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Symbiotic potential of endophytic fungi from native orchids with histological insights and growth responses in *Dendrobium* hybrid

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Abstract. *Sukamto DS, Avivi S, Nurcahyanti SD, Restanto DP. 2025. Symbiotic potential of endophytic fungi from native orchids with histological insights and growth responses in* Dendrobium *hybrid. Biodiversitas 26: 6389-6398.* Endophytic fungi play an essential role in orchid development by facilitating nutrient exchange and supporting early growth through peloton formation in root tissues. This study evaluated the symbiotic potential of eight fungal isolates obtained from native orchids in Jember, East Java, Indonesia, and examined their effects on peloton induction, root colonization, and seedling growth of a *Dendrobium* hybrid. Each isolate was identified morphologically and tested through inoculation assays, histological analyses, and seedling performance measurements. Four isolates—*Ceratobasidium* (1), *Rhizoctonia* sp. (3 and 4), and *Tulasnella* (6)—successfully colonized the root cortex and produced pelotons, whereas isolates 2 (*Fusarium*) and 8 (*Cladosporium*) caused pathogenic symptoms, including root and leaf rot, and were excluded from growth evaluation. Growth assays showed that *Rhizoctonia* isolate 4 produced the strongest symbiotic response, increasing shoot number by 175% and shoot length by more than tenfold relative to controls, followed by moderate enhancements from isolates 1, 5, 6, and 7. These findings show that native orchid-associated endophytes possess strong mycorrhizal potential and can be developed as effective bioinoculants to enhance the early growth of *Dendrobium* hybrids. This study provides a foundational step toward utilizing locally sourced symbiotic fungi to support orchid propagation and conservation efforts.

Keywords: *Dendrobium,* endophytic fungi, in vitro culture, orchid mycorrhiza, peloton

INTRODUCTION

Orchids are one of the groups of tropical ornamental plants with high economic value and remarkable biodiversity. The economic potential of orchids in Indonesia is enormous, due to their popularity and the numerous benefits they provide (Damanik et al. 2018; Rosanti and Widianjaya 2018). This great potential is not only an economic asset but also an important genetic resource for orchid development. Indonesia has two types of orchids: species orchids and hybrid orchids. Hybrid orchids are produced through crossbreeding, exhibiting a wide range of traits and a broad market share. The selection of superior offspring in hybrid orchids is mass-produced through in vitro culture or tissue culture techniques (Arobaya 2022; Sundari et al. 2023). Species orchids refer to wild orchids that represent naturally occurring taxa, occurring either as epiphytes or terrestrials in their native habitats, and are not the products of hybridization (Sebastian et al. 2021). Species orchids thrive in forests with suitable humidity, light intensity, and soil conditions suitable for their growth (Prawira et al. 2019). The uniqueness of species orchids makes them a valuable germplasm resource in conservation and plant breeding efforts, although their existence is increasingly threatened by deforestation, climate change, and overexploitation.

Despite their great potential, orchid propagation still faces fundamental challenges. Particularly from seeds, there is an absolute need for symbiosis with mycorrhizal fungi for germination and early growth (Phillips et al. 2020). Orchid seeds are small and lack endosperm, so they rely on symbiotic fungi to obtain carbon and essential nutrients during the early stages of life (Dearnaley et al. 2017). This dependence on mycorrhizae continues into the adult stage, although at a different level (Schweiger et al. 2018). Thus, the presence of mycorrhizal fungi is crucial in the orchid life cycle.

Mycorrhizal fungi that form symbiotic relationships with orchids generally live as endophytes in root tissues, and some are able to form distinctive structures called pelotons within root cortex cells. These pelotons facilitate nutrient transfer between fungi and plants, serving as an important indicator of successful symbiotic relationships. Several fungal genera, such as *Rhizoctonia*, *Ceratobasidium*, and *Tulasnella*, have been reported as orchid mycorrhizal symbionts (Meng et al. 2019a,b,c; Bell et al. 2020; Gao et al. 2020; Soelistijono et al. 2020; Zhang et al. 2020). However, the effectiveness and functional roles of each isolate vary significantly depending on the host species and environmental conditions, and some endophytic fungi may even exhibit pathogenic behavior. Therefore, accurate identification combined with experimental evaluation is essential to determine which isolates genuinely promote orchid growth and are suitable for development as bioinoculants.

Exploring mycorrhizae in orchid roots is the first step in research aimed at understanding the role of each mycorrhizal species in orchid growth. Previous studies show that the initial stages generally focus on isolating and identifying fungal partners from orchid roots to obtain mycorrhizal species suitable for reinoculation (Soelistijono et al. 2017; Soelistijono et al. 2020). However, confirmation of successful symbiosis is still commonly assessed only through plant growth responses, with limited use of histological verification such as direct observation of pelotons within cortical tissues (Vierheilig et al. 1998; Dearnaley et al. 2017). Furthermore, the effectiveness of fungal isolates in promoting orchid growth—especially in hybrid orchid tissue culture systems—remains rarely evaluated comprehensively and systematically, despite evidence that different mycorrhizal species can significantly influence germination, seedling performance, and stress tolerance (Decruse et al. 2018; Meng et al. 2019a; Zhang et al. 2020). Integrating these three aspects—accurate isolation, histological confirmation, and functional assessment—is therefore essential to understand the mechanisms of symbiosis and to identify the most potent fungal candidates for bioinoculant development in orchid cultivation.

Previous studies have mostly focused on the identification of symbiotic fungi without direct testing on plantlet growth, or conversely, conducted growth trials without adequate histological analysis (Meng et al. 2019c; Gao et al. 2020). This gap has limited knowledge regarding the functional roles of endophytic fungi in supporting orchid growth, particularly in hybrid orchids. Based on this research gap, this research was conducted to explore the potential of Indonesian orchid root endophytic fungi as bioinoculants in hybrid orchid tissue culture. This study aimed to explore the symbiotic potential of endophytic fungi isolated from the roots of native Indonesian orchids as bioinoculants in hybrid orchid tissue culture.

MATERIALS AND METHODS

Research location and sampling site

The research was conducted at the Botany Laboratory, Universitas PGRI Argopuro, Jember, Indonesia. Fungal identification was performed at the Microbiology Laboratory, Universitas Jember, and at the Sriwijaya Orchid Greenhouse located in Sumbersari District, Jember. Root samples were collected from *Phalaenopsis amabilis* growing vigorously and frequently blooming as epiphytes on mango trees (*Mangifera indica*) in Sumbersari District, Jember (-8.193425 S, 113.721004 E), and from *Dendrobium crumenatum* naturally germinating and growing on rain trees (*Samanea saman*) in Silo District, Jember (-8.236205 S, 113.847580 E). These coordinates represent the natural habitats of *P. amabilis* and *D. crumenatum* in Jember, East Java, Indonesia (Figure 1). For fungal isolation, three root samples were collected from each orchid species. Sample selection was based on the needs of endophytic fungi in roots that play a role in the growth of mature orchid species and their frequent flowering, as well as the needs of endophytic fungi involved in the natural germination of orchid species.

Procedures

Isolation of endophytic fungi

Endophytic fungi were isolated using a modified method of Soelistijono et al. (2017) and Zumri et al. (2017). Fresh roots were cut transversely and observed under a compound microscope at low magnification to confirm the presence of fungal structures within the tissue. Surface sterilization was performed by immersing the root segments in 70% ethanol for 30 seconds, followed by three rinses in sterile distilled water (30 sec each). The samples were then immersed in 0.5% NaOCl for 10 min and rinsed three times with sterile distilled water (1 min each) (Herrera et al. 2010). These modified exposure times followed standard protocols to ensure effective surface sterilization. The roots were cut into 2 cm segments, and five segments were placed onto PDA medium and incubated at 27°C for 7 days. Emerging fungal colonies were subcultured onto fresh PDA plates to obtain pure cultures, which were further incubated at 27°C for 7 days.

Morphological observation

Macroscopic observations were conducted by examining the color and morphology of fungal colonies grown on PDA medium. Microscopic observations were performed by preparing culture slides following the method of Matthapan et al. (2018). Sterile glass slides and V-shaped glass rods were arranged in sterile Petri dishes, after which a 1×1 cm agar block was placed at the center of each slide as the growth substrate. A sterile needle was used to inoculate the fungal colony onto the agar block, and a sterile cover slip was positioned over it. Sterile water was added to the Petri dish to prevent desiccation, and the preparations were incubated at 26°C for 2×24 hours. On the second day, the cover slip was transferred onto a glass slide containing a drop of lactophenol cotton blue for microscopic examination and morphological identification of the fungal species. Observations were carried out using an Olympus CX43 microscope at 400× magnification. This method is appropriate for genus-level identification; however, morphological and microscopic approaches inherently possess limited taxonomic resolution.

In vitro fungal inoculation on Dendrobium *hybrid plantlets*

Hybrid *Dendrobium* plantlets (Ranjaswari × Prigen Indah) germinated and maintained in vitro for one year were used for inoculation assays. Plantlets were carefully removed from culture bottles, washed with sterile distilled water to remove residual medium, and grouped into sets of five per sterile culture bottle. Each bottle represented one replicate, and three replicates were prepared for each treatment. Control plantlets (without inoculation) were maintained under the same conditions.

For fungal treatments, a 5 mm² block of PDA medium containing actively growing fungal mycelium was added to each bottle. Plantlets were incubated under in vitro conditions for one week, after which they were transferred onto sterilized *Platycerium coronarium* root substrates (kadaka) and maintained under controlled growth conditions. This procedure ensured consistency between treatments and allowed the effect of fungal inoculation to be compared against non-inoculated controls.

Histological observation of roots

Colonization of orchid roots by endophytic fungi was assessed using a modified Vierheilig staining method (Vierheilig et al. 1998). Two-centimeter root segments were placed in test tubes and cleared in 10% KOH at 90°C for 1 hour. The samples were rinsed in distilled water, then immersed in 5% acetic acid for 2 hours at room temperature. Following rinsing, the roots were stained in a solution of black ink and lactoglycerol (1:5, v/v) for 2 hours, rinsed again, and destained in lactoglycerol for 24 hours. The presence of pelotons within cortical cells was used as an indicator of symbiotic fungal colonization.

Plantlet growth

Plantlet growth was observed along with the pathogenicity of endophytic root fungi. Pathogenicity was observed for 21 days, while growth was observed for 84 days. Growth was observed in terms of shoot length, new shoot growth, root growth, and new root length.

Data analysis

Growth data of *Dendrobium* plantlets, including the number and length of new shoots and roots, were collected over a 12-week observation period. The data were analyzed quantitatively using one-way analysis of variance (ANOVA) to evaluate the effects of different fungal isolates on plantlet growth. When significant differences were detected, mean comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level to identify the best-performing fungal isolates in promoting growth. All statistical analyses were performed using IBM SPSS Statistics version 27.

Figure 1. A. *Dendrobium crumenatum* in Silo District, B. *Phalaenopsis amabilis* in Sumbersari District, Jember, East Java, Indonesia

RESULTS AND DISCUSSION

Endophytic fungi identification

The results of the study showed that endophytic fungi were successfully isolated and identified from the roots of Indonesian orchid species. The roots of *Phalaenopsis amabilis* and *Dendrobium crumenatum* that had sprouted and grown naturally were collected, and the fungi were then cultivated on PDA medium. Based on the identification, 8 types of fungi were found based on morphological characteristics (Table 1; Figure 2). Microscopic identification of endophytic fungi isolated from the roots of *P. amabilis* and *D. crumenatum* (Table 2).

Histological observation of roots

The formation of pelotons in the root cortex tissue is the main indicator of mycorrhizal symbiosis in orchids. Therefore, histological observation of roots was conducted after inoculation with endophytic fungi to assess the ability of each isolate to form a functional symbiotic relationship. The results of the histological observation can be seen in the following Figure 3.

The effect of inoculation of endophytic fungi on the roots of *Dendrobium* hybrid orchids

Therefore, isolates 2 and 8 were excluded from further growth tests due to their potential to harm the host plant (Figure 4). This underscores the importance of initial screening stages to evaluate the biological safety aspects of endophytic fungi before proposing them as bioinoculants. The results of the pathogenicity tests are presented in Figure 5. The observations indicated that six non-pathogenic isolates (isolates 1, 3, 4, 5, 6, and 7) were selected to evaluate their effects on the growth of *Dendrobium* plantlets. The parameters measured included the number and length of new shoots, as well as the number and length of new roots, monitored over a period of 84 days. The growth of plantlets inoculated with these isolates was observed periodically, and significant differences in several growth parameters were detected, as shown in Table 3. These growth differences are further illustrated in Figure 6. Isolate 4 showed the best results in terms of the number and length of new shoots. The average number and length of new roots were observed in the treatments with isolates 1 and 4. Variations in the effects between isolates. Isolate 4 (*Rhizoctonia* sp.) had the most significant effect on plantlet growth, with the highest number of new shoots (2.75) and the longest shoot length (1.39 cm). Comparison of the effects of isolate 4 and control can be seen in Figure 7.

Discussion

In this study, isolation was performed on the roots of two Indonesian orchid species, *P. amabilis* and *D. crumenatum*, which are known to grow and flower naturally in tropical habitats. A total of eight fungal isolates were successfully cultivated from sterilized root tissue.

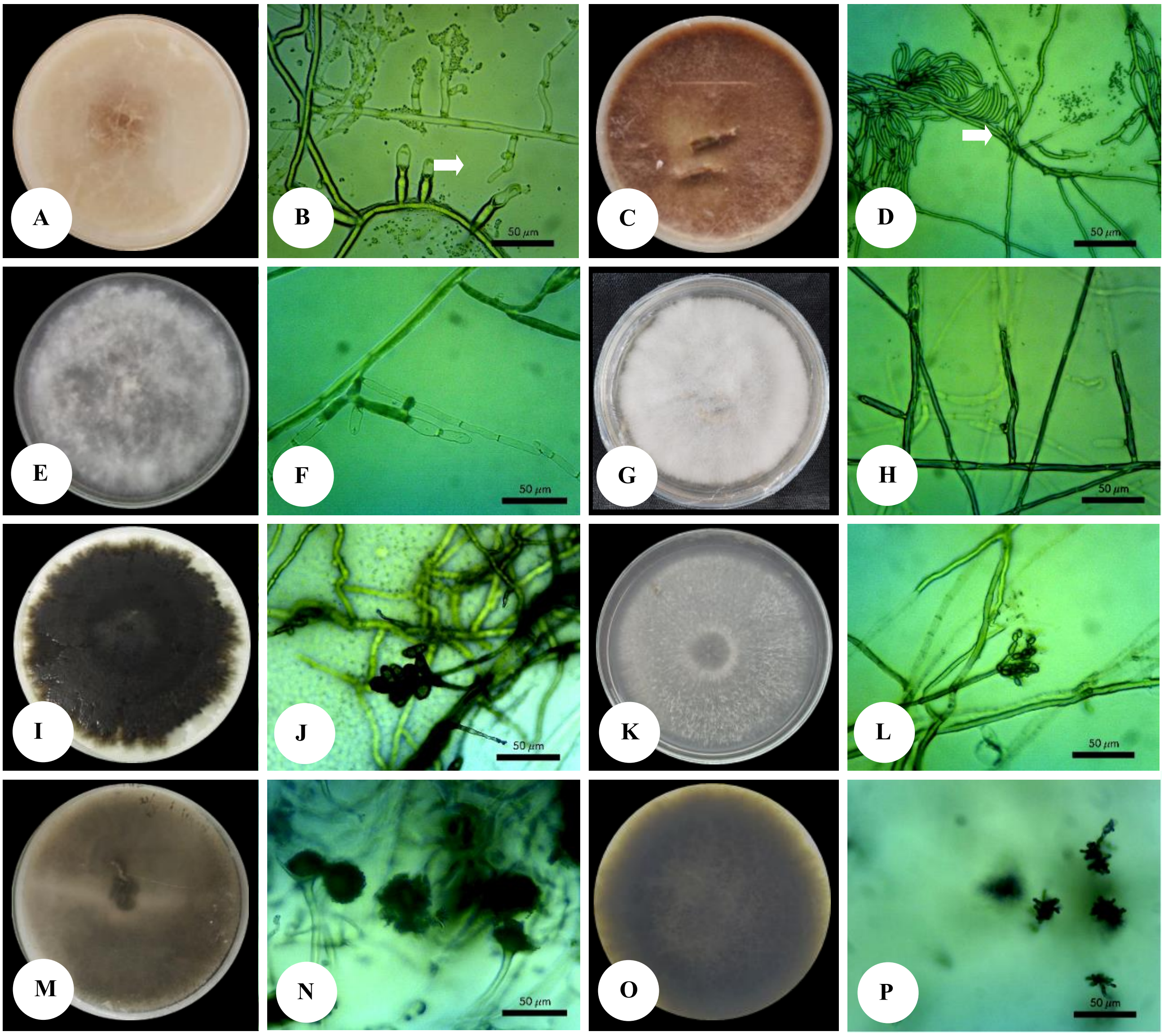

Figure 2. Macroscopic and microscopic morphology of 8 isolates of endophytic fungi from the roots of *P. amabilis* and *D. crumenatum* orchids: A. Macroscopic hyphal colony (*Ceratobasidium* sp.); B. Basidium (white arrow) (*Ceratobasidium* sp.); C. Macroscopic hyphal colony (*Fusarium* sp.); D. Microscopic crescent-shaped conidia (white arrow) (*Fusarium* sp.); E. Macroscopic hyphal colony (*Rhizoctonia* sp.); F. Hyphae 900 (white arrow) (*Rhizoctonia* sp.); G. Macroscopic hyphal colony (*Rhizoctonia* sp.); H. Hyphae 900 (white arrow) (*Rhizoctonia* sp.); I. Macroscopic hyphal colonies (*Curvularia* sp.); J. Microscopic conidia (white arrow) *Curvularia* sp.; K. Macroscopic hyphal colonies (*Tulasnella* sp.); L. Microscopic conidia (white arrow) (*Tulasnella* sp.); M. Macroscopic hyphal colony (*Rhizopus* sp.); N. Microscopic conidia (white arrow) (*Rhizopus* sp); O. Macroscopic hyphal colony (*Cladosporium* sp.); P. Microscopic conidia (white arrow) (*Cladosporium* sp.)

Table 1. Morphological colonies on PDA of endophytic fungi isolated from the roots of *Phalaenopsis amabilis* and *Dendrobium crumenatum*

Host	Isolate/Character	Colony color	Reserve colony	Growing zone
Phalaenopsis amabilis	Isolate 1	White to pale cream	White with a hint of pink	None
	Isolate 2	White to pink	Pink	Concentric growth
	Isolate 3	White	Yellowish white	None
Dendrobium crumenatum	Isolate 4	White	White with a hint of orange	None
	Isolate 5	Blackish-gray	Black	Concentric growth
	Isolate 6	White	Yellowish white	None
	Isolate 7	Dark gray	Dark gray	None
	Isolate 8	Black	Black	Concentric growth

Table 2. Microscopic identification of endophytic fungi isolated from the roots of *P. amabilis* and *D. crumenatum*

Isolate	Colony Surface	Shape of conidia/ Sporangium	Shape of phialid	Radial furrow	Hyphae	Genus	Potential
Isolate 1	Soft and fluffy like cotton	-	-	-	Septate	*Ceratobasidium*	Symbiotic
Isolate 2	Cottony and fluffy	Simple, cylindrical to flask-shaped	Simple, cylindrical to flask-shaped	Present	Septate	*Fusarium*	Pathogenic
Isolate 3	Soft and fluffy like cotton	-	-	-	Septate with characteristic branching at approximately 90°	*Rhizoctonia*	Symbiotic
Isolate 4	Fluffy like cotton	-	-	-	Septate with characteristic branching at approximately 90°	*Rhizoctonia*	Symbiotic
Isolate 5	Velvety to floccose	Curved or crescent-shaped	Cylindrical, Conidiophores are erect	Present	Septate	*Curvularia*	Symbiotic
Isolate 6	Soft and fluffy cotton	-	-	-	Septate	*Tulasnella*	Symbiotic
Isolate 7	Fluffy, cottony	Round sporangia borne on upright sporangiophores	-	-	Aseptate	*Rhizopus*	Symbiotic
Isolate 8	Velvety	Ovoid/ Elongated	Cylindrical	Present	Septate	*Cladosporium*	Pathogenic

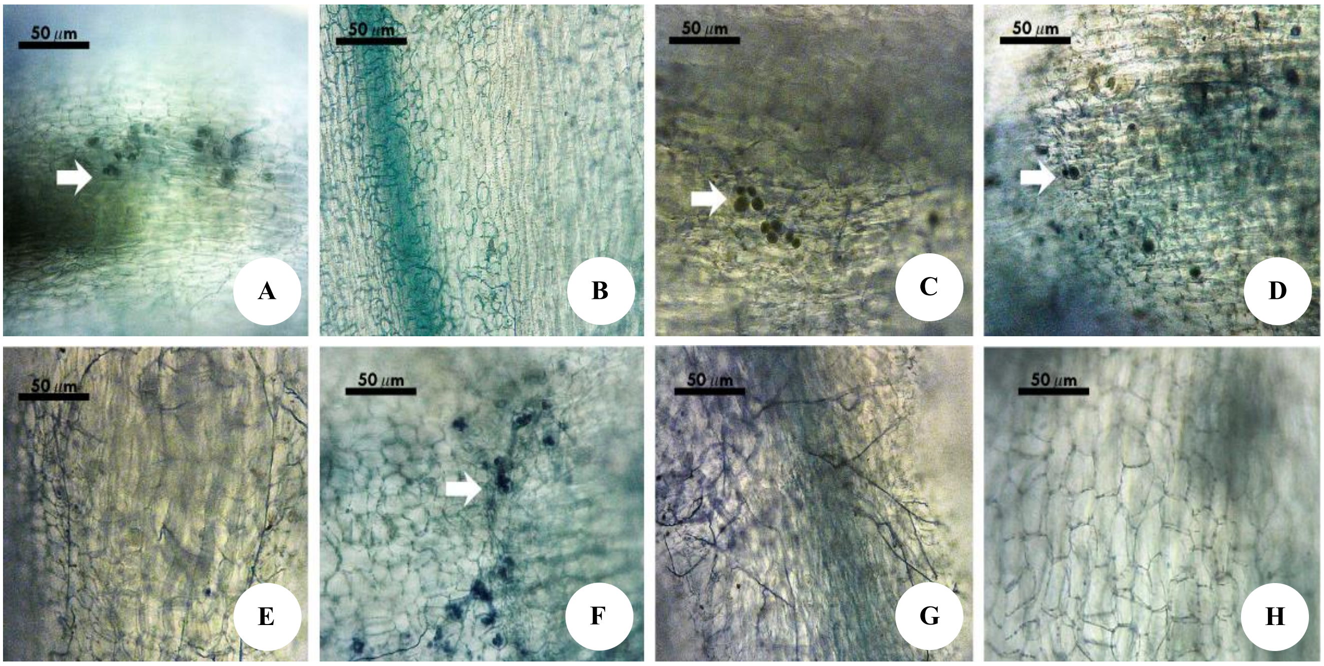

Figure 3. Histology of roots after inoculation with isolates 1 to 8, in order from A to H. Roots containing peloton are visible in roots with bioinoculants isolates 1, 3, 4, and 6 (white arrow)

Characterization was conducted using two approaches: macroscopic and microscopic, to obtain an initial identification of the fungal genus found. Macroscopic observations were conducted by assessing colony morphology on PDA medium, including macroscopic vegetative characteristics such as colony color, reserve color, colony surface texture, presence of a growing zone, and radial furrow patterns (dos Reis et al. 2022; Wulandari et al. 2025). Microscopically, observations focused on the structure and color of hyphae, the presence of monilioid cells, branching patterns, the shape and color of conidia, and the presence of phialides (Bhuiyan et al. 2021; Eldina et al. 2021). These characteristics serve as indicators to reinforce genus identification.

These microscopic morphological differences are the main basis for determining the genus of the fungus in question. A single-cell monilioid isolate after 15 days of culture (Decruse et al. 2018) is morphologically similar to *Ceratobasidium*. *Ceratobasidium* colonies on culture media such as PDA typically grow as thin, spreading colonies, white to cream in color, and appear smooth or slightly hairy depending on the isolate. These colonies usually do not exhibit prominent coloration and do not form distinct sexual reproductive structures in culture. Their hyphae are septate, hyaline, and branch at angles approaching 90°, with relatively small diameters. *Ceratobasidium* hyphae also have distinctive features, including dolipore septa with imperforate parenthesomes and the absence of clamp connections, distinguishing them from most other Basidiomycota fungi (Li et al. 2025). *Ceratobasidium* is the teleomorphic form (sexual phase), so basidia are present on its hyphae, a characteristic of the Basidiomycota phylum (Rungjindamai and Jones 2024).

Isolate 2 is *Fusarium* sp. The genus *Fusarium* is characterized by septate conidia that are fusiform or crescent-shaped, known as macroconidia. These conidia have a basal cell shaped like a foot and an apical tip resembling a beak. Macroconidia can form in separate pustular structures called sporodochia or in cohesive mucilaginous masses known as pionnots. The spore clusters from these structures typically appear in cream, salmon pink, or orange colors (Pitt and Hocking 2013).

Isolates 3 and 4, which do not produce conidia but have septate hyphae and characteristic colony structures, were identified as *Rhizoctonia* sp. *Rhizoctonia* is a group of fungi with fairly specific morphological characteristics that are easily recognizable under a microscope. Their hyphae are septate and typically branch at obtuse angles, approximately 90°, with a relatively large diameter compared to other fungi. Near the hyphal branching points, there are usually characteristic transverse septa. One important characteristic of *Rhizoctonia* is that it does not form asexual spores (conidia) under normal conditions, so it is often referred to as sterile mycelium.

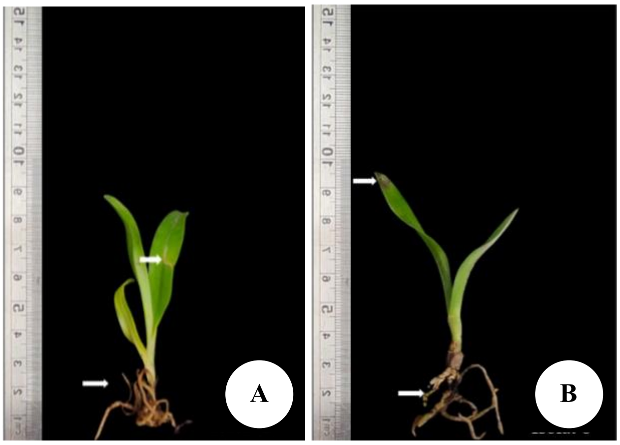

Figure 4. Symptoms of disease caused by A. Isolate 2 and B. Isolate 8 on orchid plants. Roots and leaves are rotten (white arrows)

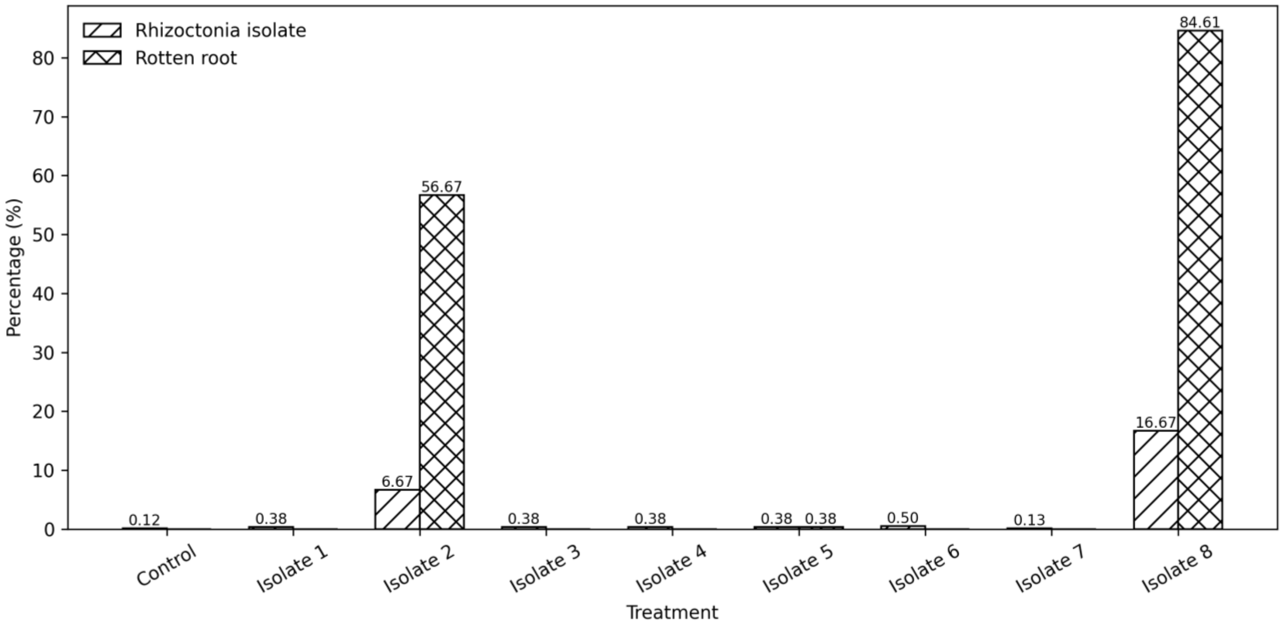

Figure 5. Pathogenicity test results of endophytic root fungi on *Dendrobium* hybrid orchid plantlets, showing the percentage of rotten leaves and rotten roots after inoculation. From the control to Isolate 8, most isolates showed minimal pathogenic effects; however, Isolate 2 and Isolate 8 exhibited notably high percentages of root and leaf rot, indicating potential pathogenicity

Table 3. Effect of endophytic root fungal isolates on the growth parameters of *Dendrobium* hybrid plantlets after 84 days of inoculation

Treatment	Number of new shoots	Length of new shoots (cm)	Number of new roots	New root length (cm)
Control	1.00±0.00^{c}	0.11±0.05^{d}	0.00±0.00^{c}	0.13±0.04^{c}
Isolate 1	1.75±0.29^{b}	0.50±0.10^{bc}	0.75±0.25^{a}	0.20±0.06^{b}
Isolate 3	2.75±0.35^{a}	1.39±0.22^{a}	0.50±0.29^{b}	0.50±0.08^{a}
Isolate 4	1.50±0.28^{b}	0.32±0.07^{cd}	0.25±0.18^{b}	0.38±0.05^{a}
Isolate 5	1.75±0.25^{b}	0.36±0.09^{bc}	0.75±0.20^{a}	0.38±0.06^{a}
Isolate 6	1.00±0.00^{c}	0.25±0.08^{cd}	0.25±0.10^{b}	0.38±0.04^{a}
Isolate 7	1.25±0.29^{c}	0.58±0.11^{b}	0.25±0.14^{b}	0.38±0.05^{a}

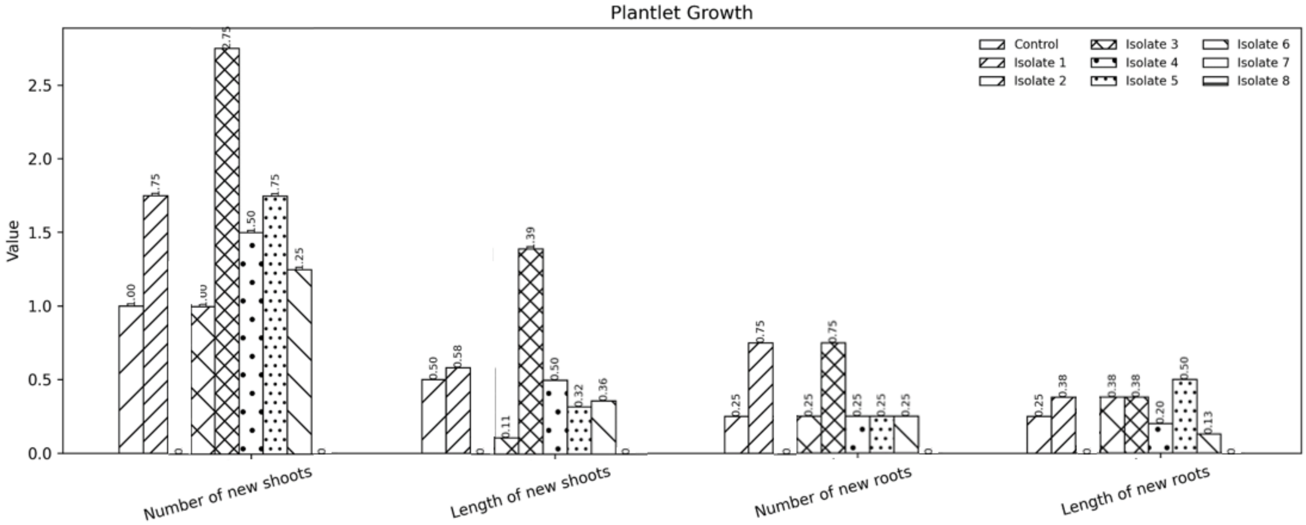

Figure 6. Growth of *Dendrobium* hybrid orchid plantlets at day 84 after inoculation with non-pathogenic endophytic root fungal isolates (Isolates 1, 2, 3, 4, 5, 6, 7, and 8) compared to the control. Growth parameters observed include the number and length of new shoots, the number of new roots, and the length of new roots

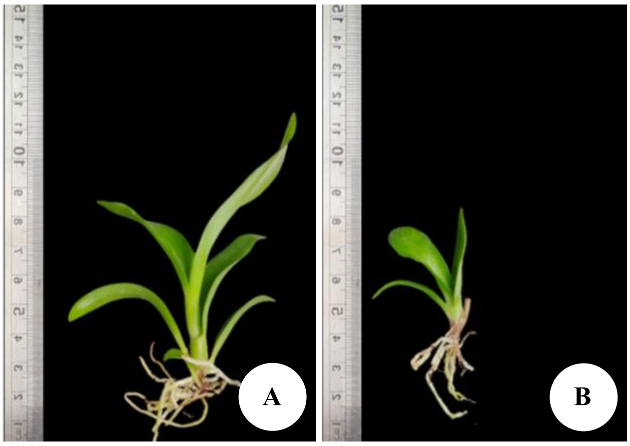

Figure 7. Growth of orchid plantlets following A. inoculation with endophytic isolate 4, compared to B. the uninoculated control

Additionally, each hyphal cell contains more than one nucleus (multinucleate), which is an important characteristic for further identification (Wang et al. 2023; Sayem et al. 2024). During its life cycle, *Rhizoctonia* can also form a survival structure called sclerotia, which are dense masses ranging in color from brown to black, enabling the fungus to survive under unfavorable environmental conditions (Suryantini et al. 2015).

By comparison, isolate 5 showed black ovoid conidia without phialides and septate hyphae, which are consistent with the features of *Curvularia* sp. The colonies were dark in color and exhibited velvety mycelium growth on the surface of the medium (Priwiratama et al. 2024). Conidiophores are erect, straight to flexible, septate, often geniculate (producing conidia sympodially), and occasionally nodulose. Conidia are elliptical, often curved or crescent-shaped, rounded at the tip or occasionally slightly tapering toward the base, pale brown, medium reddish-brown to dark brown, with 3-10 (usually 3-5) septa, and smooth to dotted conidial walls. The hilum is prominent in some species (Heidari et al. 2018; Priwiratama et al. 2024).

Isolate 6 has septate hyphae, morphologically resembling *Tulasnella* sp. Its septate hyphae have two nuclei per cell, lack clamp connections, and feature dolipore septal pore structures with non-perforated parenthesomes. The colony is white to cream-colored and grows uniformly on PDA medium (Ma et al. 2024). Another important characteristic is the formation of monilioid cell chains, whose shape and branching pattern form the basis for morphotype classification. These monilioid cells can be globose, ellipsoidal, barrel-shaped, or subglobose and form branched chains with varying numbers of cells. Some isolates produce masses containing monilioid chains in the medium, referred to as "mass clusters of monilioid cell chains." (Fujimori et al. 2019).

Isolate 7 was identified as *Rhizopus* sp. *Rhizopus* fungi have aseptate hyphae and are coenocytic, meaning they contain multiple nuclei within a single cytoplasm without intercellular wall separators. Its colonies are white-grayish in color at the beginning of growth, then turn black when the spores have matured. Its reproductive structure consists of a sporangium, which is a round sac containing spores (sporangiospores) located at the tip of the sporangiophore or spore stalk. The sporangiophore grows upright from the mycelium tissue. Additionally, *Rhizopus* has rhizoids, root-like structures that attach to the substrate and absorb nutrients, as well as stolons, horizontal hyphae that connect one sporangiophore to another.

Isolate 8, which has non-septate hyphae and round black conidia, was identified as *Cladosporium* sp. (Pereira et al. 2022). *Cladosporium* colonies are known to grow rapidly and form dense colonies with characteristic color variations, such as olive green, brownish green, or grayish black. The surface of the colony may appear velvety, powdery, or resemble fine cotton, depending on the species and growth conditions. Meanwhile, the underside of the colony is generally darker due to the production of melanin pigment, which is also a characteristic of this dematiaceous fungus group. Microscopically, *Cladosporium* has septate hyphae and is grayish-brown in color, belonging to the dematiaceous fungi group due to the presence of melanin pigments in its cell walls. This fungus produces conidiophores, which are spore-producing structures that grow upright, branched, and have a rough surface. The conidia produced are elongated to cylindrical in shape, septate, and arranged in long, fragile chains.

In this study, histological observations were performed on *Dendrobium* hybrid plantlets inoculated with eight endophytic fungal isolates. Cross-section root preparations were observed under a microscope to identify the presence of pelotons. The results showed that pelotons formed clearly in the roots of plantlets inoculated with isolates 1, 3, 4, and 6, while isolates 2, 5, 7, and 8 did not show peloton formation in the cortex tissue. This indicates that only four of the eight isolates successfully formed the characteristic structural association of orchid mycorrhiza.

The isolates that formed the peloton consisted of isolates 1, 3, 4, and 6, which were identified as *Ceratobasidium* sp., *Rhizoctonia* sp., and *Tulasnella* sp., which are widely known as typical orchid mycorrhizae. The formation of the peloton by these four isolates is consistent with previous literature, reinforcing the evidence that these species are the main symbionts in tropical orchids. These results suggest a possible adaptive role as endophytes capable of forming non-pathogenic symbiotic relationships with host plants.

In contrast, isolate 2 (*Fusarium* sp.), isolate 5 (*Curvularia* sp.), isolate 7 (*Rhizopus* sp.), and isolate 8 (*Cladosporium* sp.) did not show any peloton formation. This may indicate that although these isolates are capable of colonizing root surfaces or outer tissues, they do not progress to the intracellular infection stage that is mutually beneficial. Therefore, these isolates cannot be categorized as functional mycorrhizae at this stage of observation.

Overall, these histological results confirm that not all endophytic fungi successfully isolated have the ability to form pelotons. Only certain isolates are capable of forming true symbiotic relationships through this characteristic structure. Therefore, the presence of pelotons can serve as an important indicator in selecting potential endophytic fungi for use as bioinoculants for orchids, both in in vitro cultivation and ex situ conservation.

Inoculation of endophytic fungi on hybrid orchid plantlets is a strategy aimed at identifying potential root symbionts capable of enhancing plant growth in vitro. In this study, eight previously identified endophytic fungal isolates were inoculated into *Dendrobium* hybrid plantlets resulting from the cross between Ranjaswari × Prigen Indah. The impact was evaluated using two approaches: pathogenicity assessment and plantlet growth parameters.

The eight fungal isolates were inoculated into hybrid *Dendrobium* plantlets derived from tissue culture. During the inoculation process, pathogenicity tests were conducted to evaluate whether the isolates could cause disease in the host plants. The test results showed that isolates 2 and 8 caused root and leaf rot. The observed root rot rate in this study reached 56.67% for isolate 2 and 84.61% for isolate 8, while the leaf rot rates were 6.67% and 16.67%, respectively. In addition, *Fusarium* infection in *Dendrobium chrysotoxum* has been reported to induce tip necrosis and leaf desiccation as early as seven days after planting, with disease incidence reaching 60%. Such infections severely restrict seedling growth and can intensify under warm and humid conditions, ultimately spreading to as much as 80% of the plants (Yang et al. 2024).

Based on morphological identification, both isolates belong to the genera *Fusarium* sp. and *Cladosporium* sp., which are widely known as plant pathogens. There is research reporting that *F. oxysporum* KB-3 can behave as a mycorrhizal fungus of the orchid *Bletilla striata* (Jiang et al. 2019). Meanwhile, *Fusarium* as a pathogen is consistent with previous reports stating that some *Fusarium* strains are pseudo-endophytic but can become opportunistic pathogens depending on host and environmental conditions (Sarsaiya et al. 2020; Li et al. 2021). Similarly, *Cladosporium* is pathogenic to plants (El-Dawy et al. 2021).

The absence of visible pathogenic symptoms in plants may be attributed to several factors, one of which is the presence of antagonistic fungal species that suppress pathogen development, allowing the plants to remain healthy (Sofian et al. 2025). This is consistent with findings by Matloob et al. (2020), who reported that the combination of *Glomus intraradices* and *Pseudomonas fluorescens* significantly reduced the severity of *Fusarium chlamydosporum*-induced disease by up to 13.3% and enhanced root and shoot length. The application of both single and combined bioagents provided effective protection against wilt disease, comparable to the efficacy of chemical fungicides such as Beltanol. Similarly, endophytic fungi such as *Aspergillus niger*, *Penicillium chermesinum*, and *Talaromyces verruculosus* demonstrated strong antagonistic activity against *Fusarium* species, with inhibition levels reaching up to 61.79% and increasing with higher filtrate concentrations, highlighting their potential as biological control agents (Nuaimy and Hawar 2024).

In addition, this isolate also increased the number and length of new roots. This effect is likely due to *Rhizoctonia's* ability to form efficient pelotons in the root cortex tissue, thereby improving nutrient absorption. Isolates 1 and 6 (*Ceratobasidium* sp. and *Tulasnella* sp.) also showed moderate increases in the number and length of shoots, but still below isolate 4. Isolates 3 (*Rhizoctonia* sp.), 5 (*Curvularia* sp.), and 7 (*Rhizopus* sp.) showed lower effects, with growth parameter values similar to the control.

In general, plantlets inoculated with certain endophytic fungi showed better growth than controls (without inoculation), indicating that most isolates have potential as symbiotic agents that support the vegetative regeneration of hybrid orchids.

Orchid plantlets inoculated with specific endophytic fungi exhibited significantly improved growth compared to non-inoculated controls, indicating that most isolates have potential as effective symbiotic agents for supporting the vegetative regeneration of hybrid orchids. These findings are consistent with previous studies showing that biofertilizers such as arbuscular mycorrhizal fungi, *Candida tropicalis*, and *Trichoderma* enhance plant growth by increasing root length, dry weight, stem number, leaf area, and chlorophyll content. In addition, biofertilizer application has been shown to improve plant resistance to pathogens by boosting defense-related enzyme activities (polyphenol oxidase and peroxidase) and total phenolic content, while also enhancing phosphorus uptake biologically and promoting sustainable agriculture through reduced use of chemical fertilizers (Aljuboori et al. 2022; Al-Zaidi an Al-Mharib 2025; Risman and Sungthongwises 2025).

The combination of macroscopic and microscopic characteristics is very important in ensuring the initial classification of endophytic fungi. The discovery of genus variations such as *Curvularia*, *Fusarium*, *Rhizoctonia*, *Ceratobasidium*, *Tulasnella*, *Rhizopus*, and *Cladosporium* shows the diversity of endophytic fungal communities that can be found in the roots of tropical orchids. However, not all endophytic fungi are mutualistic. For example, two isolates identified as *Fusarium* sp. and *Cladosporium* sp. exhibit pathogenic properties toward *Dendrobium* plantlets, characterized by root and leaf decay.

Conversely, some isolates that do not show pathogenic symptoms have the potential to be used as bioinoculants in in vitro culture, especially isolates from the genera *Rhizoctonia*, *Ceratobasidium*, and *Tulasnella*, which show a positive contribution to orchid growth parameters.

In conclusion, this study successfully isolated eight endophytic fungi from the roots of *Phalaenopsis amabilis* and *Dendrobium crumenatum*, of which four isolates—*Ceratobasidium* (1), *Rhizoctonia* (3 and 4), and *Tulasnella* (6)—formed pelotons in the root cortex, confirming their symbiotic compatibility. Pathogenicity tests showed that isolates 2 (*Fusarium*) and 8 (*Cladosporium*) induced severe root and leaf rot (up to 84.61%), while the remaining six isolates were non-pathogenic. Growth assays demonstrated that *Rhizoctonia* isolate 4 produced the strongest response, increasing shoot number by 175% (2.75 vs. 1.00 in control) and shoot length more than tenfold (1.39 cm vs. 0.11 cm), along with substantial improvements in root formation. These results indicate that locally sourced endophytic fungi, particularly *Rhizoctonia* isolate 4, possess significant potential as bioinoculants for enhancing early growth of *Dendrobium* hybrids.

This study was limited by the use of a single hybrid host, a relatively small isolate set, and morphological rather than molecular confirmation for all fungal identities; therefore, future research should include multi-host evaluations, molecular and genomic identification, and greenhouse or field trials to verify long-term symbiotic performance and strengthen the development of reliable indigenous fungal bioinoculants for orchid cultivation. Collectively, this study provides one of the first integrated evaluations of endophytic fungi from native Indonesian orchids using both histological confirmation and growth assays, offering new insights into their symbiotic potential and establishing a foundation for broader application in orchid cultivation.

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