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Antagonistic Test of Endophytic Fungi from Black Pepper (*Piper nigrum* L.) against *Fusarium oxysporum* the Main Cause of Fusarium Wilt in Chili Plants (*Capsicum annuum* L.)

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***Abstract**. Fusarium wilt, caused by* Fusarium oxysporum, *is a major constraint in chili (*Capsicum annuum *L.) cultivation due to its severe impact on plant health and yield. Excessive use of synthetic fungicides to control this disease has resulted in environmental pollution and the development of resistant pathogens. This study aimed to identify endophytic fungi from black pepper (*Piper nigrum *L.) and evaluate their antagonistic potential against* F. oxysporum *as an eco-friendly biological control strategy. The novelty of this research lies in the exploration of endophytic fungi from black pepper, a crop not commonly studied as a microbial reservoir for chili disease control, providing new insight into cross-host endophytic interactions and their potential application in sustainable plant protection. Endophytic fungi were isolated from the stems, roots, and leaves of healthy black pepper plants, while* F. oxysporum *isolates were obtained from diseased chili plants. All isolates were cultured on Potato Dextrose Agar (PDA) and tested using a dual culture assay under a completely randomized design (CRD) with five treatments and six replications. Inhibition percentage and colony growth were observed for seven days, and antagonistic mechanisms such as competition, antibiosis, and parasitism were examined microscopically. Four endophytic fungi—*Trichoderma sp., Gliocladium sp., Aspergillus niger, *and* Aspergillus flavus*—showed antagonistic activity against* F. oxysporum. *The highest inhibition rates (60–75%) were observed in* Gliocladium sp. *and* Trichoderma sp., *primarily through competition and antibiosis mechanisms. These results demonstrate the potential of black pepper-derived endophytic fungi as novel, effective, and environmentally safe biocontrol agents, offering an innovative approach to developing sustainable alternatives to synthetic fungicides in integrated plant disease management systems.*

***Keywords**: Biological control, Black pepper (Piper nigrum L.), Chili (Capsicum annuum L.), Endophytic fungi, Fusarium oxysporum*

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INTRODUCTION

Chili pepper (Capsicum annuum L.) is one of Indonesia's most economically significant horticultural commodities, valued for its pungent flavor, aroma, and vibrant color. The growing population and changing dietary preferences have driven a continuous increase in chili consumption. According to the Central Bureau of Statistics (BPS, 2023), national chili production reached approximately 1.55 million tons in 2023, marking an 11.5% increase compared to the previous year. Despite these gains, chili production fluctuates seasonally, particularly during the rainy season, when increased humidity favors the development of fungal pathogens. Among these, Fusarium oxysporum—the causal agent of Fusarium wilt—remains a major constraint to chili productivity, especially in red curly chili varieties cultivated on moist or sloping lands (Iqbal et al., 2024). The pathogen spreads efficiently through contaminated soil, water, and farming tools, leading to significant economic losses.

Fusarium oxysporum infects the plant's vascular tissue, obstructing xylem vessels and disrupting water transport, resulting in symptoms such as chlorosis, unilateral wilting, vascular browning, and plant death (Dewi et al., 2021). Severe infection reduces fruit size and overall yield, and under conducive environmental conditions, disease incidence can reach epidemic levels. Conventional management strategies largely depend on synthetic fungicides; however, repeated application often leads to the emergence of resistant pathogen strains and detrimental environmental effects, including soil and water contamination (Elsayed et al., 2022). Consequently, sustainable and environmentally friendly control alternatives are urgently required to ensure stable chili production.

In recent years, endophytic fungi have gained considerable attention as potential biological control agents due to their unique ecological interactions and biochemical versatility. These microorganisms inhabit internal plant tissues without causing harm to their hosts and are capable of synthesizing diverse bioactive metabolites such as enzymes, siderophores, antibiotics, and volatile organic compounds with strong antifungal properties (Strobel et al., 2003; Bacon & White, 2016). Several studies have demonstrated that endophytic fungi isolated from chili plants can effectively suppress F. oxysporum, reducing disease severity by 58–83% through soil or foliar application (Dewi et al., 2021). For example, Aspergillus spp. endophytes enhanced plant defense responses and inhibited fungal growth by more than 80% in both in vitro and in vivo conditions (Elsayed et al., 2022). Similarly, bacterial biocontrol agents such as Bacillus subtilis and Bacillus velezensis have shown antagonistic activity against F. oxysporum while promoting chili plant growth (Iqbal et al., 2024). These studies highlight the central role of microbial endophytes in sustainable plant protection strategies.

Despite these advancements, limited studies have investigated the potential of endophytic fungi from black pepper (Piper nigrum L.) as biocontrol agents against chili pathogens. Previous work has shown that P. nigrum hosts a rich and diverse assemblage of fungal endophytes capable of vertical transmission through seeds (Sreeja et al., 2019) and that its root-associated microbes, including endophytic bacteria, exhibit strong antagonism against soil-borne pathogens such as Phytophthora capsici (Ngo et al., 2020). Given these findings, exploring P. nigrum endophytes represents a novel approach that could uncover unique bioactive interactions and cross-host biocontrol potential. Therefore, this study aims to evaluate the antagonistic activity of endophytic fungi isolated from P. nigrum against F. oxysporum infecting chili plants, providing new insight into the utilization of black pepper–derived endophytes for sustainable disease management in chili cultivation.

The findings from this research are expected to contribute directly to the development of biofungicide formulations based on Trichoderma sp. and Gliocladium sp., the two isolates showing the highest inhibition rates (60–75%) in vitro, as demonstrated in this study. These promising isolates could serve as core active ingredients in bioprotective formulations targeting Fusarium wilt in chili plants. Further greenhouse and field-scale evaluations are essential to assess their colonization efficiency, persistence in the rhizosphere, and interaction with chili plant physiology under variable environmental conditions. The integration of such endophyte-based biofungicides into integrated pest management (IPM) programs would not only reduce dependency on chemical fungicides but also enhance soil microbial balance and plant resilience. Ultimately, this approach aligns with sustainable agriculture objectives by promoting eco-friendly, effective, and long-lasting disease suppression systems suitable for tropical agroecosystems like Indonesia.

LITERATURE REVIEW

Fusarium wilt, caused by *Fusarium oxysporum*, is a destructive soil-borne disease that affects a wide range of crops, including chili (*Capsicum annuum* L.). The pathogen infects the plant's vascular system, leading to xylem blockage, wilting, chlorosis, and eventual death (Dewi et al., 2021). Yield losses due to Fusarium wilt in chili can reach 40–80% under favorable humid and warm tropical conditions (Iqbal et al., 2024). Traditionally, chemical fungicides such as carbendazim, benomyl, and mancozeb have been used for its management; however, continuous and excessive use has led to environmental contamination, soil microbiome disruption, and the emergence of resistant *Fusarium* strains (Elsayed et al., 2022). Consequently, researchers have sought alternative, eco-friendly approaches such as the application of endophytic fungi that can naturally suppress pathogenic activity while promoting plant health.

Endophytic fungi are microorganisms that inhabit internal plant tissues without causing apparent harm and often contribute to the host's defense through the production of secondary metabolites and enzymes with antimicrobial properties (Strobel et al., 2003; Bacon & White, 2016). They synthesize bioactive compounds including antibiotics, siderophores, and volatile organic compounds (VOCs) that can inhibit the growth of phytopathogens through mechanisms such as antibiosis, parasitism, and nutrient competition. Previous studies have shown that endophytic *Aspergillus* spp. from chili plants significantly reduced the severity of Fusarium wilt, achieving inhibition rates of up to 83% under laboratory and greenhouse conditions (Elsayed et al., 2022). Similarly, *Bacillus subtilis* and *B. velezensis* isolates were reported to suppress *F. oxysporum* infection in chili while simultaneously enhancing plant growth and nutrient uptake (Iqbal et al., 2024). These findings reinforce the role of endophytic microbes as sustainable alternatives in integrated disease management systems.

Trichoderma sp. is among the most studied and widely used fungal genera for biological control due to its high adaptability, rapid growth, and multiple antagonistic mechanisms. According to Mary et al. (2022), *Trichoderma* spp. suppress root rot and wilt pathogens through the secretion of lytic enzymes such as chitinases, glucanases, and proteases that degrade fungal cell walls. Additionally, *Trichoderma* produces antibiotic compounds like peptaibols and gliotoxins and induces systemic resistance in host plants (Pasalo et al., 2022). Similarly, *Gliocladium* sp., another member of the

Hypocreaceae family, has demonstrated strong antifungal activity by producing gliovirin and viridian, metabolites known to inhibit spore germination and hyphal elongation in pathogens such as *Fusarium* and *Colletotrichum* (Hidayat et al., 2020; Rahma & Karimah, 2021). Both fungi have been applied successfully as soil inoculants and seed treatments, reducing disease severity in tomato, banana, and onion crops by more than 60% (Aini & Martina, 2024).

Research on *Aspergillus* species has also shown their dual role in agriculture. *Aspergillus niger* exhibits moderate antagonistic potential against soil-borne pathogens and produces industrially valuable enzymes such as amylases and proteases (Erdiansyah & Zaini, 2023). However, *Aspergillus flavus* poses biosafety concerns due to its ability to produce aflatoxins, despite its capacity to synthesize antifungal compounds that can suppress plant pathogens (Patyal et al., 2023). Hence, its application as a biocontrol agent requires rigorous screening for non-toxigenic strains before use in agriculture (Lindawati & Rini, 2019; Mongi et al., 2020).

While endophytic fungi from chili and other host plants have been extensively studied, limited attention has been given to those derived from black pepper (*Piper nigrum* L.), a crop known for its rich microbial diversity. Studies by Sreeja et al. (2019) revealed that *P. nigrum* harbors a variety of fungal endophytes capable of seed transmission, suggesting stable vertical inheritance and ecological adaptability. Moreover, its root-associated microbes have demonstrated antagonism against *Phytophthora capsici*, a major pathogen in pepper plants (Ngo et al., 2020). These findings imply that endophytes from black pepper could exhibit cross-host antagonistic activity, offering untapped potential for controlling chili pathogens. Therefore, the current study’s focus on isolating endophytic fungi from *P. nigrum* and evaluating their antagonistic activity against *F. oxysporum* introduces a novel perspective in biocontrol research and contributes valuable insight into sustainable plant protection strategies.

RESEARCH METHODS

Time and Pzlace of Research

This research was conducted over a period of 6 months from September 2024 to January 2025 at the IHPT (Plant Pest and Disease Science) Laboratory, Faculty of Agriculture, Mulawarman University.

Materials and Equipment

The materials used in this study were the pathogenic fungus *Fusarium oxysporum* from infected chili plants and endophytic fungi from healthy pepper plants. Media included PDA (*Potato Dextrose Agar*), 70% alcohol, distilled water, spiritus, and methylene blue. The equipment used included enkes, petri dishes, Erlenmeyer flasks, Bunsen burners, aluminum foil, inoculation needles, tweezers, measuring cups, microscopes, beaker glasses, microscope slides, autoclave covers, haemocytometers, scissors, cotton, name labels, plastic wrap, tissues, and cameras.

Experimental Design

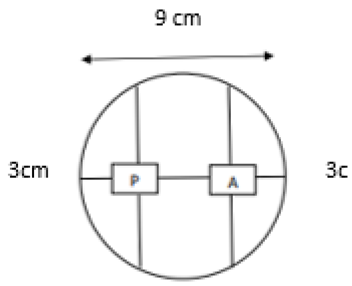
This study used a Completely Randomized Design (CRD) with 5 treatments and 6 replications. The treatments consisted of P0 (control), P1 (*Fusarium oxysporum* vs. endophytic fungus 1), P2 (*Fusarium oxysporum* vs. endophytic fungus 2), P3 (*Fusarium oxysporum* vs. endophytic fungus 3), and P4 (*Fusarium oxysporum* vs. endophytic fungus 4).

Research Procedure

This study began with the collection of endophytic fungi samples from healthy pepper plants collected from Salo Bandang Village and Batuah Village, Muara Badak District, as well as Fusarium oxysporum pathogen samples from chili plants showing symptoms of fusarium wilt in Lempake, North Samarinda District. Endophytic samples were taken from the roots, stems, and leaves of healthy plants, while wilting symptoms in chili plants were characterized by yellowing leaves, stunted growth, and the appearance of brown rings at the base of the stem. All equipment used, such as petri dishes and Erlenmeyer flasks, were sterilized in an oven at 121°C for 15–20 minutes, and metal tools such as inoculation needles and forceps were sterilized by heating over a Bunsen burner. Potato Dextrose Agar (PDA) medium was prepared from a mixture of boiled potatoes, agar-agar, and sugar, then sterilized in an autoclave at 1.5 atm pressure for 30 minutes.

Endophytic fungi were isolated by cutting 1 cm sections of the roots, stems, and leaves of healthy plants, then sterilizing them sequentially using 5% NaOCl, 70% alcohol, and rinsing with sterile distilled water before planting them on PDA medium. Meanwhile, pathogenic fungi were isolated from symptomatic chili plant tissue using a similar sterilization procedure but with 2% NaOCl. All isolates were incubated at 25–30°C for 5–7 days and observed daily. Fungal identification was performed macroscopically by observing color, shape, texture, and colony growth, as well as microscopically by observing hyphae and conidia using a microscope. Isolate purification was performed by subculturing colonies with different morphologies onto new PDA media.

Antagonism testing was performed in vitro using the direct opposition method, which involved growing endophytic fungi and F. oxysporum opposite each other on Petri dishes using the following formula:



A = Isolat Jamur Endofit

P = Patogen *F. oxysporum*

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

Explanation

I : Inhibition percentage

r1 : Radius of the pathogen colony whose growth direction is away from the antagonistic fungal colony

r2 : Radius of the pathogen colony whose growth direction is toward the antagonistic fungal colony.

RESULTS AND DISCUSSION

Characteristics of the Pathogenic Fungus

Fusarium oxysporum is a well-known soil-borne pathogen responsible for causing wilt disease in various crops, including chili, tomato, banana, and shallot. Based on the isolation from symptomatic chili plant tissue, the fungus grew well on *Potato Dextrose Agar* (PDA) medium, exhibiting distinct morphological characteristics. Macroscopically, the fungal colony was white with sparse mycelial growth that spread laterally and upwards in a concentric pattern. The mycelium had a cottony appearance, and the colony surface was flat and dense.

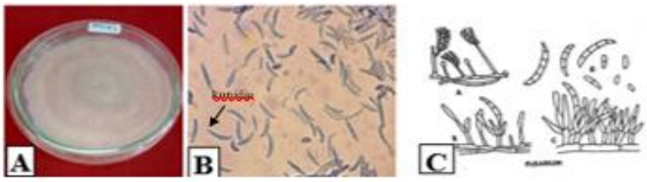


Figure 1. (a) *Fusarium oxysporum* colony, (b) *Fusarium oxysporum* conidia, (c) *Fusarium oxysporum* (Barnett and Hunter, 1972).

Microscopically, the fungus displayed characteristic features, including septate (segmented), unbranched, hyaline (transparent) hyphae, and sharp, colorless conidia. The crescent or boat-shaped structure of the conidia is consistent with descriptions of *F. oxysporum* in previous literature (Barnett & Hunter, 1972). Additionally, this fungus is known to form macroconidia with 3–5 septa, oval to kidney-shaped microconidia, and thick-walled chlamydospores that act as survival structures in the soil. In terms of pathogenicity, *F. oxysporum* infects plants through the roots and attacks the vascular tissue, causing necrosis and disrupting the water transport system. This ultimately leads to the classic symptoms of wilting in the plant. These visual characteristics could be observed within seven days after inoculation, by which time the pathogen colony had fully developed on the Petri dish. The visual representation of the colony and its microscopic structures are shown in figure 1.

Characteristics of the Endophytic Fungus

***Trichoderma* sp.**

Based on macroscopic observations of *Trichoderma* sp. growth, colonies began developing upon inoculation. By the seventh day, the colonies had spread uniformly across the entire surface of the Potato Dextrose Agar (PDA) medium, completely filling the 9 cm diameter Petri dish. The colonies appeared white to dark green with a smooth texture and a concentric growth pattern, spreading laterally and upwards in a circular manner (Figure 2a). This demonstrates the rapid and aggressive growth capability of *Trichoderma* sp. under favorable *in vitro* conditions.

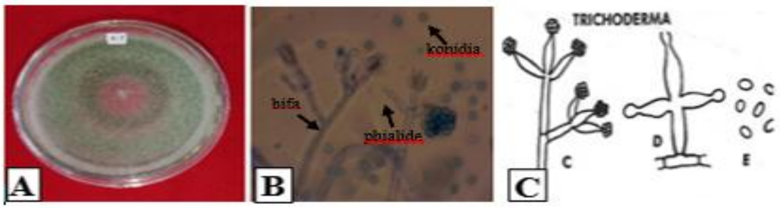


Figure 2. (a) *Trichoderma* sp. colony, (b) *Trichoderma* sp. conidia, hyphae, and phialides, (c) *Trichoderma* sp. (Barnett and Hunter, 1972).

Microscopically (Figure 2b), *Trichoderma* sp. shows erect, branched conidiophores arranged vertically on a cushion-like structure. The phialides appear short, thick, and flask-shaped, arranged in clusters at the tips of the conidiophores. The conidia are spherical to oval, pale green, and smooth-walled, distributed in groups. The hyphae are septate, branched, and hyaline, which are characteristic structures of *Trichoderma* sp. (Aini and Martina, 2024). *Trichoderma* sp. is a well-known antagonistic fungus commonly found in soil and serves as a bio-control agent against various plant pathogens. As a saprophyte, *Trichoderma* sp. thrives in soil ecosystems by utilizing nutrients from organic matter. Environmental factors such as temperature and pH also influence its growth. According to Doo et al. (2023), the optimal temperature for *Trichoderma* sp. growth is in the range of 20–28°C with an optimal pH of 4.5–5.5.

Its ability to suppress plant pathogens is supported by several antagonistic mechanisms, including competition for space and nutrients, and the production of antimicrobial compounds that can inhibit pathogen growth (Pasalo et al., 2022). *Trichoderma* sp. also produces various hydrolytic enzymes such as chitinase, cellulase, and protease, which are capable of degrading the cell wall components of pathogenic fungi, thereby directly damaging the pathogen's structure. In addition, the production of bioactive compounds like antibiotics and peptides strengthens its role as a biological control agent (Doo et al., 2023; Sopialena, 2015). Therefore, the potential of *Trichoderma* sp. in biological control is very promising for application as an environmentally friendly alternative for managing plant diseases.

***Gliocladium* sp.**

Based on macroscopic observations of *Gliocladium* sp. growth, the colonies began to grow uniformly on PDA medium and completely filled the 9 cm diameter Petri dish by the seventh day after inoculation. The colonies appeared white to greenish with a smooth texture and spread radially in all directions (Figure 3a). This rapid and uniform growth pattern indicates that *Gliocladium* sp. possesses a high degree of adaptability and strong potential as an antagonistic agent under *in vitro* conditions.

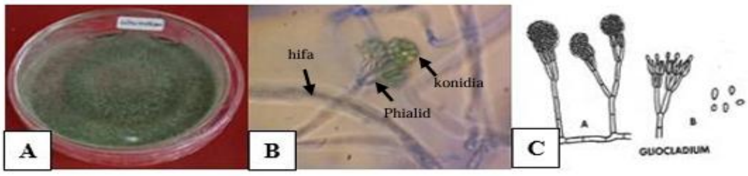


Figure 3. (a) *Gliocladium* sp. colony, (b) *Gliocladium* sp. conidia, hyphae, phialides, (c) *Gliocladium* sp. (Barnett and Hunter, 1972).

Microscopic observations at 400x magnification (Figure 3b) showed that the conidiophores of *Gliocladium* sp. were septate and branched, forming a characteristic compact brush-like (penicillate) structure. The branching of the conidiophores formed a spiral arrangement consisting of 4–5 conidia clusters. The conidia were spherical to slightly flattened, measuring 4.5–6 µm x 3.5–5 µm, tightly arranged at the tip of the phialides, and were hyaline (colorless). The hyphae of this fungus were septate and grew aggressively, which supports its ability to colonize plant tissue and compete with pathogenic fungi (Rahma & Karimah, 2021).

Gliocladium sp. belongs to the family Hypocreaceae and the order Hypocreales, and is classified in the class Sordariomycetes. As an endophytic fungus, *Gliocladium* sp. is capable of symbiotically colonizing plant tissues such as roots, stems, and leaves without causing any noticeable disease symptoms. This ability makes it a potential candidate for the biological control of plant pathogens. One of its biological advantages is its ability to produce secondary metabolite compounds like gliovirin and viridian, which act as natural antibiotics. These compounds are known to inhibit pathogen growth, reduce sporulation, and disrupt the metabolic processes and spore germination of pathogens (Hidayat et al., 2020). With its ability to produce antimicrobial compounds and a growth structure that supports rapid colonization, *Gliocladium* sp. has high potential as an environmentally friendly biological control agent in both conventional and organic farming systems.

Aspergillus niger

Macroscopic observations revealed that the colonies of the fungus *Aspergillus niger* were dark black with a white margin, while the reverse side of the colony appeared brown. The colonies spread irregularly in all directions and had a coarse, granular surface texture. The colony growth was rapid, forming dense clusters on the surface of the *Potato Dextrose Agar* (PDA) medium (Figure 4a). These characteristic features indicate the adaptive capability and aggressive growth of *A. niger* under *in vitro* conditions.

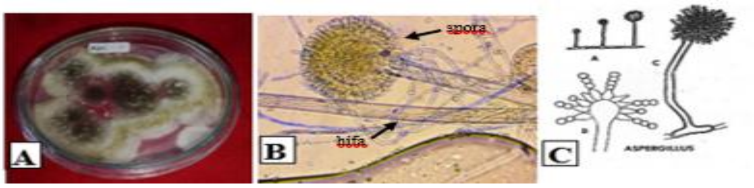


Figure 4. (a) *Aspergillus niger* colony, (b) *Aspergillus niger* hyphae and spores, (c) *Aspergillus niger* (Barnett and Hunter, 1972).

Microscopically (Figure 4b), *Aspergillus niger* displayed long, erect, and hyaline (colorless) conidiophores with smooth to slightly rough walls. The tip of the conidiophore formed a large, spherical to semi-spherical vesicle, where the phialides and metulae were arranged biseriately (in two layers). The phialides were tightly packed around the vesicle and produced dark brown to blackish conidia. These conidia were spherical to oval, had a smooth surface, and were arranged in long chains, forming a distinct dark conidial head visible under the microscope. The hyphae of *Aspergillus niger* were hyaline, septate, and unbranched, showing rapid linear growth (Erdiansyah & Zaini, 2023). *Aspergillus niger* is a fungal species belonging to the division Ascomycota and is widely known for its diverse roles, both as a plant and human pathogen and as an important agent in industrial biotechnology. This fungus is commonly found in environments rich in organic matter, such as soil, plant debris, and decaying food (Sopialena et al., 2021). In an industrial context, *Aspergillus niger* has great potential for the production of enzymes and secondary metabolite compounds, such as citric acid, glucoamylase, and protease, which are widely used in the food, pharmaceutical, and agricultural fields. The characteristic colony color, the complex structure of the conidiophores and conidia, and the rapid growth on culture media make *Aspergillus niger* a fungus that is easily identified morphologically. Additionally, its distinct microscopic structures, including the large vesicle and the organized phialide-metulae arrangement, serve as important indicators in the laboratory identification of this species.

Aspergillus flavus

Macroscopic observations of the *Aspergillus flavus* isolate showed that the fungal colonies had a yellowish-green color on the upper surface and a yellow to brown color on the reverse side. The colony texture was coarse, resembling velvet or sand, with growth spreading in all directions to form clustered colonies (Akhsan et al., 2021). The colony surface appeared raised and had a fine texture, indicating a characteristic feature of this species. The visual characteristics of the *Aspergillus flavus* colonies can be seen in figures 5a and 5c.

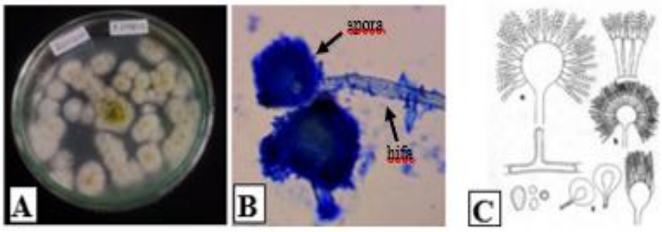


Figure 5. (a) *Aspergillus flavus* colony, (b) *Aspergillus flavus* hyphae and spores, (c) *Aspergillus flavus* (Barnett and Hunter, 1972).

Microscopic examination using methylene blue staining at 400x magnification with an optilab (Figure 5b) revealed that *Aspergillus flavus* has short, hyaline (colorless), and rough-walled conidiophores. The tip of the conidiophore forms a spherical to subglobose vesicle, where the metulae and phialides grow. The conidia are arranged in long chains, forming a characteristic conidial head. The conidia are spherical to semi-spherical, rough-walled, and yellowish-green. The hyphae of *Aspergillus flavus* are septate and branched, consistent with the morphological characteristics of the genus *Aspergillus* (Lindawati & Rini, 2019). *Aspergillus flavus* is a multicellular mold widely known as a major contaminant of food and agricultural products. This fungus is able to grow optimally at temperatures of 10–35°C and a pH of 2–9. It can also adapt to environments with high humidity and low water activity, such as on sweet potatoes, fermented shrimp paste, and other fermentation substrates (Mongi et al., 2020). These physiological characteristics demonstrate that *Aspergillus flavus* has a high survival capability in humid food storage environments, making it a significant organism in food contamination and toxicology studies.

Colony Diameter of Endophytic Fungi

The growth of endophytic fungal colonies isolated from pepper plants (*Piper nigrum* L.) was observed for seven days on PDA medium, with daily measurements of colony diameter. Based on the data in Table 1, all isolates showed a progressive increase in colony diameter, although with different growth rates. Two isolates, *Trichoderma* sp. and *Gliocladium* sp., exhibited the fastest colony growth, reaching a maximum diameter of 9 cm by the seventh day. This rapid growth became apparent between days 5 and 6, when the colonies nearly filled the entire surface of the Petri dish.

Table 1. Endophytic Fungi Colony Diameter (cm)

Information	Isolate	Day						
		1	2	3	4	5	6	7
Endofit	*Aspergillus niger* MB	2	3,4	4,5	5,6	7,9	8,2	8,5
	Trichoderma sp. MB	2,9	4,4	6,1	7	7,7	8,5	9
	Aspergillus flavus S	1,6	2,5	3,6	5,3	6,3	7,5	8,4
	Gliocladium sp. S	2,6	4,6	6,3	7,2	7,9	8,6	9
	Aspergillus niger S	1,8	2,7	4	5,8	6,7	7,6	8,5

Notes:

- Fungi with the code (MB) were obtained from the Muara Badak location.
- Fungi with the code (S) were obtained from Batuah village, Samarinda.

The colony growth rate of *Trichoderma* sp. aligns with the findings of Mary et al. (2022), who reported that this species has a fast colonization capacity and high antagonistic activity against plant pathogens. This makes *Trichoderma* sp. an excellent candidate for developing biofungicides. Meanwhile, *Gliocladium* sp. also demonstrated a competitive growth capability, supported by its mechanisms of antibiosis and nutrient competition (Mary et al., 2022). *Aspergillus niger* isolates from two different locations, Muara Badak (MB) and Batuah (S), also showed stable colony growth, with diameters reaching 8.5 cm by the seventh day. Although not as fast as *Trichoderma* sp., this isolate has potential as a producer of industrial enzymes like amylase and gluconase (Patyal et al., 2023). Its stable growth indicates good adaptation to the growth medium, even though its antagonistic effectiveness is relatively lower compared to the previous two isolates. In contrast, *Aspergillus flavus* showed the slowest colony growth among all isolates, reaching a diameter of 8.4 cm on the seventh day. Patyal et al. (2023) reported that *A. flavus* has the potential to produce bioactive compounds, but it is also known to produce aflatoxins. Therefore, the application of *A. flavus* as a biological agent must be carefully considered through strict molecular selection to ensure its safety in agricultural practices.

Endophytic Fungal Spore Density

The spore density was calculated for five endophytic fungal isolates at a 106 dilution, revealing a variation in the number of spores among the species, as shown in Table 2 below.

Tabel 2. Spore Density Calculation (Units)

Fungi	Spore Density
Aspergillus niger (MB)	7,3 X 10^6
Trichoderma sp.	8,6 X 10^6
Aspergillus flavus	7,8 X 10^6
Gliocladium sp.	8,9 X 10^6
*Aspergillus nige*r (S)	7,7 X 10^6

Notes:

- Fungi with the code (S) are from a pepper plant isolate from Samarinda (Batuah).
- Fungi with the code (MB) are from a pepper plant isolate from Muara Badak.

Gliocladium sp. isolate showed the highest spore density at 8.9×106 spores/mL, followed by *Trichoderma* sp. at 8.6×106 spores/mL. The high spore density in both of these isolates indicates their consistent and high sporulation ability, which is crucial for their effectiveness as biological agents. This aligns with research by Pasalo et al. (2022), who reported that *Trichoderma* sp. has a high sporulation capacity and produces lytic enzymes vital for pathogen control. Conversely, isolates from the genus *Aspergillus*, such as *A. flavus* and *A. niger* from both locations, had lower spore densities, ranging from 7.3×106 to 7.8×106 spores/mL. Although these values are lower than those of *Gliocladium* sp. and *Trichoderma* sp., they are still within a range that allows for effective mycelial growth and spread. According to Kurniawati et al. (2021), media and environmental adaptation can influence the sporulation ability of *A. flavus*, especially when using alternative substrates like rice flour. Overall, spore density is a critical parameter for evaluating the potential of endophytic fungal isolates as biological agents. A high spore count contributes to rapid colonization and environmental resilience. Therefore, *Gliocladium* sp. and *Trichoderma* sp. can be considered excellent candidates for developing environmentally friendly biopesticides based on endophytic fungi.

Endophytic Fungal Antagonism Mechanisms

Observations of the antagonistic mechanisms of endophytic fungi against *Fusarium oxysporum* were conducted for seven days after inoculation. Based on the results (Table 3), all tested endophytic fungal isolates demonstrated competition as the primary form of antagonistic interaction. The isolates of *Trichoderma* sp., *Gliocladium* sp., and *Aspergillus flavus* also showed antibiosis, while parasitism was not observed in any of the isolates.

Table 3. Endophytic Fungal Antagonistic Mechanisms

Endophyte	Antagonism mechanism		
	Competition	Parasitism	Antibiosis
Trichoderma sp.	+	-	+
Gliocladium sp.	+	-	+
Aspergillus niger	+	-	-
Aspergillus flavus	+	-	+

Notes:

- The presence of an antagonistic mechanism is denoted by (+).
- The absence of an antagonistic mechanism is denoted by (-).

The competition mechanism was evident through the dominance of the endophytic fungal colonies over the media surface, which limited space and nutrients for the pathogen. This was seen in the faster and more expansive growth of the endophytic fungal colonies, which inhibited the development of *F. oxysporum*. This finding is consistent with the research of Hidayat et al. (2020), who noted that antagonistic fungi like *Trichoderma* and *Gliocladium* can inhibit pathogens by dominating the growth media and competing for resources. In addition to competition, the antibiosis mechanism was also identified in *Aspergillus flavus*, *Trichoderma* sp., and *Gliocladium* sp., as indicated by a clear zone between the endophytic fungal and pathogen colonies. This zone suggests the production of secondary metabolites with antifungal properties. According to Lubis and Ikbal (2022), antibiosis results from metabolites such as toxins or lytic enzymes that disrupt pathogen growth. In *Aspergillus flavus*, this antibiosis is likely caused by compounds like aflatoxin or other aromatic compounds that can cause structural damage to the pathogen's hyphae.

Inhibition Percentage

The inhibition percentage of endophytic fungi against *Fusarium oxysporum* was observed for seven days after inoculation, revealing an increase in antagonistic ability over time. The five endophytic fungal isolates tested—*Aspergillus niger* (MB and S), *Aspergillus flavus, Trichoderma* sp., and *Gliocladium* sp. *showed varying inhibition rates, with the highest effectiveness achieved by Gliocladium sp.*(43.18%) and *Trichoderma* sp.(42.29%) on the seventh day.

Table 4. Inhibition Percentage of Endophytic Fungi Day 1 to Day 7 After Inoculation

Treatment	Average (Arcsin Transformation)						
	1	2	3	4	5	6	7
Aspergillus niger MB	23,31 a	27,36 a	32,55 b	34,68 ab	35,37 ab	37,22 ab	40,13 a
Trichoderma sp.	24,84 a	32,44 b	34,83 c	37,02 b	37,81 b	39,14 b	42,29 b
Aspergillus flavus	23,31 a	27,91 a	30,22 a	34,69 ab	35,21 a	37,14 a	40,18 a
Gliocladium sp.	25,93 a	33,45 b	33,04 bc	37,62 b	38,13 b	39,67 b	43,18 c
Aspergillus niger S	22,34 a	26,21 a	31,60 ab	34,51 a	35,36 ab	37,24 ab	41,57 b
BNT		2,28	1,86	1,41	1,7	2,43	1,51

Notes:

- Fungi with the code (S) were isolated from a pepper plant in Batuah, Samarinda.
- Fungi with the code (MB) were isolated from a pepper plant in Muara Badak.

From day one to day two, no significant differences were observed between treatments, indicating that antagonistic activity was still uniform in the early phase. However, from day three to day seven, a significant increase was noted, particularly for *Trichoderma* sp. and *Gliocladium* sp., which showed better initial colonization and secondary metabolite production compared to other isolates. The mechanisms of competition and antibiosis were key to the antagonistic activity of these two isolates. *Gliocladium* sp. excelled in competing for space and nutrients in the rhizosphere and was capable of producing antifungal compounds like gliovirin and viridin, which effectively damage the pathogen's hyphal structure. Meanwhile, *Trichoderma* sp. demonstrated rapid colonization and produced antibiosis compounds such as peptaibols and gliotoxins, as well as inducing a systemic resistance response in the plant.

Despite showing reasonably good antagonistic activity, all isolates exhibited an inhibition percentage below 50%, which is categorized as a moderate level. Based on existing classifications, an inhibition rate of 30–49% is considered moderate, while ≥50% is deemed high. This indicates that the effectiveness of these endophytic fungi in inhibiting *F. oxysporum* is still at a minimal threshold and not yet optimal. The reasons for this low inhibition rate may include the slower growth of the endophytic fungi compared to the pathogen and the suboptimal induction of bioactive compound production. The complexity of the interactions between the endophytic fungal isolates, the pathogen, and environmental conditions also influences the inhibition results.

Overall, *Gliocladium* sp. and *Trichoderma* sp. are the isolates with the highest antagonistic potential against *F. oxysporum*, but further testing is needed to enhance their effectiveness, whether through the selection of superior isolates, optimization of the culture medium, or more efficient bio-agent formulation approaches.

The antagonistic test results showed that all endophytic fungal isolates from black pepper (*Piper nigrum* L.) exhibited inhibitory effects against *Fusarium oxysporum*, the causal agent of Fusarium wilt in chili (*Capsicum annuum* L.). Among the isolates tested, *Gliocladium* sp. and *Trichoderma* sp. demonstrated the highest inhibition percentages, 43.18% and 42.29%, respectively, categorized as moderate but biologically significant inhibition levels. These results are consistent with those reported by Mary et al. (2022), who observed that *Trichoderma* spp. rapidly colonize plant roots and suppress pathogens through nutrient competition and antibiotic production. Similarly, *Gliocladium* sp. exhibits a strong antibiosis mechanism through the secretion of antifungal metabolites such as gliovirin and viridin (Hidayat et al., 2020). The findings reinforce that both species possess the physiological and biochemical characteristics required to serve as effective biological control agents in chili cultivation systems.

The observed antagonistic mechanisms included competition for space and nutrients, as well as antibiosis. The rapid radial growth of *Trichoderma* sp. and *Gliocladium* sp. colonies on PDA medium limited the growth of *F. oxysporum*, indicating their ability to dominate the rhizosphere environment. Additionally, the formation of clear inhibition zones between endophytic and pathogenic fungal colonies suggested the secretion of antifungal compounds. These findings align with the results of Pasalo et al. (2022), who emphasized that *Trichoderma* sp. produces extracellular hydrolytic enzymes such as chitinase, cellulase, and glucanase that degrade the pathogen’s cell wall, while *Gliocladium* sp. synthesizes antibiotic metabolites that interfere with fungal spore germination and hyphal elongation. Although *Aspergillus niger* and *A. flavus* also showed moderate antagonism, their potential as bioagents should be carefully evaluated due to safety concerns, particularly aflatoxin production in *A. flavus* (Patyal et al., 2023).

High spore density was observed in *Gliocladium* sp. (8.9×10^6 spores/mL) and *Trichoderma* sp. (8.6×10^6 spores/mL), indicating their suitability for large-scale inoculum production and formulation. High sporulation capacity is a desirable trait for biocontrol agents because it improves formulation stability, viability during storage, and colonization ability after application to the rhizosphere or phyllosphere, enabling rapid establishment and effective competition against plant pathogens.

CONCLUSION

This study successfully isolated four endophytic fungal species from black pepper (*Piper nigrum* L.), namely *Trichoderma* sp., *Gliocladium* sp., *Aspergillus niger*, and *Aspergillus flavus*, all of which exhibited antagonistic activity against *Fusarium oxysporum*, the causal agent of Fusarium wilt in chili plants (*Capsicum annuum* L.). Among the isolates, *Gliocladium* sp. and *Trichoderma* sp. demonstrated the highest inhibition rates, supported by their high spore density—8.9×10^6 spores/mL and 8.6×10^6 spores/mL, respectively—indicating strong potential for large-scale inoculum production and stable bioformulation. These findings confirm that endophytic fungi from pepper plants possess significant potential as eco-friendly biological control agents. Their use represents a sustainable alternative to synthetic fungicides, contributing to environmentally responsible disease management strategies in chili cultivation.

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