.* This research aimed to study the effect of fermentation using *Phanerochaete chrysosporium* with different incubation times on nutritive value, digestibility, fermentation parameters, and methane emissions of cacao pod husk. The treatments tested were: F0: without incubation; F1: 7 days of incubation time; F2: 14 days of incubation time; F3: 21 days of incubation time and F4: 28 days of incubation time. The variables observed were the quality of nutritional value, fiber fraction, in vitro rumen digestibility, gas production and methane gas concentration. The results showed that the treatment increased the crude protein value, reduced crude fiber and increased the Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) values as the incubation period increased. Treatment effectively increased digestibility with the highest obtained at F4 and the lowest at F1. The treatment also increased the total ammonia (NH3-N) and Volatile Fatty Acid (VFA) concentrations in the rumen with a pH that was still within normal levels. The total concentration of VFA or NH3 in F4 was higher than in other treatments. The gas production value and methane gas concentration can be reduced by *P. chrysosporium* treatment with an incubation time of up to 28 days. It was concluded that the nutritional quality and digestibility of cocoa pod husk could be improved but were still relatively low, even though the pH, NH3-N and VFA Total values were in the normal range.

Keywords: Cocoa pod husk, incubation time, in vitro digestibility, methane, *Phanerochaete chrysosporium*

INTRODUCTION

The supply of components for animal feed has become more scarce recently. This is caused, among other things, by the decreasing amount of land available for development and production of forages and local food plants such as natural field grasses and legumes as a result of habitation and land use for agriculture. In order to lessen dependence on the usage of conventional feed, it is required to explore potential new resources that might be used as alternative diets in place of all or part of the forage. One such solution is to use leftover food crops or plantation crops (Samadi et al. 2016). According to Edo et al. (2023), cocoa *(Theobroma cacao* L.) is one of the plantation commodities that produces a significant amount of biomass or by-products. After the Ivory Coast and Ghana, Indonesia is the world's third-largest cocoa producer (Witjaksono and Asmin 2016). In 2022, the total land of cocoa plantations in Indonesia was estimated to be about 1.5 million hectares with a total cocoa production of 667.300 tons (Badan Pusat Statistik 2023). Considering that the cocoa pod husk makes up roughly 75% of the total cocoa fruit (Baidoo et al. 2022), Indonesia produced over 500.475 tons of cocoa pod husk in 2022.

Cocoa pod husk, commonly discarded as agricultural waste, can be repurposed through biotechnological processes, supporting the circular economy by reducing pollution and improving resource efficiency (Ouattara et al. 2021). Its potential use as animal feed could lower livestock production costs (Makinde et al. 2019). However, its high fiber (29.3%) and low protein (9.66%) content limits its nutritional value (Pratama et al. 2018). Additionally, it contains 12-19% lignin (DM), which is significantly higher than rice straw, making it less digestible (Laconi and Jayanegara 2015). Diets with 20-30% unprocessed cocoa pod husk reduce animal performance and digestibility in cattle, goats, and poultry (Ozung et al. 2022). Cocoa pod husk also contains theobromine (6.79 mg/100 g DM), which imparts a bitter taste, decreasing feed palatability and intake (Nguyen and Nguyen 2017; Alanon et al. 2016). High doses of theobromine may cause metabolic disturbances, such as muscle tremors, *tachycardia*, rapid breathing, and diarrhea. Therefore, to enhance its value as feed, cocoa pod husk must undergo physical, chemical, or biological treatment to improve its nutritional quality before being incorporated into animal diets (Laconi and Jayanegara 2015).

In an effort to improve the nutritional content of cocoa pod husk, fermentation bioconversion using white rot fungi appears to be a promising strategy, especially since the small to medium scale feed industry has great potential to adopt this technology (Syahrir et al. 2017; Yakin et al. 2020). *Phanerochaete chrysosporium*, a species of white rot fungi capable of decomposing lignin, was found to be beneficial in enhancing the nutritional content (crude fiber and crude protein) of cocoa pod husk, according to Laconi and Jayanegara (2015). According to Sun et al. (2022), *P. chrysosporium* is a microbe that can degrade lignocellulosic materials selectively. It first breaks down the lignin component before moving on to the cellulose component. The fungus employs cellulose and hemicellulose as carbon sources. This fungus can also develop at relatively low temperatures, with a temperature range of 36-40°C, making it acceptable for application in various fermentation processes that create heat (Cordero et al. 2017).

The fermentation process using *P. chrysosporium* is particularly advantageous because it targets the lignin content, which is the primary barrier to the digestibility of cocoa pod husk (Yan et al. 2022). By breaking down lignin, the fungus enhances the accessibility of cellulose and hemicellulose, which are more digestible and nutritious for livestock. This process not only improves the feed's nutritional profile but also increases its palatability, making it more acceptable to animals. Furthermore, the ability of *P. chrysosporium* to operate at relatively low temperatures makes it a cost-effective and energy-efficient solution for large-scale fermentation processes (Liu et al. 2019).

Determining the optimal fermentation period is crucial for producing effective bioprocessed products. Fermentation can reduce lignin while increasing cellulose content. The success of fermentation is measured by nutrient loss and the cellulose-to-lignin ratio. This study examines the effect of *P. chrysosporium* application timing on the nutritional value and in vitro digestibility of cocoa pod husk.

MATERIALS AND METHODS

Fermentation process

A total of 30 sample units of fermented cocoa pod husk ingredients with different incubation times were used in this research. The cocoa pod husk used in this research was obtained from farmers and collected from various locations in Aceh Province. Before fermentation, the cocoa pod skin is first dried in the Sun for 2 days, then ground using a cutter mill with a 1 mm sieve. All cocoa pod husk (89% dry matter) that had been ground was homogenized for fermentation. Before use, *P. chrysosporium* was first cultured by culturing on MEA (Malt Extract Agar) media, then stored in slant agar media in a test tube and stored at 4°C in the refrigerator to maintain its viability before application. The next stage was adaptation to a solid medium based on cocoa pod husk to adapt the fungus to the target substrate, which then ends with inoculation into cocoa pod husk.

For each treatment unit, 3% white rot fungus *P. chrysosporium* in liquid media with spores at 10^6 was added to 200 g of cocoa pod husk powder, containing 40% dry matter through the addition of water. A mixture of 3% molasses and 0.2% urea that has been dissolved is also added to the fermented cocoa pod husk, then stirred thoroughly until homogeneous. All samples were stored at room temperature for aerobic fermentation at different times, i.e., 0, 7, 14, 21, and 28 days. Each treatment consisted of 6 repetitions. After the fermentation period, all samples were dried in an oven at 60°C for 48 h.

Chemical composition and fiber fraction determination

AOAC methods refer to the analytical methods and procedures developed and validated by the Association of Official Analytical Chemists (AOAC) for the accurate and reliable analysis of various substances. Latimer (2023) employed to ascertain the nutritional composition of the samples, comprising measurements of dry matter (DM), organic matter (OM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE). The subsequent steps outline the concise procedures for identifying each constituent in chemical analysis. The DM content was evaluated by subjecting the samples to a temperature of 105°C in an oven for 24 hours. The ash concentration was determined by incinerating samples weighing 300-500 mg in a furnace at a temperature of 500°C for duration of 3 hours. The organic matter (OM) content was determined by excluding the dry matter (DM) and ash. The CP content was determined using the standard Kjeldahl's method, which involved the operations of destruction, distillation, and titration. The CF content was determined by subjecting 0.3-0.5 g samples to boiling in 3 ml of acid and 10 mL of alkali solutions for 30 minutes each. The CF content was measured by extracting the lipids using a Soxhlet device. The NFE value was determined using the equation NFE = 100% - (ash content + CP + CL + CF). The determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was conducted using the procedure described by Van Soest et al. (1991). Samples weighing 100 mg were subjected to boiling in neutral detergent and acid detergent solutions for 1 hour each, respectively. The NDF analysis in this study was conducted without the use of α-amylase and sodium sulfite. The hemicellulose content was calculated by subtracting the NDF content from the ADF content. The neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) contents were determined using the method described by Licitra et al. (1996), utilizing the remaining residue from the previous analysis of NDF and ADF. The sample analysis was performed twice to ensure accuracy and reliability.

In vitro procedure

Theodorou et al. (1994) conducted an in vitro analysis in which cocoa pod husk treatments were subjected to McDaugall’s buffer mixture and rumen fluid. The rumen fluid was collected in the morning from a cow with a rumen fistula at the Animal Science Faculty of IPB University. The cow was kept specifically for research. Before rumen fluid was collected, cows were given a controlled diet to ensure the desired rumen composition for research. The rumen fluid was strained through four layers of gauze before use. A serum tube with a volume of 125 mL was prepared for filling. It included a sample weighing 0.75 g and was supplemented with 75 mL of buffered rumen fluid. The volumetric ratio of rumen fluid to buffer was 1:4. Afterwards, an incubation process was carried out for 48 hours at a temperature of 39°C in a water bath. Serum bottles were sealed with aluminum crimp seals and butyl rubber stoppers. The gas generation was quantified using a gas-tight syringe at certain time intervals of 2, 4, 6, 8, 12, 24, 36, and 48 hours after incubation. On each occasion when the quantity of gas generated was determined, the region was manually agitated. The quantification of methane was conducted utilizing gas chromatography (GC) Shimadzu, model GC8. The method used is in accordance with SNI 9224-1: 2023 concerning methods for measuring emissions of methane gas (CH_4) and nitrous oxide (N_2O). In order to separate the liquid portion from the solid residue, the serum bottles were subjected to centrifugation after 48 hours of incubation. The content of total volatile fatty acids (VFA) and ammonia, as well as the pH, were determined by analyzing the supernatant collected after a 48-hour incubation using the Conway microdiffusion method, as outlined in the study by Jayanegara et al. (2016). The VFA concentration of acetate, propionate, butyrate, and valerate was measured using the Gas Chromatography (GC) method, specifically the IKM 13 (GC-FID) instrument, as described by Kristensen et al. (2000). The values of in vitro dry matter digestibility (IVDMD); in vitro organic matter digestibility (IVOMD) were determined by subtracting the remaining dry matter (DM) and organic matter (OM) from the original DM and OM before the incubation process. The in vitro incubation was performed in three iterations, with two serum bottles utilized in each iteration. To implement this approach, a randomized complete block design was utilized for treating the experimental unit, with runs being treated as the blocks (replicates). For control purposes, two bottles containing buffered rumen fluid without any substrate were also incubated and regarded as blanks.

Data analysis

The data were subjected to statistical analysis using analysis of variance (ANOVA) with the following model:

$$Yij = \mu + \alpha i + \beta j + \varepsilon ij$$

The observed measure, denoted as Yij, is influenced by the general mean (μ), the treatment effect (αi), and the block (βj). The study reported significant differences between treatments ($P<0.05$) and further analyzed these differences using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Chemical composition

Table 1 presents the nutritional quality of cocoa pod husk that was fermented using *P. chrysosporium* for varying lengths of time. Statistical study revealed that the treatment of *P. chrysosporium* had a highly significant impact on the crude protein content of cocoa pod husk ($P<0.01$). The F4 treatment, with a fermentation duration of 28 days, had the highest crude protein concentration, specifically measuring 13.85% (Table 1). The crude protein content showed a linear increase, with a slight decrease observed in the F2 treatment. Syahrir et al. (2017) found that the crude protein content of cocoa pod husk could be increased through fermentation treatment employing a combination of *P. chrysosporium* and *Pleurotus ostreatus* at a ratio of 8 and 15 g/kg, respectively. According to a study conducted by Laconi and Jayanegara (2015), the inclusion of *P. chrysosporium* at a concentration of 3% can enhance the crude protein content of cocoa pod husk. The rise in crude protein content during solid-state fermentation is a result of microorganisms being cultivated in lignocellulosic material. This cultivation allows yeast to utilize carbohydrates from starch, which are broken down into glucose during the degradation process. The microorganisms the use the glucose to synthesize mycelium biomass (Terefe et al. 2021).

According to Kumla et al. (2020) there is a direct relationship between the amount of mycelium and the protein content. Increasing the number of mycelia leads to an increase in protein content. Among the cultivated strains, *P. chrysosporium* exhibited the highest mycelial biomass concentration in submerged fermentation (*H. annosum*, *P. chrysosporium, P. ostreatus, T. versicolor,* and *L. lepideus*) (1.03 g 100 mL^{-1}) after 14 days (Irbe et al. 2023). The decline in crude protein seen over the 14-day incubation period is attributed to protein degradation occurring during the fermentation process. Proteolytic bacteria were found to be active and responsible for breaking down the protein present in the feed. Figaj et al. (2019) suggested that certain proteins in the substrate undergo degradation, or proteolysis, by both plant and microbial protease enzymes, resulting in the formation of non-protein nitrogen (NPN) molecules, such as amino acids and ammonia. Supplementing the fermentation media with nitrogen can enhance the growth of mycelium in *P. chrysosporium.*

Table 1. Nutritive value of cocoa pod husks fermented by using *Phanerochaete chrysosporium* at different incubation times

Nutritive values	F0	F1	F2	F3	F4	P-value	SEM
DM (%)	88.33±0.07^c	88.91±0.09^b	85.96±0.10^d	89.68±0.38^a	90.21±0.11^d	<0.01	0.29
OM (%)	89.47±0.06^a	88.16±0.08^b	87.29±0.10^c	85.83±0.10^d	85.01±0.09^e	<0.01	0.30
CP (% DM)	10.16±0.04^e	13.44±0.14^d	11.14±0.18^c	12.98±0.20^b	13.85±0.08^a	<0.01	0.27
CF (% DM)	30.09±0.51^a	34.44±0.91^a	32.96±1.27^a	30.06±0.42^b	30.55±0.70^b	<0.01	0.47
EE (% DM)	1.25±0.05^b	1.25±0.03^b	1.01±0.04^c	1.74±0.08^a	1.13±0.10^{bc}	<0.01	0.05
Ash (% DM)	9.31±0.05^e	10.53±0.07^d	10.92±0.08^c	12.71±0.10^b	13.53±0.07^a	<0.01	0.28
NFE (% DM)	49.19±0.51^a	42.88±0.79^a	41.4±1.37^b	42.55±0.53^b	40.94±0.69^b	<0.01	0.81

Note: F0: without incubation, F1: 7 days of incubation time, F2: 14 days of incubation time, F3: 21 days of incubation time and F4: 28 days of incubation time. DM: dry matter, OM: organic matter, CP: crude protein, CF, crude fiber, EE: extract ether, NFE: nitrogen-free extract, superscript letter in the same row indicates a significant difference between groups by Duncan's test (P<0.01)

The substrate's crude fiber content (Table 1) exhibited an initial increase during a 7-day fermentation period, followed by a progressive decline during a fermentation period of 14-28 days (P<0.01). The study saw a lower reduction in crude fiber compared to the study conducted by Mustabi et al. (2020), i.e., 17.22%. The fungus *P. chrysosporium* extracts the nutrients present in the substrate and breaks them down into simpler forms through catabolism. To ensure its continued existence, the fungus undergoes a biodegradation process, which involves the fungal hyphae making contact with the surface of the substrate and forming resilient colonies. Once reached, the fungus readily decomposes the fiber constituents. During the 7-day long fermentation treatment, the rise in crude fiber content occurred as a result of the fungus cell walls themselves undergoing fermentation. Fesel and Zuccaro (2016) state that chitin is a crucial constituent of the cell walls of most of fungi. Chitin is a polysaccharide that serves as the primary constituent of the exoskeleton found in insects and arthropods.

The dry matter and ash content varied significantly (P>0.05) between treatments. The dry matter content remained slightly stable between treatments, ranging from 88-90% (Table 1). It is anticipated that no evaporation occurs during fermentation when the substrate is placed in a closed polyethylene bag and then opened once the substrate is completely covered with mycelium. The increased dry matter content is most likely due to the limited nutrient availability and the short incubation period (Ruvuga et al. 2022). Degrading enzymes, such as ligninolytic enzymes, do not perform well when degrading other fiber components, such as lignin. This is supported by Kumla et al. (2020), who used only easily fermentable carbohydrate sources during the fermentation process. In contrast, lignin components that are difficult to break down remain in the substrate. The increased lignin content in the substrate in this study was due to ligninolytic enzymes failing to break down lignin molecules optimally. Ligninolytic enzyme activity promotes fungal growth and is dependent on nutrition availability and media type. In this study, we did not give a source of slow-release carbohydrates derived from grains, but rather energy from molasses to kickstart the fermentation process at the start of incubation (Wajizah et al. 2015). Because ash is a component of the cell wall, the fiber content is proportional to it. An increase in crude fiber concentration doesn't directly improve ash content; it can lead to changes in the overall composition of a feedstuff, potentially affecting ash levels indirectly.

The concentrations of ether extract and NFE content showed a substantial reduction (P<0.01) as incubation time progressed (Table 1). This phenomenon occurs because the yeasts use simple carbohydrates as an energy source and the lipase enzyme uses of lipids (Azhar et al. 2017). Amara and El-Baky (2023), demonstrated that fungi consume soluble cell components for their activities throughout the fermentation process. Hidayat et al. (2022) reported that the non-nitrogen extracts from cocoa pod husk fermented with *G. lucidum* decreased in content from 47.8 to 42.1%, and the lipid content reduced from 0.77 to 0.41%.

Fiber fraction and protein fraction in fiber

The NDF concentration of cocoa pod husk waste was found to be significantly affected (P<0.01) by the administration of *P. chrysosporium* with varying incubation durations, according to an analysis of variance (Table 2). The NDF content fluctuated; treatment F4 (81.20%) showed a higher NDF content than all treatments (Table 2). Comparing this study to Laconi and Jayanegara (2015), the NDF content data is higher, at 80.7%.

In Table 2, it can be seen that there was a decrease in NDF levels but it was not significant, so it cannot be claimed that the use of this fungus is effective in reducing NDF. This statement is supported by the NDF levels in the F4 treatment which show a significant increase in NDF compared to the F0 treatment. This increase is believed to be a result of cellulolytic microbial activity, which initially utilizes the cell contents (NDS) in the substrate, leading to a corresponding increase in cell walls (NDF). The rise in NDF levels signifies that the activity of cellulolytic microorganisms is inadequate in decomposing intricate molecules into simpler ones. According to Gonzalez and Aranda (2023), the growth rate of microorganisms is influenced by a decrease in nutritional availability and the buildup of metabolic byproducts that hinder growth. Inefficient cellulolytic bacteria hinder the activity of the cellulase enzyme in breaking down the cellulose and lignin present in cell walls (NDF). As a result, the conversion of these complex molecules into simpler ones is insufficient, leading to a rise in the proportion of the cell wall (NDF). According to Dewiyanti et al. (2022), cellulolytic bacteria utilize the cellulase enzyme to break down cellulose by hydrolysis. There are dynamics in the NDF figures in this research. On day 0, the NDF value of cocoa pod shells was 74.93%. On day 7 there was an increase to 80.55%, possibly due to degradation of the non-fiber fraction and accumulation of fibrous microbial biomass from *P. chrysosporium*. Furthermore, on the 14th and 21st days, the NDF value decreased to 73.70 and 72.75% due to the activity of fungal ligninolytic enzymes, which effectively degrade lignocellulose. However, on the 28th day the NDF value rose again to 81.20%, which was thought to be caused by the accumulation of microbial biomass, repolymerization of lignin compounds, and a reduction in easily degradable substrates.

In this study, the cocoa pod husk ADF level increased significantly (P<0.01) from 61.53 to 72.59%, with the highest value occurring in the F4 treatment (28 days of incubation) (Table 2). This information indicates that the ADF rises with the length of the incubation period. The present study's data is less than that of (Lesmana et al. 2020) i.e., 84,07%. The ADF levels increased consistently from treatment F0 to F4, rising from 61.53 to 72.59%. This indicated that during fermentation, there was a relative accumulation of cellulose and lignin components, which are the main constituents of ADF. This increase was not solely due to the formation of more ADF, but rather because most of the non-ADF components, such as sugars, starches, and soluble proteins, had been degraded by the enzymatic activity of the fungus, making the ADF fraction appear to increase proportionally. As an energy source, lactic acid bacteria activity degrades non-fiber carbohydrates but not ADF, which influences the increase in ADF value on the substrate and results in an increase in ADF percentage. This finding is consistent with the research conducted by Tai et al. (2015), which suggested that the rise in ADF concentrations was due to microorganisms utilizing organic materials that were simple to metabolize during the fermentation process, resulting in an accumulation of unused organic materials like ADF.

In Table 2, it was observed that the hemicellulose content underwent significant changes, reflecting the dynamics of fiber component degradation during fermentation (P<0.01). The data showed that the highest hemicellulose content was detected in the F1 treatment (Table 2), which was probably caused by the initial decomposition of lignin, so that the hemicellulose fraction became more accessible and was detected at a higher level. According to Hartulistiyoso et al. (2022), the liberation of lignin from cellulose results in an increase in the hemicellulose content. However, in treatments F2 and F3, there was a significant decrease in hemicellulose content, indicating that active degradation of hemicellulose had occurred due to the enzymes produced. Reduction in hemicellulose levels due to the enzymatic action of *P. chrysosporium,* namely the production of ligninase. This enzyme is capable of breaking down lignin, thereby disrupting the linkages of cellulose and hemicellulose (Li et al. 2022; Giri and Sharma 2020). Zhang et al. (2023), suggest that the degradation of lignin will facilitate the breakdown of cellulose and hemicellulose. Meanwhile, in the F4 treatment, the slight increase observed could have been attributed to fluctuations in enzymatic activity or possible substrate limitations and changes in fermentation conditions, which affected the degradation effectiveness. The findings of this study indicate a decrease in F3 treatment compared to the research conducted by Mayangsari et al. (2021), i.e., 4.23%.

The Neutral Detergent Insoluble Crude Protein (NDICP) content of cocoa pod husk significantly increased after being incubated for 14 days in the F2 treatment (P<0.01) (Table 2). The F2 treatment, which involved incubation for 14 days, had the highest Acid Detergent Insoluble Crude Protein (ADICP) content. This indicates that the 14-day incubation period had a higher concentration of ADICP compared to other treatments, similar to the concentration observed in NDICP. A fraction of the protein present in plants is located within the cellular structure known as the cell wall (Pratama et al. 2022). Nevertheless, rumen bacteria exhibit a rather restricted use of this protein in comparison to the protein present in the cytoplasm. The feed evaluation approach categorizes protein as NDICP (Neutral Detergent Insoluble Crude Protein) and ADICP (Acid Detergent Insoluble Crude Protein) (Licitra et al. 1996; Samadi et al. 2023). The NDICP and ADICP are protein fractions that exhibit insolubility in neutral detergent and acid detergent, respectively. While NDICP slowly breaks down or remains intact in the rumen, ADICP is considered completely indigestible and does not provide amino acids in the lower gastrointestinal tract (Jayanegara et al. 2017). Consequently, ADICP contributes to the low protein quality in the diet. The composition includes proteins associated with lignin, complexes of tannins and proteins, and proteins that have been degraded by heat (Licitra et al. 1996). Neutral Detergent Insoluble Crude Protein (NDICP) and Acid Detergent Insoluble Crude Protein (ADICP) are types of crude proteins that are tightly attached to cell walls or resistant to breakdown in the rumen. As a result, they have low digestibility, meaning they are not easily broken down and utilized by the animal's digestive system (Wang et al. 2016). The fiber component is typically employed as a negative indicator of feed quality since it denotes the portion of the feed that is indigestible.

In vitro digestibility of fermented cocoa pod husk

The results showed that the in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) of fermented cocoa pod husk using *P. chrysosporium* increased significantly in all treatments (P<0,01) (Figure 1). This figure illustrates the range of IVDMD, which is 33.14 to 42.85%. In particular, the findings of this study are inferior to those of Nurhaita et al. (2019), i.e., 55.89%. Despite achieving an increase in IVDMD, the percentage remained below 50%. Consequently, it can be concluded that the dry matter digestibility of cocoa pod husk is comparatively low on the whole. The treatment with a 28-day incubation period exhibited the greatest IVDMD, at 42.85% (Figure 1). The reaction involving F4 (28 days of incubation) generated the crude protein (Table 1), which impacted the substrate's dry matter digestibility. The ability of *P. chrysosporium* to manufacture cellulase and ligninase enzymes demonstrates that it can enhance the quality of cocoa pod husk (Suryadi et al. 2022). IVDMD is affected by the protein content of the nutrition as the solubility and resistance to degradation of proteins vary depending on the source (Wang et al. 2016). As the percentage of digestibility in feed increases, the quality of the feed also improves. The proportion of feed constituents, chemical composition, protein content ratio, and percentage of fat and minerals influence dry matter digestibility values (Gebremariam and Belay 2021).

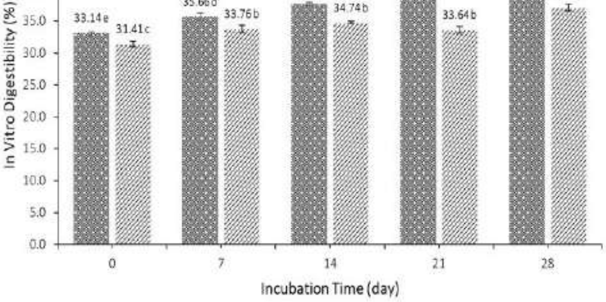

Figure 1. IVDMD and IVOMD values of cocoa pod husks fermented by using *Phanerochaete chrysosporium* at different incubation times

The in vitro organic matter digestibility (IVOMD) values obtained in this study ranged from 31.41% to 39.09% (Figure 1). The F4 treatment (28 days of incubation) exhibited the greatest IVOMD value. The digestibility value of organic matter corresponds to the digestibility value of dry matter. Maranatha et al. (2020) found that the digestibility of organic matter is strongly linked to the digestibility of dry matter, as a portion of dry matter is composed of organic matter. Ndaru et al. (2022) elucidated that the digestibility of dry matter has an impact on the digestion of organic matter. A reduction in dry matter digestibility will lead to a corresponding fall in organic matter digestibility. In this study, the digestibility value of organic matter was found to be greater than the value reported by Hidayat et al. (2022), which was 33.38%. Karimizadeh et al. (2017) stated that the digestibility of feed is a reliable indicator of its quality. In other words, the higher the digestibility of a certain type of feed, the higher its quality.

After 7 days of incubation, *P. chrysosporium* began producing ligninolytic enzymes such as lignin peroxidase and manganese peroxidase (Konan et al. 2024), which initiate the breakdown of the complex chemical bonds within lignin. At this stage, a preliminary increase in IVDMD and IVOMD was observed due to the partial degradation of lignin. With continued incubation to 14 days, enzymatic activity intensified, leading to more extensive lignin degradation and the disruption of cellulose and hemicellulose structures previously shielded by lignin. Consequently, the digestibility of the feed substrate increased significantly, a testament to the power of collaboration in scientific research. At 21 days of incubation, lignin degradation progressed deeply, with a substantial reduction in lignin content, thereby producing a further increase in IVDMD and IVOMD. By 28 days, *P. chrysosporium* had achieved near-optimal lignin decomposition, resulting in the highest recorded increases in IVDMD and IVOMD. However, extending the incubation period beyond this point poses a risk of excessive degradation of other essential nutrients. Biochemically, the improvement in feed quality is attributed to the ligninolytic enzymes' capacity to cleave the aromatic structure of lignin and break the cross-linkages between lignin and polysaccharide fibers (Gharechahi et al. 2023). This enzymatic action enhances microbial access to cellulose and hemicellulose in the cocoa pod husk, thereby facilitating ruminal digestion.

pH values and ammonia (NH_3-N)

Accurate pH values play a very important role in supporting rumen microbes to degrade feed optimally. Fibre will be digested slowly at a pH of <6.2 and will be dangerous for livestock if the pH decreases to <5.4 (Darwin and Blignaut 2019). The pH value in our study was 6.75-6.77 and is in accordance with the ideal pH value as stated by Kitkas et al. (2022), i.e., 6-7 (Figure 2). Although the IVDMD and IVOMD values in the F4 treatment (28 days of incubation) increased, the pH value of the rumen fluid was still in the normal range (Figure 2).

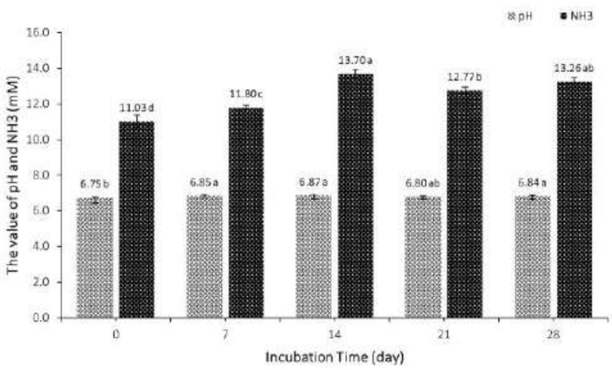

Figure 2. pH values and ammonia (NH_3-N) gas concentration of cocoa pod husks fermented by using *Phanerochaete chrysosporium* at different incubation times

Table 2. Fiber fraction and protein fraction in the fiber of cocoa pod husks fermented by using *Phanerochaete chrysosporium* at different incubation times

Parameters	F0	F1	F2	F3	F4	P-value	SEM
Fiber fraction							
NDF (%)	74.93±2.55^{bc}	80.55±1.69^{ab}	73.7±2.29^{c}	72.75±0.61^{c}	81.20±2.08^{a}	0.01	1.05
Hemiselulosa (%)	13.41±3.16^{a}	15.01±1.63^{a}	8.73±2.46^{ab}	2.58±0.70^{b}	8.60±2.02^{ab}	<0.01	1.21
ADF (%)	61.53±0.76^{d}	65.54±0.65^{c}	64.97±0.95^{c}	70.17±0.75^{b}	72.59±0.46^{a}	<0.01	0.79
Protein fraction in fiber							
NDICP (% DM)	9.60±1.06	9.70±0.75	11.12±0.51	9.42±0.15	8.52±0.25	0.10	0.31
NDICP (% CP)	66.19±2.27^{b}	71.62±1.53^{a}	63.36±1.99^{b}	65.24±0.69^{b}	73.25±1.91^{a}	<0.01	1.02
ADICP (% DM)	10.17±0.84^{bc}	9.09±0.37^{c}	11.47±0.23^{ab}	10.00±0.31^{bc}	11.97±0.51^{a}	<0.01	0.28
ADICP (% CP)	54.35±0.66^{d}	58.27±0.56^{c}	55.84±0.77^{d}	62.94±0.89^{b}	65.49±0.43^{a}	<0.01	0.83

Note: F0: without incubation, F1: 7 days of incubation time, F2: 14 days of incubation time, F3: 21 days of incubation time and F4: 28 days of incubation time. NDF: neutral detergent fiber, ADF: acid detergent fiber, NDICP: neutral detergent insoluble crude protein ADICP: acid detergent insoluble crude protein, superscript letter in the same row indicates a significant difference between groups by Duncan's test (P<0.01)

Organic acids play a crucial role in regulating the pH value of the rumen and maintaining the balance of bacterial populations within it. Based on the findings of Suiryanrayna and Ramana (2015), organic acids can accelerate the reduction in pH. Kara et al. (2018) demonstrated that organic acids, specifically formic and propionic acids, affect the breakdown of proteins, causing them to be converted from complicated structures into simpler and more soluble forms. In order to ensure the proliferation of microorganisms, pH of the rumen must be maintained within the range of 6-7.

The concentration of ammonia/NH_3-N is directly proportional to the number of microbes in the rumen. Ammonia is a product resulting from fermentation activity in the rumen, the result of protein degradation from feed and is a source of nitrogen which is quite important for rumen microbial synthesis (Tahuk et al. 2016). The results showed that the NH_3-N value of fermented cocoa pod husk using *P. chrysosporium* had a significant effect ($P<0.01$). In this study, there was a linear increase in NH_3-N concentration along with the incubation time up to 14 days, but there was a decrease at an incubation time of 21 days, then increased again at an incubation time of 28 days (Figure 2). The NH_3-N value in this study was 11.03-13.70 mM, in accordance with that reported by McDonald et al. (2002), which ranges from 6-21 mM. Ruminants need a balanced NH_3-N value to digest fiber optimally. The high NH_3-N value in the F4 treatment may be due the fast rate of protein degradation, causing NH_3-N production to increase. The high or low digestibility of protein can increase the degradability and fermentability of feed ingredients in the rumen so that the resulting NH_3-N concentrations are high (Permana et al. 2022). After incubation for 7 days, the activity of proteolytic and lignolytic enzymes produced by *P. chrysosporium* began to break down the complex bonds between protein and lignin, increasing in NH_3-N levels. This increase continued significantly on day 14, as enzymatic activity increased and organic matter decomposition released more nitrogen in the form of ammonia. The decrease in NH_3-N production in the F3 treatment (21 days of incubation) is thought to be due to limited nutrition, disrupted mycelium growth, and the laccase enzyme not working optimally (Zhang et al. 2022). After the incubation time reaches 28 days in F4 treatment, the crucial role of enzyme activity in degrading cell walls increases and releasing monosaccharides to be used by microorganisms as substrates during metabolic activities (Rho et al. 2020) is highlighted. The NH_3-N value in this research is lower than the research reported by Iflijar et al. (2021) namely 14.70 mM. Putri et al. (2021) explained that the factors influencing the increase in NH_3-N production were protein sources in ratios that were easily degraded by rumen microbes and high feed energy, and high growth of rumen microbes.

Total VFA and VFA partial concentration

The average VFA production between *P. chrysosporium* treatments with different fermentation times can be seen in Table 3. The results of the analysis of variance show that the fermentation time has a very significant effect ($P<0.01$) on the concentration of total VFA and partial VFA of cocoa pod husk. VFA production in this study ranged from 74.90 to 125.70 mM (Table 3). According to McDonald et al. (2002), the range of total VFA that supports rumen microbial growth is 70-150 mM. The rumen fluid VFA concentration value of the research feed ingredients meets these parameters. Rumen microbial activity alters the rumen metabolic profile, particularly VFA produced by carbohydrate breakdown.

The total concentration of volatile fatty acids (VFA) showed a significant increase with prolonged incubation time. The lowest concentration was observed in the F0 treatment (control) at 74.90 mM, while the highest was recorded in the F4 treatment at 125.70 mM. This increase indicates that fermentation by *P. chrysosporium* progressively enhanced total VFA production. The efficiency of substrate decomposition by fungus into VFA appeared to improve with extended fermentation duration (Li et al. 2017); the *P. chrysosporium* fungus produces lignolytic and hydrolytic enzymes which gradually break down the complex components of the substrate into simple molecules. Microbes then ferment these molecules to become VFA. With increasing fermentation time, enzymatic activity and microbial fermentation take place more optimally, so that VFA accumulation increases significantly. The statistical significance among treatments suggests that the changes in total VFA concentration were not random, but rather a result of the different fermentation treatments applied.

Table 3. VFA total and VFA partial concentrations of cocoa pod husks fermented by using *Phanerochaete chrysosporium* at different incubation times

VFA contents	F0	F1	F2	F3	F4	P-value	SEM
VFA total (mM)	74.90 ± 3.23^{d}	95.23 ± 2.45^{c}	110.17 ± 2.16^{b}	113.09 ± 2.67^{b}	125.70 ± 1.96^{a}	<0.01	3.40
VFA Partial							
C_2 (mM)	43.12 ± 6.10^{a}	18.9 ± 3.72^{c}	18.66 ± 3.37^{c}	32.71 ± 3.28^{ab}	26.28 ± 1.72^{bc}	<0.01	2.36
C_3 (mM)	8.79 ± 1.25^{a}	3.89 ± 0.79^{c}	4.10 ± 0.73^{c}	7.53 ± 0.90^{ab}	5.69 ± 0.49^{bc}	<0.01	0.51
iC_4 (mM)	0.74 ± 0.11^{a}	0.65 ± 0.12^{a}	0.59 ± 0.15^{ab}	0.34 ± 0.05^{bc}	0.24 ± 0.02^{c}	0.01	0.05
nC_4 (mM)	4.48 ± 0.56^{a}	2.36 ± 0.49^{b}	2.33 ± 0.44^{b}	3.25 ± 0.40^{ab}	2.13 ± 0.18^{b}	<0.01	0.24
iC_5 (mM)	1.28 ± 0.19^{a}	0.96 ± 0.20^{ab}	0.86 ± 0.24^{abc}	0.60 ± 0.08^{bc}	0.38 ± 0.02^{c}	0.01	0.09
nC_5 (mM)	0.59 ± 0.08^{a}	0.36 ± 0.08^{b}	0.34 ± 0.09^{b}	0.36 ± 0.04^{b}	0.23 ± 0.01^{b}	0.02	0.04

Note: F0: without incubation, F1: 7 days of incubation time, F2: 14 days of incubation time, F3: 21 days of incubation time, and F4: 28 days of incubation time. C_2: acetic acid, C_3: propionic acid, iC_4: isobutyric acid, nC_4: n-butyric acid, iC_5 = iso-valeric acid, nC_5 = n-valeric acid, superscript letter in the same row indicates a significant difference between groups by Duncan's test ($P<0.01$)

VFA partial showed a more complex pattern (Table 3), where C2 (acetate) experienced a sharp decrease from F0 to F1 and F2, then increased again at F3 and decreased slightly at F4, while C3 (propionate) also showed a similar trend; the initial sharp decrease in C2 and C3 could be caused by the dominance of lignin and cellulose degradation into unstable compounds at the start of fermentation, indicating that although total VFA increases, certain fractions such as butyric acid (iC4 and nC4) and valeric acid (iC5 and nC5) tend to decrease with fermentation time, suggesting transformation into other compounds—this dynamic pattern is important to consider because, according to Cheng et al. (2022), acetic acid is the most abundant product in rumen fermentation and its proportion, along with that of propionate and butyrate, reflects the type of fermentable substrate, where acetate is dominant in high-fiber diets and propionate in glucogenic substrates (Hua et al. 2022), and to achieve optimal energy efficiency, the molar balance between ketogenic (acetate and butyrate) and glucogenic (propionate) VFAs must be adjusted accordingly (Mullik et al. 2024). The findings of this study, as presented in Table 3, indicate that the quantity of butyrate in terms of moles is quite modest, with a range of just 2.13 to 4.48 mM in total VFA generation.

Overall, although the total VFA increased, most of the partial components such as C2, C3, and branched chain VFA (iC4, iC5, nC4, nC5) showed fluctuations or decreases. This suggests that the increase in total VFA is likely contributed to the accumulation of other fractions or subsequent metabolic reactions that alter the overall volatile fatty acid profile. A negative correlation can be seen between the increase in total VFA and the decrease in iC4 and iC5, which may be due to changes in microbial composition during fermentation or more selective enzymatic activity towards certain compounds. Thus, fermentation not only increases the quantity, but also changes the quality or type of volatile fatty acids formed, which is important to understand in the context of the nutritional value and use of fermented feed ingredients.

Total gas production

Figure 3 displays the production data for cocoa pod husk gas production for each treatment. The gas analysis results indicated a positive correlation between the concentration of feed materials and the amount of gas produced. This suggests that the capacity of rumen bacteria to break down and process feed items is growing. The gas production rate exhibited a sluggish pace from hour 0 to hour 12. This indicates that the rumen microorganisms are still in the process of acclimating to the new environment, resulting in a somewhat sluggish rate of gas generation. An appreciable surge becomes apparent during the 24 to 48-hour period, as rumen microbes in this phase exhibit optimal gas production due to their transition into the exponential growth phase and successful adaptation to the new environmental conditions. Additionally, the substrate in their surroundings remains abundantly available. According to Paul et al. (2023), gas production data nearly doubled when the observation time interval was extended to 12 and 24 hours.

Rumen fermentation produces not only VFA but also gases such as CO_2, CH_4, NO_2, NO, N_2 and approximately 3.5% O2 (Mccauley et al. 2020). The amount of gas produced largely depends on the nutritional composition of the diet. Treatment F0 exhibited the highest gas production (Figure 3), which is attributed to its elevated levels of organic matter and NFE compared to other treatments (Table 1). The high NFE content reflects abundant non-structural carbohydrates such as starch and sugars that are readily fermented by rumen microbes, resulting in substantial gas production. However, the high gas production observed in F0 did not correspond to the highest digestibility. This is likely due to its low CP content (Table 1), which may limit fermentation efficiency despite the high fermentation of non-structural carbohydrates generating more gas. Conversely, treatments F1 through F4, characterized by increased CP and variable CF contents (Table 1), showed reduced gas production but improved digestibility.

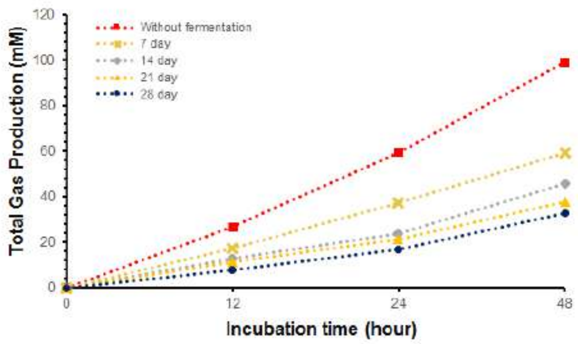

Figure 3. Total gas production formed over 48 hours of cocoa pod husk fermented using *Phanerochaete chrysosporium* at different incubation times

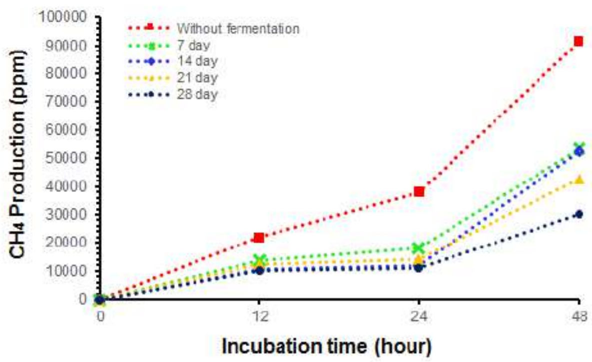

Figure 4. Methane (CH_4) gas concentrations formed over 48 hours of cocoa pod husks fermented using *Phanerochaete chrysosporium* at different incubation time

The increase in CP from F1 to F4 is hypothesized to provide a better nitrogen supply for rumen microbes, enhancing fermentative activity and supporting substrate degradation. This aligns with the understanding that protein fermentation produces less gas than carbohydrate fermentation. Furthermore, the elevated CF content in F1 and F2, likely derived from less fermentable fiber fractions such as lignin, contributed to the reduced gas production. The presence of crude fiber and anti-nutritional factors, such as tannins, has been reported to inhibit rumen enzymes and microbial activity, thus lowering total gas production (Sari et al. 2022). This phenomenon is supported by Sui (2018), who demonstrated a direct correlation between degradation rate values and total gas production (Figure 3). Additionally, treatments F1 to F4 exhibited an inverse relationship between incubation time and total gas output; longer incubation periods resulted in decreased gas production. This suggests that incubation treatments increasing fiber and protein content to a balanced level enable more efficient fermentation, resulting in significantly improved digestibility despite lower gas production than F0.

Methane (CH_4) gas concentrations

The generation of CO_2 gas and two different forms of VFA, butyric and acetic acid, are strongly associated with the synthesis of methane gas. In the F0 treatment (no incubation), there was a rise in methane gas (Figure 4). This results from the waste from cocoa pod husk having a high acetic acid content (Table 3). A stoichiometric reaction occurs when a large amount of hydrogen gas is produced during the process of generating acetic acid. Methane gas is created first, using hydrogen gas and carbon dioxide as precursors (Zabranska and Pokorna 2018). Aside from that, the link between protozoa and methanogenic bacteria in the synthesis of methane results in an increase in methane output. Methanogenic bacteria produce enzymes that catalyze the reduction of CO_2 and H_2 in the rumen, which is the basic process by which methane is formed. Microorganisms known as methanogens actively contribute to the production of methane (Volmer et al. 2023). While between 63 and 80% of methanogens still function freely in the rumen, as many as 20 to 37% of methanogens are in symbiosis with protozoa (Volmer et al. 2023).

The treatment with the shortest methane gas production after 28 days of incubation was F4 (Figure 4). This result is attributed to the lower concentration of butyric acid in F4 compared to the other treatments (Table 3). The inoculation with *P. chrysosporium* reduced the butyric acid content, which likely contributed to the decreased methane production. *P. chrysosporium* has the ability to generate polyunsaturated fatty acids (PUFA), which may influence microbial community structure and metabolic activity during fermentation (Whiteford et al. 2021). It is hypothesized that PUFA can affect alterations in the partial VFA proportion profile within the rumen. Upon entering the rumen fluid, a lipolysis and biohydrogenation process is initiated in the fat (Huda et al. 2023). According to Tran et al. (2017), the relatively brief half-life of PUFA in rumen fluid can be attributed to their immediate biohydrogenation process. Saturated fatty acids, which are produced as a byproduct of acetic acid and butyric acid synthesis, undergo this process (Talan et al. 2022). Cellulolytic microorganisms are the primary agents involved in the biohydrogenation of unsaturated fatty acids (Zhang et al. 2020). Hydrogenation, the process by which the hydrogen used to saturate the fat, can impede the methane gas synthesis reaction, resulting in a reduction in methane gas production.

Methane production was highest at 0 days, followed by a step-by-step decrease at 7, 14, 21, and the lowest value at 28 days of incubation. Cocoa pod husk was at 0 days incubation rich in lignin and other intricate compounds that not only hindered digestibility but also led to fermentable substrates that promoted hydrogen (H_2) production. Methanogenic archaea utilize this hydrogen to produce methane. After 7 days of incubation with *P. chrysosporium*, the initial lignin degradation started through ligninolytic enzymes like lignin peroxidase and manganese peroxidase, altering the chemical composition and reducing the availability of methanogenic substrates. With longer incubation (14, 21, and 28 days), lignin degradation was more effective, further limiting the substrate available for the synthesis of methane. Degradation of lignin can also produce phenolic compounds, some of which are antimicrobial characteristic and could potentially suppress the growth and activity of methanogenic microorganisms. Methanogenesis was thus progressively suppressed with increased incubation periods.

In conclusion, fermentation treatment using *P. chrysosporium* fungus was able to improve the chemical quality of cocoa pod husk such as increasing crude protein even though there was an increase in the NDF and ADF values. The increase in dry matter digestibility and organic matter digestibility is still relatively low even though the pH, NH_3 and VFA Total values are in the normal range. The treatment was able to reduce the value of total gas production and methane gas production from cocoa pod husk. Based on the data that has been collected, it was found that the F4 treatment is the best treatment of all those tested. To maximize the potential of *P. chrysosporium* fungus, it is recommended to carry out physical and chemical treatment first to increase the digestibility value.

ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to the DRPM, Ministry of Education, Culture, Research, and Technology (*Kemdikbudristek*), Indonesia for their invaluable support in funding this study through the WCR Research Scheme 2023. Without their financial assistance, this research would not have been possible. We also extend our sincere thanks to LPPM Universitas Syiah Kuala, Banda Aceh, Indonesia for their unwavering administrative support throughout this project.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 288-297 DOI: 10.13057/asianjagric/g090130

A competitive business model for export-oriented salted egg micro, small, and medium enterprises in the Japanese market

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Manuscript received: 21 March 2025. Revision accepted: 28 May 2025.

Abstract. *Mahbubi A, Fatoni A, Abimanyu NM. 2025. A competitive business model for export-oriented salted egg micro, small, and medium enterprises in the Japanese market. Asian J Agric 9: 288-297.* Japan offers a promising opportunity for Indonesian salted egg Micro, Small, and Medium Enterprises (MSMEs), given that the price of eggs in Japan is more than double that in the domestic market, and the country ranks as the second-largest consumer of eggs globally. This study seeks to formulate a competitive business model for salted egg MSMEs in the Japanese market. This study employs an exploratory approach, involves 60 participants, and incorporates analyses of comparative and competitive advantages, empathy maps, and business model canvas. This study identifies end users and retailers as primary consumer segments. The value proposition featured eggs with an average weight of 70 g, medium salt content, a slightly paler eggshell, and a vibrant egg yolk. Key activities included 11 operations, particularly on improving the monitoring system. Essential resources comprise cages, salt that meets the Indonesian National Standard (SNI) 01-3556-2000 standards, clean water sources, and processing facilities. The key partners include farmers, testing institutions, and Japanese MSMEs. Channels use direct and indirect exporting. The proposed model is of utmost importance, as it underscores the need importance for fostering collaborative partnerships between supply chain actors. The cost structure delineates the expenses related to cultivation, processing, and delivery. The revenue model is structured around profit-sharing agreements.

Keywords: Business model, comparative advantage, competitive advantage, poultry, salted egg

Abbreviations: MSMEs: Micro, Small, and Medium Enterprises

INTRODUCTION

Indonesian poultry products have not rapidly developed in the international market. One of the main obstacles, Indonesia is not considered free from the negative effects of avian influenza outbreaks. Consequently, many countries have imposed restrictions on importing poultry meat and fresh eggs from Indonesia. However, Indonesia has successfully exported processed poultry products with various requirements, including traceability of poultry origin. Processed poultry products from Indonesia have been successfully exported, including chicken nuggets of the following brands: Fiesta fried chicken, Fiesta spicy wings, Fiesta spicy chick, Fiesta delistripe, Fiesta karage, and Fiesta schnitzel. Additional brands include Sunny gold chicken nuggets tempura, Sunny gold-spicy karage, and Sunny gold-karage black peppers. Moreover, salted eggs from *Anas platyrhynchos* f. *domesticus*, which are processed from Indonesian egg products, have successfully entered the ASEAN market.

Currently, a limited number of salted egg Micro, Small, and Medium Enterprises (MSMEs) have achieved international market penetration or export status for salted eggs in the global market. Only one MSME consistently exported salted eggs to Singapore. The international market for salted eggs, particularly the Japanese market, remains untapped, with prices that are more favorable than those in the domestic market. The price of salted eggs in Japan is JPY 500 (IDR. 47,500) per pack of six eggs, significantly exceeding the average price of salted eggs in Indonesia of 200 yen (IDR. 21,000) per pack of six eggs. The 2023 International Egg Commission survey also reported that Japan ranks second after Mexico in egg consumption, with each individual consuming 339 eggs annually in 2022 (The Mainichi 2023). In addition, 30% of the foreign workers in Japan originate from China and Indonesia, which generally favor salted eggs. Salted eggs in Japan are predominantly imported from China.

These circumstances are crucial to promote the entry of salted egg MSMEs into the Japanese market. Serrano et al. (2018) and Nurfarida et al. (2022) stated that internationalization is essential for MSMEs to ensure their survival through increased revenue and to maintain the long-term viability of agricultural MSMEs, including those in the salted egg industry. The expansion of export-oriented MSMEs in this sector could generate positive societal effects by creating new job opportunities in the livestock industry. They may also contribute to environmental sustainability by enforcing strict hygiene standards for export products and thereby reducing pollution.

The OECD (2018) identified limited resources, particularly in finance and detailed information on foreign markets, as one of the primary obstacles for export MSMEs. Child et al. (2022) posited that MSMEs intending to enter the international market must consider the characteristics of the company itself, domestic economic conditions, and the economic landscape of the export destination country. Bertrand et al. (2022) identified internal factors such as insufficient resources and assets, the absence of strategic partnerships, and the lack of research and development as primary obstacles for MSMEs in emerging countries seeking to engage in export activities. MSMEs also need to comprehend regulations in international business, especially the various rules in export-destination countries.

MSMEs must design salted egg export business models to be competitive in the global market. According to An et al. (2022), modeling assists MSMEs in creating value. Moradi et al. (2021) stated that designing a business model becomes a crucial driver of innovation within MSMEs. Fitriasari (2020) further explained that business model design translates strategy into a blueprint. Subsequently, the business model must be financed through external or internal funding and must ultimately be implemented in an actual business venture. Therefore, this study aimed to develop globally competitive salted egg MSMEs, particularly those in Japan.

Islam et al. (2023) reported that the main focus of internationalization research is on large companies from developed countries, while research on the internationalization of MSMEs from developing countries is still relatively under-explored, even though MSMEs provide many jobs. They also revealed that MSMEs contribute to more than half of industrial employment. Chaldun et al. (2022) reported that sustainable business model design in relation to MSMEs' internationalization has not been widely explored. Specifically, Child et al. (2022) reported that the choice of partners and destination countries for MSMEs' exports has not been widely studied.

MATERIALS AND METHODS

Study area

This study used a mixed method. A quantitative method was used to assess comparative and competitive advantage, while a qualitative approach was used to explore customer insight and create a competitive business model. The data of this study used primary and secondary data. Primary data were obtained from salted egg consumers in Tokyo, Japan (Figure 1.A) and salted egg MSMEs in Blitar, Brebes, and Karawang, Indonesia (Figure 1.B). Secondary data were obtained from the United Nations Commodity Trade Statistics Database (UNCOMTRADE) and the Ministry of Trade of the Republic of Indonesia.

Data collection

This study interviewed 60 participants, consisting of 40 salted egg consumers in Tokyo, Japan (coded C1 to C40) and 20 salted egg MSMEs, including 13 in Brebes, Central Java, 4 in Blitar, East Java, and 3 in Karawang, West Java (coded M1 to M20). The research participants were selected based on certain criteria, such as consumers in Japan, both native residents and immigrants who have purchased salted eggs more than once, and salted egg MSMEs who have marketed their products to other regions or have a Veterinary Control Number (NKV) certificate.

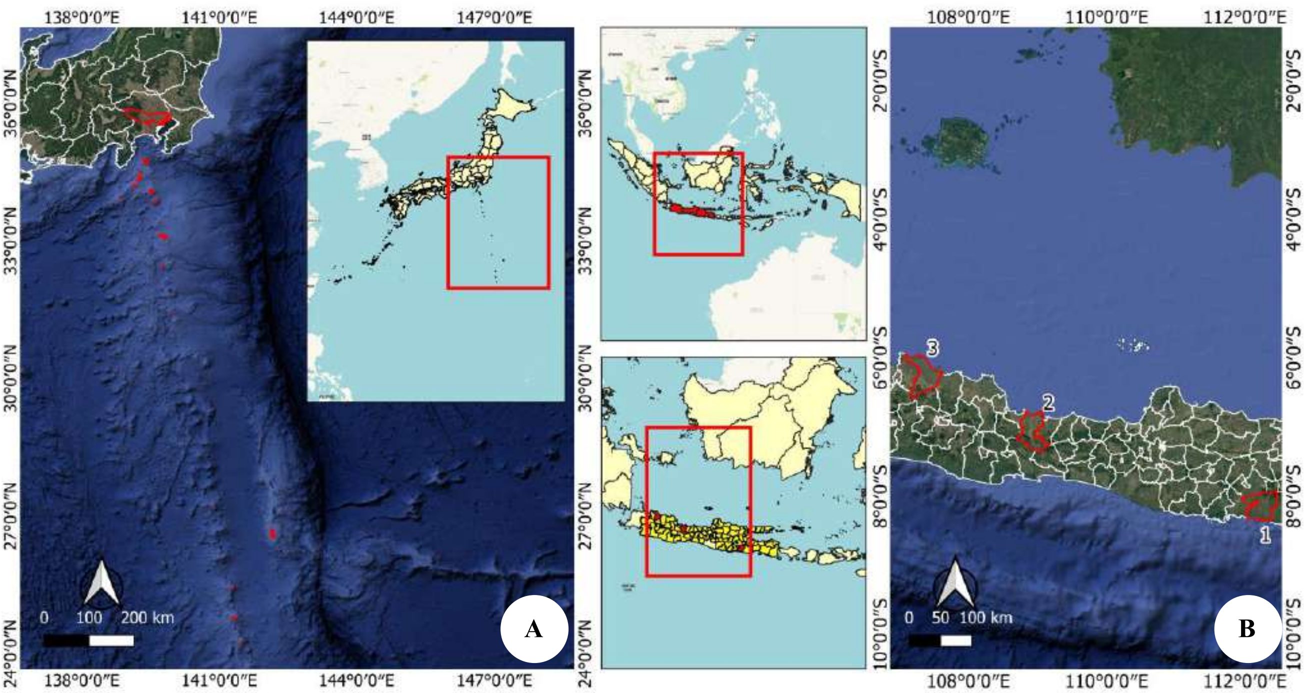

Figure 1. A. Location of salted egg consumer survey in Tokyo, Japan, B. Location of salted egg MSMEs Survey in 1. Blitar, 2. Brebes, and 3. Karawang, Indonesia

Questions for consumers refer to aspects of customer insight from the X-Plane, including seeing, hearing, thinking and feeling, saying and doing, getting, and sensing. The findings at this stage were presented on an empathy map. Moreover, questions for MSMEs adopt aspects of the business model from Osterwalder and Pigneur (2010), including value propositions, core activities, core resources, core partners, customer relationships, customer segments, channels, cost structures, and revenue streams. The findings at this stage were presented in a business model canvas by integrating with various aspects of value innovation from Kim and Mauborgne (2005).

Data analysis

Data analysis employs various analytical methods: (i) Revealed Comparative Advantage (RCA) for comparative advantage, (ii) Export Product Dynamic (EPD) and Trade Specialization Index (TSI) for competitive advantage, (iii) empathy maps, business model canvas, and value innovation framework.

Revealed Comparative Advantage (RCA)

RCA compares a country's salted eggs market share to the global salted eggs market share. The underlying rationale for this method is that a country's export performance is predominantly determined by its relative competitiveness with similar products manufactured in other countries, assuming that other factors influencing export growth remain constant. Calculations using the RCA method were performed using the following formula:

C = (Xij / Xj) / (Xiw / Xw)

Where:
C : RCA value
Xij : Export value of salted egg commodities from country j to the international market
Xj : Total export value of all commodities from country j to the international market
Xiw : Export value of salted egg commodities from all countries to the international market
Xw : Total export value of all commodities from all countries to the international market
i : Salted egg commodities
j : Salted egg exporting countries to the international market
w : All countries or the world

When the Revealed Comparative Advantage (RCA) value exceeds one (RCA>1), a country possesses a comparative advantage above the world average, indicating strong competitiveness for the commodity in question. Conversely, if the RCA value is less than one (RCA<1), a country exhibits a comparative advantage below the world average, suggesting weak competitiveness for the commodity. RCA value is directly proportional to the level of competitiveness or advantage a country holds for a specific commodity.

Export Product Dynamic (EPD) and Trade Specialization Index (TSI)

The EPD is an analytical methodology employed to evaluate and identify products or commodities that possess the highest competitive advantage and exhibit rapid growth within a country's export trade flow. Market position can be ascertained by utilizing the total export share (X) and the export commodity share (Y). By applying the EPD analysis method, it is possible to determine whether a country's commodity exports to the destination country are dynamic. There are four categories: rising stars, falling stars, lost opportunities, and retreats. The systematic approach to calculating a country's export market share and product market share is as follows.

X-axis: Growth in export market share

$$\frac{\sum_{t-1}^{t}\left(\frac{Xij}{Wij}\right) t\ X100\% - \sum_{t-1}^{t}\left(\frac{Xij}{Wij}\right) t-1\ X100\%}{T}$$

Y-axis: Growth in product market share

$$\frac{\sum_{t-1}^{t}\left(\frac{Xt}{Wt}\right) t\ X100\% - \sum_{t-1}^{t}\left(\frac{Xt}{Wt}\right) t-1\ X100\%}{T}$$

Where:
Xij : Value of Indonesian salted egg exports to export destination countries
Xt : Total value of exports of all Indonesian commodities to export destination countries
Wij : Value of salted egg exports from all countries to export destination countries
Wt : Total value of exports of all commodities from all countries to export destination countries
T : Number of years analyzed
t -1 : Previous year
I : Salted egg commodities
J : Indonesia and/or all countries

Furthermore, the TSI analysis aims to assess Indonesia as an exporter or importer of salted eggs. The TSI formulation is as follows:

TSI = (Xia – Mia) / (Xia + Mia)

Where:
Xia : Value of Indonesia's salted egg exports
Mia : Value of salted egg imports in Indonesia

Empathy Map, Business Model Canvas (BMC), and Value Innovation

An empathy map is a tool developed by X-PLANE to explore customer utility. This map shows the consumer experience, which is the basis of a company's value creation. The BMC is a tool designed to construct and explore a business concept that can create, deliver, and capture value. The BMC comprises a single page with nine boxes or blocks, which are used to analyze how a company conceptualizes revenue generation.

Value innovation is the simultaneous pursuit of differentiation and low cost, generating significant value increases for both customers and the company. Given that customer value is derived from the utility of an offering minus its price and company value is derived from the price of an offering minus its cost, value innovation is achieved only when the entire system of utility, price, and cost is aligned. This framework connects the empathy map and BMC element.

For this analysis, we adopted Miles et al.'s (2019) qualitative approach, which encompasses data condensation, presentation, and conclusions. Validity tests employ triangulation, and reliability tests use kappa statistics. Coding using the NVIVO Software yielded a kappa coefficient of 0.86, indicating excellent agreement.

RESULTS AND DISCUSSION

Competitive and comparative advantage of Indonesian salted eggs in the international market

In 2023, the World Trade Organization (WTO) identified Indonesia, along with four other ASEAN nations, as one of the top 30 exporters of goods globally. This classification aligns with the findings of Hoang (2020), who highlighted Vietnam, Thailand, Malaysia, and Indonesia as prominent suppliers of agricultural commodities in the global market. According to Hardi et al. (2021), nearly all Indonesian provinces exhibit strength in non-oil and gas exports, with 16 of the 34 provinces consistently showing these advantages throughout the year. Half of these provinces possess a comparative advantage index in double digits, significantly contributing to the national non-oil and gas export sector. Purwono et al. (2022) emphasized the critical role of competitive and comparative advantages in shaping international market competition in Indonesian animal and vegetable products.

Furthermore, Indonesia holds a robust position in the global salted egg market. The trade specialization index in Table 1 indicates that since 2017, Indonesia has not only fulfilled domestic requirements but has also become a notable exporter of salted eggs. Brebes, the center of salted egg production in Indonesia, produces approximately 142 million salted eggs annually, valued at 12 million USD (Humam et al. 2018). Brebes-salted eggs have achieved national recognition, with brands such as Randy Jaya, HTM Jaya, Pandhawa, and Romlah gaining prominence. Sumekar et al. (2021) attribute the competitive advantage of Indonesian salted eggs, particularly those from Brebes, to the diverse range of products and their superior quality.

The Export Product Dynamic (EPD) depicted in Figure 2 illustrates that Indonesia's salted egg market position in the export destination country is categorized as a "falling star." This classification suggests that while the market share of Indonesian salted eggs is on the rise, products lack dynamism in the international market. The "falling star" status of salted eggs parallels the position of other Indonesian agricultural commodities in the global market, such as nutmeg in the Japanese and German markets (Purba et al. 2021), coffee beans in the Japanese and Belgian markets (Hotman and Malau 2022), and shrimp in the Japanese market (Wati and Aini 2022). Notably, the "falling star" position is preferable to the "retreat" and "lost opportunity" positions (Mardiah and Widyastutik 2020).

Indonesian salted eggs exhibited a limited comparative advantage, as evidenced by the Average Revealed Comparative Advantage (RCA) value of 0.006 from 2012 to 2023 (Table 1). The highest RCA value was recorded in 2021, at 0.035, whereas the lowest RCA values, each at 0.000, were observed in 2013, 2016, 2017, 2022, and 2023. The marked decline in RCA values during these years suggests minimal or no export of salted eggs by Indonesia. This observation aligns with the findings of Abdullah and Rosjadi (2021), who reported a weak comparative advantage for Indonesian animal products in the international market. Sharma et al. (2023) noted that the competitiveness of Indonesian meat is low, with an RCA value of 0.490. Previously, Sutawi et al. (2019) reported a weak comparative advantage for Indonesian beef in the ASEAN market, with an RCA value of 0.000.

Japanese customer insights into salted eggs

Customer insights are instrumental in guiding businesses towards informed decision-making, the development of offerings, and effective engagement with target audiences. In the realm of the agribusiness sector, a thorough analysis of customer motivations, preferences, and challenges is the cornerstone of strategic decisions that align with market demand. These insights function as safeguards that minimize the risks associated with investments in product development or market expansion. Customer insights also contribute to revenue growth by directing resources into areas of significant customer interest, thereby allowing businesses to surpass competitors in term of revenue and profit (Chi et al. 2024). In addition, customer insights are pivotal for bridging the gap between customer expectations and business offerings. While businesses strive to meet or exceed customer expectations, there is often a disparity between customer desires and delivered services. Customer insights are essential to address this gap by ensuring that the customer's voice is recognized and acted upon. Kumar et al. (2023) emphasized that enhancing the customer experience can strengthen sustainable competitiveness.

Table 1. RCA and TSI values of salted eggs

Year	RCA	TSI
2012	0.013	-1
2013	0.000	-1
2014	0.001	-1
2015	0.006	-0.94
2016	0.000	-1
2017	0.000	1
2018	0.003	0.90
2019	0.005	1
2020	0.002	0.96
2021	0.035	1
2022	0.000	1
2023	0.000	1
Average	0.006	0.192

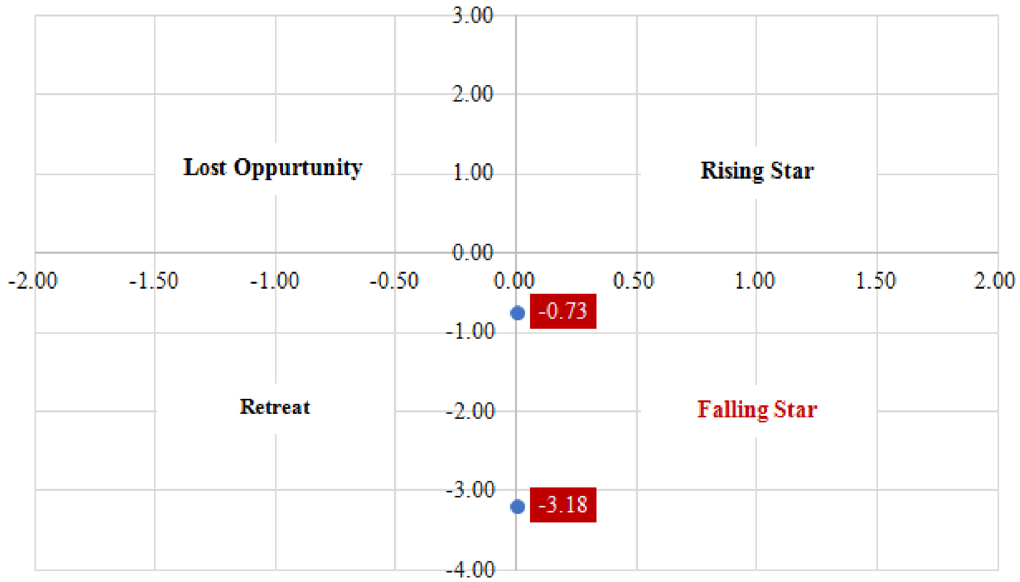

Figure 2. EPD of salted egg

The empathy map depicted in Figure 3 provides insights into Japanese consumers' perceptions of salted eggs. Salted eggs are typically associated with duck eggs, even though salted chicken eggs are commercially available. According to Suretno et al. (2021), salted duck eggs are larger than their chicken counterparts and possess wider pores, which facilitate the penetration of salt throughout the egg. In Japan, consumers recognize that salted eggs are derived from ducks and chicken eggs. Japanese consumers commonly purchase salted eggs in Gyoumo supermarkets, Asian minimarkets, and Indonesian stores, such as Sariraya. Salted duck eggs in the Japanese market are characterized by a paler shell color and vibrant yolks and are priced at 430 yen per pack of six eggs in Indonesian stores, whereas other stores offer them at 500 yen in plastic box packaging.

"I saw salted chicken and duck eggs sold in supermarkets, with predominantly salted duck eggs. This salted egg had a paler shell and a bright yolk. The packaging used plastic boxes or carton boxes containing six salted eggs with a price of 500 yen per box." (Interview with C2, C3, C6, C9, C14, C17, C23, C29, C31, C35, and C39)

Indonesian workers or students in Japan have known about salted chicken eggs, besides salted duck eggs. They perceived it from an Indonesian migrant community residing in Japan through face-to-face interactions and digital platforms. Urbinati et al. (2020) stated that virtual communities function as conduits for information exchange among members. Sharabati et al. (2022) observed that consumers exhibit the capacity for swift and efficient engagement with information shared through WhatsApp, Instagram, and Facebook groups.

"I heard from offline and online communities that salted chicken eggs are available for purchase in minimarkets, alongside the more commonly known salted duck eggs." (Interview with C1, C5, C7, C8, C13, C15, C22, C28, C30, C34, and C38)

Indonesian consumers residing in Japan have long believed that salted eggs available in the market originate from Indonesia. However, they recently discovered that these eggs originated predominantly in China and Taiwan. According to Tang et al. (2021), salted duck eggs have been a traditional food product in China and Southeast Asia for extended periods. Consumers also think and feel that both salted chicken and duck eggs possess a similar taste profile, making them practical and convenient for consumption.

"I think the salted eggs in the minimarket were from Indonesia, but this turned out to be based on labels from China and Taiwan. In my opinion, salted chicken and duck eggs have the same taste." (Interview with C10, C11, C16, C18, C19, C21, C24, and C25)

Japanese consumers state that salted eggs are more expensive than fresh eggs. However, they are considered less risky because they remain edible and retain their quality even if they are damaged. Purchases of salted eggs are typically infrequent and are often intended for use as provisions at workplaces or educational institutions, with the intention of consuming them the following day. This behavior is corroborated by the findings of Nwadi and Okonkwo (2021), who identified eggs as a staple addition to daily dietary practices.

"Salted eggs are more expensive than fresh eggs; however, they are not too risky if they break. I usually buy them on weekends, along with shopping for other products that are used on weekdays as complementary food supplies at work or college. " (Interview with C4, C12, C16, C20, C27, C30, C37, and C40)

Salted eggs serve as an alternative to traditional egg consumption and offer a substitute for fresh eggs with processed options. Salted eggs are particularly valued for their high nutritional content, which is attributed to an optimal composition of essential amino acids and a substantial fatty acid profile that includes a high percentage of polyunsaturated fatty acids and a favorable omega-6 to omega-3 ratio (Al-Obaidi and Al-Shadeedi 2016). In addition, salted eggs are classified as ready-to-eat products because of their immediate consumability; Mopera et al. (2021) categorized salted egg as a food product that is easy to consume.

"*Salted eggs are ready to be consumed as an alternative to egg consumption when working or studying*." (Interview with C25, C26, C32, C33, C36, and C38)

Consumers encounter difficulties in procuring salted eggs because of their restricted availability, as these products are predominantly found in Asian supermarkets or outlets specializing in imported goods, which are often located at considerable distances from consumers' residences. In addition, the supply of salted eggs is inconsistent, prompting consumers to purchase them weekly for occasional use and as reserves. Liu et al. (2020) demonstrated that the proximity between a consumer's home and the store offering the product significantly influences purchasing decisions. To address the challenge of limited access to salted eggs, retail stores and supermarkets should establish online platforms. Le et al. (2022) emphasized that online services can diminish the need for consumers to travel for shopping, thereby offering a viable solution to the issue of product availability.

"*Salted eggs are typically available only in Asian markets and specialty stores that offer imported goods and are located at a considerable distance from my residence. Consequently, these items are often purchased in bulk during weekend shopping trips, although they may occasionally be out of stock.* " (Interview with C1, C4, C12, C16, C18, C20, C26, C27, C30, C31, C39, and C40))

Designing a competitive salted eggs MSME business model in the Japanese market

A business model delineates the product or service that a business intends to offer, the specified target market, and the projected costs. Business models are crucial to nascent and established enterprises. Established businesses must revise their business models periodically to anticipate future trends and challenges. Ritter and Pederson (2020) illustrate the transformation of contemporary and prospective business models through the digitization of business models. The design or redesign of the business model for egg export MSMEs in the international market incorporates nine elements of the business model canvas, as shown in Figure 4.

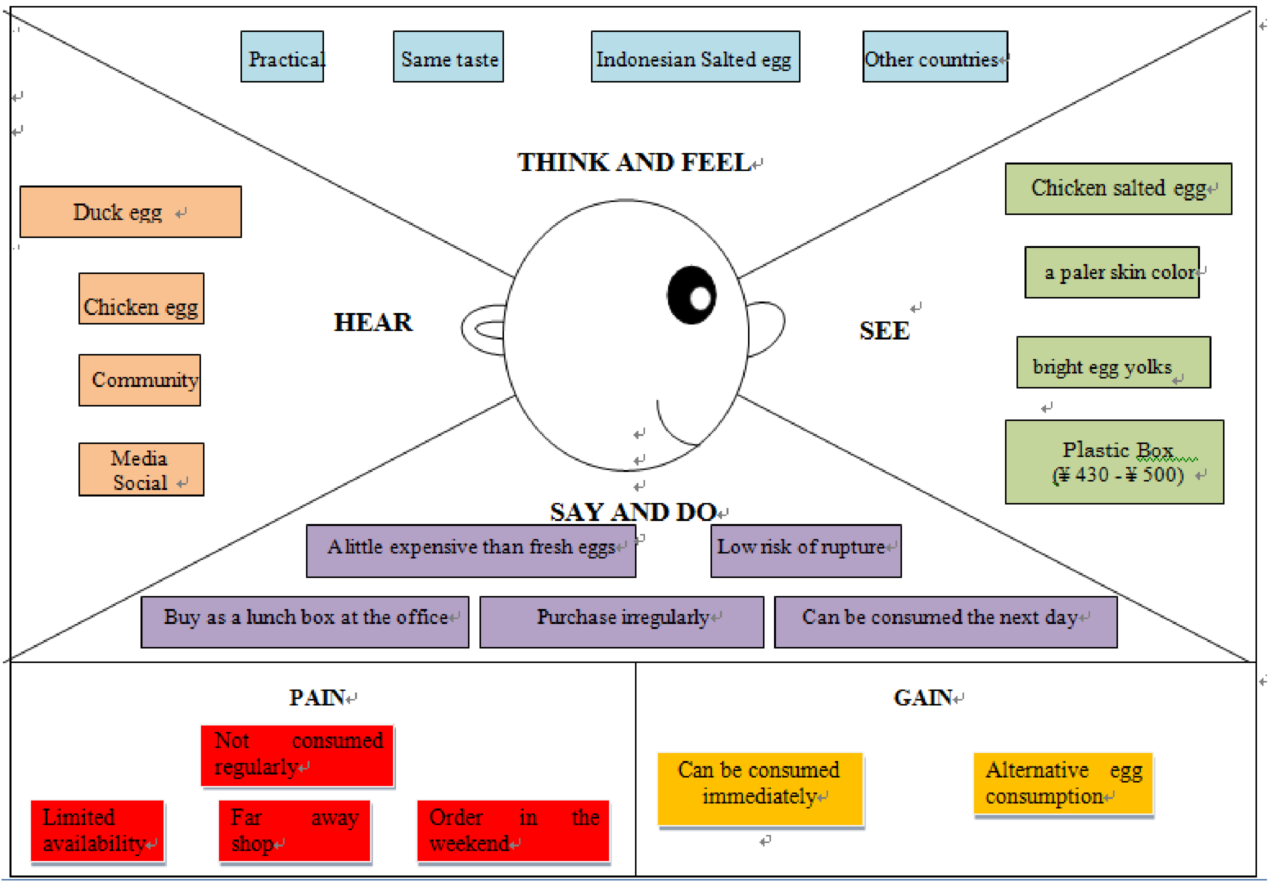

Figure 3. Empathy maps of salted egg customers

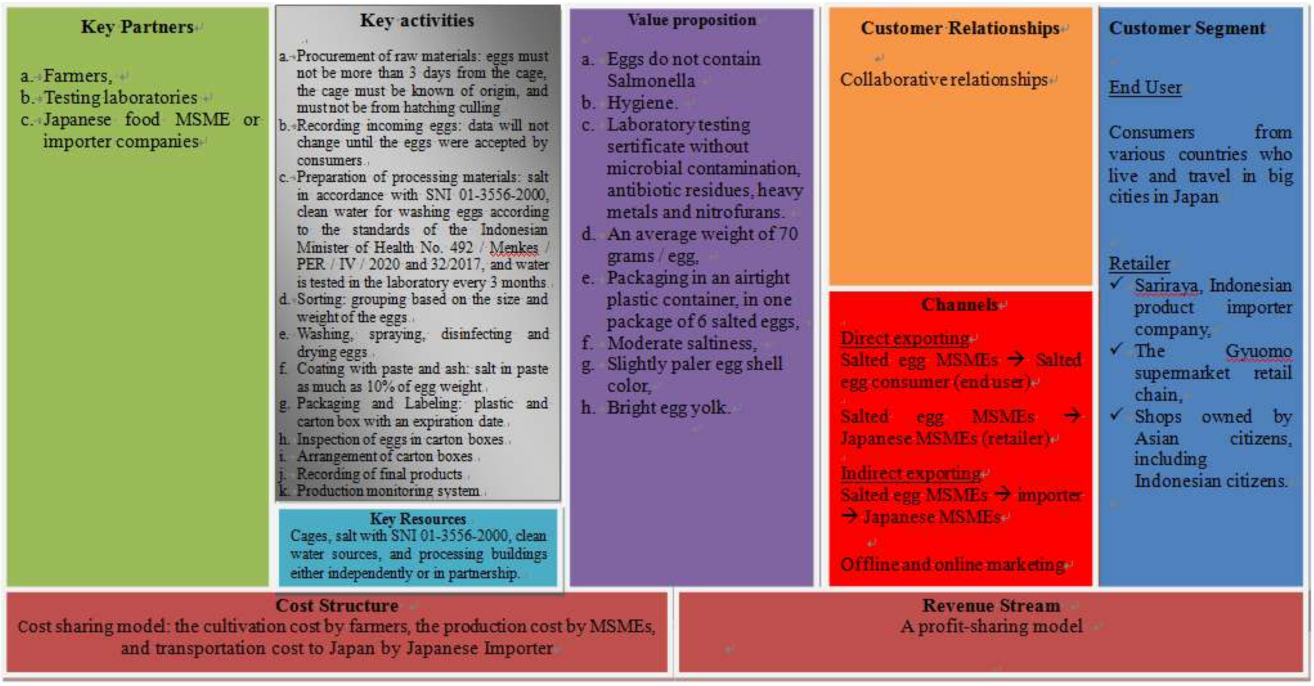

Figure 4. A competitive MSME business model of salted eggs in the Japanese market

Market segmentation is a critical strategy for business entities, particularly MSMEs, to penetrate or expand their presence in international markets. According to Xu et al. (2019), the initial step for businesses to enter the global market involves the development of a comprehensive segmentation strategy. In Japan, the primary consumers of salted eggs are individuals from diverse international backgrounds residing in or visiting major urban centers such as Tokyo and Osaka. In contrast, the local Japanese population generally prefers *onsen tamago*, a traditional dish containing half-boiled eggs. Retail market segments include the Indonesian product importer Sariraya, the Gyuomo supermarket chain, and stores operated by Asian communities, including the Indonesian society.

For MSMEs that export salted eggs, it is essential to address both regulatory compliance and consumer preferences. These compliances drive the quality performance of MSME products. Revindo et al. (2020) reported that Indonesian exporting MSMEs contributed to the high performance of product quality. Moreover, understanding consumer preferences is vital for producers to craft effective marketing strategies that resonate with consumers and evaluate whether product attributes meet consumer expectations (Shafira et al. 2021). The Japanese market requires MSMEs to provide eggs that do not contain Salmonella, adhere to stringent hygiene and sanitation protocols, and undergo laboratory testing to ensure eggs are free of microbes, antibiotic residues, and heavy metals. Matsumoto (2022) reported that over 700 food products are recalled in Japan each year, mostly due to contamination by microorganisms in violation of the Food Sanitation Act. Other causes include failure to list expiration or best-before dates, and incorrect or unlisted allergens in violation of the Japanese Food Labeling Act. Therefore, MSMEs offer a value proposition with an average egg weight of 70 g, packaging each egg in an airtight plastic container with six eggs per package, moderate saltiness, a slightly paler eggshell, and a vibrant egg yolk.

Key activities within a process are essential for the creation and delivery of a value proposition. Process innovation is a driving force for both gradual and transformative product innovation (Lee et al. 2019). Several key activities in the process of creating value for salted egg products are as follows:

a. Procurement of raw materials: Eggs must not be more than 3 days from the cage, the cage must be known of origin, and must not be from hatching culling.

b. Recording of incoming eggs: the data remained consistent until the consumers accepted eggs.

c. Preparation of processing materials: salt must meet the Indonesian National Standards (SNI) 01-3556-2000, and clean water for washing eggs must comply with the standards set by Indonesian Minister of Health No. 492/Menkes/PER/IV/2020 and 32/2017, with water quality tested in the laboratory every three months.

d. Eggs were sorted based on their size and weight.

e. Washing, spraying, disinfecting, and drying eggs.

f. Coating with paste and ash: salt in the paste should account for 10% of egg weight.

g. Packaging and labeling: plastic and carton box with an expiration date

h. Inspection of eggs in carton boxes.

i. Arrangement of carton boxes.

j. Recording of final products.

k. Production monitoring system: This encompasses ensuring water safety, maintaining environmental cleanliness, preventing contamination, maintaining sanitation facilities, preventing pollution, labeling toxic materials, ensuring employee health, and controlling pests.

The execution of these essential activities necessitates the support of key resources, including human resources, intellectual assets (e.g., brands, copyrights, patents, and customer databases), physical resources (e.g., buildings, vehicles, and equipment), financial resources, and information systems. Intellectual resources are particularly crucial for the success of MSMEs; however, they often receive insufficient attention (Ying et al. 2019). For MSMEs involved in the export of salted eggs, key resources include facilities such as cages, salt that complies with SNI 01-3556-2000, clean water sources, and processing buildings, whether operated independently or in collaboration.

Partnerships are integral to a business's success. According to Delfitriani (2019), key partners are pivotal actors in every product supply chain, ensuring that products reach consumers. Salted egg-exporting MSMEs must establish partnerships with stakeholders, including farmers, testing laboratories, Japanese food MSMEs, and international importers. Farmers provide the primary raw material (eggs). Testing laboratories assess the quality of salted egg products, whereas international MSME partners serve as distributors in the target markets.

Channels can use two ways, direct and indirect exporting through importers. Kiessling et al. (2024) found that MSMEs with direct export channels tend to internationalize more quickly than MSMEs with indirect export channels. The effectiveness of the channel is influenced by the relationship dynamics among partners or actors throughout the supply chain, from upstream to downstream, culminating in the end consumer. In addition, the effectiveness of these channels in delivering optimal services and products is contingent on the efficient utilization of resources. Companies that leverage digital technology have a greater potential to enhance digital exports (Elia et al. 2021). Digital marketing strategies can use diaspora networks for direct export. Digital marketing strategies leverage diaspora as promotional agencies, influencers, or business partners to increase the visibility of products or services in international markets and build online communities. According to Steel (2021), MSMEs can establish international business partnerships through online advertising in diasporic online groups. Diaspora Facebook groups have formed transnational online communities connected to the homeland (Mpofu et al. 2022). Salted-egg exporting MSMEs may adopt a strategy from Lashgari et al. (2018). They have introduced adoption strategies of social media in B2B, including selection, adoption, and integration processes. In the selection process, MSMEs identify the target group to determine the social media platforms and the content that will be shared on these platforms. In the media adoption process, MSMEs determine moderators to facilitate interaction with target groups, public accessibility of information, and use of online communities. Finally, in the integration phase, SMEs integrate online channels into other offline promotional channels, such as exhibitions or trade shows.

Effective partnership relationships within the supply chain are contingent upon each participant acting as a customer who must be adequately served to ensure the satisfaction of the end consumer. Various forms of these relationships include collaboration, cooperation, and coordination. According to Castañer and Oliveira (2020), collaboration involves voluntarily assisting partners in achieving their shared objectives. Coordination pertains to the establishment of common goals (IOR), whereas cooperation involves the execution of these goals. The salted egg MSME fosters collaborative relationships to achieve its mutual objectives.

Furthermore, the cost structure employed is based on a cost-sharing model, wherein cultivation costs are borne by farmers, production costs by MSMEs, and transportation costs to Japan by the Japanese importer. Xiao et al. (2020) demonstrated that cost sharing is advantageous for enhancing sustainable investment and can lead to increased profits. Chakraborty et al. (2019) stated that sharing contract costs results in higher levels of quality improvement and increased profits within the supply chain.

Finally, the revenue flow pattern adopts a value chain with a profit-sharing approach. As noted by Hidayat and Purbayati (2023), profit-sharing involves calculating the profit distribution based on the net result of total income after deducting the costs or expenses incurred to generate that income. Profit-sharing aligns with the costs incurred. Business actors incur the highest cost and receive the highest profit compared to other parties.

This business model study has managerial implications for salted egg exporting MSMEs. The business model will transform rapidly if it receives policy support from the government. According to Wasserbaur et al. (2022), policymakers play a role in supporting business models to be more competitive. This study provides policy recommendations, including the government holding MSMEs training for hygiene and halal certification, facilitating business partnerships between Indonesian and Japanese MSMEs to strengthen collaborative relationships between them, and providing incentives for exporting MSMEs.

In conclusion, the market segment MSMEs can target to export salted eggs to Japan includes international consumers residing in or visiting Japan. The value proposition for these MSMEs should focus on several key aspects: (i) egg-free Salmonella, (ii) hygiene, (iii) free from microbial contamination, antibiotic residues, and heavy metals, (iv) an average egg weight of 70 g, (v) packaging each egg in an airtight plastic container, with six salted eggs per package, (vi) offering a moderately salty taste, (vii) presenting a slightly paler eggshell color, and (viii) ensuring a bright egg yolk. The process of creating value for salted egg products involves 11 key activities, ranging from procurement of raw materials to monitoring. The essential resources for MSMEs to export salted eggs include cages, salt with SNI 01-3556-2000, clean water sources, and processing facilities, which may be operated independently or through partnerships. Salted egg export MSMEs must establish partnerships among actors. The channels for salted egg products are direct and indirect exporting. Direct export challenges require designing business model partnerships between Indonesian and Japanese food MSMEs. Therefore, collaborative relationships among actors are crucial. The cost structure is based on a cost-sharing model, whereas the income flow pattern follows a value chain with a profit-sharing model. This business model design serves as a prototype for egg MSMEs, aiming to export internationally, with a particular focus on the Japanese market. Consequently, this prototype requires validation through Participatory Action Research (PAR). Future studies may develop comparative studies with non-exporting MSMEs or exporting MSMEs in other international markets.

ACKNOWLEDGMENTS

UIN Syarif Hidayatullah Jakarta, Indonesia funded this study. We would like to thank Puslitpen LP2M UIN Jakarta for supporting this study.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 298-304 DOI: 10.13057/asianjagric/g090131

Exploring the impact of vermicomposting on growth of wheat under organic farming system

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Manuscript received: 11 September 2024. Revision accepted: 26 May 2025.

Abstract. *Barla A, Patel S, Swain SS, Parida S. 2025. Exploring the impact of vermicomposting on growth of wheat under organic farming system. Asian J Agric 9: 298-304.* Climate Resilient Agriculture (CRA) includes the sustainable use of natural resources through crop production to attain long-term higher productivity and income from farms under variable climatic conditions. Though organic farming is criticized as a lower-yielding system than the conventional system and is unable to meet the food demand of the world's population, still simultaneously it has a positive impact on the environment and on maintaining the soil ecosystem including a positive impact on human health. Therefore, consumer demand for organic products is increasing which encourages organic farming systems. This paper focuses on integrating organic farming particularly the use of organic compost in promoting soil health and wheat crop growth. In this study, vermicompost and cow dung compost were applied to observe its impact on the growth of wheat (*Triticum aestivum* L.) crops under pot experiments. A pot containing normal soil was taken as control, soil: cow dung (2:1) and vermicompost was applied in different proportions of soil and vermicompost (S: V) in 1:1, 2:1, and 4:1 ratios to evaluate the vegetative crop growth. For wheat crop, the application of vermicompost (S: V with 2:1) in soil showed better results on crop growth than the cow dung compost (S: C with 2:1). Mineral contents of leaf were also analyzed by X-ray fluorescence (XRF) study and detected more in the leaves of wheat plant raised in S: V with (2:1) than the S: C (2:1) pots. Organic farming systems can be used for superior nutritional qualities in comparison to conventional systems.

Keywords: Crop growth, mineral contents, organic farming, *Triticum aestivum*, vermicompost

INTRODUCTION

Agriculture plays an important role in the economic development of any country, and low productivity is resulted because of declining soil fertility (Mubarak et al. 2021; Salem et al. 2022). Wheat (*Triticum aestivum* L.) makes a significant contribution to one-third of overall food grain cultivation in India. India ranks second among wheat-growing countries worldwide. As a result of the "Green Revolution," India's agricultural production increased dramatically from the 1960s to the 1980s (Gupta 1996). Agrochemicals harm soil quality and pollute the environment. Healthy soil is a dynamic ecosystem enriching with microscopic to macroscopic organisms that perform several vital functions such as conversion of minerals to plant nutrients (nutrient cycling), conversion of dead and decaying matter, and improved water and nutrient holding capacity for improving crop growth and productivity. Wheat grain yield depends upon soil type, wheat genotype, persistent meteorological conditions, and their interaction. Soil quality is directly linked to crop growth, quality, and quantity of the produce. Soil supplies water, oxygen, and essential nutrients to the plants. Organic farming is a rapidly growing sector in many nations and is assessed in terms of sustainability. Everyone wants sustainable agriculture, but how to progress is not clear. Soil conditions showed the highest effect on variation in grain yield of wheat, and salinity resulted in a reduction in grain yield (Matkovic et al. 2022).

Climate Resilient Agriculture (CRA) is an agricultural approach that use the existing natural resources to induce crop growth, higher crop productivity, and farm incomes. Organic farming can enhance food quality without any adverse effect on both soil and crop produce and can ensure sustainability (Simsek-Ersahin 2010). The use of vermicompost is increasing day by day, both in developed and developing countries (Simsek-Ersahin 2011), to enhance crop yield. Organic farming has been shown to enhance different environmental components of the agroecosystem (Bulluck et al. 2002; Kramer et al. 2006). The soil's organic carbon and nitrogen content are increased by the application of compost and other organic manures. Traditional thermophilic compost is recognized as an effective method to enhance soil aggregation, fertility, and porosity, thereby improving water-holding capacity (Przemieniecki et al., 2021), increasing microbial diversity, and increasing cation exchange capacity of the soil, which results in the production of disease-free crops (Nardi et al. 2004).

Global increasing population demands the acquisition of an extensive and intensive cropping system expecting higher production (Sadowski and Baer-Nawrocka 2018). This drives for vigorous use of agrochemicals, which leads to unsustainable practices leading to soil toxicities and nutrient imbalance, which is an important drawback for sustainable production (Rojas et al. 2016). Food products containing the residues of these agrochemicals affect both livestock and human beings (Rasmussen et al. 2016; Pimentel et al. 2018). The addition of organic amendments is important to improve soil health and sustainable productivity (Hijbeek 2017; Gamage et al. 2023). Amongst the organic fertilizers, vermicompost is one of the potential sources to enhance crop growth. Earthworms are involved in producing vermicompost with other associated microbes (Abou el-Goud et al. 2021, by non-thermophilic biodegradation of organic wastes Manzoor et al. 2024). It is proven as a crucial biofertilizer that enhances crop yields by adapting to environmental stresses.

Vermicompost contains approximately 1.5-2.2% nitrogen, 1.8-2.2% phosphorus, and 1.0-1.5% potassium. Vermicompost is a substantial source of micronutrients (Adhikary 2012). Vermicompost improves plant growth by regulating soil properties (Xu et al. 2023). Vermicompost is also a well-known source of essential nutrients and promotes the yield and growth of plants when applied in small amounts (Atiyeh et al. 2002; Arancon et al. 2005). Soil organic matter is protected from biodegradation physically because of soil aggregates and soil aggregates also improve the structure of the soil and pore size distribution (Tisdall and Oades 1982; Xuan et al. 2023), all of which can affect the soil hydraulic properties. The use of vermicompost in crop fields can improve soil organic matter and reduce pesticide contamination in soils (Yen et al. 2021). In this study, vermicompost and cow dung compost were applied to observe their impact on the growth of wheat (*T. aestivum*) crops under pot experiments.

MATERIALS AND METHODS

Experiment site for wheat crop growth

A pot experiment was carried out in February 2022 to study the vegetative growth of the wheat crop in pot cultivation methods at Centurion University of Technology and Management, Bhubaneswar campus, Odisha.

Collection of cow dung manure and vermicompost

Cow dung manure and vermicompost were collected from the composting unit of Centurion University, Bhubaneswar campus.

Pot preparation

A pot containing normal soil (sandy loam soil with a pH value ranging from 6.73 to 6.85) (T0) was taken as control. Soil (S) was mixed with cow dung (C) manure (S: C) in a 2:1 (kg/kg) ratio (T1). Vermicompost (V) was applied to the soil in different proportions i.e., (S: V) in 1:1 (T2), 2:1(T3), and 4:1 (w/w) ratio (T4) and mixed properly and 15 pots were filled separately in triplicate for each treatment to evaluate the vegetative crop growth in interval of 15, 25 and 35 Days After Sowing (DAS).

Raising of seedlings

Seeds of wheat variety HD 3226 were procured from an online shopping agency. Before sowing the wheat seeds were soaked in water on overnight and sown in the soil filled pots in row manner. After 3-5 days' small wheat plants started appearing. Ten plants were randomly selected from each replication at 15 days and 25 days to measure the growth parameter. The growth of the plant in S: V treated pot in 2:1 ratio and control pot was measured at 35 days of showing (Figure 8) as other treated pots did not show any positive response in comparison to the plants raised in control pots.

Sampling and data collection

Growth parameters were recorded after 15 DAS intervals and after 25 DAS. A total of 10 plants were selected randomly from each treatment and the average plant height and leaf length were recorded in cm.

Elemental composition of leaf samples by X-ray fluorescence analyses

The leaves were collected from the T4-treated pots showing maximum vegetative growth and also from the S: C (2:1) pots with minimum vegetative growth to measure the nutritional content by X-Ray-Fluorescence Spectroscopy (XRF). Two grams of leaf samples were separately weighed for the analyses.

Statistical analysis

All determinations were carried out in triplicate. The reported values are averages of 10 plants selected from each pot. Experimental data was expressed as mean ± S. E. and one-way Analysis of Variance (ANOVA) was carried out using a One-Way ANOVA Calculator, including Tukey HSD, to determine significant differences from the control ($p \leq 0.05$).

RESULTS AND DISCUSSION

Vegetative crop growth

The vegetative crop growth was measured in an interval of 15, 25 and 35 DAS (Figures 1-7). Data depicted in Table 1 showed that the average length of the shoot at 15 DAS was measured to be more in the pot containing soil: vermicompost (2:1) with 9.86 cm followed by control soil with 9.60 cm, equal proportion of S: V (1:1) with 7.20 cm, S: V (4:1) with 7.08 cm and least growth in soil: cow dung (2:1) with 6.08 cm (Figures 1.A-1.H). The average root length of the wheat after 15 DAS in S: V (2:1) was maximum with 4.08 cm followed soil grown plants with 3.08 cm, S: V (4:1) with 2.98 cm, S:C (2:1) 2.36 cm and least in S: V (1:1) with 2.70 cm. Similarly, maximum leaf length was recorded in S: V (2:1) with 17.82 cm followed by control (soil) with 3.08 cm and least in S: C (2:1) with 6.08 cm.

Table 1. Result details of one-way analysis of variance including Tukey HSD test

Source	SS	df	MS	
Between-treatments	141.2256	1	141.2256	F = 36.61978
Within-treatments	30.8523	8	3.8565	
Total	172.078	9		

Average length of the shoot at 25 DAS was also measured to be more in the pot containing S: V (2:1) with 14.50 cm followed by control soil with 12.20 cm, equal proportion of S: V with 11.70 cm, S: V (4:1) with 11.80 cm and least growth in S: C (2:1) with 9. 60 cm. The average leaf length of the wheat at 25 DAS was measured a maximum of 17.40 cm in the control condition followed by S: V (2:1) with 13.70 cm and least in S: C (2:1) with 9.80 cm. Standard Deviation (SD), and standard error were calculated and the error graph is shown in Figure 2.

The findings of another work revealed that vermicompost-applied soil positively influenced the growth and productivity performance of wheat cultivars (Ahmed et al. 2022). Previous studies revealed the positive influence of the combined application of vermicompost with other organic fertilizers influences the morpho-physiological and yield characteristics under drought stress conditions (Aboelsoud and Ahmed 2020; Hafez et al. 2020).

A one-way ANOVA calculator is used, including the Tukey Honestly Significant Difference (HSD) test, which gives a statistically significant result; this indicates that at least one group differs from the other groups. The f-ratio value is 36.61978 and the *p*-value is .000305 (Table 1). The result is statistically significant under the value of significance level at $p < .05$.

It was observed that the growth of the wheat plants was maximum in T2 followed by T0 pots. Therefore, the height of the plant and leaf length was measured after 35 DAS. Table 2 revealed that the average plant height grown in the T2 pot after 35 DAS was 26.30 cm in comparison to T0, with an average height of 22.60 cm. Average leaf height was also higher in the case of the T2 pot than T0, with 20.9 and 18.70 cm, respectively. Other treated pots were not taken into consideration as the plant growth did not show any positive response on the growth parameter in comparison to the control condition (Figure 3).

Figure 3 revealed that the average height of the plant grown in T2 pot after 35 DAS was 26.3 cm in comparison to T0 pot with an average height of 22.6 cm. Average leaf height was also higher in the case of the T2 pot than T0, with 20.9 and 18.7 cm, respectively. From the error bar graph it is shown that plant growth in T0 and T2 shows more growth than T1, T3 and T4 treatments. Therefore, T1, T3 and T4 treated pots were not taken into consideration as the plant growth did not show any positive response on the growth parameter in comparison to the control condition.

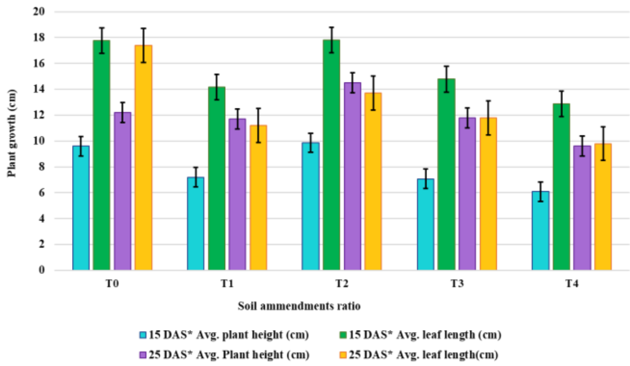

Figure 2. *Triticum aestivum* crop growth after 15 DAS on different soil composition

Table 2. Average plant height and leaf length of wheat in T0 and T2 pots at 35 DAS

Soil treatment	35 DAS*	
	Avg. plant height (cm)	Avg. leaf length(cm)
T0	22.60	18.70
T2	26.30	20.90

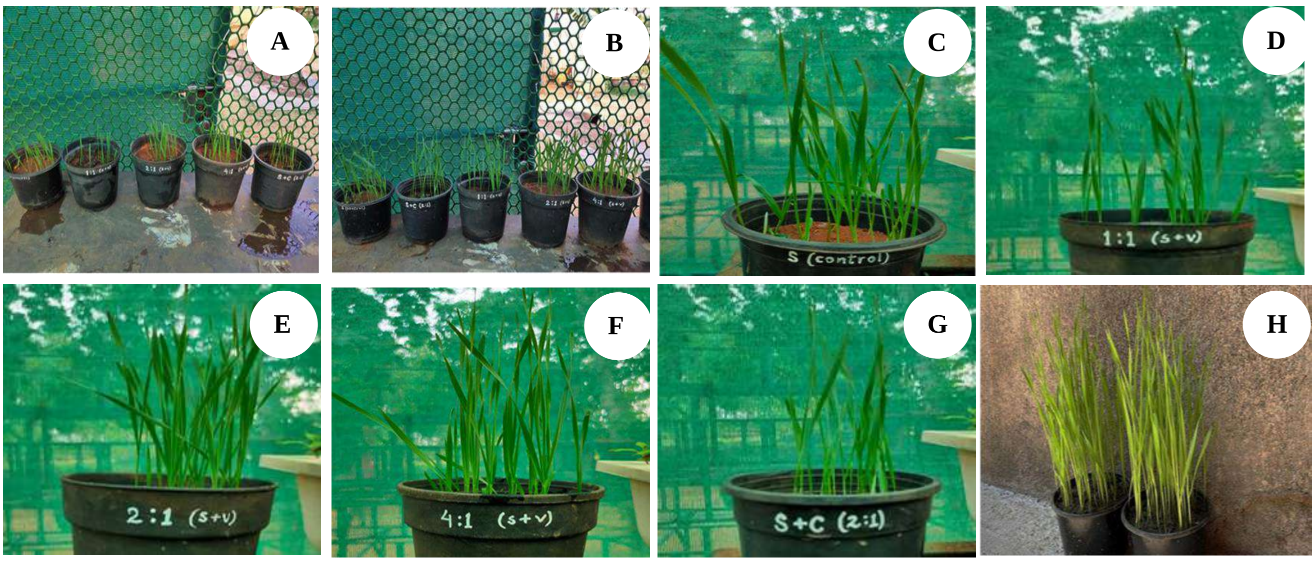

Figure 1. Wheat seedlings growth: A. After 7 DAS; B. After 15 DAS; C. After 25 DAS; D. Wheat grass growth in S: V (1:1) after 25 DAS; E. Wheat grass growth in S: V (2:1) after 25 DAS; F. Wheat grass growth in S: V (4:1) after 25 DAS; G. Wheat grass growth in S:C (2:1) after 25 DAS; H. Wheat grass growth in control and S: V (2:1) after 35 DAS

Table 3. Mineral contents in leaves of T0 (Control), T2 (S:V::2:1) and T4 (S:C::2:1) grown plants (%)

Compounds	Soil (control)	S:V (2:1)	S:C (2:1)
SiO_2	19.23	15.52	11.01
P_2O_5	6.23	7.37	7.6
SO_3	2.7	4.12	5.01
CI	6.32	5.25	14.26
K_2O	63.9	65.28	60.48
TiO_2	0.5	0.40	0. 22
MnO	0.36	0.14	-
Fe_2O_3	1.21	1.8	1.22

Table 4. Mineral contents in leaves of T0 (Control), T2 (S:V::2:1) and T4 (S:C::2:1) grown plants (ppm)

Compounds	Soil (control)	S:V (2:1)	S:C (2:1)
V_2O_5	67.3	86.5	71.1
CuO	102.3	141.3	105.1
ZnO	-	534.2	-
Br	36.5	47.5	-
Rb_2O	127.9	141.3	130.3
Zr_2O	68.5	77.7	-
Eu_2O_3	203.7	261.5	383.8

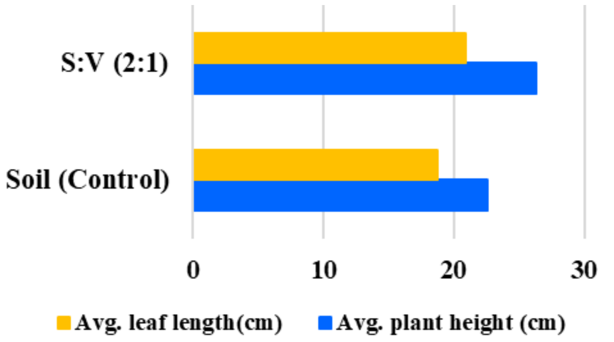

Figure 3. Comparative wheat crop growth after 35 DAS on T0 and T2

Element analyses of wheat leaves in T2 and T4 pots by XRF method

The elemental composition of leaves from both pots was measured by XRF analyses. Data in Table 3 revealed that 15 different elemental compounds were detected in the leaves of wheat plants after 15 DAS raised in T2. K_2O was detected in the maximum amount of 65.28% followed by SiO_2, P_2O_5, CI, SO_3, and Fe_2O_3 with 15.52, 7.37, 5.25, 4.12, and 1.8%, but the leaves collected from T4 pots were detected with 11 different elements with a maximum of K2O 60.48 followed by CI, SiO_2, P_2O_5, SO_3, Fe_2O_3, and TiO2 with 14.26, 11.01, 7.6, 5.01, 1.22 and 0.22 % respectively. MnO is not detected in leaves of T4 (S: C::2:1) grown plants.

ZnO was detected in leaves of T2 (S:V::2:1) with 534.2 followed by Eu_2O_3 (261.5 ppm), CuO and Rb_2O with 141.3ppm, V_2O_5 with 86.5 ppm, Zr_2O with 77.7 ppm, Eu_2O_3 with 383.8 ppm. In the leaves of T4 (S:C::2:1) grown plants, different trace elements were detected, including Rb_2O at 130.3 ppm, followed by CuO with 105.1 ppm and V_2O_5 with 71.1 ppm. In T4 treatment ZnO, Br, and Zr_2O are not detected (Table 4). XRF graph represented the elemental composition in leaf samples in soil (control), S:V (2:1) and S:C (2:1) grown pots (Figures 4-6).

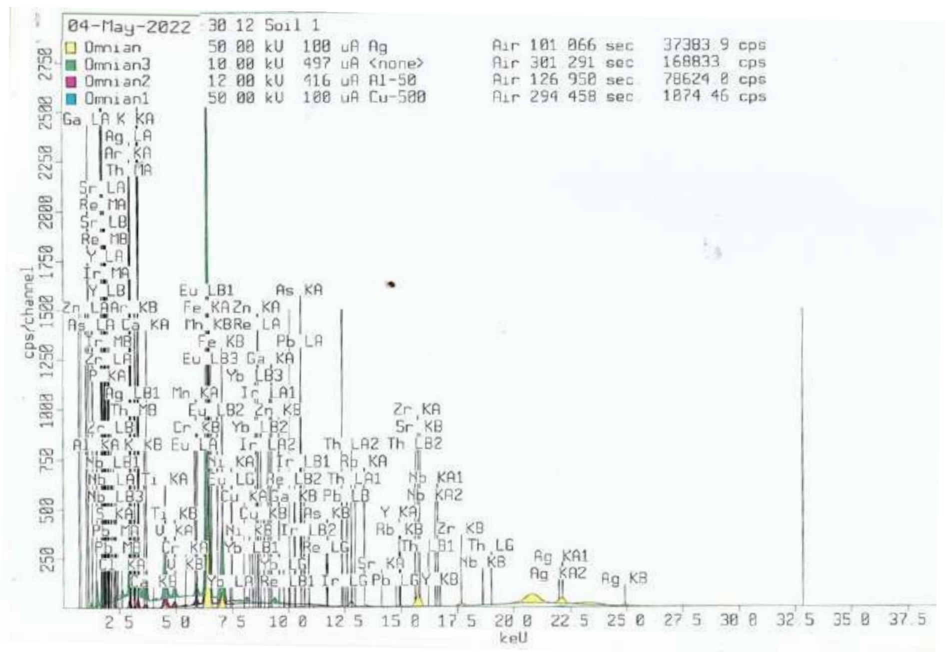

Figure 4. XRF graph showing mineral contents of leaf samples grown in normal soil (control) containing pots

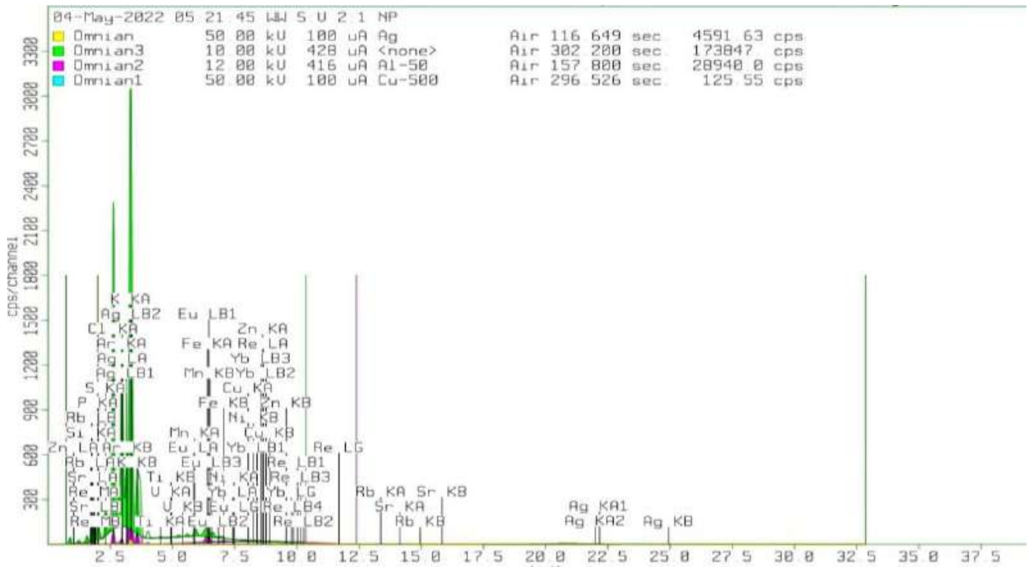

Figure 5. XRF graph showing mineral contents of leaf samples in S: V (2:1) grown pots

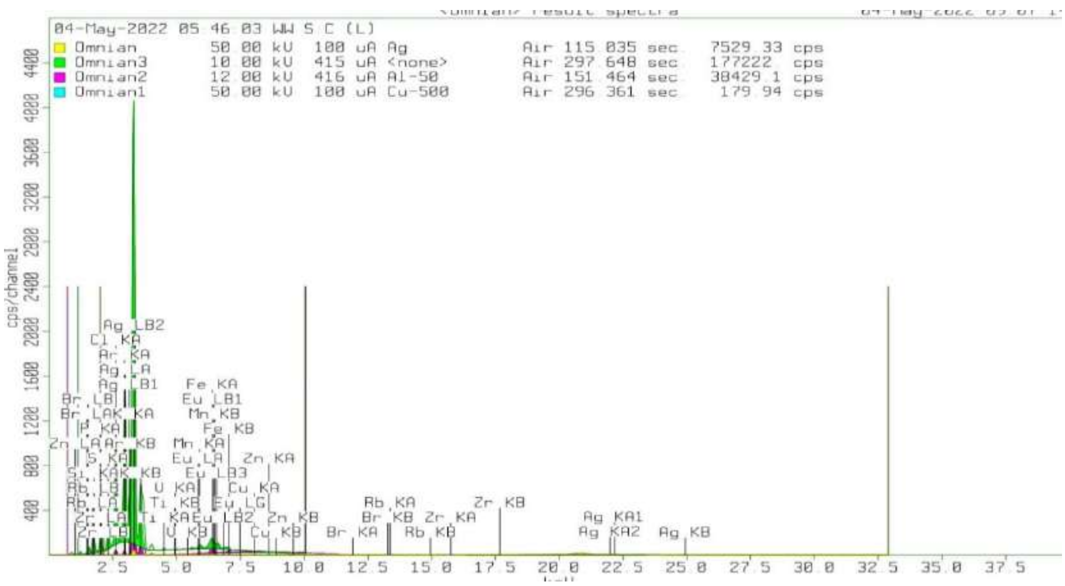

Figure 6. XRF graph showing mineral contents in wheat leaves in S:C (2:1) grown pots

The organic farming systems maintain plant vigor, yield (Burkhard et al. 2009) and quality with positive, acceptable environmental impacts (Tilman et al. 2002; Bhaskaran and Krishna 2009). This experiment shows a significant impact of vermicompost on wheat crop growth in T2, but the leaf length was more in the T0 than T4 pot, which was found to be similar to the findings of Trewavas (2004). Organic agriculture has been criticized as low-yielding and less efficient than conventional agriculture in its use of land and resources (Trewavas 2004). Several yield trial comparisons between organic and conventional farming systems have shown significantly lower yields for organic systems (Stanhill 1990; Ryan et al. 2004). In another study, grain yield, the weight of the kernel, test weight, and protein content were reported to be unaffected by the tillage system (Carr et al. 2003). It was reported that application of vermicompost alone, without any addition of inorganic fertilizer, cannot enhance productivity significantly (Ryan et al. 2004). Another study in 30 villages in Patiala and Faridkot Districts of Punjab also reported that the total variable cost per acre for wheat cultivation under the organic farming system was less as compared to the inorganic method, but the lower yield of the organic wheat crop was compensated by its higher market price (Singh and Grover 2011). The total variable cost for organic wheat cultivation has been also found less in comparison to the inorganic method of cultivation (Singh and Grover 2011).

Organic wheat cultivation, therefore, has been found more profitable but simultaneously the reduction in crop productivity poses a very serious challenge in terms of food security for the growing population of the nation. Significant increases in micronutrient content were reported in the soil after vermicompost application (Hussain et al. 2018). The amount of nitrogen in the soil increased significantly (Sreenivas et al. 2000), and the potassium and phosphorus amounts were also increased (Venkatesh 1998). It is also revealed that the growth parameters in clay soil at 50% field capacity mixed with sandy soil at 100% field capacity showed (109.34 t/ha) highest in the combined use of organic matter at the rate of 15g/2 kg with vermicompost at the rate of 5 g/2 kg (Ahmed et al. 2022). Selected Integrated Soil Fertility Management practices (ISFM) were studied at different watersheds and central highlands of Ethiopia, in the 2021-2022 cropping seasons to improve the soil quality and increase crop production. A combination of vermicompost, urea, and NPS fertilizers can help farmers in reducing their inorganic fertilizer requirements by 50 percent (Habtamu et al. 2024).

Vermicompost significantly affects plant growth (Blouin et al. 2019) because it might contain Plant Growth Promoting Rhizobacteria (PGPR) (Gopinath and Prakash 2014), which supports wheat growth, increases the grain yield, and is found as a good alternative to using chemical fertilizer in terms of economic benefits for winter wheat growth, and yield (Öksel et al. 2022). Integrated Soil Fertilization Management (ISFM) practices have been shown to improve soil quality and crop productivity. New evidence shows vermicompost and effective microorganisms can improve wheat drought tolerance by regulating photosynthetic efficiency, nutrient acquisition, root H+-pump activity, and membrane stability, thereby overcoming drought-related challenges (Talaat and Abdel-Salam 2024). Vermicompost treatments show improved vegetative growth and yield attributes of the wheat cultivar in a specific amount. Vermicompost, rich in macro-nutrients and micro-nutrients, can be used with chemical fertilizers to reduce doses and is a beneficial biofortification source. It indirectly acts as a biocontrol agent through phytohormone synthesis and increases nitrogen and phosphorous uptake. In this study soil vermicompost in a 2:1 ratio resulted in better vegetative growth and showed more elements in comparison to S:C (2:1) treated pots, which might be due to the availability of optimum level of mineral contents required for the wheat crop in comparison to other treatments.

Vermicompost significantly affects plant growth (Blouin et al. 2019) by PGPR which supports wheat growth, increases the grain yield, and is found as a good alternative to using chemical fertilizer in terms of economic benefits for winter wheat growth and yield (Öksel et al. 2022). In another study, PGPR has been isolated from vermicompost (Prakash and Hemalatha 2013), therefore in this study, PGPR may be present in the vermicompost, which may be responsible for the growth of the plants along with the rich nutritional composition of vermicompost. In this study, PGPR treatment had not been given, but the vermicompost may contain the plant growth promoting rhizobacteria according to Prakash and Hemalatha (2013), which may contribute in enhancing the wheat crop growth or the nutrient rich vermicompost may enhance the plant growth.

In conclusion, the present experiment was conducted to study the application of organic manure to the growth of the wheat plant (*T. aestivum*). In this experiment, it was observed that the highest root leaf and shoot length were observed in the S:V (2:1) ratio, other ratios were not found beneficial for wheat crops and the least growth was noticed in S:C (2:1) treated pots. It was also noticed that cow dung compost is not beneficial for wheat crop growth, rather a soil vermicompost in 2:1 ratio showed more impact on crop growth. From this study, it can be concluded that the growth and yield are dependent on the adaptations of the cultivars, and poorly adapted cultivars might be partly responsible for lesser growth and lower yields in the organic farming system. These results demonstrated that vermicompost has a positive response over the animal-based manure i.e., cow dung compost. Vermicompost and effective microorganisms are effective in alleviating environmental stresses. The productivity depends upon the variety, which should adapt to the particular conditions in organic systems then, organic farming will be better to show its full potential as the high-yielding system alternative to conventional systems of agriculture.

ACKNOWLEDGEMENTS

The authors are thankful to Centurion University of Technology and Management, India for providing the opportunities to carry out this work.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025
Pages: 305-315

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090132

Exploring the role of indigenous arbuscular mycorrhizal fungi for sustainable soil bio-conservation from Napun Gete Reservoir, Sikka District, Indonesia

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Manuscript received: 28 February 2025. Revision accepted: 30 May 2025.

Abstract. *Jeksen J, Zubair H, Ahmad A, Tenriawaru N. 2025. Exploring the role of indigenous arbuscular mycorrhizal fungi for sustainable soil bio-conservation from Napun Gete Reservoir, Sikka District, Indonesia. Asian J Agric 9: 305-315.* Soil conservation through biological methods, particularly the use of indigenous Arbuscular Mycorrhizal Fungi (AMF), presents a promising approach to sustainable land management. However, there is limited information on the role of indigenous AMF as agents of soil bio-conservation. This study aimed to explore and evaluate the effectiveness of indigenous AMF from Flores in enhancing soil conservation in the upper region of the Napun Gete Reservoir. A split-plot experimental design was employed, with soil depth (K1: 0-25 cm and K2: 25-50 cm) as the main factor and land unit types (13 units) as subplots. The AMF application followed a randomized block design with five inoculum doses of indigenous Flores AMF: control, 5 g per plant, 10 g per plant, 15 g per plant, and 20 g per plant. The result showed that a total of four AMF genera were identified, including *Glomus* (81.98%), *Acaulospora* (13.29%), *Gigaspora* (4.68%), and *Entrophospora* (0.05%). The highest spore density was recorded in land units with slopes >40%, Andisol soils, and secondary forest land use. Indigenous AMF application significantly affected mycorrhizal, soil, and crop cultivation variables. AMF treatment notably improved soil physical, chemical, and biological properties. This research confirms the potential of indigenous AMF as a viable agent for biological soil conservation, offering a sustainable approach to land management in the study area.

Keywords: Andisols, biological conservation techniques, fungal diversity, soil microbiology, sustainable land management

INTRODUCTION

Soil degradation, a significant environmental concern, is primarily driven by improper land management practices, which accelerate the depletion of soil health and its ability to support sustainable agricultural activities (Nugroho 2024). In the upper Napun Gete Reservoir area, local communities practice land clearing for upland rice cultivation, typically by burning fields 2-3 months before the rainy season. This technique, repeated annually, exposes the soil to environmental forces, leading to significant land degradation. The practice occurs on various terrain types, including steep slopes that often exceed 40%. Preliminary studies have documented erosion rates in the region ranging from 0.05 to 1,719.72 tons per hectare per year, highlighting a wide variation from light to severe erosion. This broad range of erosion intensity indicates the varying degrees of soil degradation, which, if left unaddressed, could severely affect the ecological balance and functionality of the Napun Gete Reservoir in the future.

The challenge of combating soil degradation in such regions requires not only the identification of effective soil conservation methods but also the implementation of strategies that can restore soil health and prevent further degradation. Lal et al. (2021) emphasize the necessity of rehabilitation and the adoption of resilient and sustainable soil conservation practices to mitigate the long-term impacts of soil erosion. While traditional conservation methods, such as vegetative cover, physical-mechanical techniques, and chemical interventions, have been widely applied (Blanco-Canqui and Lal 2008; Kurniawati et al. 2024), there remains an untapped potential for using biological conservation methods, particularly those involving soil microorganisms (Amallia et al. 2023). Among these, Arbuscular Mycorrhizal Fungi (AMF) have emerged as promising agents for improving soil structure and fertility, yet their application in soil conservation remains relatively underexplored (Jafarpoor et al. 2022). Gharemahmudli et al. (2021) suggest that using native microorganisms, specifically those adapted to the local environment, could offer a novel and practical approach to soil and water conservation in regions suffering from degradation.

AMFs have garnered significant attention in recent years due to their ability to enhance soil structure and fertility, particularly in sustainable agricultural systems. Research by Singh et al. (2022) underscores the potential of AMF to mitigate soil degradation by improving soil aggregation and nutrient availability. Lehmann et al. (2017) highlight that mycorrhizal fungi secrete polysaccharides that form organic acids, which bind soil particles into micro aggregates and stabilize larger soil aggregates. Furthermore, studies by Al-Maliki and Ebreesum (2020) have demonstrated that combining AMF with algae can further enhance soil aggregate formation. Gao et al. (2019) found that AMF-induced glomalin production positively correlates with improved soil aggregate stability. These findings suggest that AMF plays a crucial role in stabilizing the soil environment, supporting microbial communities, and contributing to the sequestration of carbon, all of which are essential for mitigating the effects of soil degradation.

Despite the promising potential of AMF as soil bioconservation agents, research specifically investigating their role in soil degradation and conservation, especially in tropical regions such as Flores Island, remains limited. The application of AMF in soil conservation efforts in Flores is still underexplored, and further studies are needed to assess the presence and effectiveness of indigenous AMF species in the region. This study aimed to investigate the role of indigenous AMF in soil bio-conservation in the upper Napun Gete Reservoir area. Through a comprehensive analysis, this research can provide valuable insights into the presence and potential application of AMF in the region, contributing to a better understanding of their role in sustainable soil management. These findings may also help develop biological soil conservation techniques tailored to the unique environmental conditions of the Napun Gete Reservoir area, offering a model for applying AMF-based conservation strategies in other degraded regions.

MATERIALS AND METHODS

Research time and location

The Napun Gete Dam, constructed in 2021, is the first and only dam on Flores Island, located at coordinates 8°32'57"S 122°35'0.6"E, within the administrative boundaries of Ilinmedo Village, Waiblama Sub-district, Sikka District, Flores Island, East Nusa Tenggara Province, Indonesia, spanning an area of 160 hectares, the dam has a storage capacity of 11.22 million cubic meters (PUPR 2021). The Napun Gete Dam is situated within the Nebe River Basin, which covers a total area of 23,786.17 hectares, and the upstream region of the dam is located in the villages of Natarmage, Tanarawa, and Tua Bao, encompassing an area of 3,223.21 hectares.

The research on the application of CMP (Conservation Management Practices) was conducted in the upstream area of the Napun Gete Dam, specifically in Natarmage Village, Waiblama Sub-district, Sikka District, Indonesia (coordinates 8°36'14.13"S 122°34'3.93"E), at an elevation of 391 meters above sea level, from September 2023 to January 2024. The study area experiences an average annual temperature of 21°C and an annual rainfall of 1,500 mm. The soil in this region is classified as Andisols, with a clayey texture consisting of 28% sand, 34% silt, and 38% clay.

AMF exploration

Exploration activities were carried out in the upstream area of the Napun Gete Dam in February 2023. The exploration followed an experimental design using a split-plot design, where the main plots represented the soil depth factor, which consisted of two depth ranges: 0-25 cm and 25-50 cm. The subplots represented the land unit factor, which comprised 13 land units. According to land use mapping (Figure 1.A) in the upstream area of the Napun Gete Dam, three types of land use were identified: secondary forest, mixed gardens, and shrubland. Based on slope mapping (Figure 1.B) in the study area, slopes were categorized into three classes: 0-25%, 25-40%, and >40%. Soil type mapping (Figure 3) revealed two primary soil types in the area: Andisols and Alfisols. Overlaying the three maps above resulted in 14 land unit combinations (Figure 2). However, one land unit was categorized as a settlement area and was eliminated, leaving 13 land units for further analysis.

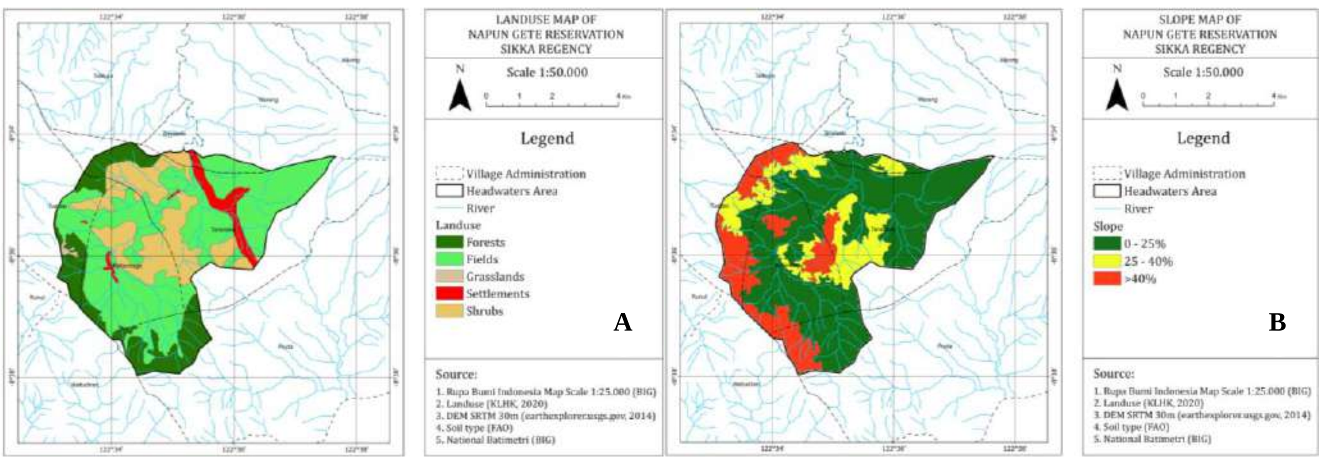

Figure 1. A. Sampling land map, B. Slope map

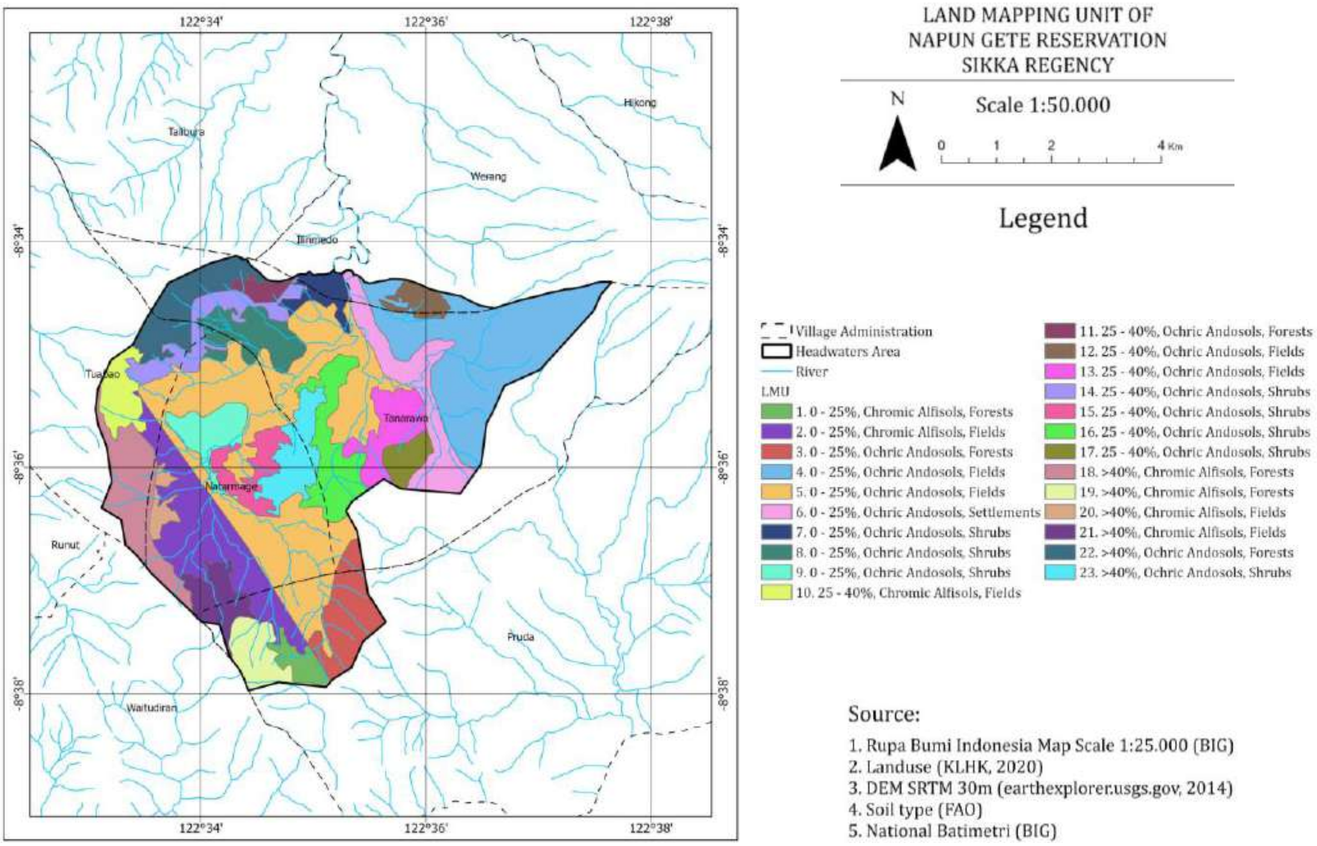

Figure 2. Land unit map

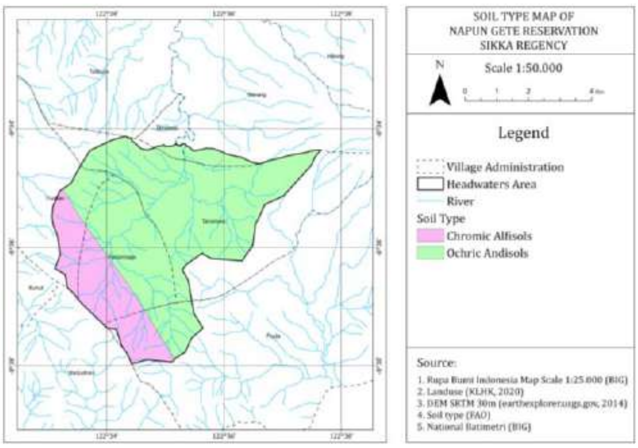

Figure 3. Soil type map

The details of the 13 land units were as follows: U1, located on a 0-25% slope, features Alfisols and secondary forest. U2, with the same slope range, also had Alfisols but was characterized by a mixed garden. U3, located on a 0-25% slope, had Andisols and was covered by secondary forest. U4, within the same slope range, also had Andisols and a mixed garden. U6, found on a 0-25% slope, comprised Andisols and shrubland. Moving to a 25-40% slope, U7 features Alfisols and a mixed garden, while U8, with a similar slope, has Andisols and a secondary forest. U9, also on a 25-40% slope, consists of Andisols and a mixed garden. Within this slope range, U10 contained Andisols and shrubland. U11, located on a slope greater than 40%, had Alfisols and a secondary forest. Alfisols and mixed gardens were found in U12, which also had a slope over 40%. U13, on a slope greater than 40%, had Andisols and secondary forests, while U14, also with a slope greater than 40%, consisted of Andisols and shrubland.

Soil sampling was repeated three times at each land unit, resulting in 78 soil samples (13 units × 2 depths × 3 replications). A 500 g soil sample was taken from the rhizosphere of each unit. Next, to identify the species and abundance of soil spores (AMF), spores were separated from the soil using sieving and centrifugation methods, as described by Brundrett et al. (1996). After isolation, spores were identified using microscopic methods, focusing on morphological characteristics such as shape, color, and ornamentation. The identification of spores was based on comparisons with spore standards from Brundrett et al. (1996), INVAM (2023a), and Pérez and Schenck (1990). This process allowed for determining the types and quantities of AMF spores in the soil samples, providing valuable data on the distribution and abundance of these vital soil microorganisms in the region.

Preparation of inoculum for indigenous Flores AMF

AMF (Colonization Mycorrhizal Inoculum) indigenous Flores was produced on sterile fine sand media. The planting medium was mineral soil once used for cultivating corn in dry fields that was sterilized using an autoclave, and the media were allowed to cool for 24 hours before use. The inoculum preparation took place in pots using 1 kg of sterile soil, with corn plants (INVAM 2023b) serving as the host plant. This process was specifically targeted towards the most dominant AMF species identified in the exploration activities conducted in the upstream area of the Napun Gete reservoir, namely the Glomus genus. The number of pots used was determined based on the research requirements, with two pots containing 100 spores each. The planting media was regularly watered throughout its growth period to maintain moisture. However, at six weeks of plant growth, watering was halted for two weeks to enhance spore growth. Fertilization was carried out using a phosphorus-free Yoshida solution. The fertilizer dosages were as follows: 10 mL of solution during the first week, 20 mL each week during weeks 2-3, and 20 mL twice a week from 4 to 6 weeks.

At eight weeks or two months of age, AMF inoculum was ready to be harvested. Harvesting the inoculum involved cutting the host plant at the base of the stem. Subsequently, the corn plant roots were chopped into small 1 cm pieces, then reintroduced into the pot and mixed thoroughly. This process yielded the AMF inoculum, which was then ready for use (Brundrett et al. 1996). The careful management of soil sterilization, watering schedules, fertilization, and the timing of inoculum harvest ensured the optimal growth of the mycorrhizal spores, highlighting the importance of controlled environmental conditions for successful AMF production.

AMF application experimental design

The experiment employed a randomized block design with a single factor pattern consisting of five inoculum doses of indigenous Arbuscular Mycorrhizal Fungi (AMF) from Flores (M): (i) control, with no AMF inoculum added (M0), (ii) 5 g per plant of AMF inoculum (M1), (iii) 10 g per plant of AMF inoculum (M2), (iv) 15 g per plant of AMF inoculum (M3), and (v) 20 g per plant of AMF inoculum (M4). Each treatment was replicated four times, resulting in 20 experimental units. The experiment utilized pots measuring 24 cm in height and 30 cm in diameter, with a soil capacity of 8 kg per pot. The experimental area covered 8 × 6 m. The indicator plant used was the Ciherang variety of upland rice, with seven seeds planted per pot. The application of AMF inoculum was performed simultaneously with the planting of seeds. An approximately 5-10 cm deep hole was made using a wooden stick, and AMF inoculum was manually placed into the soil. The hole was then partially covered with soil, and the rice seeds were planted in the same hole, followed by full coverage of the hole with soil.

Mineral fertilization was applied at 50% of the recommended standard dose, which included urea at 100 kg ha^{-1}. Fertilization was carried out in three stages: 40% of the urea dose (0.16 g per plant) was applied at planting, 30% (0.12 g per plant) at 15 Days After Sowing (DAS), and 30% (0.12 g per plant) at 30 DAS. Additionally, SP-36 was applied at 50 kg ha^{-1} (0.12 g per plant) and KCl at 50 kg ha^{-1} (0.12 g per plant) at planting time. The rice plants were harvested 126 Days After Planting (DAP). Pest control and weed management were carried out throughout the plant's growth period. The design and treatment aimed to investigate the effect of various AMF inoculum doses on the growth and yield of upland rice.

Soil properties analysis

Soil properties were evaluated at the beginning and end of the experiment by collecting topsoil samples from a depth of 0 to 20 cm. The soil samples were air-dried, homogenized, and passed through a 2 mm sieve. Soil pH was determined using the electrometric method (pH in H2O). Organic carbon content was measured using the Walkley and Black (1934) method. Total nitrogen was determined by the Kjeldahl (ACIAR 1990) method, while available nitrogen (NH4+, NO3-, NO2) was assessed using the potassium chloride (KCl) extraction (Lisle et al. 1991) method. The available phosphorus content was quantified using the Olsen (Olsen et al. 1954) method, and the total phosphorus and potassium content was determined using the 25% HCl extraction (Sudjadi et al. 1971) method. All soil property determinations followed the protocols Horwitz and International (2000) outlined.

This comprehensive analysis of soil characteristics provided essential insights into the soil's nutrient status and potential for supporting plant growth under different experimental treatments.

AMF properties

The infectivity of Arbuscular Mycorrhizal Fungi (AMF) was assessed using a staining method based on the protocol established by Brundrett et al. (1996). This method involved using specific stains that allow for the clear visualization of fungal structures within plant roots, thereby facilitating the evaluation of the degree of fungal colonization.

Additionally, the sporulation of arbuscular mycorrhizal fungi was determined using the wet-sieving method, following the guidelines set by Brundrett et al. (1996). The ability of AMF to sporulate efficiently was crucial for their long-term survival and colonization success. The UV-Vis spectrophotometric method was employed to quantify the presence of glomalin, a glycoprotein produced by AMF that plays a significant role in soil aggregation and stability, as Wright and Upadhyaya (1998) described.

Statistical analysis

All the results of exploration activities, particularly the data on the abundance of Arbuscular Mycorrhizal Fungi (AMF), the effects of indigenous flores arbuscular mycorrhizal fungi on AMF, were evaluated through Analysis of Variance (ANOVA) with a split-plot design to assess the number of spores present. Tukey's post-hoc test was conducted at a significance level 0.05 to determine the differences between treatments. Additionally, correlation analysis were performed to explore the relationships between various parameters, such as spore abundance, soil properties, and plant performance. The statistical analysis was carried out using the IBM SPSS software package, version 23.0, which offers robust data analysis and hypothesis testing tools.

RESULTS AND DISCUSSION

Exploration of AMF species at Napun Gete Reservoir Watershed

The exploration of AMF species within the Napun Gete Dam's upstream area revealed several genera, including *Glomus*, *Acaulospora*, *Gigaspora*, and *Entrophospora* (Figure 4). Among these, the *Glomus* genus was the most dominant, accounting for 81.98% of the total AMF population, followed by *Acaulospora* at 13.29%, *Gigaspora* at 4.68%, and *Entrophospora* at a minimal 0.05%. These findings highlight that *Glomus* species exhibited a broad distribution across the entire upstream area of the Napun Gete Reservoir, consistently detected in soil samples collected from 0-25 and 25-50 cm depths. In contrast, *Acaulospora*, *Gigaspora*, and *Entrophospora* were often absent in some collected samples. This observation aligns with the findings of Herawati et al. (2024), who noted that in dryland ecosystems, *Glomus*, *Acaulospora*, and *Gigaspora* dominate AMF populations. In contrast, *Entrophospora* and *Scutelospora* are found in much smaller quantities.

Furthermore, it was also suggested that *Glomus*, *Acaulospora*, and *Gigaspora* dominate in dry environments due to their superior adaptive capacity (Sefrila et al. 2023). These genera were endomycorrhizal fungi well-suited to survive in arid conditions, exhibiting greater spore germination rates and resilience than *Scutelospora* and *Entrophospora*. This adaptability likely contributes to *Glomus's* persistence and widespread occurrence in the Napun Gete watershed, indicating its ecological significance in maintaining soil health and supporting plant growth in this environment. The ability of these fungi to thrive in challenging conditions further emphasizes the importance of their role in ecosystem functioning, particularly in regions with variable and dry climates (Herawati et al. 2021).

These findings indicate that the abundance of AMF spores was significantly higher in secondary forests than in other land uses, primarily due to vegetation regeneration. The regrowth of plants in secondary forests increases the availability of organic carbon. It restores host plants for mycorrhizal fungi, which fosters the growth and reproduction of AMF spores. This aligns with previous research by Reyes et al. (2019), which found that soil characteristics, such as organic matter content, pH, aluminum concentration, cation exchange capacity, and calcium have strong relationships with abundant *glomerospora* and AMF species composition. The presence of an organic litter layer in secondary forests further supports the growth of mycorrhizal fungi by providing a steady source of energy and nutrients. This is consistent with findings by Chen et al. (2023), who noted that slope gradients affect AMF spore abundance, with lower spore counts generally found in steeper slopes due to less stable soil conditions. However, they also observed that other factors, such as soil stability, water availability, and nutrient levels, in combination with vegetation cover, are crucial in determining AMF populations and activity across varying slope gradients.

Regarding soil type, Andisols exhibited higher fungal spore counts compared to Alfisols. This can be attributed to the higher organic matter content in Andisols, which generally supports greater fertility and fungal activity. The results of this study demonstrate that soil properties, such as organic matter content, pH, and phosphorus fixation, play a crucial role in shaping the abundance and community composition of AMF spores. Furthermore, while slope gradients can affect fungal abundance, various factors-including vegetation cover and soil stability-determine the extent of AMF proliferation. However, Andisols also exhibit higher phosphate fixation rates, which may limit phosphorus availability to both plants and mycorrhizal fungi. Alfisols, while having lower organic matter content and fertility, showed different patterns in spore abundance, indicating that soil chemical properties significantly influence fungal populations. Secondary forests, through their ability to regenerate vegetation and provide an organic layer of litter (Wiraatmaja et al. 2024), create favorable conditions for the growth of mycorrhizal fungi.

AMF application

AMF abundance

An Analysis of Variance (ANOVA) showed a highly significant effect on AMF abundance (Figure 5). The highest spore abundance reached 110 spores per 10 g of soil in the treatment with indigenous AMF from Flores at 20 g pot^{-1} (M4), 79.77% higher than the control treatment without AMF (M0). The treatment without AMF (M0) significantly differed from the other AMF treatments. Further statistical tests on AMF spore abundance are shown in Figure 6. These findings underscore the potential of indigenous AMF strains in significantly enhancing spore population density and, by extension, their ecological function in promoting plant growth and soil health.

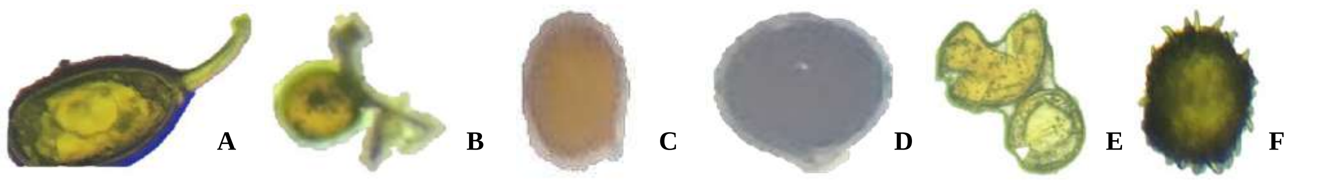

Figure 4. AMF spores identified under a binocular microscope (400X magnification): A. *Acaulospora foveata* Trappe & Janos, B. *Acaulospora undulata* Sieverd., C. *Glomus mosseae* (T.H.Nicolson & Gerd.) Gerd. & Trappe, D. *Glomus etunicatum* W.N.Becker & Gerd., E. *Gigaspora margarita* W.N.Becker & I.R.Hall, F. *Entrophospora infrequens* (I.R.Hall) R.N.Ames & R.W.Schneid.

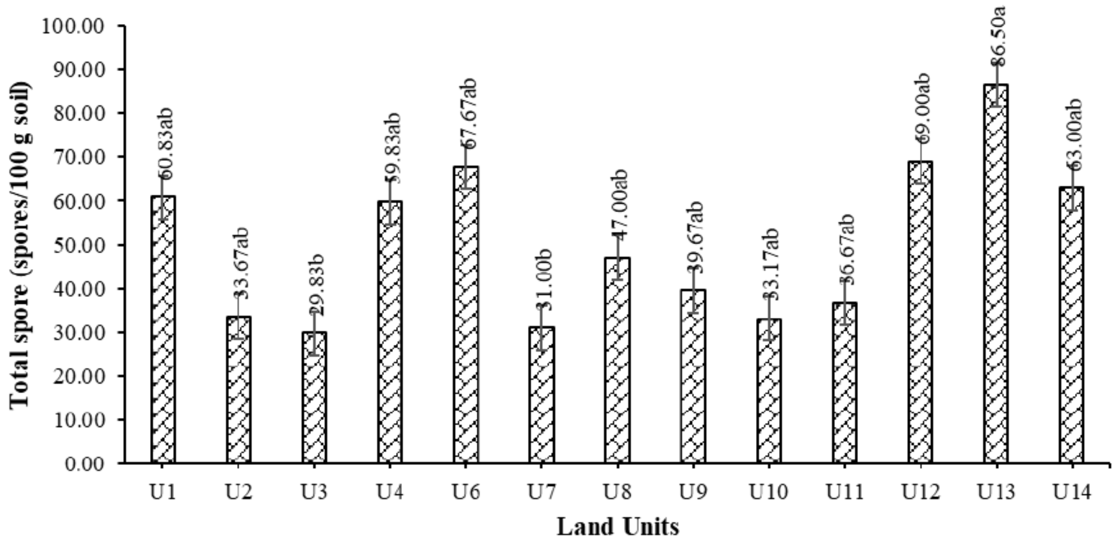

Figure 5. Effect of land unit on the number of AMF spores

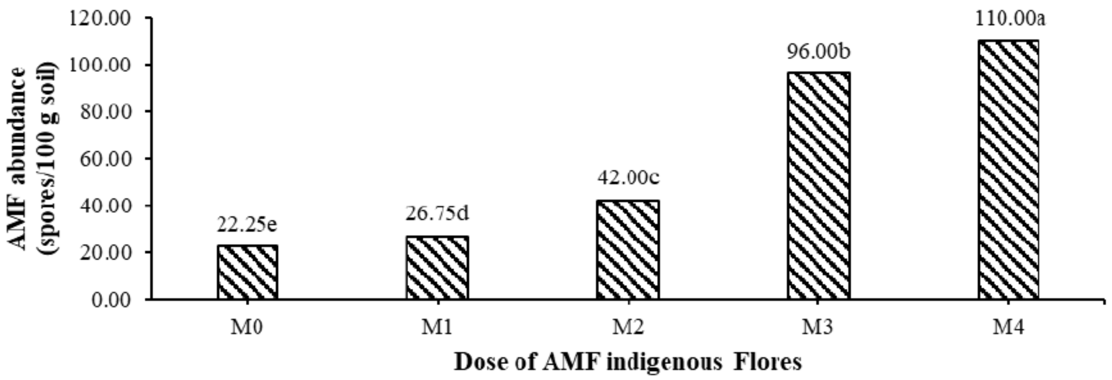

Figure 6. Effect of AMF on abundance

The abundance of Arbuscular Mycorrhizal Fungi (AMF) is influenced by three main factors: host plant availability, climate, and soil nutrients. First, compatible host plants are crucial, as they provide carbohydrates that stimulate AMF spore formation. Certain crops like rice, maize, and legumes support AMF, making plant selection an important strategy to enhance fungal activity in agroecosystems. Second, environmental conditions such as humidity and temperature also play a role. High humidity and moderate temperatures promote AMF growth and sporulation, while drought and extreme heat reduce it (Mohamed et al. 2023). The study area's average humidity of around 85% suggests favorable conditions for AMF development. Third, soil nutrient content-especially phosphorus-affects AMF presence. AMF tends to thrive in nutrient-poor soils where plants depend more on symbiosis to access nutrients. Soil analysis from the study area reveals low nitrogen, phosphorus, and potassium levels, with slightly acidic pH, indicating a low-fertility environment suitable for AMF proliferation. The combination of appropriate host plants, supportive climate, and nutrient-poor soil makes the study area a promising habitat for native AMF species (Dierks et al. 2021).

Percentage of root infection by AMF

The percentage of root infection by Arbuscular Mycorrhizal Fungi (AMF) is a crucial indicator for evaluating the effectiveness of the mutualistic symbiosis between mycorrhizal fungi and plant roots. The results exhibited that the percentage of root infection by indigenous AMF from Flores showed a highly significant effect. The highest infection rate reached 88.04% in the treatment with 20 g of indigenous AMF per pot (M4), an increase of 81.84% compared to the untreated control (M0). The treatment without AMF (M0) significantly differed from the 20 g per pot AMF treatment (M4), while other treatments did not show significant differences. The results of the post-hoc test on AMF's role in root infection percentage are presented in Figure 7. In the M0 treatment, infection was observed, indicating the natural presence of AMF at the study site. Despite soil sterilization at 121°C, AMF survived, highlighting its resilience.

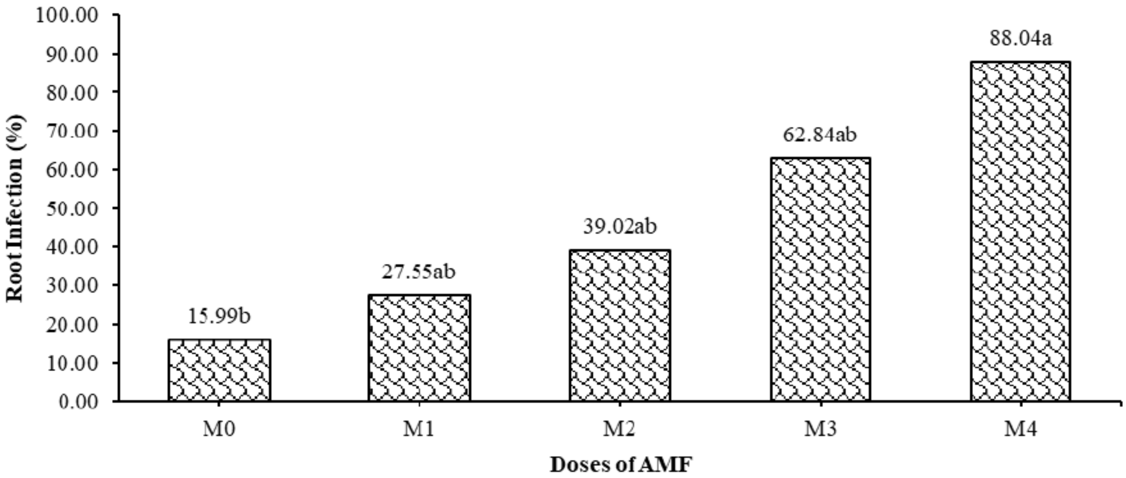

Figure 7. Effect of AMF on the percentage of root infection

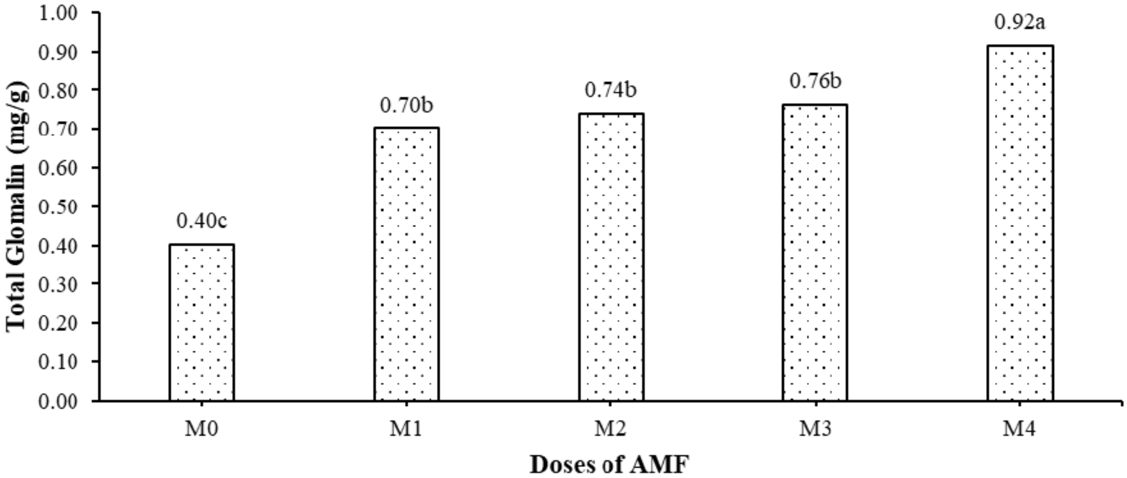

Figure 8. Effect of AMF on total glomalin

Root infection by Arbuscular Mycorrhizal Fungi (AMF) occurs through the penetration of fungal hyphae into the cortical root tissue. The hyphae form specialized structures known as arbuscules and vesicles within the cortical cells. Arbuscules function as nutrient exchange points between fungi and plants, while vesicles serve as nutrient reserves (Tedersoo et al. 2020). This process provides mutual benefits: the plant gains access to additional nutrients, especially phosphorus, while the fungi receive carbon sources from the plant's photosynthesis. According to Dowarah et al. (2022), the percentage of root infection by AMF is significantly influenced by several factors, including plant species, soil nutrient availability, environmental conditions, and organic matter and fertilizers. Research by Paymaneh et al. (2023) and Fall et al. (2022) indicates that combining organic fertilizers and AMF inoculation can enhance root infection percentage while improving soil physical, chemical, and biological properties.

Total glomalin

Glomalin is a soil protein that AMF hyphae produce and is a vital component of soil organic matter. One of the primary roles of glomalin is maintaining soil aggregate stability. Stable aggregates are crucial for preventing erosion and supporting root systems. Based on the results of the ANOVA, total glomalin showed a significant effect. The highest value was recorded at 0.92 mg g^{-1} soil in the treatment with 20 g of indigenous AMF per pot (M4), representing an increase of 56.10% compared to the untreated control (M0). The treatment without AMF (M0) significantly differed from the other AMF treatments. The post-hoc test results on the role of AMF in total glomalin are presented in Figure 8.

This aggregate stability enhances the soil's ability to retain water, prevents nutrient leaching, and provides optimal conditions for root growth. Matos et al. (2021) highlighted that glomalin plays a significant role in improving soil quality by enhancing soil structure, increasing organic matter content, reducing fertility decline, and boosting microbial activity, thus aiding in the restoration of heavy metal-contaminated soils.

Table 1. Soil analysis results after treatment

AMF	pH	Organic-C (%)	Total-N (%)	Available N		Total-P (mg/100 g)	Available-P (ppm)	Total-K (mg/100 g)
				NO_3^- (ppm)	NH_4^+ (ppm)			
M0	5.58[b]	1.63[e]	0.16[e]	1.34[c]	1.94[b]	26.12[e]	8.79[b]	17.14[c]
M1	5.43[c]	2.06[d]	0.36[c]	2.58[ab]	4.0[ab]	31.47[d]	9.31[ab]	20.92[ab]
M2	5.55[bc]	2.63[b]	0.30[d]	3.48[a]	3.06[ab]	33.35[c]	10.17[ab]	20.24[b]
M3	5.81[a]	2.56[c]	0.39[b]	2.45[b]	4.02[ab]	34.70[b]	10.65[ab]	22.21[a]
M4	5.20[d]	2.79[a]	0.45[a]	3.31[ab]	5.23[a]	37.17[a]	11.51[a]	21.03[ab]

Note: Treatment means followed by the same letter are not significantly different

Glomalin production is influenced by several factors, including soil type and conditions, agricultural practices, and the presence of AMF (Arbuscular Mycorrhizal Fungi). Soils with low disturbance levels, such as organic farming systems, tend to have higher glomalin levels. In contrast, intensive soil cultivation can reduce AMF populations, lowering glomalin production. Adding organic fertilizers such as compost and biochar has also been reported to increase the total glomalin content in the soil. In agricultural soils, the glomalin content varies between 0.30-0.70 mg g^{-1} of soil (Hossain 2021).

Glomalin, produced by AMF, is significant in physical, chemical, and biological soil conservation efforts. Singh et al. (2022) stated that glomalin plays a role in improving the soil's physical, chemical, and biological properties, helping to rehabilitate land degraded by activities such as mining, erosion, intensive farming, excessive salinity, deforestation, and grazing.

Soil characteristics

Based on the laboratory analysis results presented in Table 1, it was found that the chemical content of the soil showed different variations across the treatments. The post hoc test results indicated significant differences between the control treatment (M0) and the other treatments. The M0 treatment significantly differed from all other treatments for all the tested soil chemical properties except for the soil pH variable. Additionally, an increase was observed when comparing the initial soil analysis results with the post-treatment soil analysis results in Table 1. This suggests that the indigenous AMF treatment from Flores applied in the experiment effectively improved the soil's chemical properties.

As a result of the soil acidity value, the best treatment was found in the indigenous AMF treatment of 15 g pot^{-1} (M3), which showed the highest pH result, namely 5.81. Based on the soil-chemical property evaluation criteria the Soil Research Institute set, this value (M3) was slightly acidic, while other treatments fall into the acidic category. The results showed that soil acidity tended to decrease, and only the M3 treatment showed consistent results compared to before and after the indigenous Flores AMF treatment. This is because this study used mineral fertilizers such as urea, SP-36, and KCl, which were applied to all treatments. Based on the research by Robertson and Groffman (2024), when urea fertilizer is applied to the soil, it undergoes hydrolysis by the enzyme urease, producing ammonium (NH_4^+) and carbon dioxide (CO_2). This process can temporarily increase soil pH due to the formation of alkaline ammonium ions. Potassium ions can replace hydrogen ions in the soil colloid complex, increasing the H $concentration^+$ in the soil solution and making the soil pH more acidic (Kurniawan et al. 2023).

The application of indigenous flores AMF treatment on soil organic carbon showed significant results. The best treatment was found in the indigenous flores AMF treatment of 20 g pot^{-1} (M4), which showed the highest soil C-organic result, 2.79%. According to the Soil Research Institute, the soil-chemical property evaluation standards, M4 is classified in the moderate category. Meanwhile, all other treatments, except for M0, are also categorized as moderate, while M0 is categorized as low (BPT 2005), though they have varying percentages of C-organic. The results of further tests on the role of AMF in soil C-organic are presented in Table 1. According to Basiru and Hijri (2024), AMF plays an essential role in soil organic carbon dynamics and uniquely transfers carbon (C) obtained from its host plants into the soil. This carbon can be labile organic matter (easily decomposed) or recalcitrant organic matter (hard to decompose). This process results in different impacts on soil organic carbon reserves. Labile materials tend to accelerate the carbon cycle because they decompose quickly, while recalcitrant materials contribute to long-term carbon storage, thus increasing carbon stability in the soil. According to Ren et al. (2021), AMF inoculation can be an effective agricultural practice in increasing soil C-organic.

As the percentage of infection and AMF abundance increases, the nitrogen content in the soil also increases, both in the form of total N and nitrate (NO_3^-) and ammonium (NH_4^+) uptake. The best results for total N, NO_3^-, and NH_4^+ content from indigenous flores AMF treatment were found in the 20 g pot-1 treatment (M4), with results of 0.45%, 3.31 ppm, and 5.23 ppm, respectively. According to the soil-chemical property evaluation criteria by the Soil Research Institute, the total N content in all treatments, except for M0, is moderate (BPT 2005). However, the % total N varies between treatments. When comparing the best treatment (M4) to the untreated treatment (M0), there was an increase of 65.36, 59.67, and 62.87%, respectively. The results of further tests on the role of AMF in total N, NO_3^-, and NH_4^+ content are presented in Table 1. The increase in nitrogen content is due to AMF forming an external hyphal network that extends deep into the soil, outside the root zone of plants. This network helps absorb nitrogen from sources complex for plant roots to access, such as organic compounds bound to soil particles. AMF hyphae increase the soil contact area, enhancing nitrogen uptake (Suwardi et al. 2023). Nitrogen increases not only through hyphae but also through the mineralization of organic nitrogen. AMF can interact with other soil microorganisms to accelerate the decomposition of organic material. In this process, organic nitrogen compounds are converted into inorganic forms such as ammonium (NH_4^+) or nitrate (NO_3^-), which plants can absorb. The stimulation of microbial activity by AMF can encourage soil microorganisms involved in the nitrogen cycle, such as free-living nitrogen-fixing bacteria (Azotobacter) or nitrifying bacteria. The interaction between AMF and soil microbes accelerates processes that support the increased availability of total N.

Furthermore, according to Qiu et al. (2022), the presence of AMF significantly reduces nitrogen (N) and phosphorus (P) losses in the soil. According to Mulyadi and Jiang (2023), the combined application of AMF and nitrogen (N) fertilizers has benefitted rice farming systems significantly. This combination improves soil physicochemical properties, such as C-organic content and total glomalin, and positively impacts rice's plant growth, crop yields, and nitrogen absorption efficiency. In line with the main role of AMF, which is to increase phosphorus (P) content in the soil, this study also showed an increase in total P and available P content after treatment. Overall, all treatments showed an increase in total P and available P content, with a significant difference between M0 and all treatments. The highest increase in total P and available P was observed in the M4 treatment, with values of 37.17 mg/100 g and 11.51 ppm, respectively. According to the soil-chemical property evaluation criteria set by the Soil Research Institute, total P content in all treatments is categorized as moderate, and available P content increased from low to moderate levels before and after the treatment (BPT 2005). This is due to AMF's ability to produce phosphatase enzymes that can release P-bound to soil minerals or organic materials that are difficult for plants to access, making it available to plants. Furthermore, the increase in P occurs due to the expansion of the hyphal network, as AMF has external hyphal structures that can reach soil areas outside the plant root zone (rhizosphere). These hyphae expand the plant's capacity to absorb phosphorus from the soil, especially from forms of phosphorus that are difficult for plant roots to access directly (Etesami et al. 2021). Research on maize plants has shown that AMF inoculation increases the uptake of phosphorus, nitrogen, and other micronutrients (Qin et al. 2022; Peng et al. 2023).

Effect of AMF on upland rice plant yield

Based on the ANOVA results, the dry weight of grain ha-1 showed a very significant effect. The highest yield reached 5.77 tonnes in the 20 g pot^{-1} AMF native Flores treatment (M4), or an increase of 51.86% compared to no treatment (M0). Figure 9 shows that the treatment without AMF (M0) significantly differed from the other AMF treatments. The results of tests on the role of AMF on total glomalin are presented in Figure 9.

The accumulation of symbiosis between AMF (Arbuscular Mycorrhizal Fungi) and plants increases plant production. This is in line with the research findings of Ishaq et al. (2021) that plants inoculated with AMF significantly increase soil-available phosphorus (P), plant growth, and corn crop yield compared to non-inoculated plants, as supported by the study of Putra et al. (2020) which stated that AMF can improve the growth and yield of chili plants. AMF plays a vital role in enhancing plant growth and yield through several mechanisms, namely, improving nutrient absorption through AMF hyphae that can extend the root absorption area. This allows the plant to absorb all macro and micronutrients, especially phosphorus (P), which is typically hard to obtain in dry land. Second, improving soil structure via glomalin production contributes to soil aggregates' stability. A better soil structure increases the soil's capacity to retain water and maintain moisture, which is crucial in dryland rice fields that often face water shortages. Third, by stimulating growth hormones, AMF affects plant metabolism, which can ultimately boost the production of hormones such as auxins, cytokinins, and gibberellins in the host plant. These hormones play a role in stimulating root and vegetative part growth, thus improving productivity. Fourth, through increasing the organic carbon content and soil microbial activity, AMF symbiosis with plants can enhance the organic carbon content in the soil through contributions from root and root exudate-derived organic matter. Soil microorganism activity also increases due to the abundance of carbon available as an energy source for their growth, supporting soil fertility and plant health. Additionally, according to the research findings of Zou et al. (2021), it is said that AMF can regulate physiological and molecular responses to drought stress and counteract oxidative damage caused by drought by enhancing antioxidant defense systems.

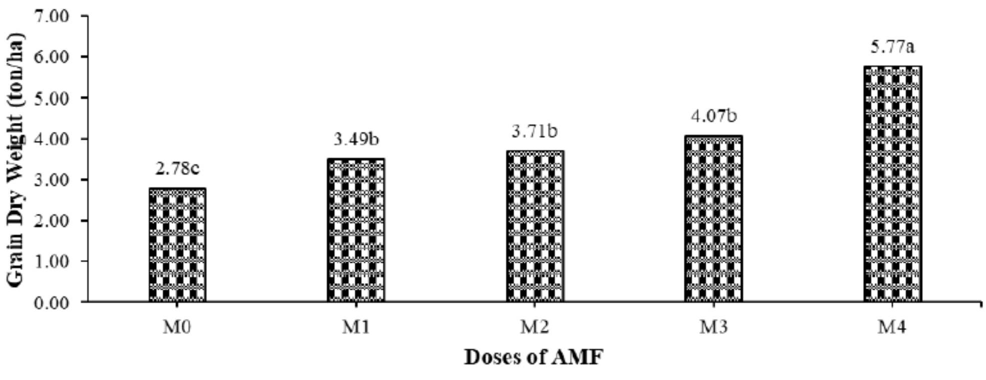

Figure 9. Effect of AMF on Grain Dry Weight (GDW)

In conclusion, in this study, a total of four genera, namely *Glomus* (81.98%), *Acaulospora* (13.29%), *Gigaspora* (4.68%), and *Entrophospora* (0.05%), were explored in the upstream area of the Napun Gete Dam. The highest number of spores was found in land units with a slope greater than 40%, andisol soil types, and secondary forest land use. In the application phase, indigenous AMF from Flores contributed significantly to the results among all the observed variables, including mycorrhiza, soil, and crop cultivation. Based on the statistical result, AMF can improve soil's physical, chemical, and biological properties. Therefore, it can be concluded that this study has successfully discovered the biological soil conservation approach model by utilizing indigenous AMF as a soil bio-conservation agent. Further research is needed to develop a large-scale indigenous AMF biological fertilizer product for broader soil conservation efforts.

ACKNOWLEDGEMENTS

The author thanks Drs. Sabinus Nabu, Chairman of the Nusa Nipa Higher Education Foundation, the academic community of Universitas Nusa Nipa, and the Tana Ai indigenous community, especially those in Waiblama Sub-district, Natarmage Village, for their support and contributions, which made this research possible.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 316-325 DOI: 10.13057/asianjagric/g090133

Evaluating constraints and prospects of black soldier fly frass for sustainable *Coelogyne pandurata* acclimatization

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Manuscript received: 10 April 2025. Revision accepted: 31 May 2025.

Abstract. *Kusuma R, Wulandari TM, Lariman, Samsurianto, Nugroho RA, Manurung H, Aryani R, Rudianto. 2025. Evaluating constraints and prospects of black soldier fly frass for sustainable* Coelogyne pandurata *acclimatization. Asian J Agric 9: 316-325.* The black orchid (*Coelogyne pandurata* Lindl), a critically endangered species endemic to Borneo, faces significant threats from habitat destruction and over collection. In vitro propagation provides an effective conservation strategy; however, the acclimatization phase remains a major hurdle. This study assessed the potential of Black Soldier Fly Larvae (BSFL) frass as an organic amendment for the ex-vitro acclimatization of *C. pandurata*, in comparison with traditional substrates (husk charcoal, cocopeat, and fern roots), with and without supplemental fertilizer. Over 56 days, growth parameters including survival rate, plant height, leaf production, and shoot emergence were evaluated. While husk charcoal showed the highest height gain (3.23 ± 0.31 cm), cocopeat with 2 g L^{-1} of fertilizer resulted in superior leaf (3.47 ± 2.06) and shoot (2.49 ± 0.49) development. Despite its high nitrogen and micronutrient content, BSFL frass underperforms when used alone because of its poor structure and rapid nutrient release but may be beneficial as a supplement. These findings highlight the importance of the media types and nutrient balance in successful orchid acclimatization. This research not only supports sustainable conservation practices for *C. pandurata* but also promotes the upcycling of organic waste via BSFL frass, contributing to environmentally responsible horticultural applications.

Keywords: Acclimatization, black orchid, black soldier fly frass, conservation, plant tissue culture

Abbreviations: BSFL: Black Soldier Fly Larvae, CITES: Convention on International Trade in Endangered Species, C/N ratio: Carbon-nitrogen ratio, DAP: Day After Planting, EM4: Effective Microorganisms 4, NPK: Nitrogen Phosphorus Potassium, PKM: Palm Kernel Meal

INTRODUCTION

Orchids (Orchidaceae) are among the most diverse flowering plants, with over 700 genera (Wong and Peakall 2022) and 28,000 species globally (Awasthi et al. 2023; Awasthi et al. 2024; Chachad et al. 2024). Their unique floral morphology and ecological relationships have intrigued botanists and horticulturists for decades. One particularly captivating species is the black orchid (*Coelogyne pandurata* Lindl), which is notable for its vibrant green petals and distinctive black lip (Hartini 2019). Endemic to Borneo, especially East Kalimantan and parts of Malaysia, *C. pandurata* faces near extinction due to deforestation, land conversion, and overcollection (Lestari and Deswiniyanti 2015; Puspitaningtyas 2020; Widiarsih and Dwimahyani 2023). Its short flowering period (5-6 days) (Sabran et al. 2003), and limited cross-pollination success (Hartati et al. 2019) further complicate conservation efforts.

As per the Convention on International Trade in Endangered Species (CITES) Appendix II-listed species (Wahyudiningsih et al. 2018), black orchid conservation is imperative, representing both species protection and broader biodiversity goals. In addition to their ecological importance, orchids have cultural and economic value. In vitro propagation, through tissue culture, provides a viable conservation approach (Semiarti 2018), allowing mass production of disease-free, genetically stable plantlets in controlled environments (Lai and Lai 2019; Lengkong et al. 2023; Manohar et al. 2024).

However, the acclimatization phase, transitioning plantlets from in vitro to ex-vitro conditions, remains a critical bottleneck (Ziraluo 2021; Nimavat and Parikh 2024). During this stage, plantlets encounter fluctuating light, temperature, humidity, and nutrient availability, leading to water stress, pathogen exposure, and nutrient imbalances (Phillips and Garda 2019; Baby et al. 2024; Eisa et al. 2025). Therefore, substrate selection is vital and requires optimal water retention, aeration, and nutrient supply. Common orchid substrates, namely: charcoal, fern roots, coconut fiber, and moss, may lack sustainability or sufficient nutrients (Haniva 2020; dos Santos and Smozinski 2021; Nuammee et al. 2024).

To address this, researchers have turned to nutrient-enriched organic amendments such as frass from Black Soldier Fly Larvae (BSFL) (*Hermetia illucens* (Linnaeus, 1758)) (Triwijayani et al. 2023; Odongo et al. 2024; Siddiqui et al. 2024). The BSFL frass, a byproduct of larvae fed on organic waste, is rich in nitrogen, phosphorus, potassium, and micronutrients with biostimulant properties (Addo et al. 2022; Abd Manan et al. 2024). Its effectiveness has been demonstrated in various crops, including *Brassica juncea* (L.) Czern. (Kesumaningwati et al. 2023), *Brassica rapa* L. (Agustiyani et al. 2021), *Solanum lycopersicum* L. (Salomon et al. 2025), *Triticum turgidum* L. (Boudabbous et al. 2023), *Glycine max* (L.) Merr. (Yudistira et al. 2025), and *Lactuca sativa* L. (Dzepe et al. 2022), enhancing growth, nutrient uptake, and resilience against pathogens.

Despite its documented benefits in agriculture, the BSFL frass remains underutilized in orchid propagation, particularly in the acclimatization of endangered species such as *C. pandurata*. Existing studies on black orchid acclimatization have shown promising results using moss, ferns, and mixtures of wood charcoal and coconut fiber (Adi et al. 2014; Zakiah and Turnip 2023). Optimal outcomes have been reported for combinations of moss, charcoal, coconut fiber, ferns, and humus (Astarini et al. 2015; Zakiah et al. 2023). Nutrient analyses of BSFL frass indicate pH 4-9, organic C > 15%, C/N ratio < 25, NPK > 2%, and Fe < 500 mg/kg (Agustin et al. 2023; Barrantes-Sandoval et al. 2024), supporting its potential as a biofertilizer.

Nevertheless, orchid-specific studies assessing the physiological and morphological effects of BSFL frass are scarce. Literature largely overlooks its use in ornamental or endangered plant species, thereby creating a gap in conservation-oriented horticultural research. While the BSFL frass provides nutrients, its properties and nutrient release dynamics in orchid substrates are not well understood.

This study aimed to evaluate the use of BSFL frass as an organic amendment in the acclimatization of *C. pandurata*, comparing its effectiveness with that of conventional substrates such as husk charcoal, fern roots, and cocopeat, combined with different fertilization levels. By measuring the survival rate, growth performance, and plant development, this study investigated whether BSFL frass can serve as a sustainable medium component.

The novelty of this study lies in applying insect-derived organic waste to conserve endangered orchids, offering dual benefits: improved ex situ acclimatization and waste valorization. These findings will support the sustainable use of insect-based biofertilizers in horticulture while addressing biodiversity conservation.

MATERIALS AND METHODS

Materials

The materials used in this study were BSFL (CV Ahasa Larva Group, Samarinda, East Kalimantan, Indonesia), black orchid, fungicides and bactericides (Zephyr+ 80WP, CV Javamas Agrophos, Yogyakarta, Indonesia), water, red plastic, chicken pellets (PT Japfa Comfeed, Tbk, Indonesia), palm kernel meal (PT REA Kaltim Plantation, East Kalimantan, Indonesia), EM4 (PT. Songgo Langit Persada, Indonesia), cocopeat, husk charcoal, fern roots, Gandasil® (PT Kalatham Corporation, Bekasi, West Java) fertilizer, distilled water, tissue, 70% alcohol, and masks.

Population and research variable

The plant population used in this study was the black orchid population, which was obtained from Kersik Luway National Park, Sekolaq Darat Sub-district, West Kutai District, East Kalimantan, whereas the sample used was 48 black orchid individuals to represent the black orchid population. Two dependent and independent variables were used in this study. The dependent variable was black orchid plants, whereas the independent variables consisted of various media (M): BSFL frass (M1), husk charcoal (M2), fern root (M3), cocopeat (M4), and three concentrations of Gandasil ® fertilizer (F). 0 g L^{-1} (F0) and 1 g L^{-1}. (F1), and 2 g L^{-1} (F2). Current research was conducted with a Completely Randomized Design (CRD) with 2 factors. The first factor was the type of media, while the second factor was the concentration of Gandasil® D Fertilizer (three levels). Each treatment was repeated four times.

Procedures

BSFL frass production

The BSFL frass was obtained from two months of BSFL rearing fed fermented Palm Kernel Meal (PKM) and chicken pellets. PKM fermentation was performed by mixing PKM with EM4, water, and sugar in containers. The ratio used was that every 1 kg of PKM was mixed with 65 mL of EM4, 32 g of sugar, and 1 L of water. The ingredients were homogenized in a container and covered with plastic until no air space was left. The fermentation process lasted for 2 weeks before the meal was ready for use. The fermented meal was mixed with chicken pellets in a 1:1 ratio and provided as BSFL feed (Nugroho et al. 2024). The BSFL rearing was carried out for approximately two months to obtain good-quality frass. The resulting frass was collected weekly and air dried. The dried frass was then sterilized using an autoclave 121°C, 15 psi for 30 min, and ready to be used for a planting medium nutrient content.

Planting media preparation

A planting container was used to plant the orchids. The planting media to be used (BSFL frass, cocopeat, husk charcoal, and fern roots) were dried in the sun until dry, and sterilized by autoclaving at 121°C, 15 psi for 30 min (Elguera et al. 2025). The sterilized media were then cooled and dried. Fifty grams of each medium was placed into the planting container. To support the evaluation data, BSFL frass, cocopeat, husk charcoal, and fern roots were analyzed for C-organic, total N, C/N ratio, P_2O_2, K_2O, Mg, and Mn percentage.

Acclimatization process

During the acclimatization stage, the black orchid that was ready (good overall health, free from contamination, discoloration, or deformities) for acclimatization was removed from the culture bottle using sterilized long tweezers. The planlets were placed on a tray, prepared and filled with water. The planlets were cleaned of the remaining agar media, soaked with fungicide and bactericide solutions, rinsed with water until clean, and then drained. The drained planlets were then initially measured and acclimatized to the prepared media.

The planlets that were planted were then watered and covered with plastic for five days. During the time the planlets were in the hood, the orchid planlets were watered every two days in the morning or evening using a hand sprayer. After five days, the plastic lid was opened slightly after the planlets appeared healthy, and the plastic lid was opened completely. The application of gandasil® fertilizer according to the treatment (no fertilizer, 1%, and 2%) and measurement of test parameters were performed 14, 28, 42, and 56 Days After Planting (DAP).

Growth parameters

The percentage of life, plant height gains, number of leaves, and shoot growth were measured at 14, 28, 42, and 56 DAP. The percentage of life was calculated by counting the number of individuals that live and grow using the following formula (Putri et al. 2022):

$$\text{Percentage of life} = \frac{\text{Number of living orchids}}{\text{Number of orchids planted}} \times 100\%$$

Plant height gain was measured using a ruler from the base of the plant to the tallest leaf (monocots) (Mubarok et al. 2024), and the number of leaves was counted on each plant, starting from the bud leaves to the leaves that had opened (Zhang et al. 2017). For shoot growth, the number of buds was determined for each plant using a previously described method (An et al. 2021).

Data analysis

The data obtained from the measurement of the parameters were analyzed using SPSS version 24 (SPSS, Inc. USA). All data were statistically analyzed using Two-Way Analysis of Variance. Any significant differences were further evaluated using the Duncan Multiple Range Test at a significant level of $P < 0.05$ to determine differences in plant growth parameter data.

RESULTS AND DISCUSSION

Nutrient analysis of the growing media used in this study revealed significant variations in organic matter, macronutrients, and micronutrients, which could influence the acclimatization of *C. pandurata* (Table 1).

The current analysis found that BSFL frass exhibited a high total nitrogen content (5.00%) but a relatively low C/N ratio (4.03), indicating rapid nitrogen availability. However, its phosphorus (4.19%) and potassium (1.27%) contents were lower than those typically required for optimal plant growth. In contrast, husk charcoal showed the highest C-organic content (25.93%), but a lower nitrogen level (0.70%) and an extremely high C/N ratio (37.04), which may limit nitrogen availability for plant uptake.

Furthermore, cocopeat showed the highest organic carbon content (38.55%), with a moderate nitrogen concentration (1.19%) and a C/N ratio of 32.40. These characteristics suggest that cocopeat can serve as a stable organic amendment with slow nutrient release. Similarly, fern roots contained a relatively balanced C-organic content (24.14%) and nitrogen (1.11%), with a moderate C/N ratio (21.82), potentially making them suitable for supporting gradual nutrient supply and microbial activity.

Micronutrient analysis further indicated that BSFL frass contained the highest levels of magnesium (1.28%) and manganese (8.45%), which are essential for enzymatic functions and photosynthesis. Although husk charcoal and cocopeat are rich in organic carbon, they have relatively low micronutrient concentrations. The fern root contained the highest Mn levels (9.24%), which could enhance plant stress tolerance.

Furthermore, the results in Table 2 illustrate the height gain (cm) of *C. pandurata* over 56 Days After Planting (DAP) under different medium compositions, with and without fertilizer application. A key observation is that the treatment group M2F0 (Husk charcoal without fertilizer) exhibited a significantly higher height gain at 28 DAP (1.83 ± 0.13 cm) and at 56 DAP (3.23 ± 0.31 cm) than to other groups. Similarly, M2F1 and M2F2 (husk charcoal with fertilizers) showed a consistent increase in height gain, suggesting that husk charcoal may provide optimal aeration and nutrient retention for root establishment.

In contrast, M1F0 (frass without fertilizer) displayed one of the lowest height gains throughout the study, indicating that frass alone may not be sufficient to support growth. However, fertilization improved height gain across all groups. The M4 groups (cocopeat) also showed improved height gain when supplemented with fertilizers, particularly M4F1 (1 g L^{-1} fertilizer), which achieved 2.60 ± 0.46 cm at 56 DAP (Figure 1). This suggests that the water-holding capacity of cocopeat, coupled with fertilization, positively influences growth.

Table 1. Nutrient composition of different acclimatization media for *Coelogyne pandurata*

Samples	Total (%)						
	C-organic	N total	C/N ratio	P_2O_2	K_2O	Mg	Mn
BSFL frass	20.14	5.00	4.03	4.19	1.27	1.28	8.45
Husk charcoal	25.93	0.70	37.04	0.23	0.42	0.50	7.91
Cocopeat	38.55	1.19	32.40	0.09	0.22	0.28	8.28
Fern root	24.14	1.11	21.82	0.10	0.31	0.36	9.24

Table 2. Mean ± Standard Error (SE) of the height gain of *Coelogyne pandurata* acclimated with various medium compositions 56 Days After Planting (DAP)

Groups	Height gain (cm)			
	14 DAP	**28 DAP**	**42 DAP**	**56 DAP**
M1F0	1.46 ± 0.76^{a}	1.35 ± 0.00^{a}	1.55 ± 0.55^{a}	0.91 ± 0.52^{a}
M1F1	1.73 ± 0.60^{a}	1.55 ± 0.13^{a}	2.80 ± 0.33^{a}	1.45 ± 0.45^{a}
M1F2	1.55 ± 0.55^{a}	1.41 ± 0.64^{a}	2.19 ± 0.22^{a}	1.36 ± 0.45^{a}
M2F0	0.79 ± 0.79^{a}	1.83 ± 0.13^{b}	2.27 ± 0.45^{a}	3.23 ± 0.31^{c}
M2F1	2.00 ± 0.76^{a}	1.52 ± 0.10^{b}	1.45 ± 0.45^{a}	2.90 ± 0.47^{c}
M2F2	2.00 ± 0.76^{a}	1.71 ± 0.16^{b}	2.19 ± 0.22^{a}	2.48 ± 0.38^{c}
M3F0	1.88 ± 0.68^{a}	1.76 ± 0.06^{ab}	2.33 ± 0.32^{a}	2.19 ± 0.22^{bc}
M3F1	1.88 ± 0.68^{a}	1.45 ± 0.11^{ab}	2.37 ± 0.56^{a}	2.79 ± 0.39^{bc}
M3F2	2.58 ± 0.89^{a}	1.41 ± 0.06^{ab}	1.55 ± 0.54^{a}	2.52 ± 0.27^{bc}
M4F0	1.88 ± 0.68^{a}	1.69 ± 0.05^{b}	2.45 ± 0.43^{a}	2.00 ± 0.19^{b}
M4F1	2.48 ± 0.38^{a}	1.61 ± 0.11^{b}	1.55 ± 0.55^{a}	2.60 ± 0.46^{b}
M4F2	2.56 ± 0.53^{a}	1.58 ± 0.09^{b}	2.66 ± 0.32^{a}	2.00 ± 0.19^{b}

Note: Mean ± Standard Error (SE) followed by different superscripts (a, b) in the same column indicate significant difference at $p < 0.05$. M1: Frass, M2: Husk charcoal, M3: Fern root, M4: Cocopeat, F0: without fertilizer, F1 and F2: with fertilizer at 1 g L^{-1} and 2 g L^{-1}

A sharp decrease in height gain between 14 and 28 DAP was observed in treatments involving BSFL frass (M1 groups). Specifically, the M1F0 (BSFL frass without fertilizer) group showed a decline in height gain from 1.46 ± 0.76 cm at 14 DAP to 1.35 ± 0.00 cm at 28 DAP, while M1F1 (BSFL frass with 1 g L^{-1} fertilizer) exhibited a reduction from 1.73 ± 0.60 cm to 1.55 ± 0.13 cm over the same period. This trend revealed that BSFL frass alone, despite its high nitrogen content (5.00%), may not sustain consistent growth due to rapid nutrient release and poor structural support for root development. In contrast, husk charcoal (M2 groups) demonstrated more stable height gains, with M2F0 (without fertilizer) increasing from 0.79 ± 0.79 cm at 14 DAP to 1.83 ± 0.13 cm at 28 DAP, indicating better nutrient retention and aeration. The sharp decrease in height gain for BSFL frass treatments between 14 and 28 DAP can be attributed to the low C/N ratio (4.03) of BSFL's frass, resulting in quick nitrogen mineralization, which may initially boost growth but fails to sustain it over time (Table 1). The BSFL's frass may lack the physical properties (e.g., aeration, water retention) that are required for stable root development, hindering long-term growth.

The overall trend suggests that husk charcoal, particularly without fertilizer, facilitated the highest growth rates compared with the other substrates. Additionally, cocopeat also demonstrated effectiveness when combined with fertilization. These findings highlight the importance of substrate selection and fertilization strategies for optimizing *C. pandurata* acclimatization.

The number of new foliage formed by *C. pandurata* under different medium compositions and fertilization treatments over 56 Days After Planting (DAP) is shown in Table 3. A notable trend was observed for M2F2 (husk charcoal with 2 g L^{-1} fertilizer), M3F2 (fern root with 2 g L^{-1} fertilizer), and M4F2 (cocopeat with 2 g L^{-1} fertilizer), which displayed significantly higher foliage formation at 56 DAP. Specifically, M4F2 achieved the highest foliage production (3.47 ± 2.06), followed closely by M3F2 (1.43 ± 1.43) and M2F2 (2.87 ± 1.66). This suggests that a higher fertilizer concentration (2 g L^{-1}) positively influenced foliage development across different substrates.

Nevertheless, several treatment groups, including M1F0 (frass without fertilizer), M1F1 (frass with 1 g L^{-1} fertilizer), and M1F2 (frass with 2 g L^{-1} fertilizer), exhibited limited foliage formation, particularly at 56 DAP, when the number of new foliage remained at 0.00 ± 0.00. This indicates that frass alone may not sufficiently support foliage growth without appropriate substrate or additional supplementation.

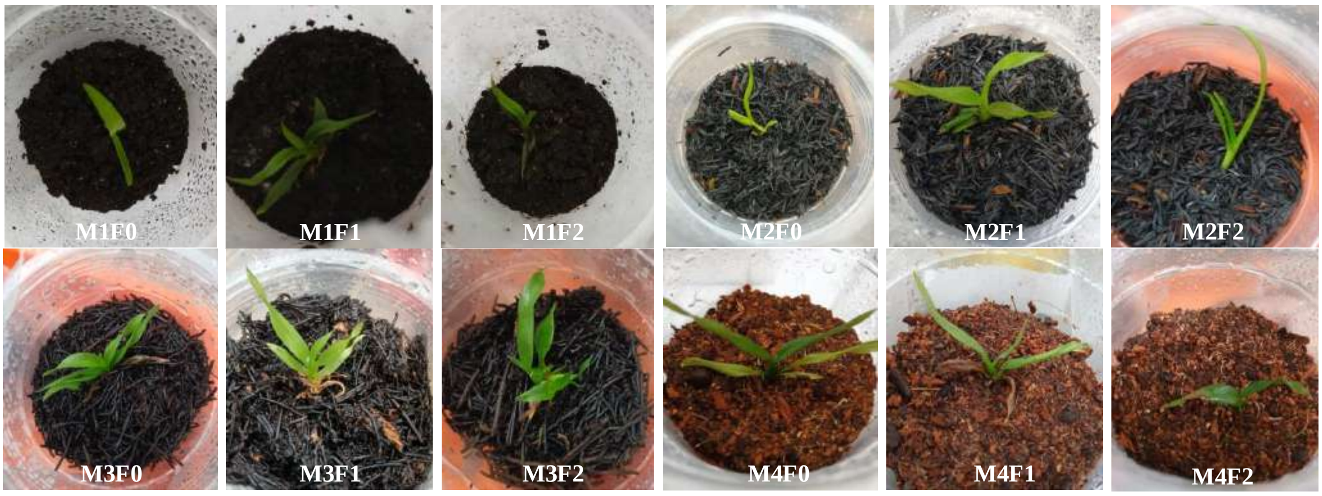

Figure 1. The *Coelogyne pandurata* acclimatized using various media and concentrations of Gandazil® fertilizer at 56 days after Planting. Note: M1: Frass, M2: Husk charcoal, M3: Fern root, M4: Cocopeat, F0: without fertilizer, F1 and F2: with fertilizer at 1 g L^{-1} and 2 g L^{-1}

Table 4. Mean ± Standard Error (SE) of the number of new shoots of *Coelogyne pandurata* acclimated with various medium compositions 56 Days After Planting (DAP)

Groups	Number of new shoots				
	0 DAP	14 DAP	28 DAP	42 DAP	56 DAP
M1F0	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M1F1	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M1F2	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M2F0	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M2F1	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M2F2	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M3F0	1.43±0.43^a	2.03±0.03^a	2.49±0.49^a	2.49±0.49^a	2.49±0.49^a
M3F1	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	1.43±0.43^a	1.43±0.43^a
M3F2	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
M4F0	0.00±0.00^a	0.00±0.00^a	1.43±0.43^a	1.43±0.43^a	1.43±0.43^a
M4F1	0.00±0.00^a	0.00±0.00^a	1.43±0.43^a	1.43±0.43^a	1.43±0.43^a
M4F2	0.00±0.00^a	0.00±0.00^a	1.43±0.43^a	2.03±0.03^a	2.49±0.49^a

Note**:** Mean ± Standard Error (SE) followed by different superscripts (a, b) in the same column indicate significant difference at $p<0.05$. M1: Frass, M2: Husk charcoal, M3: Fern root, M4: Cocopeat, F0: without fertilizer, F1 and F2: with fertilizer at 1 g L^{-1} and 2 g L^{-1}

Table 3. Mean ± Standard Error (SE) of the number of new foliage of *Coelogyne pandurata* acclimated with various medium compositions 56 Days After Planting (DAP)

Groups	Number of the new foliage			
	14 DAP	28 DAP	42 DAP	56 DAP
M1F0	4.30 ± 1.43^a	2.87 ± 1.66^a	2.87 ± 1.66^a	0.00 ± 0.00^a
M1F1	3.47 ± 2.06^a	1.43 ± 1.43^a	1.43 ± 0.43^a	0.00 ± 0.00^a
M1F2	2.87 ± 1.66^{ab}	4.07 ± 2.35^a	2.03 ± 2.03^a	0.00 ± 0.00^a
M2F0	0.00 ± 0.00^a	1.43 ± 0.43^a	3.47 ± 2.06^a	1.43 ± 0.43^b
M2F1	1.43 ± 0.43^a	1.43 ± 1.43^a	2.87 ± 1.66^a	2.87 ± 1.66^b
M2F2	0.00 ± 0.00^{ab}	0.00 ± 0.00^a	0.00 ± 0.00^a	2.87 ± 1.66^b
M3F0	1.43 ± 0.43^a	0.00 ± 0.00^a	0.00 ± 0.00^a	3.47 ± 2.06^b
M3F1	2.87 ± 1.66^a	2.87 ± 1.66^a	0.00 ± 0.00^a	1.43 ± 0.43^b
M3F2	0.00 ± 0.00^{ab}	2.87 ± 1.66^a	1.43 ± 0.43^a	1.43 ± 0.43^b
M4F0	0.00 ± 0.00^{ab}	1.43 ± 0.43^a	0.00 ± 0.00^a	3.47 ± 2.06^b
M4F1	2.87 ± 1.66^a	0.00 ± 0.00^a	0.00 ± 0.00^a	1.43 ± 0.43^b
M4F2	0.00 ± 0.00^{ab}	0.00 ± 0.00^a	3.47 ±2.06^a	3.47 ± 2.06^b

Note**:** Mean ± Standard Error (SE) followed by different superscripts (a, b) in the same column indicate significant difference at $p<0.05$. M1: Frass, M2: Husk charcoal, M3: Fern root, M4: Cocopeat, F0: without fertilizer, F1 and F2: with fertilizer at 1 g L^{-1} and 2 g L^{-1}

In addition, the study observed a decline in the number of new foliage acclimated during 14, 28, 42, and 56 Days After Planting (DAP) in *C. pandurata*, particularly in treatments involving Black Soldier Fly Larvae (BSFL) frass (M1 groups). This trend can be attributed to several factors related to substrate properties, nutrient dynamics, and plant physiological responses such as nutrient depletion and substrate limitations. The BSFL frass, despite its high nitrogen content (5.00%), exhibited a low carbon-to-nitrogen (C/N) ratio (4.03), indicating rapid nitrogen mineralization. While this initially supported early growth, the quick depletion of nutrients likely led to a subsequent decline in foliage production. The lack of structural stability in frass further exacerbated this issue, as it failed to provide sustained aeration and moisture retention necessary for root and shoot development. In contrast, substrates like husk charcoal and cocopeat, with higher C/N ratios (37.04 and 32.40, respectively), demonstrated slower nutrient release, supporting more consistent foliage growth over time.

Moreover, the absence of new foliage in certain groups at 28 and 42 DAP, particularly in those without fertilization, suggests that the initial establishment phase may require both structural support from the substrate and nutrient availability. Consistent foliage production in husk charcoal, fern roots, and cocopeat, particularly with higher fertilizer concentrations, reinforces the importance of balanced substrate selection and fertilization strategies for successful acclimatization.

The number of new shoots produced by *C. pandurata* acclimatized under different medium compositions and fertilization treatments over 56 DAP is shown in Table 4. The majority of treatments exhibited no shoot formation throughout the observation period, except a few specific treatments that showed significant responses at later stages. Among the treatments, M3F0 (fern root without fertilizer) exhibited the earliest shoot emergence at 14 DAP (1.43 ± 1.43), which remained consistent throughout the acclimatization period. At 28 DAP, M3F0 demonstrated an increase in shoot formation (2.49 ± 2.49), maintaining the highest shoot number among all treatments. Other notable responses were observed in M3F1 (fern root with 1 g L^{-1} fertilizer) and M3F2 (fern root with 2 g L^{-1} fertilizer), which showed significant shoot emergence at 42 and 56 DAP, reaching 1.43 ± 1.43 and 2.49 ± 2.49, respectively. Interestingly, M4F2 (cocopeat with 2 g L^{-1} fertilizer) also demonstrated a gradual increase in shoot production, reaching 2.49 ± 2.49 at 56 DAP, similar to M3F2. This suggests that both fern roots and cocopeat, when supplemented with appropriate fertilization, may enhance shoot initiation during acclimatization. However, treatment with BSFL frass (M1F0, M1F1, M1F2) did not induce shoot formation at any time point, indicating that frass alone may not be suitable for promoting shoot growth in *C. pandurata* under the tested conditions.

Discussion

The selection of appropriate acclimatization media significantly affected the growth and survival of *C. pandurata*. Each tested medium exhibited distinct nutrient profiles that influenced plant development. The high nitrogen content in BSFL frass supports early vegetative growth, but its low C/N ratio may lead to rapid nutrient depletion. This suggests that while BSFL frass can enhance initial growth, they may require supplementation with structurally supportive media to sustain long-term development. Current findings are in line with past studies that mentioned that substrates with low C/N ratios initially promote rapid vegetative growth due to high nitrogen availability but lead to nutrient depletion over time. Structurally supportive media (e.g., husk charcoal, cocopeat) with higher C/N ratios provide sustained nutrient release, improving long-term acclimatization success (De Stefano et al. 2022; Gebremikael et al. 2022; Chia et al. 2024).

On the other hand, husk charcoal, with its high organic carbon content and excellent aeration properties, supports root development but lacks sufficient nitrogen and phosphorus. This limitation may necessitate the addition of nitrogen-rich amendments to optimize plant growth. Cocopeat, with its high water-holding capacity, provides stable moisture conditions and benefits acclimatization. However, its low phosphorus and potassium levels indicate the need for external supplementation to sustain its metabolic function. Cocopeat is widely used in soilless cultivation systems for its high water-holding capacity and ability to maintain stable moisture conditions, which are beneficial for seedling acclimatization. Despite these advantages, cocopeat is low in essential nutrients such as phosphorus and potassium, necessitating the addition of external fertilizers to support healthy metabolic function and plant growth (Mariotti et al. 2020).

The fern roots demonstrated a balanced nutrient composition with moderate nitrogen availability and the highest Mn concentration, which can enhance stress tolerance. Its structure supports both moisture retention and aeration, making it a promising substrate for long-term acclimatization. The varying micronutrient content across media highlights the importance of selecting substrates that not only provide essential macronutrients but also support enzymatic functions and physiological stability.

Integrating nutrient-rich amendments with structurally supportive substrates can optimize acclimatization outcomes. Combining BSFL frass with cocopeat or fern roots may improve nutrient availability while maintaining adequate aeration and moisture retention. These findings emphasize the need for strategic substrate selection and supplementation to enhance the survival and growth of *C. pandurata* under ex-vitro conditions.

The significant performance of husk charcoal as a growth medium aligns with previous findings that husk charcoal provides optimal conditions for root aeration and moisture retention, thereby enhancing seedling establishment (Asadi et al. 2021; Karam et al. 2022). The porosity and structure of the husk charcoal likely contributed to improved water drainage and aeration, which are critical factors in orchid root development. Husk charcoal or biochar, is recognized for its high organic carbon content and excellent aeration properties, which support root development and improve soil structure. However, due to its inherently low nitrogen and phosphorus levels, it often requires supplementation with nutrient-rich amendments to optimize plant growth. Studies have shown that combining biochar with additives such as plant ash or effective microorganisms significantly enhances soil fertility (Luo et al. 2025; Sun et al. 2025). Additionally, the ability of husk charcoal to retain nutrients may support plant growth, especially in the absence of fertilizer.

Compared to cocopeat, which demonstrated moderate growth improvements when combined with fertilizers, husk charcoal appears to be a more efficient medium for orchid acclimatization. This contrasts with previous findings suggesting that cocopeat, when combined with organic amendments such as compost, could outperform husk charcoal owing to improved microbial activity (Adekiya et al. 2022; Younis et al. 2022; Melo et al. 2024). The discrepancies in the findings could be due to differences in the orchid species, environmental conditions, and fertilization rates used in each study.

The relatively low performance of frass alone as a substrate was unexpected, considering its high organic matter content. However, the absence of a significant height gain in M1F0 suggests that while frass may provide essential nutrients, they may lack the necessary structural properties for optimal root development. This is consistent with previous studies that highlight the importance of combining organic amendments with structurally supportive substrates to enhance plant growth (Wei et al. 2016; Li et al. 2021; Singh et al. 2022). When combined with husk charcoal or cocopeat, frass may function as an organic enhancer rather than as a primary substrate.

Another important finding of the present study was the role of fertilization in improving growth outcomes. The M4F1 (cocopeat with 1 g L^{-1} fertilizer) exhibited a significantly higher growth rate than its unfertilized counterpart, indicating that nutrient supplementation is crucial for cocopeat-based substrates. This supports the findings of previous studies on the benefits of balanced fertilization during orchid acclimatization (Hariyanto et al. 2019; Rineksane et al. 2023). These results suggest that BSFL frass alone may not be the most effective organic amendment for in vivo acclimatization, but when combined with husk charcoal or cocopeat and supplemented with fertilizer, it may enhance plant growth. Future studies should explore microbial interactions in different substrates and optimize fertilizer composition for black orchid acclimatization.

The findings of this study also suggest that fertilization plays a crucial role in stimulating foliage production in *C. pandurata* during acclimatization. The significantly higher foliage formation in M4F2 (cocopeat with 2 g L^{-1} fertilizer) than in the other treatments highlights the potential of cocopeat as a favorable substrate when supplemented with adequate nutrients. Cocopeat is known for its high water-holding capacity and its ability to retain nutrients, which may contribute to improved foliage development. This result aligns with a previous study that found that cocopeat, when combined with appropriate organic amendments and fertilization, enhanced foliage growth in orchid species because of its ability to support microbial activity and maintain adequate moisture levels (Treder 2008; Kaushal and Kumari 2020; Sachin et al. 2020).

A similar trend was observed for M3F2 (fern root with 2 g L^{-1} fertilizer) and M2F2 (husk charcoal with 2 g L^{-1} fertilizer), in which foliage production was significantly improved. The effectiveness of husk charcoal in supporting foliage development has been reported by Onggo (2013); Fadillatunnisa et al. (2023), who emphasized its role in enhancing aeration and drainage, thereby reducing the risk of root rot, while maintaining sufficient moisture for plant growth. In contrast, fern roots have traditionally been used as an orchid substrate because of their natural affinity for epiphytic species, providing both physical support and essential micronutrients (Sari et al. 2018; Tini et al. 2019).

However, the poor performance of frass-based treatments, particularly M1F0, M1F1, and M1F2, raises concerns regarding the suitability of frass as a standalone substrate for *C. pandurata* acclimatization. BSFL frass are rich in organic matter and essential nutrients, and its limited structural support and potential rapid nutrient leaching may hinder effective plant growth. This observation contrasts with previous findings by Tan et al. (2021) and Antoniadis et al. (2023), who demonstrated that frass application enhanced vegetative growth in leafy greens and fruiting plants. The difference in response could be attributed to the specific growth requirements of orchids, which typically rely on substrates that provide optimal aeration and slow nutrient release, rather than high initial nutrient availability.

One possible explanation for the lower foliage formation in the frass-treated groups is microbial activity associated with frass decomposition. Previous studies have suggested that high microbial activity in organic substrates can lead to temporary nitrogen immobilization, making it less available for plant uptake (Gannett et al. 2024; He et al. 2024; Zhang et al. 2024). This phenomenon might have contributed to the reduced foliage growth in the frass-based treatments, as nitrogen is essential for leaf expansion and photosynthetic activity. Future research should investigate microbial interactions in frass-amended substrates to determine optimal application methods for orchid acclimatization.

The significant effects of fertilization on foliage development also supports the findings of previous studies on nutrient requirements during orchid acclimatization. According to Tang (2025), balanced fertilization is necessary to support leaf initiation and overall plant vigor during the transition from in vitro to ex-vitro conditions. The results of the present study confirmed that fertilization at 2 g L^{-1} is more effective than fertilization at 1 g L^{-1}, suggesting that orchids require higher nutrient availability during the early acclimatization stages. Further studies are required to determine the long-term effects of high fertilizer concentrations on plant health and flowering potential.

Our results also suggest that fern roots and cocopeat, particularly in combination with fertilization, positively influenced the production of new shoots in *C. pandurata* during acclimatization. The superior performance of M3F0 (fern root without fertilizer) at early stages, followed by an increasing trend in M3F1, M3F2, and M4F2, highlights the potential of these substrates to provide suitable conditions for shoot development. These findings align with those of previous studies that emphasized the importance of organic substrates with good aeration properties in promoting shoot formation in orchids (Utami and Hariyanto 2020; Agarwal et al. 2023). Fern root has been widely recognized as an effective medium for orchid cultivation because of its natural ability to retain moisture while maintaining proper aeration, which is essential for root and shoot development (Hariyanto et al. 2019; Arthagama et al. 2021).

Furthermore, the role of cocopeat in enhancing shoot formation, as observed in M4F2, is consistent with previous studies that demonstrated that cocopeat improves the microenvironment for root establishment, leading to enhanced shoot initiation (Atzori et al. 2021; Wu et al. 2025). The high water-holding capacity and organic matter content of cocopeat may have contributed to the steady shoot emergence observed in this study. In addition, the increased shoot production with higher fertilizer concentrations in the fern root and cocopeat treatments is in line with a previous study, which reported that nutrient supplementation, particularly nitrogen, is crucial for shoot differentiation and meristematic activity in orchids during acclimatization (Zhang et al. 2017; Zhang et al. 2018).

In contrast, black soldier fly frass-based treatments (M1F0, M1F1, and M1F2) failed to induce shoot formation. Although frass is known for its high nutrient content, the inability to support shoot growth in this study suggests that it may not provide the necessary structural or microbial environment for effective orchid shoot initiation. BSFL frass application improves vegetative growth in leafy crops (Agustiyani et al. 2021), but its effect on orchids remains uncertain. One possible explanation is that the decomposition process of the BSFL frass leads to temporary nitrogen immobilization, making nitrogen unavailable for plant uptake during critical growth phases (Gebremikael et al. 2022). This might have limited shoot formation in *C. pandurata*, which relies on steady nutrient availability for successful acclimatization.

Moreover, the absence of new shoots in most treatments until later stages suggests that *C. pandurata* requires an extended acclimatization period for shoot differentiation. Certain orchid species may exhibit delayed shoot emergence when transitioning from in vitro to ex-vitro conditions because of physiological adjustments and environmental stress responses (Vaz and Kerbauy 2008; Teixeira da Silva et al. 2014; Kaur 2022). The delayed response in fertilized treatments (M3F1, M3F2, and M4F2) further reinforces the idea that nutrient availability plays a significant role in stimulating shoot growth, but only after the initial root establishment.

Another factor influencing shoot emergence could be microbial interactions within different substrates. While fern roots and cocopeat may support beneficial microbial communities that enhance nutrient availability and hormone regulation, frass-based treatments may have promoted microbial competition, potentially suppressing shoot development. Microbial-driven nutrient cycling in organic substrates can influence plant growth dynamics, particularly in slow-growing species, such as orchids. Future investigations should explore the microbial composition of frass-amended substrates to determine their potential impact on orchid acclimatization (Schleuss et al. 2021; Koch and Sessitsch 2024).

Additionally, the variation in shoot number among the treatments underscores the importance of optimizing substrate selection based on specific orchid growth requirements. Although husk charcoal (M2 treatments) has been widely used in orchid cultivation, its performance in this study did not yield significant shoot formation. This could be attributed to its relatively low water retention capacity, which might not be ideal for *C. pandurata*. Further research is needed to assess the combination of husk charcoal with moisture-retaining amendments to improve its efficacy as an orchid growing medium. Overall, this study underscores the importance of selecting appropriate substrates and fertilization strategies to optimize foliage development during *C. pandurata* acclimatization. Although cocopeat, fern root, and husk charcoal demonstrated promising results, frass alone appeared to be insufficient to support vigorous foliage growth. Future studies should explore the potential of combining frass with structurally supportive substrates to enhance their efficacy as organic amendments. Additionally, investigations into microbial interactions and nutrient dynamics in frass-amended substrates could provide valuable insights into optimizing organic fertilization strategies for orchid cultivation.

This study evaluated the effectiveness of Black Soldier Fly Larvae (BSFL) frass as an organic amendment for in vivo acclimatization of black orchids. The results showed that husk charcoal was the most effective growth medium, whereas cocopeat, when supplemented with fertilizer, enhanced leaf and shoot development. BSFL frass exhibited high nitrogen content but was insufficient to support optimal growth when used alone. However, combining frass with structurally supportive media, such as husk charcoal or cocopeat, has improved its effectiveness. These findings highlight the importance of selecting appropriate growing media and fertilization strategies to enhance the acclimatization success of black orchids. This study contributes to orchid conservation efforts through a sustainable approach that utilize organic waste as a nutrient source for plants.

ACKNOWLEDGMENTS

This study was supported by the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman, Samarinda, Indonesia. The authors also thank PT REA Kaltim Plantation, Indonesia, for providing palm kernel meal.

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ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 326-338 DOI: 10.13057/asianjagric/g090134

Nematicidal activity of *Trichoderma harzianum*-derived secondary metabolites against *Meloidogyne incognita* and metabolomic profiling of selected potent isolates

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Manuscript received: 27 March 2025. Revision accepted: 2 June 2025.

Abstract. *Pradana AP, Sholehah S, Andriyani DR, Hoesain M, Astuti W, Hadiani RU, Masnilah R, Adiwena M, Putri D. 2025. Nematicidal activity of* Trichoderma harzianum*-derived secondary metabolites against* Meloidogyne incognita *and metabolomic profiling of selected potent isolates. Asian J Agric 9: 326-338.* Root-knot nematodes, especially *Meloidogyne incognita*, reduce global crop yields, necessitating the development of safe and effective management approaches. While chemical nematicides can mitigate infestations, they often present environmental concerns and potentially foster resistance in target nematodes. Consequently, using antagonistic fungi, particularly *Trichoderma harzianum*, has gained traction as an eco-friendly solution. Nonetheless, the viability of *T. harzianum* under field conditions is often compromised by abiotic and biotic factors. This study investigated the nematicidal efficacy and secondary metabolite composition of four *T. harzianum* isolates (AJG, TGL, SBS, and SKS) collected from distinct geographical regions. In vitro assays revealed that the AJG and TGL isolates elicited the most potent effects, achieving >75% egg-hatching inhibition and >93% juvenile mortality at 168 hours post-treatment. Gas chromatography-mass spectrometry indicated that fatty acid esters, including methyl palmitate and methyl oleate, were the major contributors to nematicidal effects through membrane disruption. Notable differences in metabolite profiles between isolates highlight isolate-specific biochemical pathways impacting suppressive capacity. The predominance of fatty acids underscores their critical function in controlling nematodes, offering prospects for developing stable, environmentally friendly formulations. These results underscore the potential of *T. harzianum*-derived metabolites for integrated nematode management. They also emphasize the importance of isolate selection and metabolite profiling in designing targeted, sustainable strategies to combat root-knot nematodes, empowering the audience with this crucial knowledge.

Keywords: Biological control, fatty acid esters, GC-MS analysis, ovicidal activity, root-knot nematode

INTRODUCTION

Meloidogyne incognita poses a major threat to global agriculture, causing 15-20% yield losses in key crops and affecting over 2,000 plant species (Subedi et al. 2020; Tapia-Vázquez et al. 2022). Economic losses attributed to root-knot nematode infestations have been estimated at over $100 billion annually, highlighting the critical need for effective management strategies to sustain agricultural productivity and food security (Khan et al. 2021).

Chemical nematicides have traditionally dominated the management strategies employed against nematode infestations. While effective, these chemicals often have adverse environmental impacts, contribute to soil degradation, and potentially lead to the emergence of resistant nematode populations (Tudi et al. 2021; Pathak et al. 2022). Consequently, research has shifted toward environmentally friendly alternatives, including biological control strategies utilizing antagonistic fungi such as *Trichoderma* spp. (Azlay et al. 2023). These fungi have been widely adopted as biocontrol agents due to their ability to colonize root systems, inhibit pathogen growth, and stimulate plant defense mechanisms (Forghani and Hajihassani 2020; Khan et al. 2020).

Among the *Trichoderma* species, *Trichoderma harzianum* Rifai (1969) has garnered substantial attention for its effectiveness in suppressing nematode populations through mechanisms such as parasitism, antibiosis, and competition for nutrients and space (Ghazanfar et al. 2018; Sood et al. 2020). Field applications involving direct inoculation of *T. harzianum* spores or mycelium have significantly reduced nematode populations, sometimes achieving >60% suppression under favorable conditions (Feyisa et al. 2016). While concerns have been raised regarding the comparative effectiveness of *T. harzianum* versus chemical nematicides under variable environmental conditions, integrating *T. harzianum* within comprehensive Integrated Pest Management (IPM) strategies can significantly enhance its reliability and effectiveness. Successful deployment of *T. harzianum*-based strategies depends on integrating them within comprehensive IPM frameworks, supported by targeted farmer education, precise application timing, and optimized techniques. Investment in outreach programs and extension services can significantly enhance practitioner awareness, adoption, and consistent performance of biological control agents (Azlay et al. 2023).

However, the practical application of living *T. harzianum* preparations faces substantial challenges in agricultural fields. The survival and effectiveness of fungal inoculants in situ are often compromised by environmental stresses such as drought, soil pH fluctuations, UV radiation exposure, and predation by soil fauna (Soesanto et al. 2019; Khan et al. 2020). Among abiotic and biotic environmental factors, drought stress, fluctuations in soil pH, UV radiation exposure, and predation by soil fauna significantly compromise the viability and persistence of *T. harzianum* inoculants under field conditions.

Empirical studies have shown that approximately 30-40% of applied fungal inoculants experience significant viability losses within the first few weeks post-application due to abiotic stressors. Microarthropod predation and competing microorganisms also limit fungal persistence and activity, ultimately reducing efficacy to <50% of laboratory-tested levels under actual field conditions. These factors substantially reduce the practical reliability and predictability of biological control outcomes (Shahriar et al. 2022; Ayaz et al. 2023).

Recent studies have highlighted secondary metabolites from *T. harzianum* as promising alternatives to fungal biomass for nematode management. These metabolites, including volatile organic compounds, antibiotics, and enzymatic secretions, offer improved environmental stability, shelf-life, and consistent efficacy (Wang et al. 2021; Yan and Khan 2021). Laboratory assays show mortality rates of 70-80% against *M. incognita* juveniles (Khan et al. 2020; Lopes et al. 2024). Identified compounds such as 6-pentyl-α-pyrone, harzianic acid, gliotoxin, and peptaibols also exhibit antifungal, antimicrobial, and plant growth-promoting properties (Wang et al. 2021; Yan and Khan 2021). However, detailed profiling and specific mechanisms underlying their nematicidal efficacy remain limited, necessitating advancements in formulation technologies for practical agricultural use.

Initial findings on *T. harzianum* metabolites are promising, yet knowledge gaps remain regarding isolate-specific variability and nematicidal efficacy. Although regional factors might influence metabolite profiles, this study focuses on inherent metabolite diversity among isolates regardless of geographic origin. Characterizing these profiles is essential for selecting effective strains and optimizing biocontrol consistency. Secondary metabolites can be formulated as stable biopesticides or integrated into controlled-release systems, improving their practicality. Assessing their long-term ecological impacts is also necessary, despite preliminary indications of minimal negative effects (Soesanto et al. 2019; Khan et al. 2020). Therefore, this study evaluates nematicidal activity and metabolite composition from four geographically distinct *T. harzianum* isolates against *M. incognita*, identifying potent bioactive compounds for sustainable nematode management.

MATERIALS AND METHODS

Research location and experimental duration

This study was conducted at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember, East Java, Indonesia, from June to December 2024.

Isolation and culture of *Trichoderma harzianum* strains

This study examined four *T. harzianum* isolates. Isolates TGL and AJG were provided by the Laboratory of Pest and Disease Observation for Food Crops and Horticulture-Tanggul, Department of Agriculture and Food Security, East Java Province, Indonesia. They originated from the rhizosphere soils of soybean plants in the Tanggul and Ajung Sub-districts in Jember District, East Java, Indonesia. Isolates SBS and SKS were obtained from the culture collection at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember. These isolates were also originally isolated from soybean rhizosphere soils in the Sumbersari and Sukorambi Sub-districts, respectively, in Jember District, Indonesia. Pure cultures of all isolates were maintained on potato dextrose agar (HiMedia, India) in 9 cm diameter Petri dishes under controlled laboratory conditions.

Source and preparation of *Meloidogyne incognita* eggs and second-stage juveniles

This study utilized an *M. incognita* isolate obtained from the nematode culture collection maintained at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember. It was propagated on tomato plants (variety Servo F1) to preserve its purity. Nematode eggs were collected by carefully harvesting symptomatic tomato roots, gently washing them, and segmenting them into 1-2 cm pieces. Next, the root segments were vigorously agitated in a 1% sodium hypochlorite solution to release the eggs, and the resulting suspension was filtered through a 600-mesh sieve to collect them. Then, the eggs were thoroughly rinsed with distilled water to remove residual sodium hypochlorite. Second-stage juveniles (J2) were prepared by incubating the clean eggs in darkness at room temperature for 7 days, facilitating the hatching of J2 nematodes, which were then used in the experimental assays (Asyiah et al. 2025).

Production and collection of *Trichoderma harzianum* secondary metabolites

Each *T. harzianum* isolate was cultured individually in 100 mL of potato dextrose broth (PDB; HiMedia, India), adjusted to pH 6.0. The cultures were incubated at 30±2°C for 7 days on a rotary shaker set at 80 rpm. After incubation, the cultures were centrifuged aseptically at 6000 rpm, and the supernatants obtained were filtered through sterile membranes with a pore size of 0.22 µm. The filtered supernatants containing the extracellular secondary metabolites of *T. harzianum* were stored aseptically at 4°C until used in the biological assays (Moo-Koh et al. 2022).

In vitro bioassay of ovicidal and nematicidal activities

In vitro bioassays were performed to evaluate the ovicidal and nematicidal potential of *T. harzianum* secondary metabolites against *M. incognita*. Five treatments were examined: secondary metabolites from four distinct *T. harzianum* isolates and a PDB control. Each treatment comprised six replicates and followed a completely randomized design.

For the ovicidal assays, 100 *M. incognita* eggs were transferred to Petri dishes containing 5 mL of 5% (v/v) secondary metabolite solutions from respective isolates or 5% PDB as a control. The eggs were incubated in the dark, with unhatched eggs counted at 24-hour intervals for up to 168 hours. In the nematicidal assays, 100 J2 *M. incognita* were introduced into test dishes containing 5 mL of the same solutions. The mortality rates of J2 nematodes were observed and recorded at 24-hour intervals up to 168 hours.

The resulting data underwent arcsine transformation before statistical analyses. The transformed data was compared between groups using analysis of variance, followed by post-hoc Tukey's honestly significant difference (HSD) test, considering a 5% significance level (Khan et al. 2021).

Metabolomic profiling of *T. harzianum* secondary metabolites by gas chromatography-mass spectrometry

Untargeted metabolomic profiling was conducted using gas chromatography-mass spectrometry (GC-MS) on a GC-MS-QP2010 Plus system (Shimadzu, Japan). The samples were analyzed in split injection mode, with an injector temperature of 250°C. The oven temperature program began at 80°C for 2 minutes, then increased at 5°C/min up to 280°C, which was maintained for 3 minutes. The carrier gas was helium, operating at a linear velocity of 36.6 cm/sec, column flow rate of 0.99 mL/min, total flow of 4.9 mL/min, and an internal column pressure of 64.1 kPa. The split ratio was adjusted to 1:1, with a carrier gas saver-to-split ratio of 5.0 after 2 minutes.

The mass spectra were recorded in electron ionization mode with the ion source and interface temperatures both set to 280°C. The mass spectral data were collected in the full scan range of m/z 40-600 at a scan speed of 1666 scans/sec and event time of 0.40 sec. The total runtime per sample was 45 minutes. Compounds in the metabolite extracts were identified by comparing their mass spectra against the Wiley9.LIB reference mass spectral database (Asyiah et al. 2025).

RESULTS AND DISCUSSION

Effect of *T. harzianum* secondary metabolites on root-knot nematode eggs and juveniles

The secondary metabolites derived from various *T. harzianum* isolates demonstrated significant efficacy in inhibiting the hatching of *M. incognita* eggs. Notable inhibition was observed starting at 48 hours of treatment. The mean percentage of unhatched eggs was 93.33% with PDB (control), compared to 99.67% and 98.50% with secondary metabolites from *T. harzianum* isolates AJG and TGL, respectively. Statistical analysis indicated significant differences between these two isolates and the control. Conversely, the mean percentage of unhatched eggs did not differ significantly between PDB (control) and the secondary metabolites from *T. harzianum* isolates SBS and SKS at this time point.

After 96 hours of treatment, the mean percentage of unhatched eggs was significantly lower with PDB (control: 75.00%) than with the secondary metabolites from *T. harzianum* isolates AJG (84.67%) and TGL (83.67%). In contrast, the mean percentage of unhatched eggs did not differ significantly between PDB (control) and the secondary metabolites from *T. harzianum* isolates SBS (76.17%) and SKS (76.33%).

A clear change in the pattern emerged after 168 hours of treatment, with all tested *T. harzianum* isolates significantly outperforming the control. At this final observation, the mean percentage of unhatched eggs with PDB (control) had decreased drastically to 17.00% and was significantly lower than with the secondary metabolites from *T. harzianum* isolates AJG (76.17%), TGL (75.33%), SBS (63.67%), and SKS (59.50%). *Trichoderma harzianum* isolates AJG and TGL consistently exhibited the greatest efficacy, with *T. harzianum* isolate AJG exhibiting the greatest overall ovicidal activity. Further data on the percentage of unhatched *M. incognita* eggs treated with *T. harzianum* secondary metabolites is presented in Figure 1.

The secondary metabolites from *T. harzianum* isolates were also effective in inducing mortality in *M. incognita* J2. Mortality effects were evident as early as 24 hours of treatment. The mean mortality was only 0.33% with PDB (control), significantly lower than with the secondary metabolites from *T. harzianum* isolates AJG (11.50%), TGL (10.83%), and SBS (3.67%). However, the mortality rate did not differ significantly between PDB (control) and the secondary metabolites of *T. harzianum* isolate SKS (1.83%) at this early time point.

After 96 hours of treatment, the mortality rates remained significantly higher with the secondary metabolites from *T. harzianum* isolates AJG (42.67%), TGL (40.17%), SBS (22.83%), and SKS (23.17%) than with PDB (control: 1.33%). This consistent pattern persisted through to the final observation at 168 hours of treatment, with the mortality rate significantly lower with PDB (control: 3.33%) than with the secondary metabolites from *T. harzianum* isolates AJG (95.50%), TGL (93.83%), SBS (52.00%), and SKS (46.00%).

These results clearly indicate the potential of *T. harzianum*-derived secondary metabolites to control both *M. incognita* eggs and J2 effectively. Among all examined *T. harzianum* isolates, the secondary metabolites from AJG consistently demonstrated superior ovicidal and nematicidal activity, suggesting isolate-specific bioactivity and potential for practical applications in nematode management strategies. Figure 2 presents the mortality rates of *M. incognita* J2 exposed to *T. harzianum* secondary metabolites.

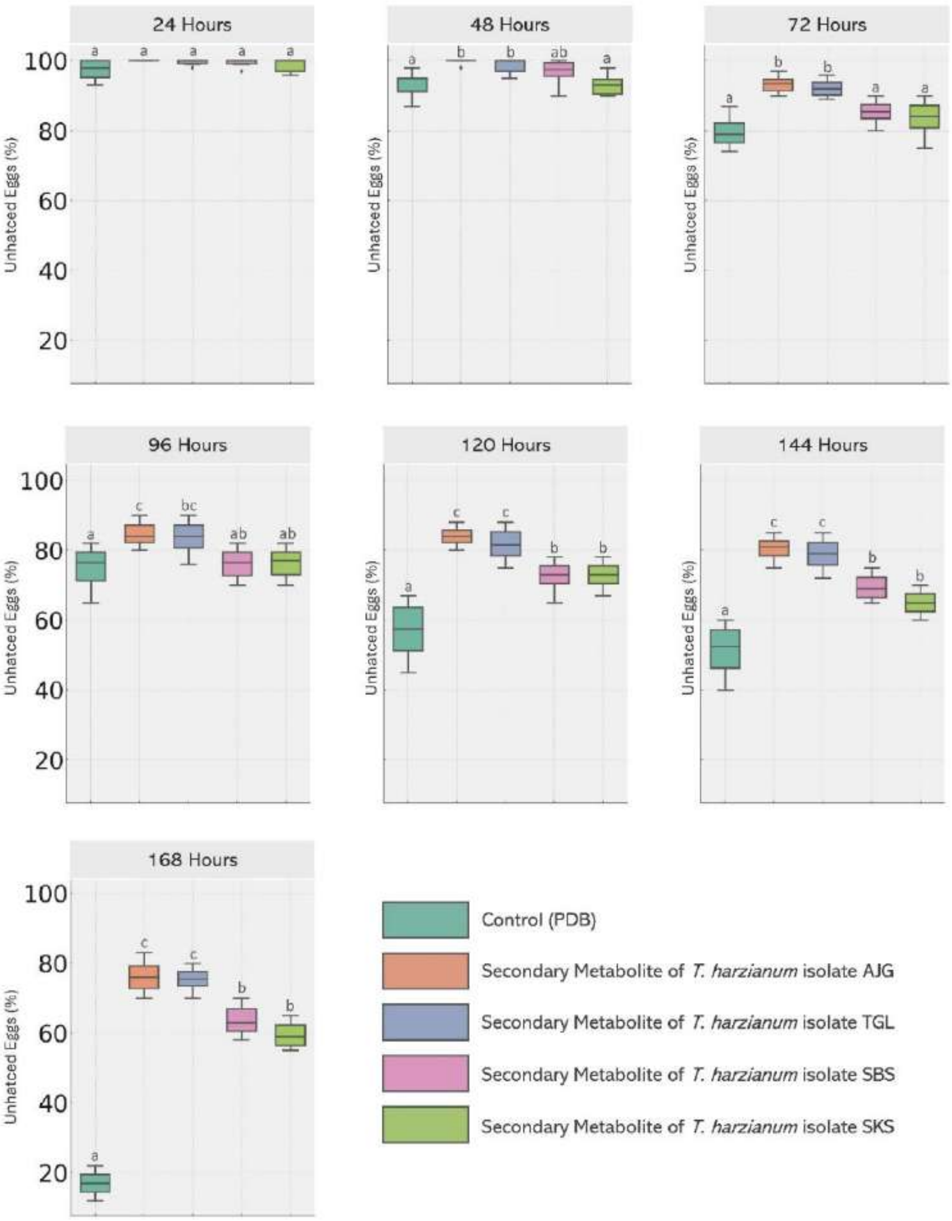


Figure 1. The ovicidal effect of *Trichoderma harzianum* secondary metabolites on *Meloidogyne incognita* eggs. Note: Groups that share at least one letter above the boxplots at the same time point are not significantly different, as determined by post-hoc Tukey's HSD tests at the 5% significance level. Shared letters indicate statistical similarity

The differential ovicidal and nematicidal activities observed among the secondary metabolites derived from different *T. harzianum* isolates could be attributed to their distinct metabolomic profiles and bioactive compound variations (Moo-Koh et al. 2022). The superior performance of *T. harzianum* isolates AJG and TGL likely results from their ability to synthesize higher concentrations of specific metabolites such as gliotoxin, harzianic acid, and other volatile organic compounds previously documented to have nematicidal properties (Rangel et al. 2021; Zaid et al. 2022). Previous research has shown that gliotoxin significantly interferes with nematode cellular metabolism, disrupting vital physiological processes such as mitochondrial function and enzyme regulation, eventually leading to high nematode mortality (Chen et al. 2020; Hamrouni et al. 2025). This research opens up exciting possibilities for further investigation into the mechanisms of action of these metabolites and their potential applications in agriculture.

In contrast, *T. harzianum* isolates SBS and SKS exhibited comparatively lower efficacy, possibly due to a lower diversity or concentration of bioactive metabolites. According to Zeilinger et al. (2016), differences in nematicidal activity among fungal isolates commonly correlate with variability in secondary metabolite synthesis, influenced by isolate-specific genetic and environmental factors during growth and metabolite production. The observed differences in bioactivity might be explained by variations in the metabolic pathways activated under similar cultivation conditions, emphasizing the importance of metabolomic characterization in selecting effective biocontrol strains (Keswani et al. 2019; Rodrigo et al. 2021).

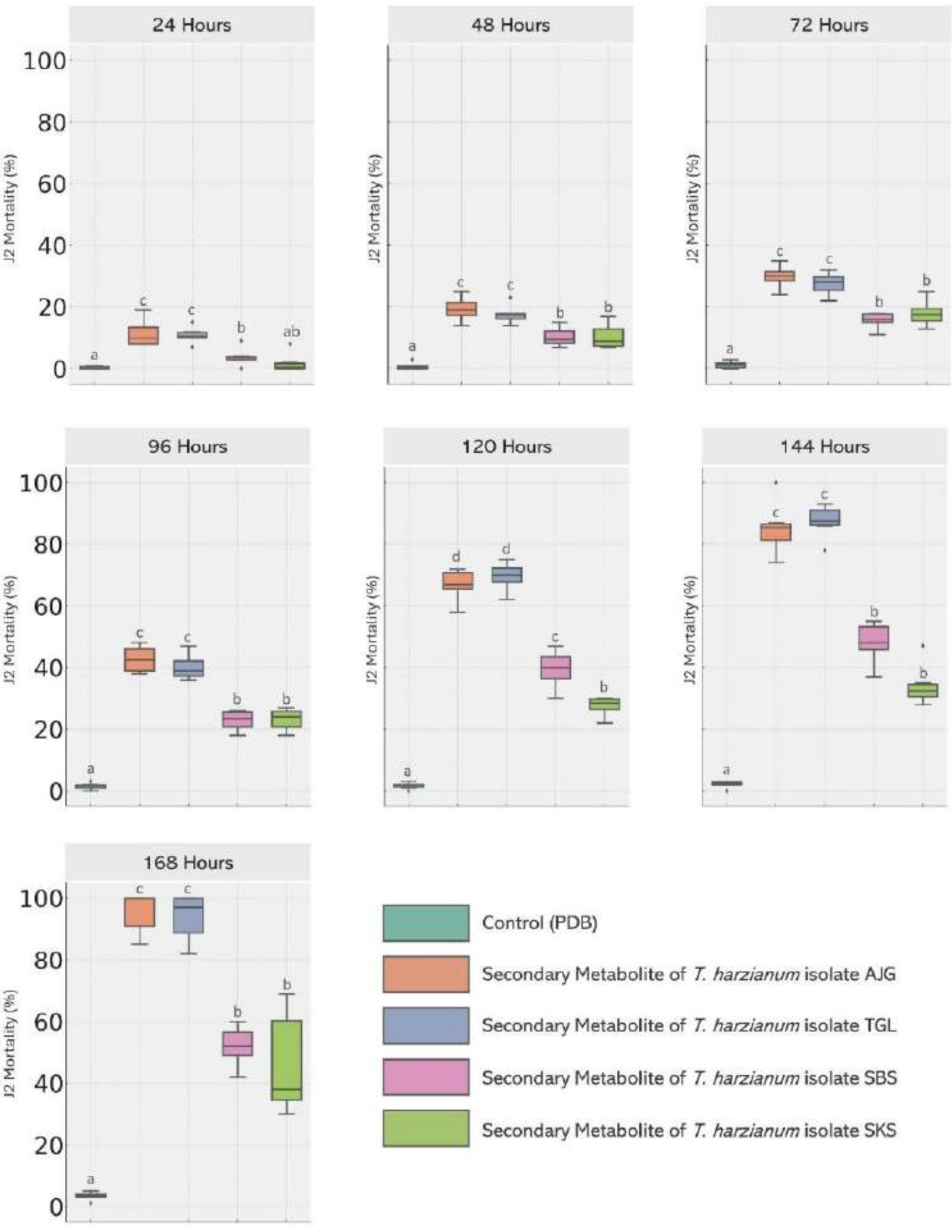


Figure 2. The nematicidal effect of *Trichoderma harzianum* secondary metabolites on *Meloidogyne incognita* J2. Note: Groups that share at least one letter above the boxplots at the same time point are not significantly different, as determined by post-hoc Tukey's HSD tests at the 5% significance level. Shared letters indicate statistical similarity

The reduced egg hatching observed with effective *T. harzianum* isolates likely involves specific fungal metabolites disrupting nematode embryogenesis and developmental signaling pathways. Previous studies indicated that metabolites such as 6-pentyl-alpha-pyrone produced by *T. harzianum* can directly interfere with the permeability and integrity of nematode eggshells, resulting in impaired embryonic development (Baazeem et al. 2021; Lopes et al. 2024). These metabolites could also indirectly affect hatching by altering the egg's surrounding chemical environment, inhibiting hatching stimulants, or enhancing the activity of natural egg inhibitors (Devi 2018).

Similarly, the high J2 mortality may reflect the effects of secondary metabolites with neurotoxic and enzyme-inhibitory properties. Some metabolites have been reported to exert direct nematicidal effects by causing irreversible damage to the nematode nervous system, resulting in paralysis and subsequent death (Yao et al. 2023). The observed variation in mortality rates among the different *T. harzianum* isolates may reflect isolate-specific differences in metabolite profiles, with highly effective isolates producing metabolites that are more potent or more readily absorbed by nematodes.

In addition to direct nematicidal and ovicidal activities, *Trichoderma*-derived secondary metabolites have been recognized for their multifaceted roles in managing plant-parasitic nematodes through induced systemic resistance (ISR) in host plants. Compounds such as harzianic acid and

6-pentyl-alpha-pyrone (6-PP), which are abundant in certain isolates of *T. harzianum*, not only exhibit direct toxicity to nematodes but also enhance plant defense responses, thereby providing integrated nematode management strategies. For instance, harzianic acid and 6-PP have been shown to induce the expression of plant defense-related genes, such as those involved in phenylpropanoid pathways, jasmonic acid signaling, and salicylic acid-dependent defenses. This systemic protection complements the direct toxicity mechanisms, reinforcing the efficacy of secondary metabolite-based biocontrol applications in agricultural practices (Rangel et al. 2021; Zaid et al. 2022).

Furthermore, the practical potential of *T. harzianum* metabolites as nematode management agents can be significantly enhanced by advancements in formulation and delivery methods. Recent research has demonstrated that formulating metabolites into nano-emulsions, encapsulated slow-release formulations, or incorporating them into bioactive carrier materials markedly improves their environmental stability, longevity, and efficacy under realistic agricultural conditions (Wang et al. 2021; Yan and Khan 2021). For example, encapsulation of gliotoxin and volatile organic compounds into biodegradable polymeric nanoparticles has shown sustained release and prolonged nematicidal activity, leading to substantial reductions in nematode populations even under challenging field conditions (Chen et al. 2020; Hamrouni et al. 2025). These advancements validate the practical use of secondary metabolites derived from *T. harzianum* and address the viability and consistency concerns associated with traditional biological control strategies.

Compound profiling of *T. harzianum* secondary metabolites via GC-MS

The secondary metabolite analysis of *T. harzianum* isolate AJG using GC-MS identified 35 distinct compounds representing diverse chemical groups such as fatty acids, alcohols, ketones, aldehydes, and hydrocarbons. Among the identified metabolites, the most abundant included 9-octadecenoic acid (Z)-, methyl ester (22.91%); hexadecanoic acid, methyl ester (22.64%); and octadecanoic acid, ethyl ester (8.78%). These compounds collectively accounted for more than half of the total peak area detected in the sample. The GC-MS chromatogram of secondary metabolites produced by *T. harzianum* isolate AJG is shown in Figure 3.

Other notable metabolites present in moderate abundances were hexadecane, 2,6,10,14-tetramethyl- (6.25%); cyclopentadecanone, 2-hydroxy- (5.35%); pentacosane (3.87%); triacontane (3.38%); and tetradecanoic acid, methyl ester (2.33%). The analysis also revealed several minor constituents, such as acetic acid, various alcohols, aldehydes, and ketones, reflecting the metabolic complexity and biochemical diversity of *T. harzianum* isolate AJG. The metabolite profile of *T. harzianum* isolate AJG is summarized in Table 1.

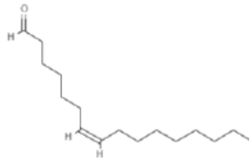
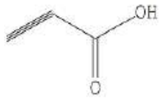
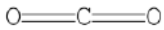
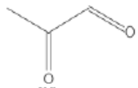
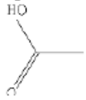
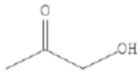
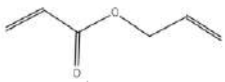
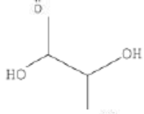
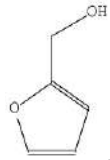
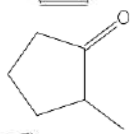
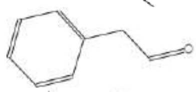
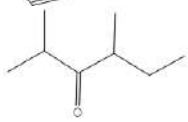
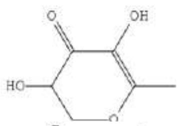
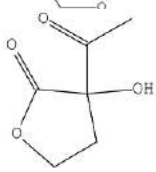
The GC-MS analysis of secondary metabolites derived from *T. harzianum* isolate TGL identified 28 distinct chemical constituents, and the GC-MS chromatogram is shown in Figure 4. Like *T. harzianum* isolate AJG, the *T. harzianum* isolate TGL produced diverse compounds, including fatty acids, esters, alcohols, ketones, aldehydes, and hydrocarbons. Among these secondary metabolites, the most abundant were 9-octadecenoic acid (Z)-, methyl ester (20.21%), and hexadecanoic acid, methyl ester (18.96%), collectively accounting for nearly 40% of the total peak area.

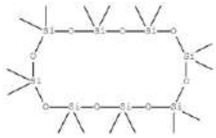
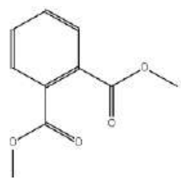
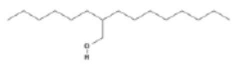
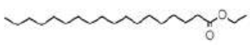
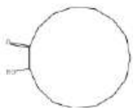
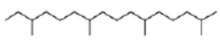
Trichoderma harzianum isolate TGL also showed notable concentrations of metabolites such as 2-furancarboxaldehyde, 5-(hydroxymethyl)- (10.47%); octadecanoic acid, ethyl ester (5.95%); acetic acid (5.85%); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (5.29%). Several other compounds, such as 2,5-dimethyl-4-hydroxy-3(2H)-furanone and hexadecane, 2,6,10,14-tetramethyl-, were present at moderate concentrations, highlighting the biochemical complexity of this isolate.

Comparatively, *T. harzianum* isolates TGL and AJG exhibited significant similarities in their major secondary metabolites, particularly in the high abundance of 9-octadecenoic acid (Z)-, methyl ester, and hexadecanoic acid, methyl ester. However, notable quantitative differences were observed. *Trichoderma harzianum* isolate AJG had slightly higher abundances of these fatty acid esters (22.91 and 22.64%, respectively) than *T. harzianum* isolate TGL. In addition, *T. harzianum* isolate AJG showed relatively higher amounts of certain hydrocarbons and ketones, such as hexadecane, 2,6,10,14-tetramethyl-, and cyclopentadecanone, 2-hydroxy-, while *T. harzianum* isolate TGL had higher relative amounts of oxygenated aromatic compounds such as 2-furancarboxaldehyde, 5-(hydroxymethyl)-, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. A comprehensive list of the identified compounds in the metabolomic profile of *T. harzianum* isolate TGL is presented in Table 2.

The metabolite profiling of *T. harzianum* isolate AJG revealed several bioactive compounds that are likely the key players in its nematicidal and ovicidal activities against *M. incognita*. Notably, the detection of hexadecanoic acid methyl ester (methyl palmitate) and 9-octadecenoic acid (Z)-, methyl ester (methyl oleate) is particularly promising, as it indicates significant potential mechanisms underlying nematode control (Lu et al. 2020; Sall et al. 2024). Previous studies have demonstrated that methyl palmitate exhibits potent nematicidal properties through mechanisms such as disruption of lipid membranes and modulation of nematode gene expression (Elbanhawy et al. 2019). Lu et al. (2020) reported that increasing concentrations of methyl palmitate significantly inhibited *M. incognita* egg hatching by up to 95.5%, supporting its crucial role in the observed ovicidal activity of *T. harzianum* isolate AJG in this study.

Table 1. The secondary metabolites identified in *Trichoderma harzianum* isolate AJG by GC-MS

Peak #	Retention time	Area percentage (%)	Molecular weight (g mol^{-1})	Compound name	Formula	CAS registry number	Chemical structure
1	0.171	0.09	238	Z-7-Hexadecenal	$C_{16}H_{30}O$	56797-40-1	
2	1.529	0.43	70	Propiolic acid	$C_3H_2O_2$	471-25-0	
3	1.636	0.06	46	Ethanol	C_2H_6O	64-17-5	
4	1.773	0.21	118	Carbon dioxide (CAS)	CO_2	124-38-9	
5	2.445	0.04	86	Butanal, 2-methyl- (CAS)	$C_5H_{10}O$	96-17-3	
6	4.772	0.62	72	Propanal, 2-oxo- (CAS)	$C_3H_4O_2$	78-98-8	
7	5.093	2.80	60	Acetic acid (CAS)	$C_2H_4O_2$	64-19-7	
8	5.356	2.07	74	2-Propanone, 1-hydroxy- (CAS)	$C_3H_6O_2$	116-09-6	
9	5.985	0.59	112	2-Propenoic acid, 2-propenyl ester (CAS)	$C_6H_8O_2$	999-55-3	
10	6.512	1.21	90	2,3-Butanediol (CAS)	$C_4H_{10}O_2$	513-85-9	
11	7.376	0.14	98	2-Furanmethanol (CAS)	$C_5H_6O_2$	98-00-0	
12	9.735	0.46	98	Cyclopentanone, 2-methyl-	$C_6H_{10}O$	1120-72-5	
13	11.464	0.21	120	Benzeneacetaldehyde (CAS)	C8H8O	122-78-1	
14	13.624	0.56	128	3-Hexanone, 2,4-dimethyl- (CAS)	$C_8H_{16}O$	18641-70-8	
15	15.464	0.17	132	anhydro - sugar	$C_5H_8O_4$	0-00-0	-
16	16.286	1.35	144	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	28564-83-2	
17	17.847	0.12	144	2-acetyl-2-hydroxy-.gamma.-butyrolactone	$C_6H_8O_4$	135366-64-2	

18	18.607	0.20	592	Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	556-68-3	
19	19.723	0.44	214	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	19780-33-7	-
20	20.470	4.13	126	2-furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	67-47-0	
21	21.778	0.99	214	Dodecanoic acid, methyl ester (CAS)	$C_{13}H_{26}O_2$	111-82-0	
22	22.252	0.97	214	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	19780-33-7	-
23	23.707	0.28	194	1,2-benzenedicarboxylic acid, dimethyl	$C_{10}H_{10}O_4$	131-11-3	
24	24.723	0.68	242	2-Hexyl-1-decanol	$C_{16}H_{34}O$	2425-77-6	
25	26.589	2.33	242	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	124-10-7	
26	29.203	2.06	268	Nonadecane (CAS)	$C_{19}H_{40}$	629-92-5	
27	31.100	22.64	270	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	112-39-0	
28	32.236	1.08	284	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	628-97-7	
29	33.369	3.87	352	Pentacosane	$C_{25}H_{52}$	629-99-2	
30	34.189	2.52	214	Tridecanoic acid (CAS)	$C_{13}H_{26}O_2$	638-53-9	
31	34.987	22.91	296	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	112-62-9	
32	36.174	8.78	312	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	111-61-5	
33	37.173	3.38	422	Triacontane	$C_{30}H_{62}$	638-68-6	
34	37.745	5.35	240	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	4727-18-8	
35	38.944	6.25	282	Hexadecane, 2,6,10,14-tetramethyl- (CAS)	$C_{20}H_{42}$	638-36-8	
Total		100					

Note: The compounds were identified by comparing their mass spectra from the chromatogram with the Wiley9.LIB mass spectral database

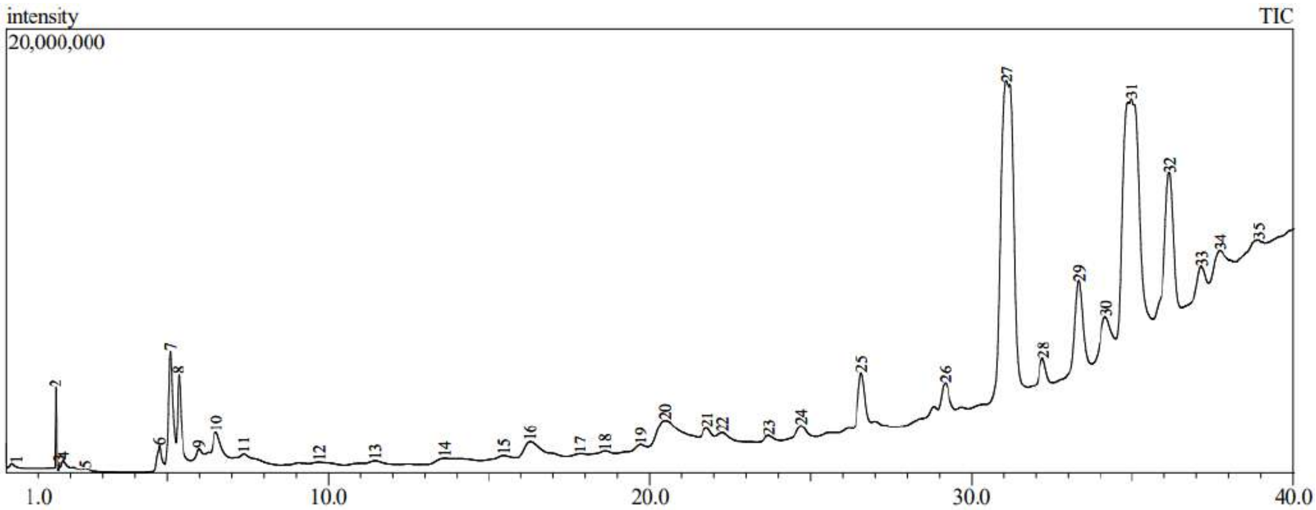
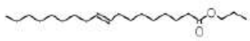
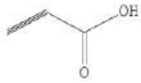
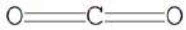
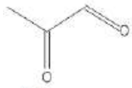
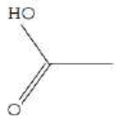
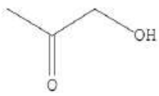
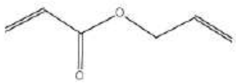
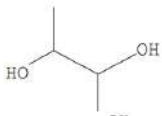
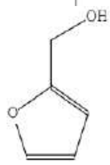
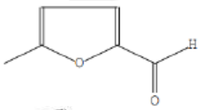
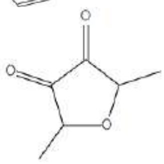
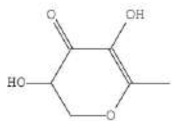
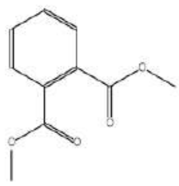
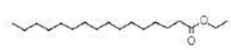
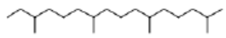
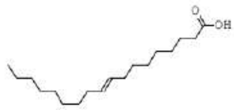


Figure 3. The GC-MS chromatogram of the secondary metabolites produced by *Trichoderma harzianum* isolate AJG

Table 2. Metabolites identified in *Trichoderma harzianum* isolate TGL by GC-MS

Peak #	Retention Time	Area Percentage (%)	Molecular Weight (g mol^{-1})	Compound Name	Formula	CAS Registry Number	Chemical structure
1	0.175	0.15	324	Oleic acid, propyl ester	$C_{21}H_{40}O_2$	111-59-1	
2	1.533	0.44	70	Propiolic acid	$C_3H_2O_2$	471-25-0	
3	1.800	0.64	118	Carbon dioxide (CAS)	CO_2	124-38-9	
4	2.466	0.25	86	Butanal, 2-methyl- (CAS)	$C_5H_{10}O$	96-17-3	
5	4.695	0.82	72	Propanal, 2-oxo- (CAS)	$C_3H_4O_2$	78-98-8	
6	5.021	5.85	60	Acetic acid (CAS)	$C_2H_4O_2$	64-19-7	
7	5.290	3.12	74	2-Propanone, 1-hydroxy- (CAS)	$C_3H_6O_2$	116-09-6	
8	5.934	1.86	112	2-Propenoic acid, 2-propenyl ester (CAS)	$C_6H_8O_2$	999-55-3	
9	6.439	3.38	90	2,3-Butanediol (CAS)	$C_4H_{10}O_2$	513-85-9	
10	7.353	2.59	98	2-Furanmethanol (CAS)	$C_5H_6O_2$	98-00-0	
11	9.500	1.13	110	5-Methyl furfural	$C_6H_6O_2$	620-02-0	
12	11.491	0.33	120	Benzeneacetaldehyde (CAS)	C_8H_8O	122-78-1	
13	13.609	3.89	128	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	3658-77-3	
14	15.463	0.75	132	Anhydro-sugar	$C_5H_8O_4$	C5H8O4	-
15	16.286	5.29	144	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	28564-83-2	
16	20.378	10.47	126	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	67-47-0	
17	23.707	0.30	194	1,2-Benzenedicarboxylic acid, dimethyl ester (CAS)	$C_{10}H_{10}O_4$	131-11-3	

18	24.760	0.15	648S	Docosanoic acid, docosyl ester (CAS)	$C_{44}H_{88}O_2$	17671-27-1	
19	26.608	0.89	242	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	124-10-7	
20	29.211	0.93	268	Nonadecane	$C_{19}H_{40}$	629-92-5	
21	31.099	18.96	270	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	112-39-0	
22	32.216	1.11	284	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	628-97-7	
23	33.388	1.89	282	Hexadecane, 2,6,10,14-tetramethyl- (CAS)	$C_{20}H_{42}$	638-36-8	
24	34.162	2.97	214	Tridecanoic acid (CAS)	$C_{13}H_{26}O_2$	638-53-9	
25	34.869	20.21	296	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	112-62-9	
26	36.133	5.95	312	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	111-61-5	
27	37.493	2.75	302	Silane, trichloro-dodecyl- (CAS)	$C_{12}H_{25}Cl_3Si$	4484-72-4	
28	37.741	2.92	282	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	0-00-0	
	Total	100					

Note: The compounds were identified by comparing their mass spectra from the chromatogram with the Wiley9.LIB mass spectral database

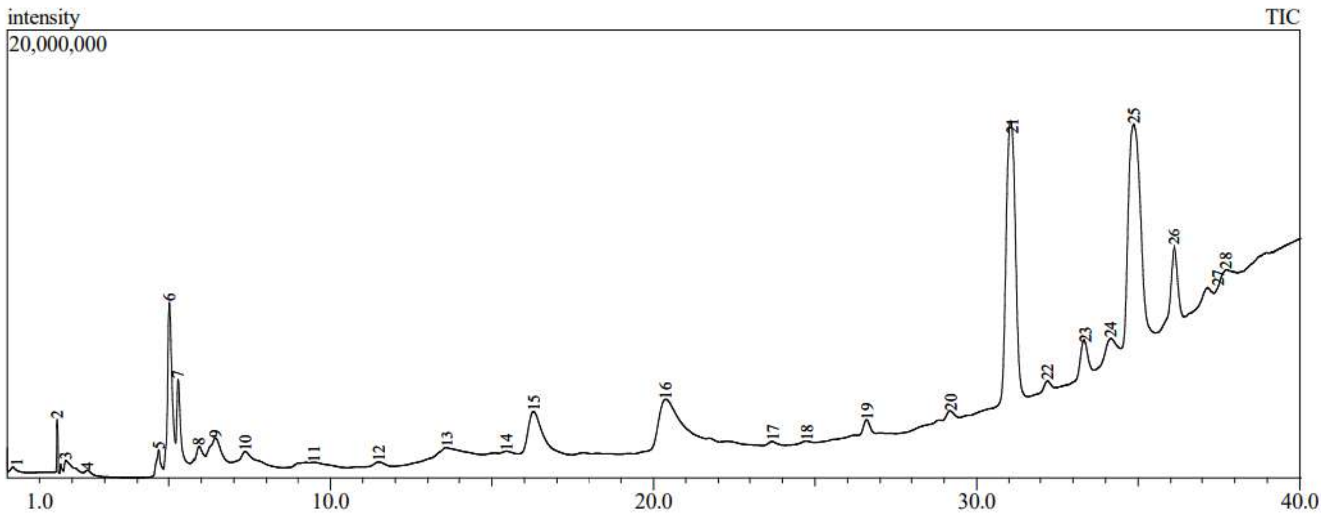


Figure 4. The GC-MS chromatogram of the secondary metabolites produced by *Trichoderma harzianum* isolate TGL

Similarly, methyl oleate, another abundant metabolite identified in *T. harzianum* isolate AJG, has been documented to interact directly with lipid membranes, increasing their permeability, which then leads to cellular disruption and nematode mortality (Asyiah et al. 2025). The substantial presence of methyl oleate (22.91%) in the metabolite profile suggests it likely contributes significantly to the high mortality rates observed in J2 nematodes treated with secondary metabolites from *T. harzianum* isolate AJG (Vieira et al. 2023). Additionally, the detection of cyclopentadecanone, 2-hydroxy-, and other cyclic ketones might further enhance the nematicidal potential due to their known antimicrobial and insecticidal activities, possibly acting synergistically with the fatty acid esters to enhance bioactivity (Helal et al. 2019; Mansour et al. 2023).

The metabolite profiling of *T. harzianum* isolate TGL also identified similar fatty acid methyl esters such as methyl palmitate and methyl oleate. However, at slightly lower abundances than for *T. harzianum* isolate AJG.

Nonetheless, *T. harzianum* isolate TGL uniquely exhibited a higher relative abundance of aromatic compounds, particularly 2-furancarboxaldehyde, 5-(hydroxymethyl)- (known as hydroxymethylfurfural), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. These aromatic metabolites have previously been shown to exhibit antimicrobial and antifungal properties by inducing oxidative stress and disrupting membranes (Saravanan et al. 2022; Sergany 2024). While these aromatic compounds might indirectly influence nematode viability by altering their microbial environment, their direct nematicidal efficacy is comparatively less documented, potentially explaining the slightly reduced performance of *T. harzianum* isolate TGL.

Furthermore, isolate-specific differences in metabolite profiles highlight the potential influence of environmental and genetic factors on metabolite synthesis. Variations in cultivation conditions and genetic background are known to significantly affect fungal secondary metabolite production, thereby influencing their biocontrol efficacy (Goyal et al. 2017; Adiwena et al. 2023). It is plausible that environmental adaptations of *T. harzianum* isolates AJG and TGL, derived from different geographical locations, contribute to their distinct metabolite compositions and resultant bioactivity. The categorization of secondary metabolites from *T. harzianum* isolates AJG and TGL into broader chemical groups provides essential insights into their potential roles in plant pest and pathogen management (Figure 5). Grouping metabolites into chemical classes facilitates understanding their potential bioactivities and possible mechanisms of action against plant pathogens, including nematodes.

The secondary metabolite profile of *T. harzianum* isolate AJG was dominated by fatty acids and their derivatives, accounting for 61.76% of the total metabolite composition. Aldehydes represented the second-largest group (13.00%), followed by ketones (12.30%), alcohols (5.97%), and hydrocarbons (5.57%). This composition highlights the prominence of lipid-derived metabolites, particularly fatty acid methyl esters, which are widely recognized for their antimicrobial, antifungal, and nematicidal properties.

Comparatively, the secondary metabolite profile of *T. harzianum* isolate TGL was also dominated by fatty acids and their derivatives (54.39%). However, this proportion was slightly lower than in *T. harzianum* isolate AJG. Additionally, *T. harzianum* isolate TGL exhibited notable differences in the relative abundance of other metabolite groups. For example, aldehydes constituted a significant portion (13.96%), whereas furans and related aromatic compounds were substantially more abundant in *T. harzianum* isolate TGL, reflecting distinct biochemical pathways active in this isolate. Alcohols and ketones were present in moderate amounts, further underscoring differences in secondary metabolite synthesis between these isolates.

Overall, these findings indicate substantial biochemical diversity between *T. harzianum* isolates AJG and TGL, which may underlie observed differences in their biological activities against *M. incognita*. The notable dominance of fatty acids and their derivatives in both isolates emphasizes their potential utility in biological control applications. However, isolate-specific variations in metabolite profiles suggest differences in their mechanisms of action and potency, supporting the need for targeted selection and optimization of isolates for effective pest management strategies.

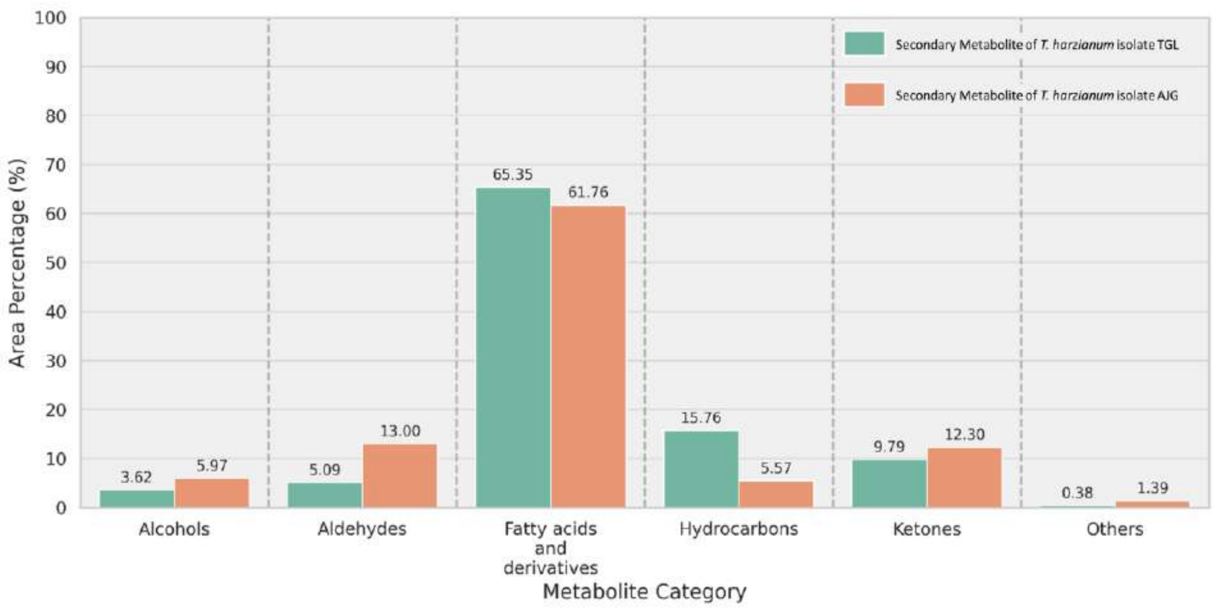


Figure 5. The classification of secondary metabolites from *Trichoderma harzianum* isolates AJG and TGL into major chemical groups

The substantial abundance of fatty acids and their derivatives in both *T. harzianum* isolates AJG and TGL suggests a strong nematicidal potential, given that fatty acid esters and related compounds have been extensively reported to disrupt nematode membranes and metabolic functions (Eloh et al. 2015). For example, hexadecanoic acid, methyl ester, and 9-octadecenoic acid, methyl ester, have previously been demonstrated to severely disrupt nematode membrane integrity, impair motility, and inhibit egg hatching (Lu et al. 2020; Fabiyi et al. 2022). Therefore, the high abundance of these fatty acid derivatives in both isolates likely contributes significantly to their overall nematicidal and ovicidal efficacy.

A notable difference between the isolates was the greater abundance of furans and aromatic aldehydes identified in *T. harzianum* isolate TGL than in *T. harzianum* isolate AJG. Aromatic compounds such as hydroxymethylfurfural and related furan derivatives found in *T. harzianum* isolate TGL have been reported to exhibit antimicrobial properties, primarily by inducing oxidative stress and disrupting membranes (Saravanan et al. 2022). While less extensively studied against nematodes, their presence in significant quantities could imply additional indirect mechanisms of pest suppression or modulation of microbial communities associated with nematodes.

Finally, the differences observed in ketone and aldehyde abundances between *T. harzianum* isolates AJG and TGL suggest that isolate-specific enzymatic and metabolic pathways influence secondary metabolite synthesis. Environmental adaptations, genetic factors, and cultivation conditions likely help shape these biochemical profiles, resulting in different levels of biological activity (Calvo et al. 2002; Keller 2019). Further studies involving more detailed metabolomic characterization and bioactivity assays could provide valuable insights into optimizing the use of these isolates for developing sustainable nematicidal formulations tailored to specific agricultural contexts.

In conclusion, this study has demonstrated the significant nematicidal activity of secondary metabolites derived from *T. harzianum* isolates against *M. incognita*, with *T. harzianum* isolates AJG and TGL exhibiting superior efficacy. Fatty acid esters, particularly methyl palmitate and methyl oleate, were identified as key bioactive compounds responsible for disrupting nematode membranes and inhibiting egg hatching. The isolate-specific differences in metabolite profiles emphasize the importance of detailed metabolomic characterization for selecting the most potent strains. These findings highlight the potential for developing effective, sustainable biological nematicides tailored to agricultural pest management.

ACKNOWLEDGEMENTS

The author sincerely thanks the University of Jember, Indonesia for financially supporting this research and publication through the MBKM-Based Research Grant (*Hibah Penelitian Berbasis Merdeka Belajar Kampus Merdeka*). This funding was awarded according to the Rector's Decree of the University of Jember No. 7554/UN25/KP/2024, dated March 20, 2024, and the Assignment Agreement No. 3202/UN25.3.1/LT/2024, dated March 21, 2024.

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