Effect of Mycorrhiza from Fern Roots (*Nephrolepis* spp.) on the Growth of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings at the Prenursery Stage

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Abstract

Palm oil is a strategic agricultural commodity that plays a vital role in the global economy, productivity heavily depends on the success of the nursery phase, especially the prenursery stage, which requires optimal growth conditions. Nutrient availability, particularly phosphorus, often becomes a limiting factor, the use of mycorrhiza is crucial because fungi are known to enhance plant nutrient absorption, especially phosphorus, through their extensive external mycelial networks. This study aimed to evaluate the effect of mycorrhiza derived from fern roots (Nephrolepis spp.) on the growth of oil palm (Elaeis guineensis Jacq.) seedlings at the prenursery stage. The research was conducted at the Experimental House and Plantation Science Laboratory II, Politeknik Negeri Pontianak, for four months (May–August 2025). The experimental design used a Randomized Block Design (RBD) with four levels of mycorrhizal biofertilizer doses: 0, 10, 25, and 35 g/polybag, each replicated four times. The results indicated that mycorrhizal application significantly affected plant height during early growth (2–6 weeks after planting) but had no significant effect on the number of leaves and stem diameter up to 12 weeks. Microscopic observations revealed arbuscular mycorrhizal colonization in oil palm roots, characterized by the presence of hyphae, vesicles, and arbuscules. Therefore, fern root-derived mycorrhiza shows potential as a natural biofertilizer that enhances early seedling growth and supports sustainable oil palm nursery management on marginal soils.

Keywords: *Fern roots, mycorrhiza, prenursery, oil palm, seedling growth.*

INTRODUCTION

Palm oil is a strategic agricultural commodity that plays a vital role in the global economy, particularly as a source of vegetable oil (Yasmin & Rosnina, 2021). Palm oil productivity heavily depends on the success of the nursery phase, especially the prenursery stage, which requires optimal growth conditions (Setyorini et al., 2020). Nutrient availability, particularly phosphorus, often becomes a limiting factor in peatland environments, which are common locations for palm oil cultivation, as low soil pH inhibits the absorption of essential nutrients (Hakim et al., 2017). In this context, the use of mycorrhiza is crucial because fungi are known to enhance plant nutrient absorption, especially phosphorus, through their extensive external mycelial networks (Widiastuti et al., 2024). The symbiotic interaction between mycorrhiza and oil palm roots enables the expansion of nutrient exploration within the soil, overcoming limitations in nutrient availability, and ultimately enhancing seedling growth during the early nursery phase (Satria et al., 2023).

The use of arbuscular mycorrhizae in oil palm cultivation has shown significant potential, not only in improving nutrient uptake but also in enhancing plant adaptation to suboptimal environmental conditions (Adinugroho et al., 2025). Arbuscular mycorrhizae form a mutualistic symbiotic relationship with plant roots, where fungal hyphae extend the range of nutrient absorption, particularly phosphorus, in soil that is often difficult for plant roots to access themselves (Satria et al., 2023). Additionally,

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mycorrhizal fungi facilitate the synthesis of plant hormones such as auxin and gibberellin, which are important for root development and better nutrient absorption (Meng et al., 2025). Mycorrhizae from fern roots (Nephrolepis spp.) can form significant arbuscular mycelial structures, which enhance the plant root's ability to subsequently absorb nutrients from liquid organic fertilizer and air (Satria et al., 2023).

This symbiotic relationship also contributes to the increased activity of soil microorganisms, which play a crucial role in the decomposition of organic matter and nutrient cycling (Satria et al., 2023). The use of appropriate planting media, such as a mixture of Ultisol subsoil with compost or vermicompost, is very important in supporting optimal oil palm seedling growth during the prenursery stage, as the planting medium functions as a vital source of nutrients and air for plant development (Hutabarat et al., 2023). This approach also helps reduce reliance on synthetic chemical fertilizers, which have negative impacts on the environment and soil quality in the long term (Ajeng et al., 2020). Mycorrhiza enhances plant growth by extending root reach through extraradical hyphae, thereby increasing the absorption of phosphorus, nitrogen, water, and dry matter (Umer et al., 2025). However, for coconut, the most direct evidence still shows an increase in colonization, physiological status, and growth, while long-term mechanistic data on rhizosphere microbiome engineering is still developing (Gusmawartati et al., 2025).

Mycorrhizal fungi act as natural biofertilizers that support air absorption, nutrition, and protection from pathogens, while expanding the plant's root system to enhance nutrient uptake efficiency (Molefe et al., 2023). Mycorrhizae can also mitigate the negative effects of toxic phenolic acids commonly found in peatlands, thereby improving plant growth(Pangaribuan, 2018). Additionally, the presence of mycorrhizae can enhance plant tolerance to abiotic and biotic stresses, which is highly relevant for oil palm grown in challenging peatland conditions (Wiriya et al., 2020). Foliar endophytic fungi, including those associated with mycorrhizae, also have the ability to convert inorganic phosphate into organic forms, further supporting plant growth, especially in phosphorus-poor peatlands (Hakim et al., 2017).

MATERIALS AND METHODS

Place and Time

This research at the Experiment House and Laboratory of Horticulture Science II, Department of Agricultural Technology, Pontianak State Polytechnic, was conducted for 4 months, starting from May to August 2025.

Materials and Tools

The materials used in this research include: PPKS palm oil seedlings, PMK soil, solid palm oil waste, NPK fertilizer (16+16+16), dolomite, biological mycorrhiza fertilizer from palm tree roots, aquades, labels, 10% KOH solution, 3% H_2O_2 solution, 60% glucose solution, 25% vinegar solution, Lactophenol Cotton Blue. The tools used in this research include: graded sieve, centrifuge, digital measuring tape, polybag 15 x 35 cm, digital scale, scale, oven, pH meter, beaker, paranet, hoe, chocolate-sized helper 18 x 28 cm, machete, ruler, writing tools, microscope, culture dish, forceps, cup, petri dish, scalpel, and soil sieve.

Research Method

This research uses an experimental design with in vitro and in vivo approaches to test the effectiveness of mycorrhiza from moss roots in enhancing oil palm growth. The study employs a non-factorial Randomized Complete Block Design (RCBD). The experiment involved four treatment levels of mycorrhizal biofertilizer derived from moss roots, with each treatment replicated four times, resulting in 16 experimental units. Each experimental unit consisted of three observation plants, giving a total of 48 plants. The treatments consisted of A0 (no mycorrhizal biofertilizer, as the control), A1 (10 g of mycorrhizal biofertilizer per polybag), A2 (25 g of mycorrhizal biofertilizer per polybag), and A3 (35 g of mycorrhizal biofertilizer per polybag).

Research Implementation

Soil Medium Preparation

Before the sterilization process, the topsoil needs to be screened to remove waste that could interfere with the media preparation. The soil used has undergone sterilization through steaming. In one process, 15 kg of soil is steamed for 3 hours. After being sterilized, the soil is placed in bags to prevent contamination by microorganisms.

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Media Preparation

The planting medium used consists of top soil with a weight of 1 kg per polybag sized 14 x 17 cm. This medium is mixed with solid palm oil waste, and then supplemented with NPK fertilizer (16+16+16) at a dose of 0.5 g per polybag and dolomite at the same dose, i.e., 0.5 g per polybag, with a total of 48 polybags used in this research and incubated for 2 weeks.

Root Rot Testing Before Application

Before application, the fern roots must be tested first, thereby strengthening the assumption that fern root establishment involves mycorrhiza, indicated by the presence of vesicles on the tested roots. Only after it is confirmed or suspected that the fern roots are infected with mycorrhiza can we apply mycorrhiza to the oil palm plants. The collection of fern root samples that have been checked for the presence of mycorrhiza and root infection in the plants. The roots are collected according to research requirements, taking the roots along with the soil to prevent the roots from drying out. Root collection is done H-2 before application to the plants, and before application, they are first cleaned of soil. Then they are cut finely and applied to the plants at the time of oil palm planting.

Application of Biofertilizer with Mycorrhiza

Application of this mycorrhizal organic fertilizer is done after the incubation of the planting medium for 2 weeks at the beginning of planting in the polybag application. The application method is by distributing the fungi roots evenly in the planting hole so that the mycorrhiza in the roots of the fungi can directly contact the oil palm roots. The application dose of mycorrhiza is usually calculated based on the root spread of the plant, or for plants in polybags adjusted to the size of the polybags.

Planting Oil Palm

The oil palm seedlings plants that have been prepared will be planted in polybags that have been filled with soil, plant the oil palm plants into the polybags with a depth of about 2 cm.

Oil Palm Root Sampling

Soil samples from the planting medium were collected by taking soil at a depth of 10 cm around the oil palm root system, then placed into plastic bags that had been named as samples. Soil samples were taken in an amount of 100 g per sample, while for root sample collection during the last week of observation at 12 weeks after planting (WAP), the oil palm root system was cut finely and not infected with fungus after cutting the roots, then placed into plastic bags that had been named as samples

Isolation and identification of mycorrhiza in oil palm root samples.

Isolation of mycorrhizal spores is carried out using two methods: wet sieving and sucrose gradient centrifugation. For the wet sieving method, 100 g of soil is weighed and dissolved in 1000 mL of distilled water. This solution is then filtered through graded sieves with diameters of 40 µm, 50 µm, and 200 µm, and collected in a centrifuge tube. This process is performed by spraying air using a spray bottle, mixing the solution with 60% sucrose, and then centrifuging at 2500 rpm for 5 minutes. The supernatant from the centrifugation is poured into an 8 cm diameter petri dish. Next, the preparation is made by taking the spores using a micropipette and placing them on a microscope slide. The spores are carefully transferred onto the glass surface, tested with aquades, and covered with a cover glass.

Root Staining

Infection of roots by mycorrhiza can be observed through root staining. The first step is to rinse the roots from each sample with running water until clean, then soak them in a 10% KOH solution for 24 hours inside a cup (volume 8 mL) until the roots change color to white or pale yellow. The 10% KOH solution functions to remove cytoplasmic fluid from the roots, making the roots pale and serving as a preservative. Next, the roots are rinsed with a 3% H_2O_2 solution for 30 minutes, then washed until clean. The 3% H_2O_2 solution helps facilitate the penetration of Lactophenol Cotton Blue during the staining process. Root staining is performed with Lactophenol Cotton Blue dissolved in 25% vinegar, then the clear or pale roots are rinsed with running water. To calculate the number of roots infected with micoriza, observe by cutting the roots into 10 pieces, each 1 cm long. Each root piece is then covered with a cover glass. The percentage of roots infected with micoriza is calculated using the following formula: The level of root infection is classified according to the guidelines from the Institute of Mycorrhizal Research and Development, USDA Forest Service, Athena, Georgia, as follows:

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- Class 1 if root infection is 0% - 5% (very low)
- Class 2 if root infection is 6% - 25% (low)
- Class 3 if root infection is 26% - 50% (moderate)
- Class 4 if infection is 51% - 75% (high)
- Class 5 if infection is 76% - 100% (very high).

Observations

The observation parameters in the research are divided into 2 parts, namely field observations and laboratory observations, including: Plant Height, Stem Diameter, Number of Leaves, and Root Infection Degree.

Data Analysis.

Data analysis is performed using ANOVA tests to determine the statistical significance of treatments, followed by further tests such as DMRT if significant differences are found.

RESULTS AND DISCUSSION

Mycorrhizal Infection Characterization on Fern Roots (*Nephrolepis* sp.)

The results of the mycorrhiza findings from the roots observed under a microscope show the mycorrhizally colonized root network, which is of the arbuscular mycorrhiza type (Figure 1). The elongated and orderly structure of the root cortex cells is clearly visible, serving as the primary colonization site for mycorrhiza. Among these cell networks, structures resembling hyphae, thin and transparent threads, are characteristic of mycorrhiza. These hyphae spread both internally and externally from the root cells, forming a network that aids in air and nutrient absorption, particularly phosphorus. The FMA symbiosis with plants involves the formation of hyphae, spores, vesicles, and arbuscules. This symbiosis provides numerous benefits to plants, such as enhancing resistance to pathogens, improving nutrient absorption, and increasing drought tolerance (Prihantoro et al., 2023).

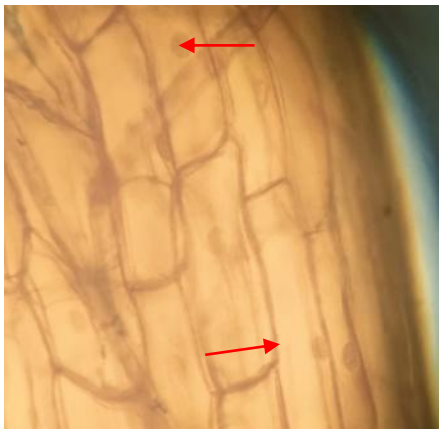


Figure 1. Mycorrhizal Infection on Fern Roots (*Nephrolepis* sp.)

Characterizing mycorrhizal infection on fern roots, particularly Nephrolepis sp., involves identifying the presence, type, and colonization level of mycorrhizal fungi, as well as environmental factors affecting this symbiosis. Mycorrhizal infection in *Nephrolepis* sp. is generally an association with arbuscular mycorrhizal fungi (AMF), but colonization levels vary greatly depending on species, location, and environmental conditions. Studies in India indicate that *Nephrolepis* exaltata has AMF associations with intermediate morphology, and AMF spores (particularly from the genera Claroideoglomus, Funneliformis, Glomus, and Rhizophagus) were found in the root zone (Muthukumar & Prabha, 2013). The AMF morphology on ferns is typically intermediate or Paris type, with colonization levels varying between 1–82% depending on species and environment (Zeng et al., 2024; Zulfita et al., 2020). Root staining with trypan blue and microscopic examination are used to detect AMF structures (arbuscules, vesicles, hyphae) (Giovannetti & Mosse, 1980).

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Plant Height

Based on the analysis of soil samples, the application of mycorrhizal bio-fertilizer from mushroom roots significantly affects the increase in oil palm height at the ages of 2 WAP, 4 WAP, and 6 WAP (Table 1).

Table 1. Average Effect of Mycorrhizal Biofertilizer on Oil Palm Seedling Height

Mycorrhizal	Weeks After Planting (WAP)					
	2	4	6	8	10	12
A0 (0 g)	1.21b	6.32b	11.60ab	17.35a	22.63a	26.47a
A1 (10 g)	2.17a	7.85a	13.11a	18.55a	24.11a	25.15a
A2 (25 g)	1.67ab	6.46b	11.29b	17.06a	22.76a	25.68a
A3 (35 g)	1.67ab	6.53b	12.74ab	17.11a	23.55a	25.74a

Note: Numbers followed by the same letter in the same column do not differ significantly in the DMRT α 5%

Based on the further α 5% DMRT test, it shows that at 6 WAP, the treatment of biofertilizer mycorrhiza 10 g/polybag is significantly different from the treatment of 25 g of biofertilizer mycorrhiza, mycorrhiza biofertilizer 35 g/polybag. However, it is not significantly different from the treatment without biofertilizer mycorrhiza. According to Simarmata et al., (2025), biofertilizer is an appropriate alternative for improving, enhancing, and maintaining soil quality, thereby significantly increasing the growth and yield as well as the quality of various plants. It indirectly helps improve soil structure, enhance the dissolution and erosion of soil organic matter, and directly increases the absorption of nutrients, air, and protects the roots from pathogens (Eliyani et al., 2022).

In addition, mycorrhiza also stimulates the formation of plant growth hormones, such as cytokinin and auxin, which play a role in cell division and elongation, thereby further optimizing plant growth (Mutiarahma et al., 2021). Research shows that mycorrhizal infection, particularly arbuscular mycorrhizal fungi (AMF), significantly enhances the growth of oil palm seedlings. This increase is evident in plant height parameters, stem diameter, leaf area, root volume, and dry weight of seedlings, especially when combined with optimal NPK fertilizer dosage (Darwis et al., 2023; Gusmawartati et al., 2025; Nizar et al., 2023; Rini et al., 2022). Mycorrhizal types such as Glomus sp. and combinations with Entrophospora sp. have been proven to most consistently increase and enhance nutrient (N, P, K, Ca, Mg, B) uptake (Rini et al., 2022; Sundram & Abdullah, 2010). This significant improvement occurs because mycorrhiza optimizes the absorption of micro and macro nutrients, such as potassium, nitrogen, and phosphorus, which are essential for the formation of new cells and tissues in plants (Jamiołkowska et al., 2021). Overall, inoculation with arbuscular mycorrhizal fungi can significantly increase plant biomass, with an increase of 47% in biomass, 16% in nitrogen concentration, 27% in phosphorus concentration, as well as 67% nitrogen uptake and 105% phosphorus uptake in all parts of the plant (Wu et al., 2024). The effectiveness of mycorrhizal biofertilizers like MZ2000 in enhancing the growth of Acacia seedlings in nurseries has shown significant potential in application for other plantation crops (Hazra & Novtiar, 2020).

Penetration of the mycorrhizal hyphae into the root cortex, forming intracellular arbuscular structures, effectively enhances plant access to nutrients that are difficult to reach, especially in environments with low nutrient availability or low pH (Simarmata et al., 2025b; Zulfita et al., 2020). In the context of tropical peatlands, which are characterized by low pH and limited nutrient availability, this mycorrhizal association becomes highly crucial for the rehabilitation and growth of certain tree species (Tawaraya et al., 2003).

This symbiotic relationship also contributes to the increased activity of soil microbes, which play a crucial role in the decomposition of organic matter and nutrient cycling (Pérez Hernández & Pérez Sato, 2022). This increase in nutrient absorption and microbial activity directly supports the biosynthesis of primary and secondary metabolites essential for plant growth, including enhanced photosynthesis rate and biomass accumulation (Ajeng et al., 2020). Arbuscular mycorrhizal fungi significantly increase seedling height by 36.50 cm in ginseng-producing plants, as well as facilitate an increase in shoot bud diameter and root collar diameter by 268% in sweetgum seedlings (Satria et al., 2023).

Stem Diameter

Based on the analysis of variance, the application of mycorrhizal biofertilizer from fern roots had no significant effect on 12 WAP regarding the increase in oil palm seedling leaf (Tabel 2).

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Table 2. Average Effect of Mycorrhizal Organic Fertilizer on the Diameter of Palm Seedlings.

Mycorrhizal	Weeks After Planting (WAP)					
	2	4	6	8	10	12
A0 (0 g)	1.56	2.73	3.36	4.27	3.11	3.72
A1 (10 g)	1.86	3.05	3.63	4.52	3.275	3.88
A2 (25 g)	2.23	3.05	3.58	4.45	3.33	3.83
A3 (35 g)	1.83	2.92	3.48	4.54	3.22	3.77

The results of this research show that the application of mycorrhizal biofertilizer does not provide a significant effect on stem diameter increment. This may be due to several factors, first, the effectiveness of mycorrhiza in increasing stem diameter usually requires a relatively long time to be visibly apparent, while the observation period used may still be too short for the diameter change to be sufficient. Other factors could come from the plant growth stage not yet entering the active stem expansion phase, so the response to biofertilizer is not optimal. The interaction between mycorrhiza and the plant also requires time to show its physiological effects, so within a certain period, its effect on stem diameter increment may not be significantly apparent.

Some studies indicate that the application of mycorrhiza on oil palm seedlings does not always have a significant effect on the number of leaves and stem diameter, especially during the pre-nursery or early growth phase. The effect of mycorrhiza on plant growth, including the number of leaves and stem diameter, often requires a longer time to become apparent. Studies show that within the first 16 weeks, mycorrhiza application did not have a significant effect on the number of leaves or stem diameter, thus requiring more time to see its benefits (Darwis et al., 2023). Some studies found that neither the mycorrhiza dose nor the planting medium composition (e.g., peat soil/top soil mixture) had a significant effect on the growth of the number of leaves and stem diameter during the pre-nursery phase (Batu et al., 2023; Lubis et al., 2019). The interaction between manure and mycorrhiza also did not show a significant effect on these parameters (Lubis et al., 2019).

Number of Leaves

The leaf of number is a parameter that counts the number of perfectly opened leaves in one plant and is measured from 2 WAP to 12 WAP. Based on the analysis of the diversity, the application of mycorrhizal biofertilizer from fern roots had no significant effect on the increase in leaf number of oil palm seedlings at 12 WAP (Table 3).

Table 3. Average Effect of Mycorrhizal Biofertilizer on the Number of Oil Palm Seedling Leaves.

Mycorrhizal	Weeks After Planting (WAP)					
	2	4	6	8	10	12
A0 (0 g)	0.22	1.44	2.00	2.61	5.19	5.83
A1 (10 g)	0.38	1.72	2.00	2.66	5.15	6.21
A2 (25 g)	0.16	1.72	2.00	2.55	5.80	6.42
A3 (35 g)	0.11	1.77	1.94	2.61	5.51	6.16

The results of this study that the application of mycorhizal biofertilizer did not show a significant effect on the increase in the number of plant keaves. This could be due to several environmental factors. Soil pH that is too acidic or extreme can inhibit the effectiveness of mycorrhiza, becouse these conditions cause nutrient absorption by the roots to become suboptimal, and even poisoning by certain elements such as aluminium can occur. Futhermore, if the soil already contains sufficiently abundant nutrients, the role of mycorrhiza in enhancing nutrient uptake becomes less critical, resulting in no significant difference in the growth of the number of leaves.

Mikoriza indeed plays a role in enhancing the absorption of nutrients, particularly phosphorus, which supports plant growth, but significant effects on leaf number are usually only observed during longer observation periods, namely between 4 and 28 weeks after planting. This indicates that the effects of mycorrhiza can be seen over a considerable period of time (Ciadamidaro et al., 2022). The effectiveness

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of mycorrhiza is highly dependent on the isolate type, dosage, and environmental conditions. Not all isolates or dosages provide the same response to certain growth parameters, including leaf number and stem diameter (Duaja et al., 2019; Sakiah et al., 2023). During the early growth phase, oil palm seedlings still rely heavily on the food reserves within the seed, rather than nutrient absorption from the soil through the roots. This results in suboptimal mycorrhiza effects because the roots have not fully developed and have not yet had much interaction with mycorrhiza ((Babalola & Adedayo, 2023; Solanki et al., 2023).

Oil Palm Root Infection Degree

Observation of mycorrhizal infection under a microscope shows the presence of arbuscular mycorrhizal infection on the roots of oil palm plants, appearing as branches and spherical structures within the roots. The vesicular structures are elongated or spherical, while the arbuscular structures are small tree-like hyphae within the host root cortex (Figure 2).



Figure 2. Micoriza Colonization on Palm Oil Root

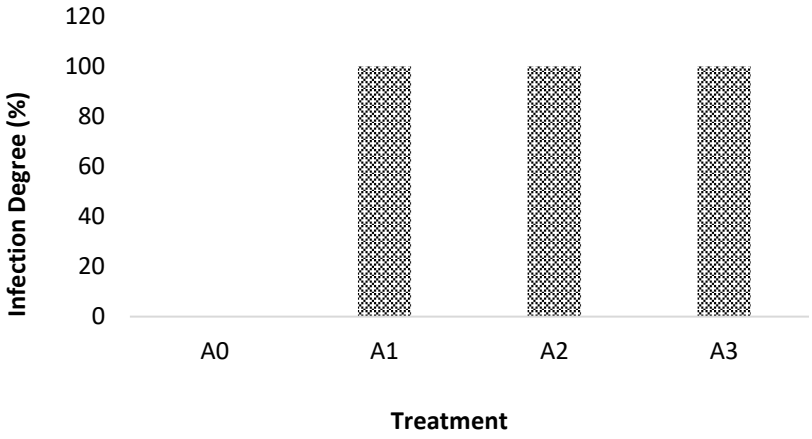


Figure 3. The Degree of Mycorrhizal Infection in Oil Palm Roots

In the Figure 3 level of mycorrhizal infection on oil palm roots, a quantitative method involving the counting of colonized root segments or the use of trypan blue staining techniques can be applied to visualize hyphae, vesicles, and arbuscules (Ifeanyi & Philippine, 2020). This method provides a direct view of the colonization level and infection intensity within the root network (Mawardhani et al., 2023). Subsequently, statistical analyses, such as ANOVA with Tukey's post hoc test, can be used to compare root colonization percentages between different treatment groups (Hazra & Novtiar, 2020; Kwashie et al., 2023; Wiriya et al., 2020). Mycorrhizal colonization can be further evaluated by cleaning the roots with 10% KOH and staining them with Trypan blue, allowing for detailed observation of hyphae, arbuscules, and vesicles under a microscope (Giovannetti & Mosse, 1980). The presence of these structures indicates the success of mycorrhizal symbiosis, which plays a role in enhancing nutrient uptake and plant resistance to environmental stresses (Prihantoro et al., 2023). Previous research also shows that roots colonized by arbuscular mycorrhizal fungi have a significantly higher colonization percentage in inoculated plots compared to controls, positively correlating with increased nutrient uptake in sugarcane (Mistry et al.,

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2023). Evaluating the intensity of AMF colonization in roots can be done by measuring arbuscules, Vesicles, and hyphae using quantitative methods such as the line-intercept method (line intersection method) on at least 100 sections of 20 root segments under a light microscope (Shi et al., 2025). The penetration of mycorrhizal hyphae into the root cortex, forming intracellular arbuscular structures, effectively increases plant access to nutrients that are difficult to reach, especially in environments with low nutrient availability or low pH (Zulfita et al., 2020; Simarmata et al., 2025). In the context of tropical peatland, which is characterized by low pH and limited nutrient availability,this mycorrhizal association becomes highly critical for the rehabilitation and growth of certain tree species (Tawaraya et al., 2003).

CONCLUSION

Application of mycorrhiza from fern roots can provide a positive effect on the height of oil palm plants during early growth, but it has not shown a significant effect on the number of leaves and stem diameter during the observation period up to 12 WAP. Mycorrhiza from fern roots can infect and colonize oil palm roots that have been given treatment.

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