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Biocontrol and growth-promoting potential of endophytic *Bacillus subtilis* NPA6 against shallot pathogens under sub-optimal land conditions

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Abstract. *Prihatiningsih N, Lestari P, Ilhami ZM, Djatmiko HA, Irwandhi. 2025. Biocontrol and growth-promoting potential of endophytic* Bacillus subtilis *NPA6 against shallot pathogens under sub-optimal land conditions. Asian J Agric 9: 607-614.* Endophytic *Bacillus subtilis* NPA6, isolated from rice roots on suboptimal land, has potential as an eco-friendly biocontrol agent for shallot diseases. This study developed liquid biopesticide formulations using coconut water and rice washing water as local carriers, characterized their chemical composition, and tested their effects on shallot disease resistance and growth. A randomized block design with four treatments (control, coconut water formula with NPA6, rice washing water formula with NPA6, and Propineb fungicide) and six replications was used. The coconut water formula contained higher N (0.68%) and Zn (0.36 mg L^{-1}), while the rice washing water formula had higher P (0.74%), K (0.52%), and Cu (0.10 mg L^{-1}). Both formulations reduced disease intensity by 34-36%, comparable to chemical fungicides, although growth and yield parameters were not significantly improved. Correlation analysis revealed a negative relationship between disease intensity and plant height, indicating that disease suppression indirectly contributes to growth maintenance. The novelty of this study lies in utilizing locally available carriers to enhance the performance of *B. subtilis* NPA6, demonstrating its potential for sustainable shallot cultivation on suboptimal land. Further research should optimize carrier combinations to strengthen both biocontrol and growth-promoting functions.

Keywords: *Bacillus subtilis* NPA6, biopesticide, endophytic bacteria, sub-optimal land, shallot

INTRODUCTION

Shallot (*Allium cepa* L.) is one of the most important vegetable commodities cultivated intensively across tropical regions. Belonging to the spice group, it is widely used as a food flavoring and traditional medicine. Optimal shallot cultivation requires a dry climate with temperatures of 25-32°C and long sunlight exposure, but production is often constrained by high rainfall, humidity, and fog, which favor pathogen development (Nanda et al. 2022). In Southeast Asia, major diseases such as purple spot (*Alternaria porri*), moler (*Fusarium oxysporum*), anthracnose (*Colletotrichum gloeosporioides*), onion mosaic virus, shoot death (*Phytophthora porri*), and downy mildew (*Peronospora destructor*) remain serious threats. Their incidence has increased under climate change (Budiarti et al. 2022). Farmers largely rely on chemical pesticides, often mixing products in single applications, which can cause environmental risks and resistance in pathogens (Istiqomah et al. 2021). This underscores the urgent need for safer, sustainable alternatives such as biopesticides (Djaenuddin et al. 2021).

Biopesticides are derived from natural sources, including microbial agents, that control plant pathogens while minimizing ecological impacts (Razaq and Shah 2022). Among them, endophytic microbes are promising because they colonize plant tissues without causing harm and often enhance host fitness (del Carmen Orozco-Mosqueda and Santoyo 2021; Bilański and Kowalski 2022). Endophytes can suppress diseases and stimulate plant growth, offering a dual function of biocontrol and growth promotion (Chaudhary et al. 2022). *Bacillus subtilis* is one of the most widely studied endophytes due to its ability to inhibit pathogens through antibiosis and competition, while also inducing systemic resistance in host plants (Mahapatra et al. 2022; Ortiz et al. 2024).

The Induced Systemic Resistance (ISR) mechanism triggered by *B. subtilis* relies on the jasmonic acid and ethylene signaling pathways, enabling plants to activate defense responses without prior pathogen infection (Wilson et al. 2023). This mechanism also stimulates auxin production, particularly Indole Acetic Acid (IAA), enhancing plant vigor (Miljaković et al. 2020). In addition, *B. subtilis* produces enzymes such as glucanases, cellulases, and proteases, as well as phytohormones, siderophores, HCN, and phosphate-solubilizing compounds, all of which suppress pathogen activity and promote growth (Afzal et al. 2019; Irwandhi et al. 2024a).

Exploration of *B. subtilis* strains from challenging environments, such as suboptimal land, may yield stress-tolerant endophytes with strong biocontrol and growth-promoting functions (Sutariati et al. 2019).

Suboptimal land in Indonesia is widespread, covering up to 70% of agricultural areas, and is characterized by high acidity, poor drainage, and nutrient limitations (Sumiahadi and Acar 2019; Kamaluddin et al. 2025). Studies have shown that endophytic bacteria from such environments can produce IAA at high levels (57-79 ppm), enhance growth, and reduce disease intensity by more than 60% (Prihatiningsih et al. 2020a). Specifically, *B. subtilis* NPA6 has shown strong antagonism against *Rhizoctonia solani* (63.54% inhibition) and produces more than 300 secondary metabolites with antimicrobial properties (Prihatiningsih et al. 2022, 2024). NPA6 exhibits strong phosphate solubilization and IAA production, moderate HCN production, and weak siderophore activity, confirming its multifunctional role (Nur et al. 2023).

Biopesticide formulation can further enhance the performance of such strains by providing supportive carrier materials. Carriers like coconut water and rice washing water contain essential nutrients, amino acids, and phytohormones that improve bacterial viability and activity (Hernandez-Tenorio et al. 2022; Irmayanti et al. 2025). Coconut water supplies nitrogen and cytokinin to stimulate bacterial proliferation and plant growth, while rice washing water contains phosphate and potassium that enhance root colonization and stress tolerance (Setyowati et al. 2023). Using locally available carriers not only lowers costs but also promotes sustainable and farmer-friendly technologies.

This study was conducted to examine the chemical characteristics of *B. subtilis* NPA6-based liquid biopesticides formulated with coconut water and rice washing water, evaluate their effectiveness in reducing shallot disease intensity and promoting plant growth under suboptimal conditions, and analyze the correlation between disease intensity and growth traits. The findings are expected to provide a scientific basis for developing *B. subtilis* NPA6 as a sustainable biocontrol agent and growth promoter for shallot cultivation in marginal environments.

MATERIALS AND METHODS

Study area

The potential test of the liquid biopesticide formula *B. subtilis* NPA6 to control major diseases in shallots was conducted in Linggasari Village, Kembaran Sub-district, Banyumas District, Central Java, Indonesia, at 117 meters above sea level with an average temperature of 28.96°C and an average humidity of 59.8%. The sub-optimal land has soil pH characteristics of 6.2 (slightly acidic category) and K_2O content of 4.03% (very low category) according to the classification of the BPSI Tanah dan Pupuk (2023). The biopesticide was characterized at the Integrated Research Laboratory, Universitas Jenderal Soedirman, Central Java. The *B. subtilis* NPA6 isolate used in this study was obtained from Petanahan, Kebumen, Central Java. The location of this study is shown in Figure 1.

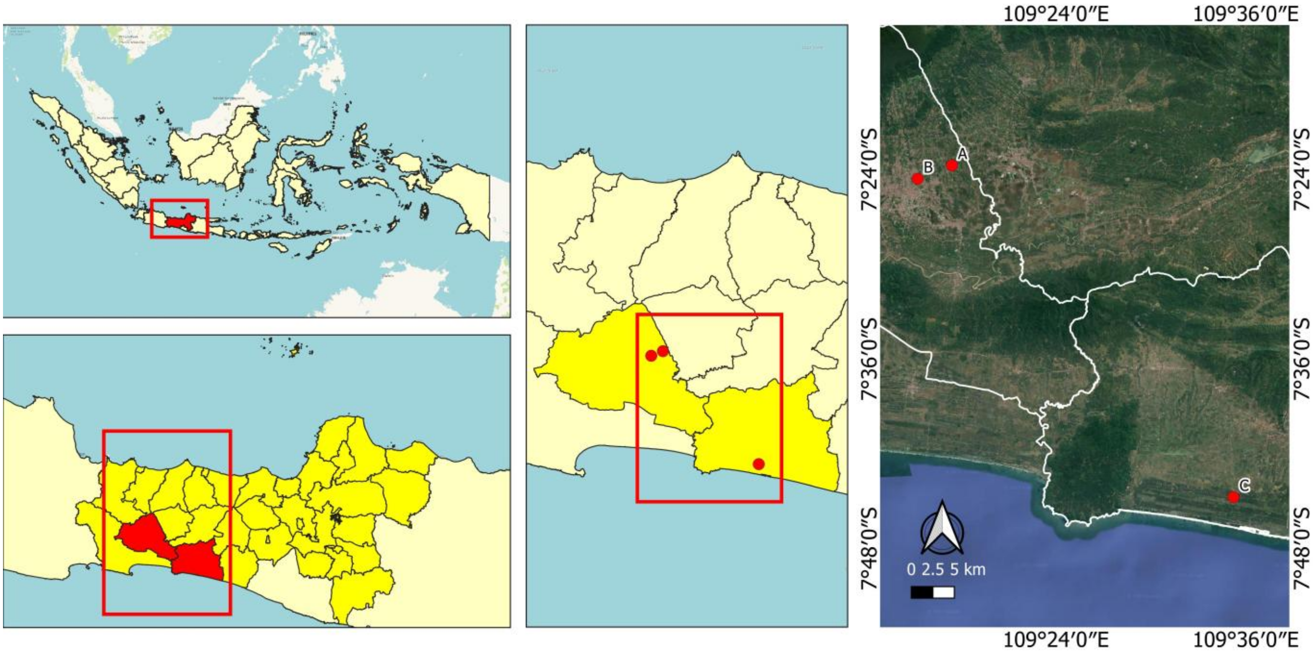


Figure 1. Biopesticide testing location in: A. Linggasari Village, Kembaran Sub-district, Banyumas District, Central Java, Indonesia; B. Biopesticide characterization; C. Isolate source in Petanahan, Kebumen, Central Java

Strain characterization and confirmation

Endophytic bacteria have been studied extensively for their morphological characteristics, including pigments, shape, edges, colony texture, and cell and endospore shape. To ensure accuracy, these characteristics are compared with reliable references such as Bergey's Manual of Bacteriology, 7th edition (Breed et al. 1957). Additionally, the biochemical properties of endophytic bacteria were evaluated through Gram staining, 3% KOH test, catalase test using 10% hydrogen peroxide, and chitinase test, as described by Lestari et al. (2017) and Prihatiningsih et al. (2020a). Endophytic bacteria NPA6 were further identified by partially sequencing the 16S, 27F, and 1492R DNA genes. Furthermore, to compare the complete nucleotide sequences of the NPA6 DNA isolate and the four other isolates, we referred to the nucleotide sequences of *Bacillus* sp. available in GenBank, this isolate identic to *B. subtilis* strain YT2HQ143571.1:4-1440 and has been analyzed using GCMS to produce secondary metabolites in the form of fatty acids and alcohols that function as antimicrobials, inhibit toxin production by pathogens, and trigger plant resistance responses to pathogens. The NPA6 isolate was prepared on Nutrient Agar (NA) medium (Merck, USA) and stored as a working culture for further testing. The isolate was tested for endophytic bacterial antagonistic activity on Potato Dextrose Agar (PDA) medium (Merck, USA) against rice fungal pathogens, specifically *R. solani*, using a double culture method with other isolates (Prihatiningsih et al. 2024).

Formulation of biopesticide

In this study, two liquid biopesticide formulations were formulated (Figure 2), each consisting of several materials as shown in Table 1. This liquid biopesticide formula comprises two primary materials: coconut water and rice washing water. Three liters of each primary material were boiled separately in different pans. After boiling, 15 g of granulated sugar and 6 g of shrimp paste were added to each pan and allowed to dissolve without stirring. After that, the boiled mixture was cooled and filtered to separate the coarse residue. Furthermore, 50 mL of *B. subtilis* NPA6 suspension was added to each filtered solution. The bacterial suspension was prepared by cultivating bacteria in Nutrient Broth (NB) medium for 24 hours, which was then homogenized using an orbital shaker (KBLee 3001) at a speed of 150 rpm at room temperature. The final formula was stored in a 250 mL bottle for chemical analysis.

Table 1. Composition of materials in each biopesticide formulation

Formula	Composition
Liquid coconut water formula	Coconut water (3 L), granulated sugar (15 g), shrimp paste (6 g), *B. subtilis* NPA6 suspension (50 mL)
Liquid rice washing water formula	Rice washing water (3 L), granulated sugar (15 g), shrimp paste (6 g), *B. subtilis* NPA6 suspension (50 mL)

Figure 2. The formula of *B. subtilis* NPA6 biopesticide is based on a liquid coconut water formula (left) and a liquid rice washing water formula (right)

Characterization of biopesticides

Chemical analysis of the liquid biopesticide formula containing *B. subtilis* NPA6 was performed immediately after formulation. The analysis included measurements of carbon (C), nitrogen (N), potassium (K), phosphorus (P), zinc (Zn), and copper (Cu). Nitrogen content was determined using the Kjeldahl method, and mineral content was determined using an atomic absorption spectrophotometer. Analysis was also performed on the suspension's pH, turbidity, odor, color, and the bacterial population (Irwandhi et al. 2025).

Research experiments

The study used a completely Randomized Block Design (RBD) with four treatments and six replications. The treatments given were P0 (control/no treatment), P1 (liquid rice washing water formula and *B. subtilis* NPA6), P2 (liquid rice washing water formula and *B. subtilis* NPA6), and P3 (Propineb-based fungicide). In this experiment, no artificial inoculation was carried out to introduce pathogens. This was done because the required pathogens were already present in the seeds or soil, considering that the land had previously been used for shallot cultivation. The population density of *B. subtilis* NPA6 in the formula was 1×10^{10} CFU mL^{-1}. Each biopesticide treatment was applied to shallots aged 11, 22, 33, and 44 days after planting (DAP). The variables observed included the incubation period, disease intensity, infection rate, control effectiveness, and plant growth variables such as number of leaves, plant height, number of bulbs, bulb biomass, and plant biomass. Samples were taken from 10 out of 54 plants per treatment. Sampling was done in a zig-zag manner in the center of the plot. The incubation period was observed daily until the first symptoms appeared, starting from the planting date, using DAP as the unit. Bacterial leaf blight disease was observed using the following formula:

∑(n x v)
Disease Intensity (DI) = -------------- x 100%
Z x N

Where:

n : The number of plants with symptoms based on the symptom's score
v : Bacterial leaf blight disease symptom's score
Z : The total number of plants observed
N : The highest symptom score

The scoring system for leaf blight symptoms is as follows, 0: indicates a healthy plant with no symptoms, 1: represents plants with 1-20% symptoms, 2: for plants with 21-40% symptoms, 3: for plants with 41-60% symptoms, 4: for plants with 61-80% symptoms, and 5: for plants showing more than 81% symptoms. The infection rate was analyzed with the Van der Plank formula:

$$r = \frac{2,3}{t_2 - t_1}\left(log\frac{1}{1-X_t} - log\frac{1}{1-X_o}\right)$$

The control effectiveness is calculated using the following formula:

$$E = \frac{DI_{control} - DI_{treatments}}{DI_{control}} \times 100\%$$

Data analysis

The treatment effect on each parameter was assessed using ANOVA with Statistical Package for the Social Sciences (SPSS) software. Significant differences were identified using the Least Significant Difference (LSD) test, with significance at P<0.05. In addition, correlation analysis was performed using the Pearson correlation test. The research data were visualized using Prism 9 software.

RESULTS AND DISCUSSION

Biopesticide traits

The results of the biopesticide character analysis, as shown in Table 2, show that the Liquid Coconut Water Formula (LCWF) and the Liquid Rice Washing Water Formula (LRWWF) have different characteristics. LCWF has a pH of 3 and is yellowish white, while LRWWF has a pH of 4 and is cloudy white. Both formulas have a fermented odor with a bacterial population of around 8.0-8.3×10^8 CFU mL^{-1}. The results of the chemical analysis of biopesticides in each formula are depicted in Figure 3, which shows that each formula has different nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), and copper (Cu) content, which can affect the performance of *B. subtilis* NPA6. LCWF tends to have higher N (0.68%) and Zn (0.36 mg L^{-1}) than LRWWF, while LRWWF has higher P (0.74%), K (0.52%), and Cu (0.10 mg L^{-1}) than LCWF.

High N content can play a role in amino acid synthesis and bacterial development (Satyantini et al. 2019). The presence of high P in LRWWF increases the secretion of root metabolites, such as mangiferin, L-tryptophan, O-phosphorylethanolamine, and methoxyindoleacetic acid, which can increase the population of biological control microbes in the rhizosphere (Cao et al. 2024). High K content can also increase plant and microbial tolerance to environmental stress by increasing enzyme production and maintaining osmotic balance (Ahanger et al. 2015). Additionally, micronutrient content, such as Zn and Cu, can increase the synthesis of IAA and stimulate lipopeptide production in *B. subtilis*. These lipopeptides can be similar to surfactin, iturin, and fengycin, which can cause lysis and cell death in pathogens (Sreedharan et al. 2023; Xu et al. 2023). The differences in nutrient composition between the two formulas indicate that each formula can have different effects on the performance of biopesticides. LCWF, which is high in N and Zn, has the potential to support rapid growth and biosynthetic activity in bacteria. At the same time, LRWWF, which is high in P, K, and Cu, can enhance root colonization and plant response to stress. A combination of both formulas or a balanced formulation of macro and micro nutrients has the potential to produce synergistic effects in enhancing the effectiveness of biopesticides and supporting overall plant growth.

Table 2. Characteristics of the biopesticide *B. subtilis* NPA6

Parameter	**Liquid coconut water formula**	**Liquid rice washing water formula**
pH	3	4
Odor	Fermentation smell	Fermentation smell
Color	Yellowish white	Cloudy white
Bacterial population	8.0 x 10^8 CFU mL^{-1}	8.3 x 10^8 CFU mL^{-1}

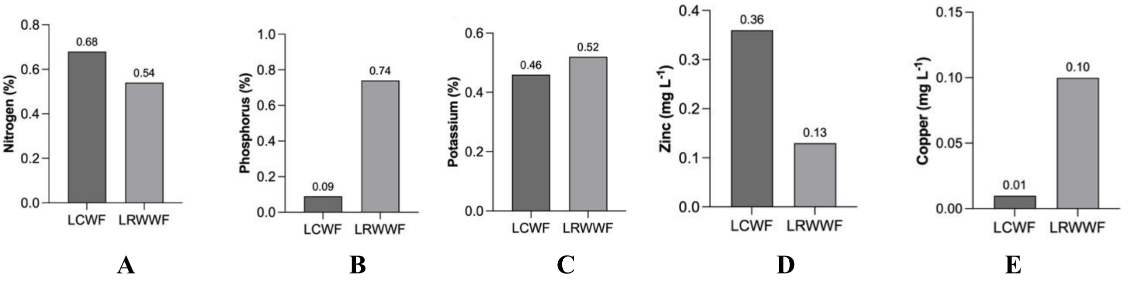


Figure 3. The nutrient content of Liquid Coconut Water Formula (LCWF) and liquid rice washing water formula (LRWWF) with *B. subtilis* NPA6. A. Nitrogen, B. Phosphorus, C. Potassium, D. Zinc, E. Copper

Table 3. Disease Intensity, infection rate, and effectiveness of twisted disease by liquid formula of *B. subtilis* NPA6 applications

Treatment	Disease intensity (%)	Infection rate (unit.day^{-1})	Effectivity (%)
P0	32.55a	0.019	-
P1	20.83b	0.012	36.01
P2	21.25b	0.012	34.72
P3	21.67b	0.012	33.43

Note: P0: Control, P1: Liquid Coconut Water Formula (LCWF) with *B. subtilis* NPA6, P2: Liquid Rice Washing Water Formula (LRWWF) with *B. subtilis* NPA6, P3: Propineb-based fungicide

Twisted disease biocontrol assessment

Twisted disease (*F. oxysporum* f.sp. *cepae*) in shallots shows typical symptoms with twisted or curled leaves due to disturbances in the roots or base, which are thought to rot widely and result in inhibition of leaf function (Supyani et al. 2021). The fungal attack begins with colonization in the plant's root area. The fungus enters the plant tissue, parasitizes, and inhibits the transport of water and photosynthesis products to all parts of the plant. The fungus also produces mycotoxins and famonirins that affect the flexibility of the plasma membrane in red onion leaves, ultimately causing the plant to bend (Prakoso et al. 2016). The results of the effectiveness test for the *B. subtilis* NPA6-based biopesticide formula are presented in Table 3. The table shows that the use of *B. subtilis* NPA6 in LCWF (P1) and LRWWF (P2) biopesticides can reduce the intensity of twisted disease compared to the control (P0), and this reduction is not significantly different from that of the Propineb-based fungicide treatment (P3).

In addition, both treatments have higher effectiveness than the P3 treatment (33.43%), with the effectiveness of P1 being 36.01% and P2 being 34.72%. These results align with previous studies, which have shown that bacteria such as *Bacillus amyloliquefaciens* can produce antimicrobial compounds that are utilized to control plant pathogens (Garay et al. 2023). Recent research has shown that *B. subtilis* NPA6, as a pathogen control, works through an antibiosis mechanism and produces secondary metabolites of up to 301 compounds. These compounds include Lyxitol, 1-thiooctyl-, estra-1,3,5(10)-triene-3,17-diol, 2-bromo-1-methyl-, and Methyl (E)-4-bromo-3-methoxy-2-butenoate (Prihatiningsih et al. 2024). Based on the review of Ramesh et al. (2024), *B. subtilis* also produces rich antifungal lipopeptides such as iturin, bacillomycin, and fengycin. These natural antifungal lipopeptides have been identified by investigating their antagonistic activity against pathogenic fungi in vitro. In general, these lipopeptides that bind to the plasma membrane result in the restructuring of the double lipid layer, thereby preventing various cellular processes. Natural antifungal lipopeptides have α-helix, β-hairpin, or sheet structures and β-hydroxy fatty acids capable of inhibiting several fungal species. Other research from Rumyantsev et al. (2023) stated that *B. subtilis* has a Plant Growth-Promoting Bacteria (PGPB) mediated plant defense mechanism associated with triggering ISR in plants. PGPB-mediated ISR occurs through microbe-associated molecular patterns, such as flagellins, lipopolysaccharides, siderophores, antibiotics, and biosurfactants, as well as Volatile Organic Compounds (VOCs), and is regulated by phytohormones, jasmonic acid and salicylic acid, ethylene, abscisic acid, as well as cytokinins and auxins.

The presence of *B. subtilis* NPA6 significantly influences the microbial composition and biological activity in the rhizosphere environment. This activity involves changes in root exudate patterns due to increased secondary metabolites such as L-tryptophan and methoxyindoleacetic acid, which can alter the microbial community around the roots (Cao et al. 2024). The increase in beneficial microbes, including phosphate-solubilizing bacteria, siderophore-producing bacteria, and HCN-producing bacteria, indicates a shift in the rhizosphere toward a more balanced and plant-health-supportive community. This is particularly important in sub-optimal land, where microbial balance is often disrupted and dominated by pathogens. These changes indicate the restoration of soil ecosystem function, where synergistic interactions between beneficial microbes and plants create a more protective and nutrient-rich root zone. Phosphate-solubilizing bacteria increase the availability of inorganic phosphorus previously bound to soil particles, siderophore-producing bacteria help chelate Fe^{3+} ions, thereby inhibiting access to essential nutrients for soil-borne pathogens (Das et al. 2025), while HCN producers act as agents of metal weathering and complexation in the substrate and enhance nutrient availability (Rijavec and Lapanje 2016).

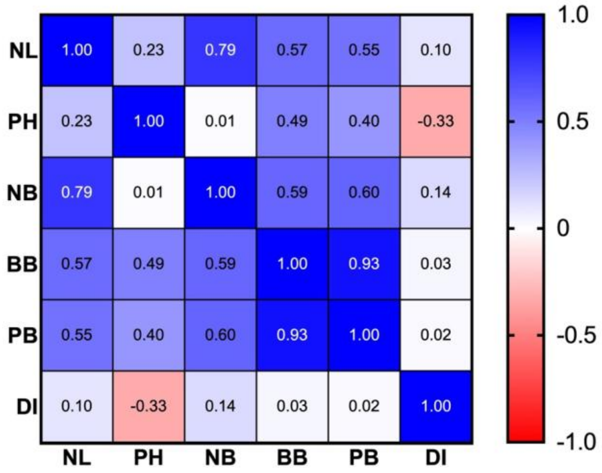
Growth and productivity of shallots

The analysis results of growth and productivity parameters, as in Figure 4, show that biopesticide treatment did not significantly affect all parameters (Figures 4.A-E). This indicates that although biopesticides can effectively control plant diseases, they have a limited impact on shallot growth and yield. This condition is indicated to occur due to the limited content of nutrients, both macro and micro, in the biopesticide formula. The NPK content in both biopesticide formulas remains below the quality standards for Indonesian liquid organic fertilizers, which require a minimum NPK content of around 2-6%. NPK content is a limiting factor in biopesticides' effectiveness in increasing shallots' growth and productivity. Carrier materials with high nutrient content can function as a source of nutrients for cultivated plants (Ma et al. 2022).

Nevertheless, analysis of the chemical properties of the soil showed positive changes, namely an increase in soil pH from 6.2 (slightly acidic) to 6.6 (neutral) with a percentage of 6.45%, an increase in N content from 1.74% (very high) to 1.96% (very high) with a percentage of 12.64%, and an increase in K_2O content from 4.03% (very low) to 4.41% (very low) with a percentage of 9.43%. This increase in soil pH indicates an improvement in the growing environment that is more conducive to nutrient availability (von Tucher et al. 2017), as well as increased activity of soil microorganisms involved in organic matter decomposition (Dohare et al. 2025). Meanwhile, the increase in K_2O content indicates an increase in potassium availability, an essential macronutrient for tuber formation, water balance regulation, and improved shallot quality (Triadiawarman et al. 2022). Although using *B. subtilis* NPA6-based biopesticides has not shown a significant direct effect on increasing shallot growth and yield, these findings can serve as a basis for developing biopesticides using an integrated approach. Biopesticides with carrier materials based on Local Wisdom Biofertilizer (LWB) can be developed. This latest research reveals that LWB contains organic C and NPK, reaching 14.80% and 4.13%, respectively (Irwandhi et al. 2025).

Pearson's correlation Analysis of growth traits in shallot

The results of the analysis of growth characteristics and disease intensity in shallots after the application of the biopesticide *B. subtilis* NPA6 are shown in Figure 5. The figure illustrates the strongest relationship Between Bulb Biomass (BB) and Plant Biomass (PB), characterized by a very high correlation coefficient (r=0.93). The r value shows that the increase in BB strongly correlates with the increase in PB. In addition, the Number of Bulbs (NB) is strongly correlated with the Number of Leaves (NL) (r=0.79). This suggests that good vegetative development in shallots will form numerous tubers (Rahmawati and Ladewa 2023). However, the growth characteristics of shallots have a low or very weak relationship with Disease Intensity (DI). DI has a negative correlation with PH (r=-0.33), indicating that the higher the DI, the greater the decrease in plant growth. This may be related to the ability of *B. subtilis* to suppress pathogens and stimulate systemic resistance (Prihatiningsih et al. 2020b). However, the correlation value is included in the correlation, showing moderate strength but not significant. This suggests that the plant's defense mechanism is likely systemic and complex, which does not directly impact the growth characteristics of shallots. In addition, DI also exhibits a weak and negligible correlation (r=0.02-0.15) with NL, NB, BB, and PB. This may indicate that the effectiveness of the *B. subtilis* NPA6 biopesticide in supporting shallot growth is not optimal. Future research will be conducted to optimize carrier materials that serve dual functions, namely as carrier materials and growth promoters for cultivated plants, in the context of climate change, to achieve net-zero farming practices (Irwandhi et al. 2024b).



	NL	PH	NB	BB	PB	DI
NL	1.00	0.23	0.79	0.57	0.55	0.10
PH	0.23	1.00	0.01	0.49	0.40	-0.33
NB	0.79	0.01	1.00	0.59	0.60	0.14
BB	0.57	0.49	0.59	1.00	0.93	0.03
PB	0.55	0.40	0.60	0.93	1.00	0.02
DI	0.10	-0.33	0.14	0.03	0.02	1.00

Figure 5. Correlation matrix between morphological traits of shallot and the effect of biopesticide application. NL: Number of leaves, PH: Plant Height, NB: Number of Bulbs, BB: Bulb Biomass, PB: Plant Biomass, DI: Disease Intensity. Color reflects the value of the correlation coefficient (r) on a scale from -1 to 1

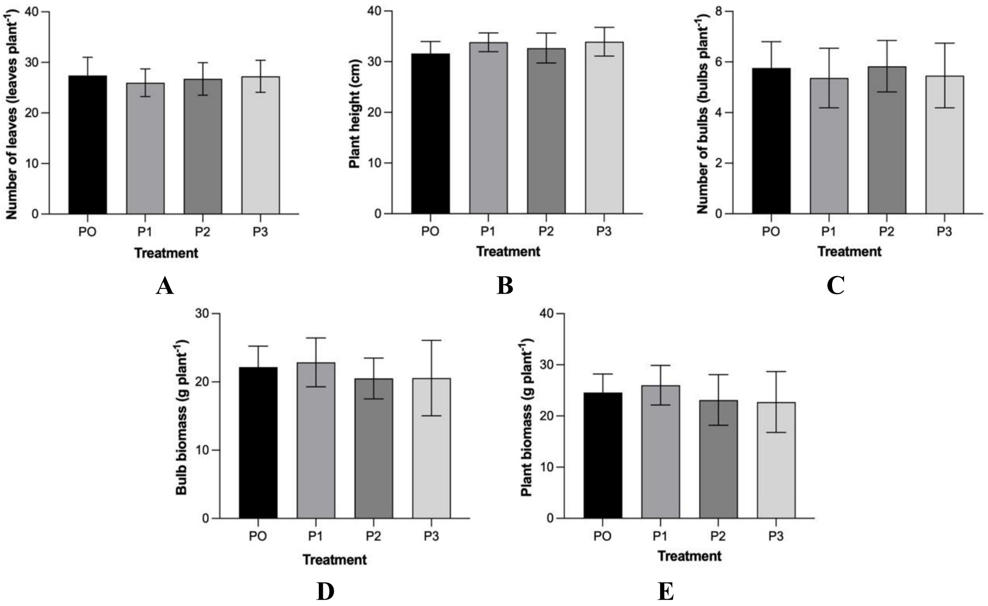


Figure 4. Morphological traits and productivity of shallot by biopesticide application. A. Number of leaves, B. Plant height, C. Number of bulbs, D. Bulb biomass, E. Plant biomass. P0: Control, P1: Liquid Coconut Water Formula (LCWF) with *B. subtilis* NPA6, P2: Liquid Rice Washing Water Formula (LRWWF) with *B. subtilis* NPA6, P3: Propineb-based fungicide

The environmental applicability of biopesticides

The use of biopesticides based on endophytic microbes such as *B. subtilis* NPA6 is considered highly applicable in plant disease management, particularly on sub-optimal land. Sub-optimal land generally has physical and chemical limitations, such as low fertility levels, extreme pH, abiotic stress, and high populations of soil-borne pathogens (Rahmasary et al. 2020). Under these conditions, the use of biological agents that can survive and remain active is essential. *Bacillus subtilis* is known for its good physiological tolerance to extreme environmental conditions. Its ability to form endospores allows the bacterium to survive for extended periods without losing viability, even under low pH, fluctuating humidity, or nutrient deficiency conditions (Agusta et al. 2023). Additionally, *B. subtilis* can adapt to sub-optimal land through mutualistic interactions with host plants and produce bioactive compounds such as antibiotics, lipopeptides, and phytohormones that support biocontrol functions and plant growth (Zhou et al. 2022; Agusta et al. 2023). Ecologically, the use of *B. subtilis*-based biopesticides is also considered safe for the environment because it leaves no chemical residues, does not cause pathogen resistance, and does not disturb the balance of soil microbes, as it is often biodegradable (Sawicka et al. 2025). This reinforces the feasibility and sustainability of using these biopesticides as part of an environmentally friendly agricultural system on sub-optimal land.

In conclusion, this study demonstrates that endophytic *B. subtilis* NPA6 formulated with coconut water and rice washing water has promising potential as a biopesticide for shallots under sub-optimal land conditions. Both formulations effectively reduced twisted disease intensity by approximately 34-36%, comparable to chemical fungicides, although they did not significantly enhance growth and yield parameters. Differences in nutrient composition between the two carriers influenced bacterial performance, with coconut water supporting higher N and Zn contents. At the same time, rice washing water provided higher P, K, and Cu levels. These formulations also improved soil chemical properties, including pH and K_2O content, indicating broader soil health benefits. The correlation analysis showed a negative relationship between disease intensity and plant height, suggesting indirect growth promotion through disease suppression. Future research should focus on optimizing carrier materials to simultaneously strengthen the biocontrol capacity and growth-promoting functions of *B. subtilis* NPA6 for sustainable shallot cultivation.

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