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Diversity of Endophytic Fungi in Leaves of Oil Palm *(Elaeis guineensis Jacq.)* Non-Infected Ganoderma (*Ganoderma boninense*)

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Abstract

Oil palm (*Elaeis guineensis* Jacq.) is a strategic plantation commodity that plays an important role in the Indonesian economy. The productivity of this plant is influenced by the health of its tissues and the presence of microorganisms that naturally inhabit it. This study aims to identify the types of fungi present in the foliar tissue of healthy plants in the PTPN IV Gunung Bayu Unit area. The research used a descriptive qualitative method, involving isolation on Potato Dextrose Agar (PDA) media, followed by macroscopic and microscopic observations. We sterilized samples with ethanol and NaOCl, then incubated them for about seven days at 28°C before purifying the cultures. We identified isolates based on colony characteristics and microscopic structures using lactophenol cotton blue dye. The results revealed four isolates, HB, HT, HM, and HG, with distinct morphological features. The HM isolate was identified as being similar to Aspergillus sp. This study's strength lies in documenting several microorganisms associated with healthy plants that could serve as sources of biological agents to enhance plant resilience. The findings provide a basis for further research, including antagonist testing and the development of environmentally friendly disease control strategies in oil palm plantations.

Keywords: Biological Control, Colony Morphology, Fungal Isolation, Microorganisms, Plant Health

1. Introduction

Indonesia is known as a major global producer of palm oil, with plantations spread across regions such as Sumatra, Kalimantan, Sulawesi, and Papua. According to data from the Central Statistics Agency (BPS) in 2022, Indonesia's oil palm plantations covered more than 14.62 million hectares, with palm oil production exceeding 45.12 million tons (Masitah et al., 2023). This commodity plays a vital role in the national economy, serving as a source of foreign exchange through exports and providing employment for millions of people in the agribusiness sector. However, this significant contribution comes with serious challenges in maintaining the productivity and sustainability of oil palm plantations (Abdillah et al., 2025).

One of the main problems in oil palm cultivation is the attack of basal stem rot disease caused by the fungus *Ganoderma boninense*. (Eltivitasari, 2021). This pathogen attacks the roots and base of the stem, disrupting water and nutrient absorption, ultimately leading to gradual plant death. (Halo, 2026) . This disease poses a serious threat because it is difficult to control, can persist in the soil for a long time, and can reinfect new plants through contaminated root remains. *Ganoderma boninense attacks* can cause plant mortality rates of up to 50–80% in heavily infested areas and reduce fresh fruit bunch production by 40–80%, depending on infestation level, plant age, and environmental conditions. These conditions result in decreased plantation productivity and cause significant economic losses for oil palm plantation operators (Saragih et al., 2023).

Efforts to control this disease have largely relied on chemical fungicides (Jha et al., 2023). However, the use of these chemicals is often ineffective in the long term, can pollute the environment, and can trigger pathogen resistance (Juariyah et al., 2018). This heavy reliance on chemical fungicides makes Ganoderma boninense increasingly difficult to control, necessitating alternative, more environmentally friendly and sustainable approaches

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to maintain the productivity of oil palm plantations in Indonesia (Khoirunnisa, 2023).

One emerging approach is the use of endophytic microorganisms as biological control agents (Kristianingrum et al., 2024). Endophytic fungi are microorganisms that live within plant tissues without causing disease symptoms and generally have a mutualistic relationship with their hosts (Mendrofa, 2021). These fungi can produce various bioactive compounds such as alkaloids, terpenoids, and phenolics that play a role in increasing plant resistance to pathogens (Ali & Samosir, 2022). Endophytes can also act through direct antagonism against pathogens or of inducing systemic resistance in plants, potentially serving as an alternative to chemical fungicides (Rianto et al., 2018).

Previous research has shown that endophytic fungi have great potential as biocontrol agents and producers of secondary metabolites that are beneficial to plants. Azim 92021) reported that endophytic fungi isolated from various plants are capable of producing antimicrobial compounds that play a role in inhibiting the growth of pathogenic microorganisms. Research (Azzahra et al., 2022) found that several endophytic fungal isolates have antagonistic activity against plant pathogens through mechanisms of competition for space, competition for nutrients, and production of inhibitory metabolites. Furthermore, Chusniasih et al. (2024) reported that endophytic fungi from healthy plant tissue exhibit high morphological diversity and can produce bioactive compounds that support plant growth and resistance to disease attacks. These results indicate that endophytic fungi can be used as environmentally friendly biological control agents in plant cultivation systems (Isti et al., 2024).

However, research on the diversity of endophytic fungi in oil palm (Elaeis guineensis Jacq.) leaves that are not attacked by Ganoderma boninense remains limited, especially in oil palm plantations at PTPN IV Gunung Bayu Unit. Most previous studies have focused more on testing the activity of endophytic fungi or identifying endophytic fungi in plants other than oil palm (Basyar & Qomar, 2023). Therefore, the novelty of this study lies in the identification and characterization of the diversity of endophytic fungi found in healthy oil palm leaves that do not show symptoms of *Ganoderma boninense attack*. The information obtained is expected to form the basis for developing further research on the use of local endophytic fungi as biological control agents to support the health and productivity of oil palm plants (Sihaan & Khoiri, 2023).

Based on these issues, studying the diversity of endophytic fungi on oil palm leaves is crucial as a potential and sustainable biological control approach. This study aims to identify endophytic fungi on oil palm (Elaeis guineensis Jacq.) leaves that are asymptomatic of basal stem rot disease. The research results are expected to contribute scientifically to the development of endophytic microorganism-based biological control strategies and support a more environmentally friendly and sustainable oil palm cultivation system.

2. Material and Methods

This research was conducted at PTPN IV Gunung Bayu Unit, Simalungun Regency, North Sumatra, from January to March 2026. The research location is at coordinates around 3°12'–3°20' North Latitude and 99°10'–99°20' East Longitude, with an altitude of approximately 50–100 meters above sea level. This study used a qualitative descriptive method to observe and describe the variation of endophytic fungi in healthy oil palm (Elaeis guineensis) leaves, or those without symptoms of Ganoderma boninense infection. This approach was chosen because it provides a natural picture of fungal morphology without experimental treatment (Hardiansyah, 2018) and is suitable for exploratory microbiological research in identify the characteristics of microorganisms macroscopically and microscopically (Mendrofa et al., 2021).

The research began with a preliminary study and preparation of tools, materials, and healthy oil palm leaf samples. of tools and samples were sterilized using an autoclave, 70% ethanol, and NaOCl to prevent contamination. Fungal isolation was carried out aseptically on Potato Dextrose Agar (PDA) plates using a laminar airflow hood, then incubated at 28°C for ±7 days and continued with isolation and purification through subculture. Observations included the number of colonies and macroscopic and microscopic characteristics using a light microscope. Observation data were documented and processed in Microsoft Excel 2021 and Microsoft Word 2021, then analyzed descriptively by comparing fungal morphological characteristics with identification literature by Watanabe (2002) and Barnett and Hunter (1998).

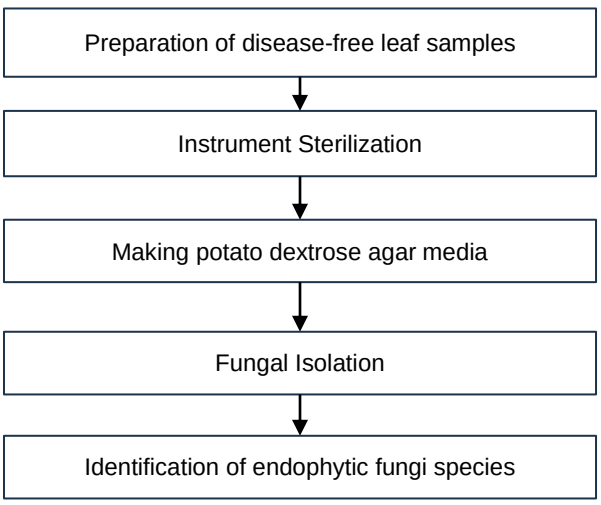


Figure 1. Research Flow Diagram

3. Results and Discussion

3.1. Field Observation Results

3.1.1. Results of Weed Vegetation Identification in Adolina

Based on the results of weed vegetation identification at PT Perkebunan Nusantara IV Regional II Adolina Afdeling II, 6 types of weeds were found, which were classified into three groups, namely broadleaf weeds, grasses, and sedges. The weed composition shows that the grass and sedge groups are dominant, with the most frequently found types being Lophaterum gracile and Kyllinga nemoralis. The dominance of these two types of weeds indicates that the environmental conditions at the research location are quite supportive of their growth, which is characterized by adequate water availability, relatively high soil moisture, and sufficient sunlight intensity to support vegetation development. The presence of relatively shaded areas on the ground surface also supports the growth of certain types of weeds, causing the distribution of weeds at the research location to be quite diverse.

3.1.2. Overview of Research Location

Research on the diversity of endophytic fungi in oil palm (Elaeis guineensis Jacq.) leaves that were not attacked by Ganoderma was conducted at PTPN IV Gunung Bayu Unit, Simalungun Regency, North Sumatra. This location is one of the oil palm plantation units that has a large planting area with environmental conditions that support the growth of various microorganisms, including endophytic fungi. The samples were healthy oil palm leaves that showed no symptoms of Ganoderma sp. attack, with normal plant physical condition and no symptoms of basal stem rot.

The research involved leaf sampling, tissue surface sterilization, endophytic fungal isolation on Potato Dextrose Agar (PDA) medium, isolate purification, and macroscopic and microscopic morphological observations. A descriptive approach was used to describe the characteristics of the endophytic fungi found, referring to microorganism identification methods commonly used in microbiological research (Hardiansyah, 2018; Mendrofa et al., 2021).

3.1.3. Data Collection and Processing Process

Data collection was conducted through laboratory observations and direct observation of healthy oil palm leaf samples that showed no symptoms of Ganoderma infection. The study was conducted from January to March 2026 at PTPN IV Gunung Bayu Unit, Simalungun Regency, North Sumatra, and continued at the Biology Laboratory, Faculty of Agriculture, University of North Sumatra. Leaf samples were purposively selected based on healthy plant condition and stored in sterile containers to prevent contamination during transport to the laboratory.

In the laboratory, leaf samples underwent surface sterilization using 70% ethanol, 2.5% NaOCl solution, and sterile distilled water. Next, the leaves were cut into approximately 1 cm × 1 cm pieces and inoculated onto Potato Dextrose Agar (PDA) medium. The cultures were incubated at approximately 28°C for seven days, then the growing fungal colonies were purified through subculture to obtain pure isolates. We assigned each isolate an identification code and monitored its growth periodically.

We obtained research data through macroscopic and microscopic observations. Macroscopic observations included color, shape, texture, diameter, and the color of the underside of the colony. Microscopic observations were conducted using a binocular microscope with Lactophenol Cotton Blue stain to observe the structure of hyphae, conidiophores, conidia, and spores. All observations were documented and analyzed descriptively by comparing the morphological characteristics of the isolates with fungal identification literature to determine the type and diversity of endophytic fungi found (Hardiansyah, 2018; Mendrofa et al., 2021).

3.2. Research Findings

3.2.1. Endophytic Fungal Isolation Results

The results of the isolation of endophytic fungi from healthy oil palm leaves (Elaeis guineensis Jacq.) that did not show symptoms of Ganoderma attack successfully obtained four isolates with different morphological characteristics. Identification based on macroscopic and microscopic observations showed that one isolate belonged to the genus *Penicillium* and the other three isolates belonged to the genus *Aspergillus*. These results indicate that healthy oil palm leaves host a variety of endophytic fungi that live symbiotically without causing disease symptoms in their host plants.

Table 1. Results of Endophytic Fungus Isolation 1

No	Isolate Code	Information
1	HB	*Penicillium sp*
2	HT	*Aspergillus sp*
3	HM	*Aspergillus sp*
4	HG	*Aspergillus sp*

Description: HB, HT, HM, and HG are codes for endophytic fungal isolates from healthy oil palm leaves (*Elaeis guineensis* Jacq.). Each isolate was observed in 4 replicates on PDA medium.

Macroscopic observations showed differences in morphological characteristics in each endophytic fungal isolate. Isolate HB (*Penicillium* sp.) has a greenish-gray colony with a white edge and a cotton-like texture, while isolates HT, HM, and HG (*Aspergillus* sp.) showed a dark green to black color with a floury or velvety texture due to the formation of abundant conidia. These characteristics are in accordance with the results of research (Suhartina, 2018) which identified *Aspergillus* as one of the endophytic fungi with green to blackish colonies and a floury texture. Quantitatively, this study obtained 4 isolates consisting of 3 *Aspergillus isolates* (75%) and 1 *Penicillium isolate* (25%). In comparison, Muhammad Abrar (2025) on oil palm roots succeeded in obtaining 30 endophytic fungal isolates

belonging to 6 genera, including *Aspergillus* and *Penicillium*. The dominance *of Aspergillus* in this study indicates that the genus adapts well in oil palm plant tissues, as also found in various studies of endophytic fungi in plantation plants (Disease & Nigrum, 2023).

Table 2. Percentage of Endophytic Fungal Genus 1

Mushroom Genus	Number of Isolates	Percentage (100%)
Aspergillus sp.	3	75%
Penicillium sp.	1	25%
Total	4	100%

The percentage of the genus was calculated based on the number of isolates obtained from a total of 4 endophytic fungal isolates that were successfully isolated from healthy oil palm leaves (*Elaeis guineensis* Jacq.) that were not infected with *Ganoderma*.

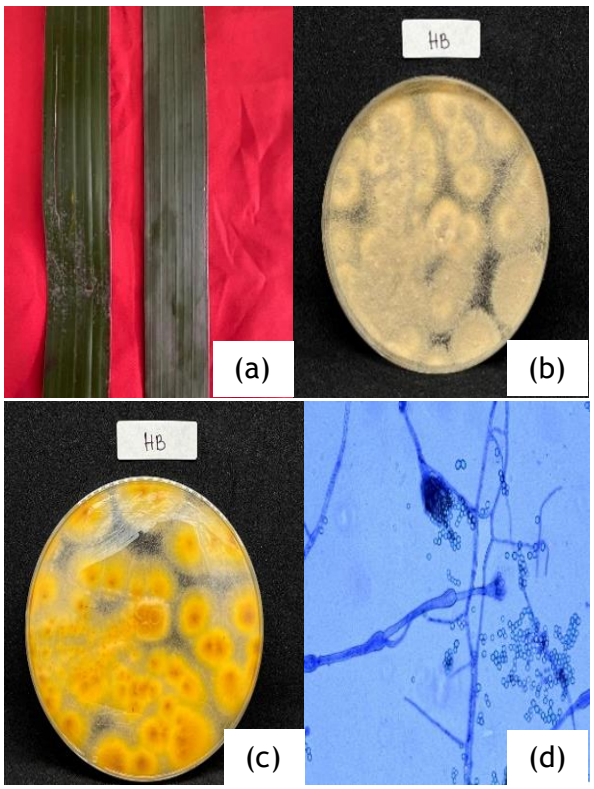


Figure 2. Penicillium: (a) Oil palm leaf (b) top surface isolate image (c) bottom surface isolate image (d) microscopic

The image shows the results of isolating endophytic fungi from healthy oil palm leaves coded HB:

a. Healthy oil palm leaf samples: Oil palm leaves used as samples were selected in healthy condition and did not show any symptoms of disease, so that the isolated fungi were confirmed to originate from the internal tissue of the plant as endophytic fungi.
b. HB isolate colony morphology (top view): HB isolates on PDA medium form round, gray-green to blackish colonies in the center with white edges. The colonies have a cotton-like texture, indicating good mycelial growth.
c. Colony morphology of HB isolate (bottom view): The underside of the colony shows a yellow to brownish pigment spread across the medium. This pigment characteristic is one of the indicators in the fungal identification process.
d. Microscopic morphology of HB isolates: Microscopic observations revealed the presence of branching septate hyphae and clusters of spores at the tips of the hyphae. These structures indicate the ability of HB isolates to reproduce asexually through the formation of conidia.

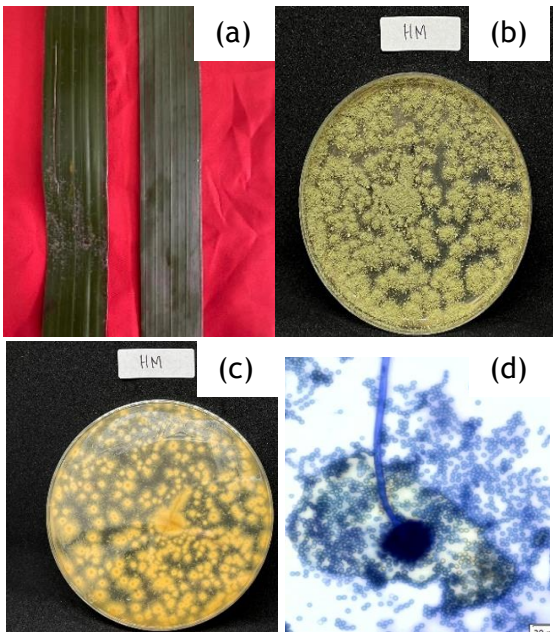


Figure 3. Aspergillus: (a) Oil palm leaf (b) top surface isolate image (c) bottom surface isolate image (d) microscopic

The image shows the results of isolating endophytic fungi from healthy oil palm leaves, coded HM:

a. Healthy oil palm leaf samples: Oil palm leaves used as samples were selected in healthy condition and free from disease symptoms so that the isolated fungi came from the internal tissue of the plant as endophytic fungi.
b. Colony morphology of HM isolate (top view): HM isolate on PDA medium formed colonies with a white outer layer and a dark green to blackish-green center. The colonies grew circularly with a cotton-like texture, indicating good mycelial growth and high spore production.
c. Colony morphology of HM isolate (bottom view): The underside of the colony shows a cream to light brown color that follows the colony growth pattern. This pigmentation can be used as a character in fungal identification.

d. Microscopic morphology of HM isolates: Microscopic observation shows branched hyphae and clusters of spherical spores tightly packed at the tips of the reproductive structures. These characteristics indicate that HM isolates reproduce asexually of forming conidia.

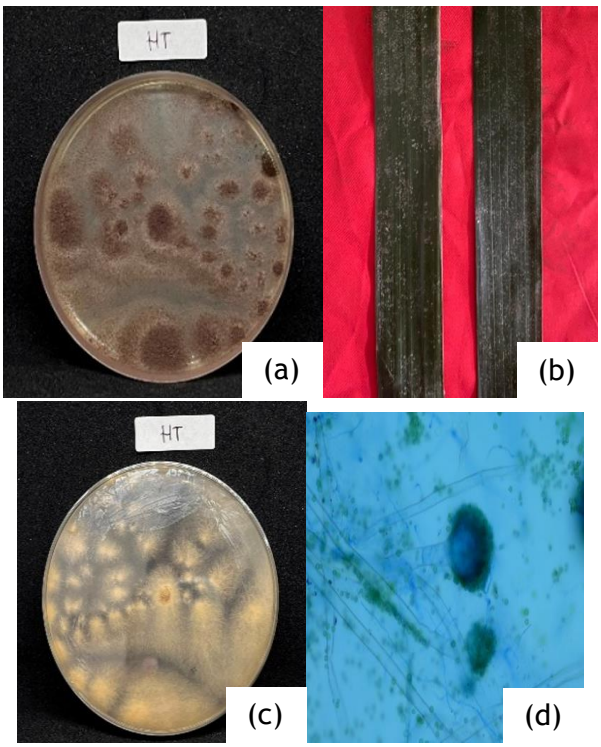


Figure 4. Aspergillus: (a) Oil palm leaf (b) top surface isolate image (c) bottom surface isolate image (d) microscopic.

The image shows the results of isolating endophytic fungi from healthy oil palm leaves coded HT:

a. Healthy oil palm leaf samples: Healthy oil palm leaves were used as a source for endophytic fungal isolation to ensure that the microorganisms obtained lived naturally within plant tissues without causing disease symptoms.
b. Colony morphology of HT isolate (top view): HT isolate on PDA medium forms dark brown to black colonies with a velvety texture. White mycelium is only visible at the edges of the colony, while the center is predominantly dark, indicating high spore formation.
c. Colony morphology of HT isolate (bottom view): The underside of the colony shows a reddish-brown to black color that follows the colony growth pattern. The deeper pigmentation indicates a difference in physiological characteristics compared to other isolates.
d. Microscopic morphology of HT isolates: Microscopic observations revealed branching septate hyphae and round to oval spores at the tips of the hyphae. These structures indicate that HT isolates reproduce asexually of forming conidia, or spores.

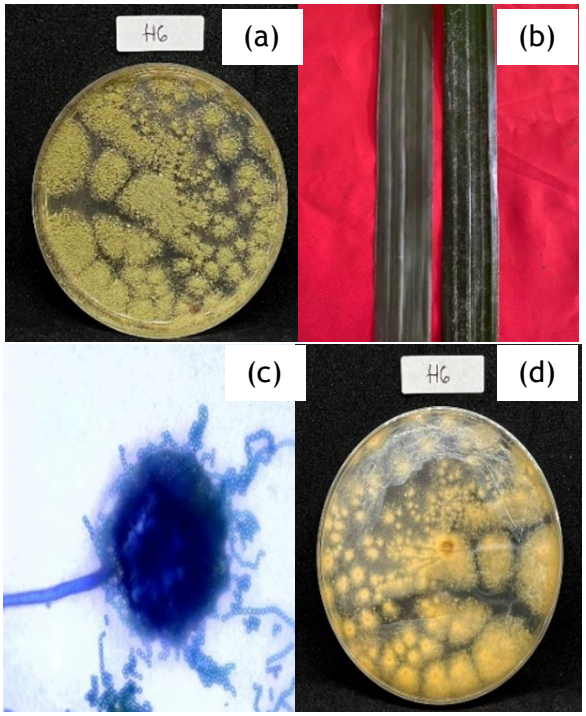


Figure 5. Aspergillus: (a) Oil palm leaf (b) top surface isolate image (c) bottom surface isolate image (d) microscopic.

The image shows the results of isolating endophytic fungi from healthy oil palm leaves, which are coded HG:

a. Healthy oil palm leaf samples: Oil palm leaves used as samples were selected in healthy condition without any disease symptoms to ensure that the isolated fungi were endophytic fungi that live in plant tissue.
b. Colony morphology of HG isolates (top view): HG isolates on PDA medium form dark green to grayish-green colonies with white edges. Colonies grow in a circular pattern and have a velvety, slightly powdery texture due to high spore production.
c. Colony morphology of HG isolate (bottom view): The underside of the colony shows a creamy to reddish-brown pigment that follows the direction of colony growth. The color difference between the upper and lower surfaces of the colony is an important characteristic in fungal identification.
d. Microscopic morphology of HG isolates: Microscopic observations revealed branching septate hyphae and tightly packed spores at the tips of the spore-bearing structures. These characteristics indicate that HG isolates reproduce asexually of forming conidia.

Based on the results, the HM isolate showed the fastest growth rate among the isolates, although the HB, HT, and HG isolates also covered the entire surface of the Petri dish by the end of the observation. This result is in line with the study (Wahid et al., 2026) which reported that *Aspergillus* isolates have faster colony growth than several other endophytic fungal genera due to their high adaptability and

nutrient utilization. Qualitatively, all isolates in this study showed good growth on PDA medium. In contrast, quantitatively, the HM isolate achieved colony coverage faster than the other three isolates, indicating differences in growth ability between isolates which are thought to be related to physiological characteristics and the efficiency of nutrient absorption from the growth medium (Allita, 2019).

Table 3. Growth of Endophytic Fungal Colonies

Day To	HB (mm)	HM (mm)	HG (mm)	HT (mm)
1	7.5 x 5.2	7.8 x 7.9	9.3 x 9.2	7.3 x 7.8
2	14.4 x 18.9	14.3 x 15.5	15.3 x 28.2	21.7 x 42
3	24.2 x 63.1	54.6 x 72.6	Full	56.4 x 52.8
4	Full	Full	Full	Full

Note: The colony is declared full if it has filled the Petri dish with a diameter of ± 85 mm.

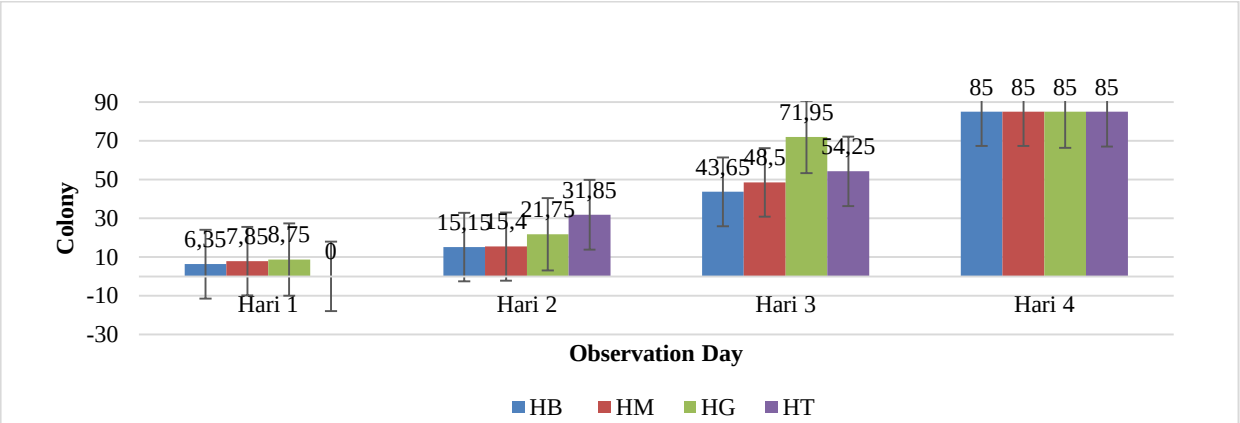


Figure 6. Growth Rate of Endophytic Fungal Colony Diameter on PDA Media

Based on observations, all endophytic fungal isolates increased in colony diameter during the incubation period. On the first day, the colony diameters of HB, HM, HG, and HT were 6.35 mm, 7.35 mm, 7.58 mm, and 7.50 mm, respectively. On the second day, these increased to 15.15 mm, 15.40 mm, 21.75 mm, and 31.85 mm. On the third day, colony growth accelerated with diameters of 43.65 mm, 48.50 mm, 71.95 mm, and 54.25 mm, respectively, before all isolates reached a maximum diameter of 85 mm on the fourth day. Qualitatively, these results are in line with research by Yogaswara et al., 2020, which reported that endophytic fungi experience rapid growth after the adaptation phase on PDA medium. Quantitatively, the colony diameter of HG isolates on the third day (71.95 mm) and all isolates on the fourth day (85 mm) showed relatively high growth compared with several endophytic fungal isolates reported by Sri Wahyuni (2019).

On the first day, the HB isolate began to grow with small colonies around the inoculation point. Growth increased on the second and third days, covering most of the PDA medium. On the fourth day, the colonies reached maximum growth, filling the entire Petri dish with a diameter of approximately 85 mm, indicating that the HB isolate had good growth capacity throughout the incubation period.

At the beginning of the observation, the HM isolate showed the formation of small, limited colonies around the inoculation point (Yurnaliza et al., 2021). Entering the second day, there was an increase in colony size, marked by the widespread spread of mycelium on the surface of the PDA medium. On the third day, the growth rate accelerated so that most of the surface of the medium began to be covered by mycelium. Furthermore, on the fourth day, the HM colony had grown optimally to fill the entire surface of the Petri dish with a diameter of approximately 85 mm, indicating that this isolate has excellent growth ability on PDA medium during the incubation period (Dewi, 2025).

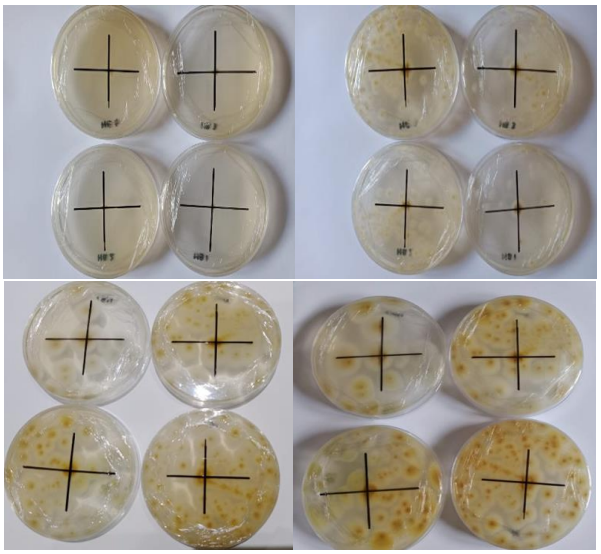


Figure 7. Documentation of endophytic fungal colony growth on PDA medium from day 1 to day 4 for the HB isolate.

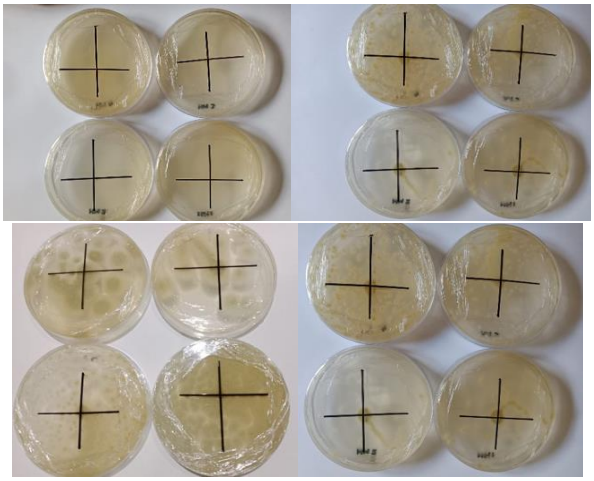


Figure 8. Documentation of endophytic fungal colony growth on PDA medium from day 1 to day 4 for HM isolate.

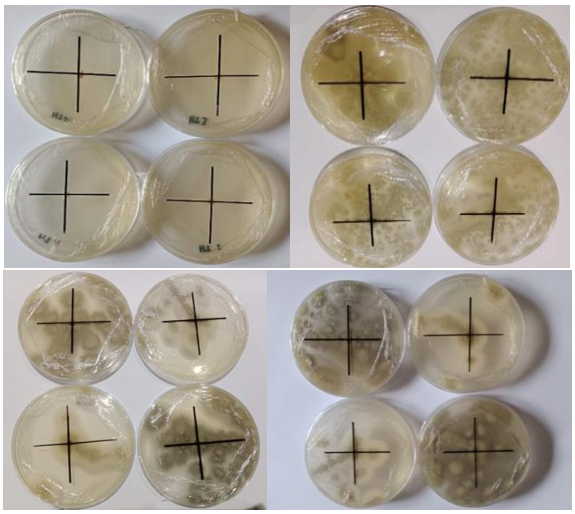


Figure 9. Documentation of endophytic fungal colony growth on PDA medium from day 1 to day 4 for the HT isolate.

On day 1, the HT isolate began to form small colonies. Growth accelerated on the second day, with a larger diameter than some of the other isolates. It then continued to grow on the third day, covering most of the PDA medium. By the fourth day, the HT colonies had filled the entire Petri dish, measuring approximately 85 mm in diameter, demonstrating good adaptability and growth in the culture medium.

On day 1, the HG isolate showed small colony growth on PDA medium. Growth accelerated on the second day and became very rapid on the third day, with a larger diameter than the other isolates. By day 4, the colonies covered the entire surface of the medium, reaching a diameter of approximately 85 mm, indicating that the HG isolate had a high growth rate throughout the observation period.

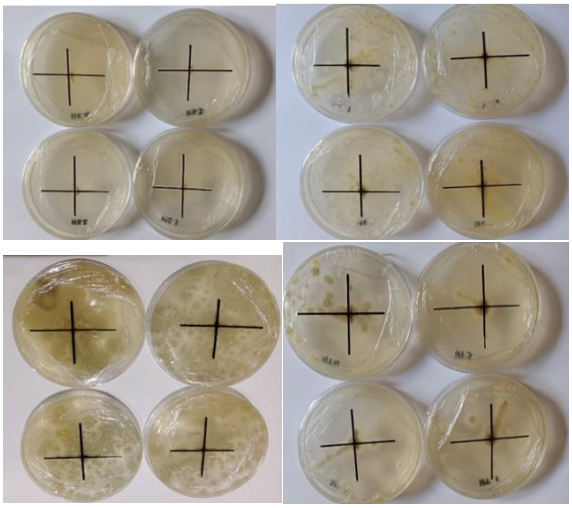


Figure 10. Documentation of endophytic fungal colony growth on PDA medium from day 1 to day 4 for the HG isolate.

4. Conclusion

Research on healthy oil palm leaves (Elaeis guineensis Jacq.) that were not infected with Ganoderma at PTPN IV Gunung Bayu Unit successfully isolated four endophytic fungi: HB, HT, HM, and HG. Observations showed that each isolate exhibited distinct macroscopic and microscopic morphological characteristics, including colony color, texture, growth pattern, and hyphal and conidial structures. Based on identification results, the isolates were classified within the genera Aspergillus sp. and Penicillium sp. All isolates grew well on Potato Dextrose Agar (PDA) medium, indicating strong adaptability to their environment. The presence and diversity of these endophytic fungi suggest that healthy oil palm leaves serve as a habitat supporting the growth of fungi with potential applications in biological control and plant health management.

This study provides preliminary insights into the diversity of endophytic fungi in healthy oil palm leaves and successfully isolated strains with potential as environmentally friendly biological control agents to enhance plant health and manage oil palm diseases. For practitioners, especially oil palm plantation managers, these findings can serve as a basis for developing endophyte-based biological control strategies. Further research should include molecular identification and antagonistic testing against Ganoderma sp. to confirm their potential application in the field.

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