BIODIVERSITAS
Volume 26, Number 4, April 2025
Pages: 1816-1825

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260431

Isolation and characterization of endophytic fungi and their antagonistic activity against *Fusarium* spp., the causal agent of yellow disease in *Piper nigrum*

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Manuscript received: 25 November 2024. Revision accepted: 12 April 2025.

Abstract. *Sofian, Suyadi, Nurhasanah, Sopialena. 2025. Isolation and characterization of endophytic fungi and their antagonistic activity against* Fusarium *spp., the causal agent of yellow disease in* Piper nigrum*. Biodiversitas 26: 1816-1826.* Yellow disease in pepper (*Piper nigrum*), caused by *Fusarium* spp., significantly impacts crop productivity, manifesting as yellowing, wilting, defoliation, and root necrosis. Endophytic fungi offer a sustainable alternative to chemical pesticides for managing *Fusarium* spp. infections. The objective of this study was isolate and characterize endophytic fungi from *Piper nigrum*, *Piper crocatum*, and *Capsicum annuum*. Fungi were obtained from roots, stems, and leaves, cultured on PDA medium, and identified based on macroscopic and microscopic traits. The result revealed that a diverse community of endophytic fungi was associated with host-specific variations in fungal taxa. *P. nigrum* exhibited the highest fungal diversity, likely due to its rich phytochemical composition. A total of six fungal genera were identified: *Trichoderma* sp., *Gliocladium* sp., *Penicillium* sp., *Aspergillus* sp., *Geotrichum* sp., and *Cephalosporium* sp., each displaying unique morphological and functional attributes. *Penicillium* sp., *Gliocladium* sp., and *Trichoderma* sp. were found in several host plants, demonstrating their adaptability and potential for pathogen suppression. Notably, *Gliocladium* sp. and *Trichoderma* sp. showed remarkably rapid growth, giving them an advantage in competing for space and resources. Antagonistic assays further confirmed their ability to inhibit *Fusarium* sp.; *Gliocladium* sp. isolated from *P. nigrum* exhibited the highest effectiveness, consistently outperforming other isolates. This remarkable performance may be attributed to its dual antagonistic strategies—competition and antibiosis. *Gliocladium* sp. (PN) demonstrates a remarkable ability to establish sustained interactions with pathogens, ensuring prolonged suppression of *Fusarium* sp. over time. This capability highlights its potential utility in managing yellow disease in *P. nigrum*.

Keywords: Antagonistic activity, biological control, endophytic fungi, *Piper nigrum*, yellow disease

INTRODUCTION

Fungal pathogens present a significant challenge to global agriculture, severely affecting crop yields and threatening food security. Among them, *Fusarium* spp. stand out as particularly harmful, causing diseases such as vascular wilt, root rot, and leaf yellowing, which result in widespread and lasting damage to economically vital crops. Their ability to persist in soil and rapidly adapt to changing environmental conditions complicates management efforts (Ekwomadu and Mwanza 2023). The widespread reliance on chemical fungicides, though effective initially, has caused serious long-term issues like environmental pollution, decreased effectiveness, and the rise of fungicide-resistant strains (Matelionienė et al. 2024). These problems highlight the urgent need for sustainable and eco-friendly strategies to manage plant diseases, especially those caused by *Fusarium* spp. (Pothiraj et al. 2021; Haruna et al. 2024).

Fusarium spp., a soil-borne fungus, is a major problem in agriculture because it can severely reduce crop yields and affect many different types of plants. In black pepper (*Piper nigrum* L.), *Fusarium* spp. are responsible for yellow disease, a severe condition characterized by symptoms such as leaf yellowing, wilting, defoliation, and root necrosis, which can ultimately lead to plant mortality (Da Luz et al. 2017). The significant losses caused by *Fusarium*-induced diseases and the difficulties in controlling this pathogen highlight the pressing need for practical and sustainable solutions (Pusztahelyi et al. 2015; Savary et al. 2019). Endophytic fungi are increasingly recognized for their potential as natural biocontrol agents due to their close association with plants and their ability to suppress pathogens through various mechanisms (Fontana et al. 2021). These fungi reside asymptomatically within plant tissues, providing several advantages to their hosts, including enhanced growth, improved stress tolerance, and increased disease resistance (Segaran and Sathiavelu 2019; Watts et al. 2023). The antagonistic potential of endophytic fungi lies in their ability to compete for space and nutrients, produce antifungal secondary metabolites, and directly parasitize pathogens (Akram et al. 2023). Such characteristics position endophytic fungi as viable alternatives within Integrated Pest Management (IPM) strategies (Parveen and Rashtrapal 2024; Ul Haq et al. 2024), providing a sustainable approach (Baron and Rigobelo 2021) to controlling soil-borne pathogens like *Fusarium* spp. (Abdallah et al. 2018; Abro et al. 2019; Muhorakeye et al. 2024).

Endophytic fungi, with their remarkable adaptability, naturally adjust to the internal environment of the host plant (Ji et al. 2022). The presence of endophytic species is strongly influenced by the microhabitat conditions of the host plant as well as the genotypes of both the host plant and the endophytic fungi (Sopialena et al. 2018). These factors interact to shape the diversity, abundance, and functional roles of endophytes within plant tissues. Environmental factors, such as soil type, climate, and nutrient availability, along with the genetic traits of the host plant, create conditions that determine the compatibility and colonization potential of specific endophytic fungi (Guerreiro et al. 2022; Wippel 2023). Similarly, the genetic characteristics of the fungi influence their ability to adapt to the host, establish beneficial relationships, and contribute to the plant's health (Alam et al. 2021). Moreover, their co-evolution with plants (Saikkonen et al. 2004; Benucci et al. 2020) and associated pathogens have enabled them to develop sophisticated antagonistic mechanisms. These include the production of bioactive compounds, which enhance their effectiveness as plant defense agents (Ancheeva et al. 2020; Fadiji and Babalola 2020).

Previous studies have demonstrated the diversity and biocontrol potential of endophytic fungi isolated from *P. nigrum* (Sopialena et al. 2018), which are well-known for their rich phytochemical composition and ecological interactions. However, there is still a significant gap in our understanding of the diversity, growth dynamics, and antagonistic mechanisms of endophytic fungi from pepper.

The aim of this study was to isolate and characterize endophytic fungi from roots, stems, and leaves of various plant species, with an emphasis on evaluating their antagonistic potential against *Fusarium* spp. causing yellow disease in black pepper (*P. nigrum*). The ultimate objective was to identify the most effective candidates for use as biocontrol agents.

MATERIALS AND METHODS

Sample collection and isolation of *Fusarium* sp.

Fungal samples were collected from diseased pepper plants (*Piper nigrum*) plants exhibited symptoms of yellow disease plants exhibiting symptoms of yellow disease in community pepper plantations in Karang Tunggal and Bukit Pariaman Villages, Kutai Kartanegara Regency, East Kalimantan, Indonesia (Figure 1). Samples were collected from diseased pepper plants (*P. nigrum*) that exhibited yellow disease, such as leaf yellowing, branch shedding, complete plant defoliation, partial root necrosis, wilted root hairs, and bluish root discoloration.

Diseased plant samples were washed under running tap water and sectioned in diseased and healthy tissues. These sections were surface sterilized by sequential immersion in 2% sodium hypochlorite (NaOCl) for 1 minute, 70% ethanol for 1 minute, and rinsed twice with sterile distilled water. The sterilized samples were blotted dry with sterile tissue paper and plated onto Potato Dextrose Agar (PDA) medium in sterile Petri dishes. Plates were incubated at 25°C for 3-5 days.

Isolation of endophytic fungi

Healthy plants of the genus *Piper*, including *Piper crocatum* (red betel), *Capsicum annuum* (red chili) and *Piper nigrum* (black pepper) were selected for endophytic fungal isolation. Samples were collected from roots, leaves, and young stems of plants that displayed no visible signs of disease. Two replicates of each plant part were collected. A total of six samples were collected from plant species.

Plant parts were washed with 70% ethanol, followed by rinsing with sterile distilled water. Sections measuring 1 × 1 cm were excised and surface-sterilized by sequential immersion in 5% NaOCl for 1 minute, 70% ethanol for 1 minute, and rinsed twice in sterile distilled water. The sterilized tissues were blotted dry with sterile tissue paper and plated onto a PDA medium in sterilized Petri dishes. Plates were incubated at 25°C for 3-7 days to allow fungal growth.

Identification of fungal isolates

After incubation, fungal colonies were identified based on macroscopic and microscopic characteristics. Macroscopic identification included observations of colony morphology, such as color, texture, growth rate, and shape. For microscopic analysis, fungal structures were stained with methylene blue and examined under a light microscope to observe hyphal types, conidia morphology, and conidiophore features.

Purification of fungal isolates

Isolated fungal colonies were purified by subculturing onto fresh PDA medium. Hyphal tips or spores were aseptically transferred using a sterile inoculating loop. Purified cultures were incubated in sealed Petri dishes wrapped with parafilm and maintained at 25°C until sufficient growth was observed for further analysis.

Figure 1. Pepper plants infected with yellow disease

Fungal propagation

Purified fungal isolates were propagated on fresh PDA medium to obtain sufficient fungal biomass. This process was repeated to ensure consistency and uniformity of fungal cultures. The diameters of fungal colonies were measured to assess growth. Measurements were taken along the longest (d1) and shortest (d2) diameters of the fungal colony, and the mean colony diameter (D) was calculated using the formula:

$$D = \frac{(d1 \pm d2)}{2}$$

In vitro antagonistic test of endophytic fungal

The in-vitro antagonistic test was conducted using the direct opposition method on 9 cm diameter Petri dishes containing PDA medium. Purified inoculants of the endophytic fungi and *Fusarium* sp. were aseptically placed side by side on the PDA medium; each inoculum was positioned 3 cm from the edge of the Petri dish. Both fungi were inoculated simultaneously to ensure uniform growth conditions. Pathogen isolates were also cultured in separate Petri dishes under the same conditions as controls. The experiment was performed with 10 replicates, and the cultures were incubated at 25°C. Observations were carried out until 7 days post-inoculation. Observations focused on the growth of *Fusarium* sp. colonies and the presence of inhibition zones between the two opposing fungal colonies. Colony diameters were measured to assess fungal growth and calculate the percentage inhibition of *Fusarium* sp. growth by endophytic fungi.

The mechanisms of antagonism were evaluated with thoroughness and precision by observing interactions such as growth competition between the pathogenic and antagonistic fungi. Observations were meticulous, focusing on features like the overgrowth of antagonistic fungi on pathogen colonies, the formation of inhibition zones, and other morphological changes indicative of antifungal activity. The antagonistic interactions between endophytic fungi and *Fusarium* sp. were assessed by measuring colony growth and observing antagonistic mechanisms such as competition and inhibition zones. The percentage of growth inhibition (I) was calculated using the formula developed by Barnett and Hunter (1972) as following:

$$I = \frac{r1 - r2}{r1} \, x \, 100\%$$

Where:

I : Growth inhibition

r1 : Radius of pathogen colony with the growing path against the antagonist fungi colony

r2 : Radius of pathogen colony with the growing path close to the antagonist fungi colony

Data analysis

Quantitative data were analyzed using Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD). Means were compared using the Least Significant Difference (LSD) test at a 5% significance level. Qualitative data, including morphological observations and antagonistic interactions, were analyzed descriptively.

RESULTS AND DISCUSSION

Isolation of *Fusarium* sp. causal agent of yellow disease of pepper

Fusarium sp. was isolated from infected plant tissues and found to be associated with yellow disease symptoms, including yellowing, wilting, defoliation, and eventual root necrosis. The pathogen was identified as the causal agent of the disease based on its consistent morphological characteristics, observed both macroscopically and microscopically (Table 1). On PDA medium, *Fusarium* sp. colonies exhibited a white, cotton-like appearance with a concentric growth pattern spreading both laterally and vertically. After seven days of incubation, fungal colonies grew to the edge of the 9 cm Petri dish, with smooth colony margins and limited aerial mycelium (Figure 2.A). These macroscopic features highlight the aggressive growth nature of *Fusarium* sp., a trait often associated with pathogenicity.

Microscopic observations revealed that *Fusarium* sp. possessed hyaline, septate hyphae, with crescent shape (sickle-shaped) conidia with sharp, pointed ends. The conidia were hyaline (non-pigmented) and formed on non-branching conidiophores (Figure 2.B). These morphological features align with authoritative fungal identification references (Shahnazi et al. 2012; Lestari et al. 2021), including Barnett and Hunter's fungal identification guide. The absence of branching conidiophores and the presence of crescent-shaped macroconidia are diagnostic characteristics that confirm the pathogen as *Fusarium* sp. (Schroers et al. 2017; Zakaria et al. 2022).

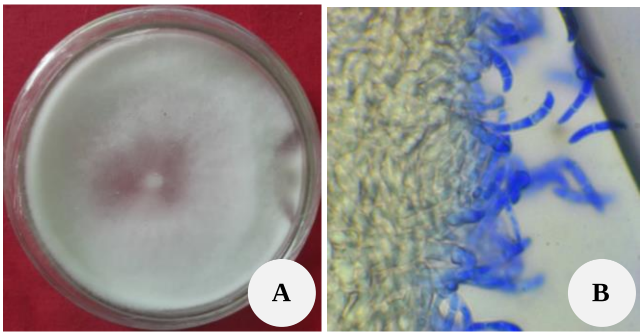

Figure 2. Pure culture of *Fusarium* sp.: A. 7 days old colony; B. Macro conidia

Table 1. Characteristic of *Fusarium* sp. on PDA medium

Characteristic	Macroscopic	Microscopic
Colony color	White	-
Spreading pattern	Spreading laterally and vertically (Concentric)	-
Mycelium shape	Cotton-like	-
Conidia shape	-	Crescent-shaped with sharp, pointed ends
Conidia color	-	Non-pigmented
Conidiophores branches	-	Non-branching
Hyphae shape	-	Septate
Hyphae color	-	Hyaline

The observed symptoms of yellow disease in pepper plants strongly correlate with the pathogenic mechanisms of *Fusarium oxysporum*, a well-documented soil-borne pathogen known to cause vascular wilting in a wide range of crops (Srinivas et al. 2019; Soleha et al. 2022). The yellowing of leaves, defoliation, and root necrosis observed in this study are hallmarks of vascular wilt disease (Ekwomadu and Mwanza 2023). The pathogen most likely enters the host plant through root wounds or natural openings, colonizing the vascular system and disrupting water and nutrient transport (Yadeta and Thomma 2013; Wang et al. 2015). The production of cell wall-degrading enzymes and mycotoxins by *Fusarium* sp. exacerbates tissue damage, ultimately leading to plant death (Perincherry et al. 2019; Ekwomadu et al. 2021). The aggressive growth observed in culture, with rapid lateral and vertical spread, underscores the pathogen's colonization efficiency and may explain the rapid progression of symptoms in infected plants (Aslam et al. 2019; Chakrapani et al. 2023).

Endophytic fungal diversity in genus *Piper*

The present study successfully identified a diverse community of endophytic fungi associated with three plant species in the genus *Piper*: *P. crocatum*, *C. annuum*, and *P. nigrum*. Each species exhibited distinct fungal taxa, indicating host-specific variations in fungal colonization (Figure 3). These findings are consistent with previous studies, showing that habitat preferences and host plant interactions influence the diversity of endophytic communities, exhibited by the distinct variations of fungi's macroscopic and microscopic characteristics.

In *P. crocatum*, two significant fungal isolates were identified: *Penicillium* sp. (Isolate 1) and *Aspergillus* sp. *Penicillium* sp. exhibited greenish colonies with yellowish pigmentation underneath, irregular margins, and hyaline, non-septate hyphae, producing green, spherical conidia. *Aspergillus* sp. formed solid black, powdery colonies characterized by septate hyphae and a brush-like arrangement of black, spherical conidia (Table 2). Three fungal isolates were observed in *C. annuum*, namely: *Gliocladium*, *Trichoderma*, and *Geotrichum* (Table 3). *Gliocladium* sp. developed white to greenish colonies with branched conidiophores and green, spherical conidia; *Trichoderma* sp. exhibited green to dark green circular colonies with smooth conidia and branching conidiophores; and *Geotrichum* sp. presented white to brownish-grey colonies with cylindrical conidia. In *P. nigrum*, four taxa were identified, including *Trichoderma* sp. with dark green colonies, *Cephalosporium* sp. displaying cream-colored colonies with single-celled cylindrical conidia, and *Penicillium* sp. (Isolate 2) showing greenish colonies with reddish undersides and velvety textures (Table 4). The morphological characteristics of these endophytic fungi align with established taxonomic descriptions, confirming their identity and significance (Saithong et al. 2010; Abdennabi et al. 2017; Sopialena et al. 2018; Hidayah et al. 2021; Syarifah et al. 2021; Fan and Shi 2024).

The observed diversity of endophytic fungi across the three host plants suggests that factors, such as plant tissue structure, chemical composition, and environmental conditions significantly influence fungal colonization patterns (Baron and Rigobelo 2021). Distinct fungal communities indicate that host plants exert selective pressures on their associated endophytes, shaping microbial composition to enhance resilience against environmental stressors and pathogen challenges (Segaran and Sathiavelu 2019; Watts et al. 2023; Manathunga et al. 2024). Endophytic fungi are recognized for their robust adaptability and secondary metabolite production, both of which play crucial roles in plant defense mechanisms (Akram et al. 2023). For instance, *Penicillium* sp. is well-documented for its production of bioactive compounds with antimicrobial properties (Rančić et al. 2006; Shaaban et al. 2016; Zerroug et al. 2018). Similarly, *Aspergillus* sp. is notable for its contributions to nutrient cycling, stress tolerance, and the regulation of plant endogenous hormones and secondary metabolites (Ismail et al. 2020; Leetanasaksakul et al. 2024). *Geotrichum* sp. also demonstrates antimicrobial activity (Manganyi et al. 2019; Wang et al. 2023) and plant growth-promoting effects (George et al. 2019), further underscoring its functional versatility. Among the endophytes studied, *Trichoderma* sp. and *Gliocladium* sp. stand out for their exceptional biocontrol potential against soil-borne pathogens like *Fusarium* spp. These fungi employ a range of mechanisms, including mycoparasitism, secretion of hydrolytic enzymes, and production of volatile organic compounds, to suppress pathogen growth (Bastakoti et al. 2017; Tyśkiewicz et al. 2022; Guzmán-Guzmán et al. 2023; Fardhani et al. 2024). Their ability to rapidly colonize plant tissues and exert long-lasting antagonistic effects makes them valuable components of integrated pest management strategies. Furthermore, the presence of *Cephalosporium* sp. reinforces the concept that endophytic fungi contribute to plant health through their antimicrobial, antioxidant, and cytotoxic activities (Farhat et al. 2023; Rollando et al. 2023).

The presence of *Penicillium* sp., *Gliocladium* sp., and *Trichoderma* sp. in multiple host plants (Tables 2-4) highlights their ecological flexibility and potential as effective agents for biological control. The occurrence of these fungi in multiple host plants demonstrates their ecological flexibility and capacity to form symbiotic associations across different environments, suggesting their suitability for use in Integrated Pest Management (IPM) systems (Corbu et al. 2023). Through mechanisms like competing for nutrients, producing hydrolytic enzymes, and secondary metabolites biosynthesis, these fungi can effectively reduce disease impacts and support plant growth and resilience.

The highest diversity of endophytic fungi was observed in *P. nigrum* (Table 4), which can be attributed to its rich and complex phytochemical composition (Sopialena et al. 2018). These bioactive compounds, including alkaloids, phenolics, and terpenoids, likely create a favorable microenvironment that supports microbial colonization and sustains diverse fungal communities (Rai et al. 2021; Su et al. 2023). The rich chemical profile of *Piper nigrum* serves both as a defense against pathogens and a catalyst for symbiotic interactions with endophytic fungi. This dynamic relationship emphasizes the role of secondary metabolites in shaping and structuring fungal communities.

Antagonistic potential

In this study, among the isolated endophytic fungi, *Gliocladium* sp. (CA) and *Trichoderma* sp. (PN) exhibited the fastest growth, nearly covering the entire Petri dish by the fourth and fifth day of incubation (Table 5), which are also observed in the previous studies (Matas-Baca et al. 2023; Yao et al. 2023; Sopialena et al. 2024; Valeria et al. 2024). In addition, *Gliocladium* sp. (CA) demonstrated faster growth, surpassing that of *Fusarium* sp., the causative agent of yellow disease in *P. nigrum,* beginning on the fifth day. This rapid growth not only enhances their ability to monopolize limited resources but also provides a competitive edge in spatial niches, a vital factor in suppressing slower-growing pathogens (Yao et al. 2023). In contrast, other fungal isolates exhibited slower growth, which may be attributed to intrinsic growth characteristics or differences in resource utilization efficiency.

Fungal colony growth is a critical parameter in assessing the antagonistic potential of endophytic fungi, as it reflects their ability to compete for space and resources, which are essential factors in biological control (Panchalingam et al. 2022; Muhorakeye et al. 2024). Rapidly growing fungi can dominate the substrate, outcompeting pathogens for nutrients and colonization sites, thereby suppressing their growth and proliferation (Mohiddin et al. 2021). The results of antagonism tests demonstrated the ability of endophytic fungi to inhibit the growth of *Fusarium* sp. through diverse mechanisms, including competition and antibiosis (Table 6).

The ecological diversity and functional specificity of these mechanisms reflect the adaptive capacity of endophytic fungi to combat diverse pathogen challenges in various environmental conditions (Akram et al. 2023). The competition involves the rapid colonization of resources and substrate space, which denies pathogens the essential conditions for growth (Panchalingam et al. 2022; Priyashantha et al. 2023; Yao et al. 2023). This physical and spatial exclusion is particularly effective when combined with rapid fungal growth. Antibiosis, on the other hand, involves the production of secondary metabolites such as antibiotics, hydrolytic enzymes, and volatile organic compounds, which directly inhibit pathogen growth and development (Tyśkiewicz et al. 2022; Fardhani et al. 2024; Leetanasaksakul et al. 2024). These dual mechanisms make endophytic fungi versatile biological control agents.

Among the tested fungi, *Gliocladium* sp. (PN) demonstrated the most effective inhibition of *Fusarium* sp. throughout the study, consistently outperforming other isolates in its antagonistic activity. The effectiveness of *Gliocladium* sp. may be due to its ability to use both competition and antibiosis against pathogens (Figure 4). *Gliocladium* sp. quickly took control of the substrate (Table 5) and likely produced secondary compounds that further inhibited the growth of *Fusarium* sp. The combined effect of these mechanisms underscores the importance of multifaceted antagonistic strategies in achieving effective biological control. These findings are consistent with previous studies that highlight the dual mechanisms of *Gliocladium* sp. as key contributors to its biocontrol efficacy against several plant pathogenic fungi including *Colletotrichum gloeosporioides* (78.75%), *Rhizoctonia solani* (75%), *Fusarium* sp. (56%), and *Phytophthora* sp. (25%) (Fardhani et al. 2024).

Table 2. Characteristics of endophytic fungi isolated from *Piper crocatum*

Morphological characteristics	*Penicillium* sp. (isolate 1)	*Aspergillus* sp.
Macroscopic		
Colony color	Greenish with yellowish reverse side	Black
Growth direction	Spreading irregularly in all directions	Spreading radially, forming clustered colonies
Colony texture	Moderately smooth	Coarse, resembling sand granules
Microscopic		
Conidia shape	Spherical	Spherical
Conidia color	Greenish	Dark brown to black
Conidiophore branching	Branched	Unbranched
Hyphae structure	Non-septate	Non-septate
Hyphae color	Hyaline	Hyaline

Table 3. Characteristics of endophytic fungi isolated from *Capsicum annuum*

Morphological characteristics	*Gliocladium* sp. (isolate 1)	*Trichoderma* sp. (isolate 1)	*Geotrichum* sp.
Macroscopic			
Colony color	White to greenish	White to dark green	White to brownish grey
Growth direction	Spreading uniformly in all directions	Spreading radially and vertically to form circular colonies	Spreading uniformly in all directions, covering the Petri dish
Colony texture	Smooth	Smooth	Smooth, even
Microscopic			
Conidia shape	Spherical	Spherical	Cylindrical
Conidia color	Dark green	Green	Blackish white
Conidiophore branching	Branched	Branched	Branched
Hyphae structure	Septate	Septate	Cylindrical
Hyphae color	Hyaline	Hyaline	Hyaline

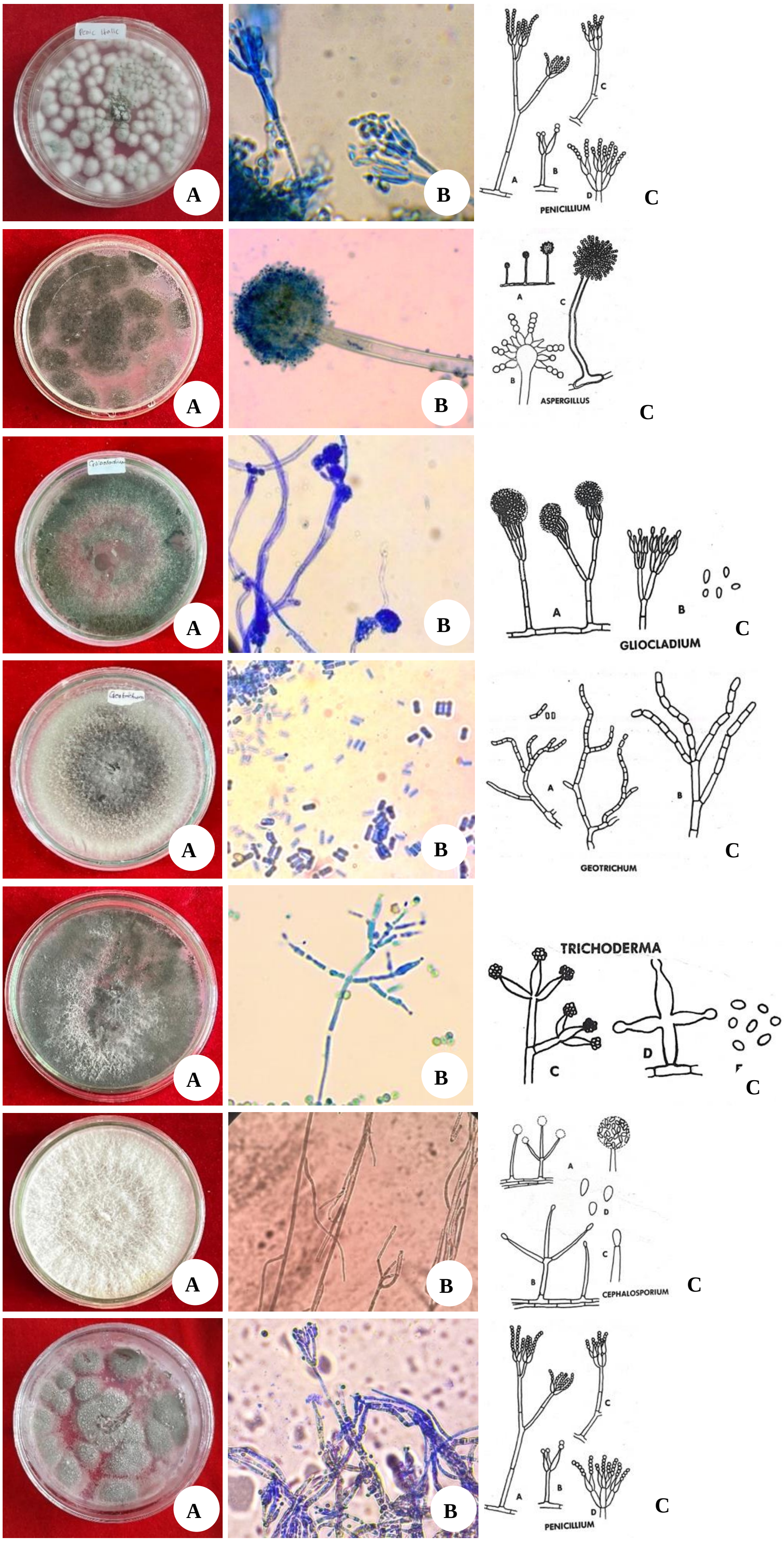

Figure 3. Endophytic fungal diversity in genus *Piper*: A. Colony; B. Microscopic observation; C. Illustrates of fungi (Barnett and Hunter 2006)

Table 4. Endophytic fungi in *Piper nigrum*

Morphological characteristics	*Trichoderma* sp. (Isolate 2)	*Cephalosporium* sp.	*Gliocladium* sp. (Isolate 2)	*Penicillium* sp. (Isolate 2)
Macroscopic				
Colony color	White to dark green	Cream	White to greenish	Greenish with pink reverse
Growth direction	Radially and vertically, forming circular colonies	Radially and vertically, covering the Petri dish	Spreading in all directions	Spreading in all directions
Colony texture	Smooth	Smooth	Smooth	Moderately smooth
Microscopic				
Conidia shape	Spherical	Cylindrical	Spherical	Spherical
Conidia color	Green	Transparent	Dark green	Green
Conidiophore branching	Branched	Branched	Branched	Branched with phialides
Hyphae structure	Septate	Septate	Septate	Septate
Hyphae color	Hyaline	Hyaline	Hyaline	Hyaline

Table 5. Fungal colony growth rate on PDA medium (cm)

Fungi	Day After Inoculation (DAI)						
	1	2	3	4	5	6	7
Penicillium sp. (PC)	2.40	4.40	5.60	7.60	7.90	8.20	8.50
Aspergilus niger (PC)	2.40	3.50	5.50	6.60	7.60	8.20	8.33
Geotrichum sp. (CA)	1.96	3.45	4.50	5.64	7.85	8.30	8.50
Trichoderma sp. (CA)	4.40	4.60	6.50	7.20	7.80	8.40	9.00
Gliocladium sp. (CA)	4.60	6.50	7.30	8.50	9.00	9.00	9.00
Cephalosporium sp. (PN)	1.04	3.45	4.80	5.65	6.86	7.50	8.50
Gliocladium sp. (PN)	4.40	5.80	6.50	7.40	8.00	8.60	9.00
Trichoderma sp. (PN)	5.00	5.70	6.70	7.30	7.80	8.80	9.00
Penicillium sp. (PN)	2.30	4.70	5.60	6.60	7.50	8.40	8.64
Fusarium sp. (PY)	5.60	6.65	8.45	8.65	8.75	8.80	9.00

Note: PC: Isolated from *P. crocatum*; CA: Isolated from *C. annum*; PN: Isolated from *P. nigrum*; PY: Pathogen of Yellow disease in *P. nigrum*

Table 6. Antagonistic mechanism of endophytic fungi

Endophytic fungi	Antagonistic mechanisms		
	Competition	Parasitism	Antibiosis
Penicillium sp. (PC)	+	-	-
Aspergilus niger (PC)	+	-	-
Geotrichum sp. (CA)	+	-	-
Trichoderma sp. (CA)	+	-	-
Gliocladium sp. (CA)	+	-	-
Cephalosporium sp. (PN)	+	-	-
Gliocladium sp. (PN)	+	-	+
Trichoderma sp. (PN)	+	-	-
Penicillium sp. (PN)	+	-	-

Notes: PC: Isolated from *P. crocatum*; CA: Isolated from *C. annum*; PN: Isolated from *P. nigrum*; PY: Pathogen of yellow disease in *P. nigrum.* Antagonistic mechanism occur (+); An antagonistic mechanism did not occur (-)

The inhibition percentage data further confirmed the efficacy of specific endophytic fungi in suppressing *Fusarium* sp. (Table 7). On the first day of observation, *Gliocladium* sp. (CA) (P3) and *Trichoderma* sp. (CA) (P3) exhibited significantly higher inhibition rates compared to other treatments. Early suppression is critical in preventing pathogen establishment and subsequent disease development (Yao et al. 2023). However, by the fourth to seventh days, *Gliocladium* sp. (PN) (P6) emerged as the most effective antagonist, maintaining its inhibitory activity up to 48%. The progressive increase in inhibition percentages observed for *Gliocladium* sp. (PN) suggests a sustained interaction with the pathogen, reflecting its ability to establish long-term dominance over *Fusarium* sp.

The consistent and robust antagonistic performance of *Gliocladium* sp. (PN) highlights its potential as a reliable biocontrol agent, capable of suppressing soil-borne pathogens such as *Fusarium* sp., the causal agent of yellow disease in black pepper. This fungus combines multiple antagonistic mechanisms, including competition for space and nutrients and antibiosis through the production of antifungal secondary metabolites (Aishwardani et al. 2024; Mindrati et al. 2024). These dual mechanisms enhance its adaptability to diverse environmental conditions and pathogen challenges, making it a versatile and effective option for Integrated Pest Management (IPM). Such biological control strategies offer a sustainable alternative to conventional chemical pesticides, reducing their environmental impact and the risk of developing pesticide-resistant pathogen strains (Fadiji and Babalola 2020; Baron and Rigobelo 2021; Fontana et al. 2021; Manathunga et al. 2024). The findings of this study highlight the ecological and functional diversity of endophytic fungi and their significant potential for biological control. The consistent inhibitory activity of *Gliocladium* sp. (PN) and its ability to sustain pathogen *Fusarium* sp. suppression over time suggests its utility in overcoming yellow disease in *P. nigrum.*

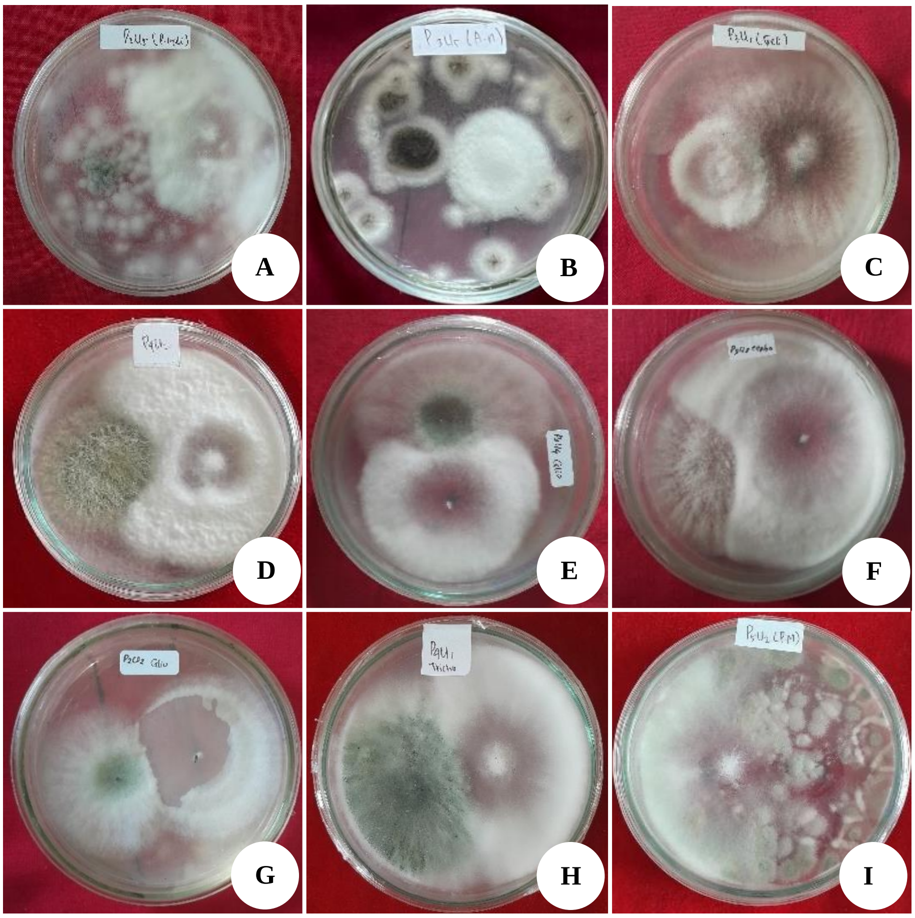

Figure 4. Antagonistic mechanism of endophytic fungi. Endophytic fungi that experience competition mechanisms: A. *Penicillium* sp. (PC); B. *Aspergillus* sp. (PC); C. *Geotrichum* sp. (CA); D. *Trichoderma* sp. (CA); E. *Gliocladium* sp. (CA); F. *Cephalosporium* sp. (PN); H. *Trichoderma* sp. (PN); I. *Penicillium* sp. (PN). Competition and antibiosis mechanisms: G. *Gliocladium* sp. (PN). Note: PC: Isolated from *P. crocatum*; CA: Isolated from *C. annum*; PN: Isolated from *P. nigrum*; PY: Pathogen of Yellow disease in *P. nigrum*

Table 7. Percentage of inhibitory activity of endophytic fungi against *Fusarium* sp.

Fungi	**Day After Inoculation (DAI)**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
P1. *A. niger* (PC)	16.92 b	15.62 a	12.39 a	13.62 a	13.63 a	16.35 a	17.11 a
P2. *Penicillium* sp. (PC)	15.16 ab	18.43 ab	14.67 a	18.99 a	26.23 b	18.08 a	25.53 ab
P3. *Gliocladium* sp. (CA)	30.11 d	25.11 a	32.13 b	32.69 c	35.96 b	36.76 b	36.55 b
P4. *Trichoderma* sp. (CA)	38.60 d	31.44 b	35.10 c	34.33 d	36.44 b	38.45 b	38.46 b
P5. *Geotrichum* sp. (CA)	28.56 cd	23.39 c	30.94 b	32.03 c	32.43 b	33.09 b	33.01 b
P6. *Gliocladium* sp. (PN)	7.55 a	24.56 c	31.22 b	35.76 d	37.68 c	38.70 b	48.07 c
P7. *Cephalosporium* sp. (PN)	10.18 ab	18.59 c	32.59 b	35.47 d	36.35 b	35.94 b	37.63 b
P8. *Trichoderma* sp. (PN)	23.27 c	22.01 ab	29.19 b	27.77 b	28.25 b	34.36 b	37.71 b
P9. *Penicillium* sp. (PN)	19.43 c	26.48 c	22.27 a	21.21 a	23.73 ab	29.31ab	32.45 b
ANOVA	*	*	*	*	*	*	*
LSD	8.23	6.92	7.83	7.75	8.66	10.05	9.11

Note: PC: Isolated from *P. crocatum*; CA: Isolated from *C. annum*; PN: Isolated from *P. nigrum*. *Significant at 95%; LSD: Least Significant Different value

In conclusion, this study highlights the diversity and functional potential of endophytic fungi isolated from *P. crocatum*, *C. annuum*, and *P. nigrum*. The fungal isolates, including *Trichoderma* sp., *Gliocladium* sp., *Penicillium* sp., *Aspergillus* sp., *Geotrichum* sp., and *Cephalosporium* sp., exhibited distinct macroscopic and microscopic characteristics and demonstrated varying degrees of antagonistic activity against *Fusarium* sp. Among these, *Gliocladium* sp. isolated from *Piper nigrum* emerged as the most effective biocontrol agent, maintaining consistent and robust inhibitory activity through mechanisms of competition and antibiosis. *Gliocladium* sp. (PN) has shown strong and consistent pathogen suppression, making it a promising option for controlling yellow disease in

Piper nigrum. This research highlights the diverse range of endophytic fungi and their significant contribution to sustainable farming, inspiring a new wave of research and application. By providing a natural and eco-friendly alternative to chemical pesticides, these fungi promote agricultural practices that are more environmentally sustainable. As valuable components of integrated pest management (IPM) systems, they contribute to long-term crop protection and the reduction of chemical inputs in agricultural systems.

ACKNOWLEDGEMENTS

The authors are thankful to The Ministry of Education, Culture, Research and Technology which has funded this research through the Doctoral Dissertation Research Grant (Hibah Penelitian Disertasi Doktor), with main contract number 135/E5/PG.02.00.PL/2023 and derivative contract 651/UN17.L1/HK/2023.

REFERENCES

Abdallah MF, De Boevre M, Landschoot S, De Saeger S, Haesaert G, Audenaert K. 2018. Fungal endophytes control *Fusarium graminearum* and reduce trichothecenes and zearalenone in maize. Toxins 10 (12): 493. DOI: 10.3390/toxins10120493.

Abdennabi R, Triki MA, Salah RB, Gharsallah N. 2017. Antifungal activity of endophytic fungi isolated from date palm sap (*Phoenix dactylifera* L.). EC Microbiol 13 (4): 123-131.

Abro MA, Sun X, Li X, Jatoi GH, Guo L-D. 2019. Biocontrol potential of fungal endophytes against *Fusarium oxysporum* f. sp. *cucumerinum* causing wilt in cucumber. Plant Pathol J 35 (6): 598-608. DOI: 10.5423/PPJ.OA.05.2019.0129.

Aishwardani N, Irawan B, Agustrina R, Yulianty. 2024. Effect of *Gliocladium* sp. and mung bean sprouts extract (*Vigna radiata* (L.) R. Wilczek) induction on Fusarium wilt in tomato plants (*Solanum lycopersicum* L.). Res Invent: Intl J Eng Sci 14 (7): 45-50.

Akram S, Ahmed A, He P, He P, Liu Y, Wu Y, Munir S, He Y. 2023. Uniting the role of endophytic fungi against plant pathogens and their interaction. J Fungi 9 (1): 72. DOI: 10.3390/jof9010072.

Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S, Yuán Y, Gǒng W. 2021. Endophytic fungi: From symbiosis to secondary metabolite communications or vice versa?. Front Plant Sci 12: 791033. DOI: 10.3389/fpls.2021.791033.

Ancheeva E, Daletos G, Proksch P. 2020. Bioactive secondary metabolites from endophytic fungi. Curr Med Chem 27 (11): 1836-1854. DOI: 10.2174/0929867326666190916144709.

Aslam S, Ghazanfar MU, Munir N, Hamid MI. 2019. Managing Fusarium wilt of pea by utilizing different application methods of fungicides. Pak J Phytopathol 31: 81-88. DOI: 10.33866/phytopathol.031.01.0482.

Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Co., Minneapolis. DOI: 10.2307/3757954.

Baron NC, Rigobelo EC. 2021. Endophytic fungi: A tool for plant growth promotion and sustainable agriculture. Mycology 13 (1): 39-55. DOI: 10.1080/21501203.2021.1945699.

Bastakoti S, Belbase S, Manandhar S, Arjyal C. 2017. *Trichoderma* species as biocontrol agents against soil-borne fungal pathogens. Nepal J Biotechnol 5 (1): 39-45. DOI: 10.3126/njb.v5i1.18492.

Benucci GMC, Burnard D, Shepherd LD, Bonito G, Munkacsi AB. 2020. Evidence for co-evolutionary history of early diverging Lycopodiaceae plants with fungi. Front Microbiol 10: 2944. DOI: 10.3389/fmicb.2019.02944.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. Mycologia 64 (4): 930-932. DOI: 10.2307/3757954.

Chakrapani K, Chanu WT, Sinha B, Thangjam B, Hasan W, Devi KS, Chakma T, Phurailatpam S, Mishra LK, Singh GM, Khoyumthem P, Saini R. 2023. Deciphering growth abilities of *Fusarium oxysporum* f. sp. *pisi* under variable temperature, pH, and nitrogen. Front Microbiol 14: 1228442. DOI: 10.3389/fmicb.2023.1228442.

Corbu VM, Gheorghe-Barbu I, Dumbravă AȘ, Vrâncianu CO, Șesan TE. 2023. Current insights in fungal importance-A comprehensive review. Microorganisms 11: 1384. DOI: 10.3390/microorganisms11061384.

Da Luz SFM, Yamaguchi LF, Kato MJ, De Lemos OF, Xavier LP, Maia JGS, de RRamos A, Setzer WN, Da Silva JKdR. 2017. Secondary metabolic profiles of two cultivars of *Piper nigrum* resulting from infection by *Fusarium solani* f. sp. *piperis*. Intl J Mol Sci 18 (12): 2434. DOI: 10.3390/ijms18122434.

Ekwomadu TI, Akinola SA, Mwanza M. 2021. *Fusarium mycotoxins*, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. Intl J Environ Res Public Health 18 (22): 11741. DOI: 10.3390/ijerph182211741.

Ekwomadu TI, Mwanza M. 2023. Fusarium fungi pathogens, identification, adverse effects, disease management, and global food security: A review of the latest research. Agriculture 13 (9): 1810. DOI: 10.3390/agriculture13091810.

Fadiji AE, Babalola OO. 2020. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. Front Bioeng Biotechnol 8: 467. DOI: 10.3389/fbioe.2020.00467.

Fan Y, Shi B. 2024. Endophytic fungi from the four staple crops and their secondary metabolites. Intl J Mol Sci 25 (11): 6057. DOI: 10.3390/ijms25116057.

Fardhani DM, Fuadiyah DA, Susila WA, Nugraheni IA. 2024. Inhibition of *Gliocladium* sp. against plant pathogenic fungus and their exoenzyme activity. Intl J Health Sci Technol 6 (1): 45-54. DOI: 10.31101/ijhst.v6i1.3312.

Farhat H, Urooj F, Sohail N, Ullah S, Aamer M. 2023. Evaluation of antimicrobial potential of endophytic fungi and GCMS metabolic profiling of *Cephalosporium sp.* and *Fusarium moniliforme*. J Exp Pathol 4 (1): 16-23. DOI: 10.33696/pathology.4.043.

Fontana DC, de Paula S, Torres AG, de Souza VHM, Pascholati SF, Schmidt D, Neto DD. 2021. Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens 10 (5): 570. DOI: 10.3390/pathogens10050570.

George TK, SubaidaBeevi S, Asok AK, Shaikmoideen JM. 2019. Plant growth promoting endophytic yeast *Geotrichum candidum* (JX 477426) from roots of *Bruguiera cylindrica*. J Microbiol Biotechnol Food Sci 9 (2): 267-272. DOI: 10.15414/jmbfs.2019.9.2.267-272.

Guerreiro MA, Kleetz J, Torres MR, Polle A, Peršoh D, Begerow D. 2022. Interaction between growth environment and host progeny shape fungal endophytic assemblages in transplanted *Fagus sylvatica*. Fungal Ecol 60: 101175. DOI: 10.1016/j.funeco.2022.101175.

Guzmán-Guzmán P, Kumar A, de los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda MDC, Fadiji AE, Hyder S, Babalola OO, Santoyo G. 2023. *Trichoderma* species: Our best fungal allies in the biocontrol of plant diseases-A review. Plants 12: 432. DOI: 10.3390/plants12030432.

Haruna SG, Yahuza L, Tijjani I. 2024. Management of Fusarium wilt of tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and related soil-borne diseases using eco-friendly methods: A review. Asian J Res Crop Sci 9 (1): 154-168. DOI: 10.9734/ajrcs/2024/v9i1257.

Hidayah BN, Herawati N, Aisah AR, Utami NR. 2021. Diversity of fungi associated with rhizosphere of healthy and diseased garlic crops. Biodiversitas 22 (3): 1433-1440. DOI: 10.13057/biodiv/d220346.

Ismail, Hamayun M, Hussain A, Iqbal A, Khan SA, Lee I-J. 2020. *Aspergillus niger* boosted heat stress tolerance in sunflower and soybean via regulating their metabolic and antioxidant system. J Plant Interact 15 (10): 223-232. DOI: 10.1080/17429145.2020.1771444.

Ji X, Xia Y, Zhang H, Cui J-L. 2022. The microscopic mechanism between endophytic fungi and host plants: From recognition to building stable mutually beneficial relationships. Microbiol Res 261: 127056. DOI: 10.1016/j.micres.2022.127056.

Leetanasaksakul K, Roytrakul S, Kittisenachai S, Lohmaneeratana K, Jantasuriyarat C, Lueangjaroenkit P. 2024. Exploring the impact of endophytic fungus *Aspergillus cejpii* DMKU-R3G3 on rice: Plant growth promotion and molecular insights through proteomic analysis. Agronomy 14 (3): 498. DOI: 10.3390/agronomy14030498.

Lestari SA, Henri H, Sari E, Wahyuni T. 2021. Microscopic characterization of *Fusarium* sp. associated with yellow disease of pepper (*Piper nigrum* L.) in South Bangka Regency. Planta Tropika J Agrosains 9 (1): 1-9. DOI: 10.18196/pt.v9i1.7753.

Manathunga KK, Gunasekara NW, Meegahakumbura MK, Ratnaweera PB, Faraj TK, Wanasinghe DN. 2024. Exploring endophytic fungi as natural antagonists against fungal pathogens of food crops. J Fungi 10 (9): 606. DOI: 10.3390/jof10090606.

Manganyi MC, Tchatchouang C-DK, Regnier T, Bezuidenhout CC, Ateba CN. 2019. Bioactive compound produced by endophytic fungi isolated from *Pelargonium sidoides* against selected bacteria of clinical importance. Mycobiology 47 (3): 335-339. DOI: 10.1080/12298093.2019.1631121.

Matas-Baca MA, Flores-Córdova MA, Pérez-Álvarez S, Rodríguez-Roque MJ, Salas-Salazar NA, Soto-Caballero MC, Sánchez-Chávez E. 2023. *Trichoderma* fungi as an agricultural biological control in Mexico. Rev Chapingo Ser Hortic 29: 79-114. DOI: 10.5154/r.rchsh.2022.11.015.

Matelionienė N, Žvirdauskienė R, Kadžienė G, Zavtrikovienė E, Supronienė S. 2024. In vitro sensitivity test of *Fusarium* species from weeds and non-gramineous plants to triazole fungicides. Pathogens 13 (2): 160. DOI: 10.3390/pathogens13020160.

Mindrati D, Fuadiyah DA, Susila WA, Nugraheni IA. 2024. Inhibition of *Gliocladium* sp. against plant pathogenic fungus and their exoenzyme activity. Intl J Health Sci Technol 6 (1): 45-54. DOI: 10.31101/ijhst.v6i1.3312.

Mohiddin FA, Padder SA, Bhat AH, Sofi NR, Waza SA, Hamid B, Parveen S, Hussain A, Bhat AN, Ali OM, Dar MS, Latef AAHA. 2021. Phylogeny and optimization of *Trichoderma harzianum* for chitinase production: Evaluation of their antifungal behavior against the prominent soil-borne phytopathogens of temperate India. Microorganisms 9 (9): 1962. DOI: 10.3390/microorganisms9091962.

Muhorakeye MC, Namikoye ES, Khamis FM, Wanjohi W, Akutse KS. 2024. Biostimulant and antagonistic potential of endophytic fungi against Fusarium wilt pathogen of tomato *Fusarium oxysporum* f. sp. *lycopersici*. Sci Rep 14: 15365. DOI: 10.1038/s41598-024-66101-1.

Panchalingam H, Powell D, Adra C, Foster K, Tomlin R, Quigley BL, Nyari S, Hayes RA, Shapcott A, Kurtböke Dİ. 2022. Assessing the various antagonistic mechanisms of *Trichoderma* strains against the brown root rot pathogen *Pyrrhoderma noxium* infecting heritage fig trees. J Fungi 8 (10): 1105. DOI: 10.3390/jof8101105.

Parveen SS, Rashtrapal PS. 2024. Integrated pest management strategies using endophytic entomopathogenic fungi. Plant Sci Today 11 (1): 568-574. DOI: 10.14719/pst.2740.

Perincherry L, Lalak-Kańczugowska J, Stępień Ł. 2019. *Fusarium*-produced mycotoxins in plant-pathogen interactions. Toxins 11 (11): 664. DOI: 10.3390/toxins11110664.

Pothiraj G, Hussain Z, Singh AK, Solanke AU, Aggarwal R, Ramesh R, Shanmugam V. 2021. Characterization of *Fusarium* spp. inciting vascular wilt of tomato and its management by a Chaetomium-based biocontrol consortium. Front Plant Sci 12: 748013. DOI: 10.3389/fpls.2021.748013.

Priyashantha AKH, Karunarathna SC, Lu L, Tibpromma S. 2023. Fungal endophytes: An alternative biocontrol agent against phytopathogenic fungi. Encyclopedia 3: 759-780. DOI: 10.3390/encyclopedia3020055.

Pusztahelyi T, Holb IJ, Pócsi I. 2015. Secondary metabolites in fungus-plant interactions. Front Plant Sci 6: 573. DOI: 10.3389/fpls.2015.00573.

Rai N, Keshri PK, Verma A, Kamble SC, Mishra P, Barik S, Singh SK, Gautam V. 2021. Plant-associated fungal endophytes as a source of natural bioactive compounds. Mycology 12 (3): 139-159. DOI: 10.1080/21501203.2020.1870579.

Rančić A, Soković M, Karioti A, Vukojević J, Skaltsa H. 2006. Isolation and structural elucidation of two secondary metabolites from the filamentous fungus *Penicillium ochrochloron* with antimicrobial activity. Environ Toxicol Pharmacol 22 (1): 80-84. DOI: 10.1016/j.etap.2005.12.003.

Rollando R, Yuniati Y, Monica E. 2023. Bioactive potential of *Cephalosporium* sp., a fungal endophyte isolated from *Phyllanthus niruri* L. Trop J Nat Prod Res 7: 2749-2755. DOI: 10.26538/tjnpr/v7i4.13.

Saikkonen K, Wäli P, Helander M, Faeth SH. 2004. Evolution of endophyte-plant symbioses. Trends Plant Sci 9 (6): 275-280. DOI: 10.1016/j.tplants.2004.04.005.

Saithong P, Panthavee W, Stonsaovapak S, Congfa L. 2010. Isolation and primary identification of endophytic fungi from *Cephalotaxus mannii* trees. Maejo Intl J Sci Technol 4 (3): 446-453.

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. 2019. The global burden of pathogens and pests on major food crops. Nat Ecol Evol 3: 430-439. DOI: 10.1038/s41559-018-0793-y.

Schroers H-J, Samuels GJ, Zhang N, Short DPG, Juba J, Geiser DM. 2017. Epitypification of Fusisporium (*Fusarium solani*) and its assignment to a common phylogenetic species in the *Fusarium solani* species complex. Mycologia 108 (4): 806-819. DOI: 10.3852/15-255.

Segaran G, Sathiavelu M. 2019. Fungal endophytes: A potent biocontrol agent and a bioactive metabolites reservoir. Biocatal Agric Biotechnol 21: 101284. DOI: 10.1016/j.bcab.2019.101284.

Shaaban M, Sohsah GE, El-Metwally MM, Abdel-Mogib M. 2016. Bioactive compounds produced by a strain of *Penicillium* sp. Intl J Sci Eng Appl 5 (6): 342-347. DOI: 10.7753/IJSEA0506.1007.

Shahnazi S, Meon S, Vadamalai G, Ahmad K, Nejat N. 2012. Morphological and molecular characterization of *Fusarium* spp. associated with yellowing disease of black pepper (*Piper nigrum* L.) in Malaysia. J Gen Plant Pathol 78 (3): 160-169. DOI: 10.1007/s10327-012-0379-5.

Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2022. Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*. Biodiversitas 23 (1): 25-32. DOI: 10.13057/biodiv/d230104.

Sopialena S, Arwita NNP, Suyadi S. 2024. Antagonist test of *Trichoderma* sp. and *Gliocladium* sp. against fungal pathogens that cause diseases on tomato plants. Jurnal Agroekoteknologi Tropika Lembab 7 (1): 78-84. DOI: 10.30872/jatl.7.1.2024.15636.78-84.

Sopialena S, Suyadi S, Sahil M, Nurdiana J. 2018. The diversity of endophytic fungi associated with *Piper nigrum* in the tropical areas: A recent study from Kutai Kartanegara, Indonesia. Biodiversitas 19 (6): 2028-2034. DOI: 10.13057/biodiv/d190607.

Srinivas C, Devi DN, Murthy KN et al. 2019. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity-A review. Saudi J Biol Sci 26 (7): 1315-1324. DOI: 10.1016/j.sjbs.2019.06.002.

Su Y, Wang J, Gao W, Wang R, Yang W, Zhang H, Huang L, Guo L. 2023. Dynamic metabolites: A bridge between plants and microbes. Sci Total Environ 899: 165612. DOI: 10.1016/j.scitotenv.2023.165612.

Syarifah, Elfita, Widjajanti H, Setiawan A, Kurniawati AR. 2021. Diversity of endophytic fungi from the root bark of *Syzygium zeylanicum*, and the antibacterial activity of fungal extracts and secondary metabolites. Biodiversitas 22 (10): 4572-4582. DOI: 10.13057/biodiv/d221051.

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Intl J Mol Sci 23 (4): 2329. DOI: 10.3390/ijms23042329.

Ul Haq I, Rahim K, Yahya G, Ijaz B, Maryam S, Paker NP. 2024. Eco-smart biocontrol strategies utilizing potent microbes for sustainable management of phytopathogenic diseases. Biotechnol Rep 44: e00859. DOI: 10.1016/j.btre.2024.e00859.

Valeria GB, Gabriela DB, Gustavo R. 2024. *Trichoderma* spp.: Characteristics and applications. J Appl Biotechnol Bioeng 11 (1): 18-22. DOI: 10.15406/jabb.2024.11.00354.

Wang H, Liu Z, Duan F, Chen Y, Qiu K, Xiong Q, Lin H, Zhang J, Tan H. 2023. Isolation, identification, and antibacterial evaluation of endophytic fungi from Gannan navel orange. Front Microbiol 14: 1172629. DOI: 10.3389/fmicb.2023.1172629.

Wang M, Sun Y, Sun G, Liu X, Zhai L, Shen Q, Guo S. 2015. Water balance altered in cucumber plants infected with *Fusarium oxysporum* f. sp. *cucumerinum*. Sci Rep 5: 7722. DOI: 10.1038/srep07722.

Watts D, Palombo EA, Castillo AA, Zaferanloo B. 2023. Endophytes in agriculture: Potential to improve yields and tolerances of agricultural crops. Microorganisms 11 (5): 1276. DOI: 10.3390/microorganisms11051276.

Wippel K. 2023. Plant and microbial features governing an endophytic lifestyle. Curr Opin Plant Biol 76: 102483. DOI: 10.1016/j.pbi.2023.102483.

Yadeta KA, Thomma BPHJ. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front Plant Sci 4: 97. DOI: 10.3389/fpls.2013.00097.

Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front Microbiol 14: 1160551. DOI: 10.3389/fmicb.2023.1160551.

Zakaria SNS, Noor NM, Ramli NWB, Anuar ISM, Ab Wahab MZ. 2022. Preliminary identification of new fungal pathogen isolated from black pepper disease. IOP Conf Ser: Earth Environ Sci 1059: 012067. DOI: 10.1088/1755-1315/1059/1/012067.

Zerroug A, Sadrati N, Demirel R, Bakli S, Harzallah D. 2018. Antibacterial activity of endophytic fungus, *Penicillium griseofulvum* MPR1 isolated from medicinal plant, *Mentha pulegium* L. Afr J Microbiol Res 12 (48): 1056-1066. DOI: 10.5897/AJMR2018.8887.