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The Role of Mycorrhizal and Humic Acid Biofertilizers in Supporting the Growth of Oil Palm Seedlings (*Elaeis guineensis* Jacq.) in the Main Nursery Stage on Ultisol Soil

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Abstract

To overcome the limitations of ultisol soils and the increasing demand for high-quality oil palm seedlings, this study evaluates a combination of humic acid and mycorrhizal biofertilizer applications with reduced NPK fertilization at the main nursery stage to increase nutrient availability and support more efficient and sustainable nursery management on marginal soils. The experiment was conducted in the Agricultural Field of the Faculty of Agriculture, University of North Sumatra using a Non-Factorial Randomized Design, consisting of mycorrhiza + humic acid), F (3/4 standard NPK + mycorrhiza + humic acid), and G (standard NPK + mycorrhiza + humic acid). The results showed that the combined application of mycorrhizal biofertilizer and humic acid significantly improved the growth of oil palm seedlings at the main nursery stage. The best growth performance was observed in treatment E (1/2 standard NPK + mycorrhiza + humic acid), as indicated by increased plant height, number of leaves, stem diameter, and both fresh and dry weights. In addition, treatments E (1/2 standard NPK + mycorrhiza + humic acid) and F (3/4 standard NPK + mycorrhiza + humic acid) improved phosphorus uptake and its efficiency, with values comparable to the standard NPK treatment (B), while also increasing root colonization, with the highest levels observed in these treatments.

Keywords: Marginal Soils, Nutrient Availability, Palm Oil, Phosphorus Uptake Efficiency, Root Colonization

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a leading Indonesian commodity with high productivity as a source of vegetable oil and a contributor to the country's foreign exchange. Currently, the area of oil palm plantations in Indonesia is growing quite rapidly (Directorate General of Plantations, 2018). As plantations expand, the need for quality planting material is also increasingly important. Seed quality significantly influences production yields, making the use of superior seeds an absolute requirement in oil palm cultivation (Tampubolon et al., 2019). The nursery stage is the initial phase that determines the success of the plants in the field. Therefore, optimal nursery management is essential. One important aspect of the nursery is the provision of quality seeds that meet growth standards, and this is greatly influenced by the planting medium used.

Ultisol soil is often used as a nursery medium. However, it has several limitations, such as high acidity

(pH <5), low base saturation (<35%), and low macro- and micronutrient content such as N, P, K, Ca, Mg, S, and Mo. Furthermore, this soil has high aluminum, iron, and manganese content. Excess aluminum levels can make phosphorus unavailable to plants because it is bound in an insoluble form.

To improve the condition of ultisol soil, ameliorants such as humic acid and mycorrhizal biofertilizers can be used. Humic acid is a chemically and biologically stable compound resulting from the decomposition of organic matter. Its functions include increasing nutrient availability, improving soil structure, and supporting soil microbial life (Hermanto et al., 2013). Wahyuningsih et al. (2017) showed that the application of humic acid can increase phosphorus availability by forming complex compounds with metal ions. The results of research by Ariyanti et al. (2019) showed that the application of 30 mL of humic acid plus 3200 g of compost from oil palm fronds increased

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plant height and leaf chlorophyll content in oil palm seedlings after four months. Lambok et al. (2016) reported that the application of 25 g of humic acid per polybag in ultisol media provided the best growth results in the main nursery stage.

In addition to humic acid, the use of arbuscular mycorrhizae also has the potential to improve seedling quality by increasing nutrient uptake by plants. Mycorrhizae are fungi that form a mutually beneficial symbiotic relationship with plant roots. The fungi obtain carbon from the plant, while the roots are assisted in absorbing nutrients that are not easily mobile in the soil, such as P, Zn, and Fe. The mycorrhizal hyphae network also plays a role in improving soil structure through aggregate formation (Listyowati, 2020).

The novelty of this research lies in the simultaneous study of the combination of humic acid and arbuscular mycorrhizae in ultisol soil media during the main nursery phase of oil palm. Previous research has generally evaluated each treatment separately. At the same time, this study emphasizes the potential synergistic effect of both in increasing nutrient availability, particularly phosphorus, in soil conditions with high aluminum saturation. This approach is expected to provide an alternative, more effective and sustainable nursery media management strategy for marginal soils.

Based on this, this study was conducted to evaluate the effectiveness of the application of humic acid and mycorrhizal biofertilizer in increasing the growth of oil palm seedlings at the advanced nursery stage (*main nursery*) on ultisol soil.

2. Material and Methods

The research was conducted at Agricultural Land, Faculty of Agriculture, University of North Sumatra (3.575° N, 98.655° E; ±25 meters above sea level) and Analysis of mycorrhizal infection at the Soil Biology Laboratory, Faculty of Agriculture, University of North Sumatra. This research was conducted from June to November 2024.

The tools used in this research were hoes, small shovels, polybags (30 x 40 cm measuring 15 kg, markers, labels, rulers or meters, ovens, vernier calipers and other laboratory tools, petri dishes, Erlenmeyer flasks, tweezers, binocular microscopes, stereo microscopes, slides, and cover slips.

The materials used in this study were ultisol soil from Aek Pancur, Tanjung Morawa; oil palm seeds of the PPKS DxP 540 variety; mycorrhizal biofertilizer from the Soil

Biology Laboratory collection of FP USU; and humic acid (Terahumic PT Artha Prima Humatindo) as the treatment material. NPKMg fertilizer 15:15:6:4, NPKMg 12:12:17:2, Kiserite. HCL 30% and trypan blue solution were used as mycorrhizal colonization infection test materials.

The study used a Non-Factorial Randomized Block Design (RAK) with 7 treatment levels, namely A: Control, B: Standard NPK, C: Mycorrhizal biofertilizer 20 g / polybag, D: Humic Acid 25 g / polybag, E: 1/2 standard NPK + 20 g mycorrhizal biofertilizer + Humic Acid 25 g / polybag, F: 3/4 standard NPK + 20 g mycorrhizal biofertilizer + Humic Acid 25 g / polybag, G: 1 standard NPK + 20 g mycorrhizal biofertilizer + Humic Acid 25 g / polybag with 4 replications, so there are 28 experimental units. Each experimental unit consists of 3 plants, so the total number of plants is 84. The parameters observed were plant height, measured from the base of the marked stem to the tip of the leaf canopy. Measurements were carried out every 2 weeks for 5 months; the wet weight of the plant was measured by removing each treatment sample, washing it, drying it, and then weighing it using an analytical balance. Weighing was carried out at harvest time; measurement of the dry weight of the crown and roots was carried out at the end of the observation by cutting the crown and roots of the plant sample. Each part of the plant was put into paper and labeled. After that, the sample was dried in an oven at a temperature of 700 C for 72 hours until a constant dry weight was reached. The sample was then weighed using a scale. Observation of the crown root ratio was calculated at the end of the observation, namely by removing the plant and separating the crown from the roots of the plant by cutting from the base of the plant. The crown and roots were then dried. The crown root ratio was calculated by comparing the dry weight of the crown and the dry weight of the roots. The crown root ratio was calculated using the formula:

$$\text{Root Crown Ratio} = \frac{\text{Dry Weight of Header}}{\text{WetWeight of Header}}$$

P uptake was measured at the end of the study. Fresh plant samples were weighed and placed in brown envelopes. They were then oven-dried for approximately 3 days at 65°C. Once dry, the leaves were ground to a size of 0.5 mm, placed in clear plastic bags, and labeled according to treatment. These samples were used for P uptake analysis in the laboratory.

P Uptake = % Plant Nutrient Content x Shoot Dry Weight

P absorption efficiency is obtained through:

$$RE = \frac{\text{gram nutrient absorption P1,P2,P3} - \text{gram nutrient absorption without fertilizer}}{\text{grams of nutrients provided}} \times 100\%$$

RE = Nutrient Uptake Efficiency (Recovery efficiency)

P1,2,3 = Treatment P1, P2, P3

P0 = Treatment P0 (without fertilizer)

The percentage of root colonization was used to assess mycorrhizal infection and was analyzed at the end of the study following the method (Trejo-Aguilar & Banuelos,

2020). The roots were cleaned, soaked in 2.5% KOH, acidified with 0.1 M HCl, stained with trypan blue, and then observed under a microscope. Colonization was

determined based on the presence of vesicles, arbuscules, and hyphae (Wijayani & Wirianata, 2021), and then the percentage was calculated using the formula:

$$\% \text{Root Colonization} = \frac{\sum \text{colonized field of view}}{\sum \text{entire field of view}} \times 100\%$$

The experimental results were analyzed using Analysis of Variance at 5% and Duncan's multiple range test at 5% using SPSS version 25.

Research Flowchart

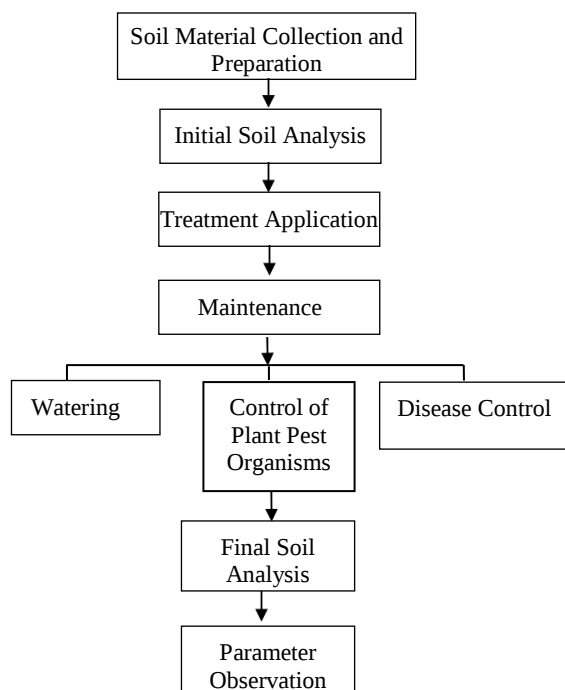


Figure 1. Research flow diagram

3. Results and Discussion

3.1. Plant Height

Based on the research results, it was found that the application of biofertilizer and humic acid significantly

increased plant height between 8 and 18 weeks after planting (WAP). The average plant height resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer can be seen in Tables 1 and 2.

The results of the study showed that the treatment of using mycorrhizal biofertilizer and humic acid caused variations in the height of oil palm plants. The combination of 1/2 standard NPK treatment + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag (E) gave better results with an average plant height of 67 cm compared to no treatment and single treatment. Compared to no treatment (control), the treatment of 1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic acid/polybag (E) increased plant height by 79.86%, while compared to the single standard NPK treatment, there was an increase of 6.06%.

Plant height growth is more optimal when fertilization uses a combination of NPK fertilizer, mycorrhizal biofertilizer, and humic acid compared to when applied separately. This result indicates that mycorrhizae and humic acid can partially replace the function of inorganic fertilizers. Mycorrhizae play a crucial role in increasing nutrient absorption through a symbiotic relationship between mycorrhizal fungi and plant roots, significantly expanding the root absorption area, particularly for phosphorus (Musafa et al., 2015). The application of mycorrhizal biofertilizer also increases the efficiency of the plant's root system by mobilizing and releasing nutrients bound to clay particles, thereby accelerating the absorption of phosphorus, which is essential for cell division and meristem tissue development. Plants inoculated with mycorrhizae form a hyphal network that helps them absorb water and nutrients from the soil more efficiently.

Table 1. Plant height at 2 - 10 MST when using mycorrhizal and humic acid biofertilizers

Treatment	Plant Height				
	2 MST	4 MST	6 MST	8 MST	10 MST
	-----cm-----				
A	29.00 ± 1.66	29.92 ± 1.83	30.17 ± 1.45	31.25 ± 1.42b	32.67 ± 1.31b
B	30.92 ± 2.04	30.92 ± 1.87	32.25 ± 2.32	34.83 ± 2.86a	42.92 ± 1.69a
C	30.92 ± 1.95	29.92 ± 3.67	32.17 ± 2.53	33.00 ± 2.39ab	33.75 ± 1.79c
D	29.33 ± 1.87	29.92 ± 1.10	31.17 ± 2.01	31.67 ± 1.41b	32.67 ± 2.05c
E	30.75 ± 3.41	30.33 ± 2.02	32.17 ± 1.93	35.50 ± 2.20a	42.42 ± 1.71ab
F	31.08 ± 1.29	31.00 ± 1.05	31.83 ± 2.20	35.25 ± 1.13a	40.50 ± 1.14ab
G	28.67 ± 0.98	30.00 ± 1.66	30.50 ± 1.35	33.92 ± 1.83ab	39.83 ± b

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$. (A: Control, B: Standard NPK, C: Mycorrhizal biofertilizer 20 g/polybag, D: Humic Acid 25 g/polybag, E: 1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag, F: 3/4 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag, G: 1 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag)

Table 2. Plant height at 12 – 18 weeks after planting using mycorrhizal and humic acid biofertilizers

Treatment	Plant Height			
	12 MST	14 MST	16 MST	18 MST
	-----cm-----			
A	33.83 ± 1.48c	34.75 ± 1.42c	35.83 ± 1.64c	37.25 ± 1.64c
B	47.92 ± 0.79a	52.33 ± 0.61ab	57.92 ± 0.50ab	63.17 ± 1.55b
C	34.75 ± 1.13c	35.50 ± 1.11c	37.08 ± 0.83c	38.58 ± 1.13c
D	34.17 ± 1.50c	35.67 ± 0.98c	36.92 ± 0.83c	38.58 ± 1.10c
E	48.42 ± 1.77a	53.83 ± 2.63a	59.58 ± 2.28a	67.00 ± 2.74a
F	45.83 ± 0.79b	51.67 ± 0.82ab	57.42 ± 0.69b	63.92 ± 0.42b
G	44.92 ± 1.91b	51.08 ± 1.50b	56.50 ± 1.40b	63.00 ± 0.94b

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$. (A: Control, B: Standard NPK, C: Mycorrhizal biofertilizer 20 g/polybag, D: Humic Acid 25 g/polybag, E: 1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag, F: 3/4 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag, G: 1 standard NPK + 20 g mycorrhizal biofertilizer + 25 g humic acid/polybag)

Adequate nutrition will support the optimal growth of oil palm seedlings compared to plants that do not receive mycorrhizal treatment. Meanwhile, the addition of humic acid also plays a role in improving soil structure and increasing the soil's ability to retain water, which is crucial for early plant growth. Humic acid can also improve soil fertility from a physical, chemical, and biological perspective. Increased soil fertility also improves plants' ability to absorb nutrients, enabling optimal plant growth,

including plant height (Tan, 2014).

3.2. Wet and Dry Weight of Plant Canopies

Based on the research results, it was found that the application of biofertilizer and humic acid significantly increased the wet and dry weight of plant crowns. The average wet and dry weight of plant crowns resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer is shown in Table 3.

Table 3. Wet and dry weight of plant crowns using mycorrhizal and humic acid biofertilizers

Treatment	Wet Weight Title	Dry Weight of Header
	-----g-----	
A (Control)	56.63 ± 8.60b	16.64 ± 3.68 b
B (NPK Standard)	212.68 ± 47.72a	72.85 ± 26.06 a
C (Biofertilizer 20 g/polybag)	39.45 ± 7.74b	10.01 ± 2.69 b
D (Humic Acid 25 g/polybag)	40.53 ± 5.84b	10.16 ± 1.05 b
E (1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	180.60 ± 24.28a	70.77 ± 19.42 a
F (3/4 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	205.70 ± 20.93a	65.79 ± 24.34 a
G (1 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	176.33 ± 29.05a	49.81 ± 10.55 a

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$.

High canopy wet weight reflects the presence of active plant tissue and supports optimal photosynthesis. Meanwhile, canopy dry weight indicates the amount of biomass formed, reflecting the efficiency of the plant in utilizing resources for tissue growth and development (Sodikin et al., 2022). Treatment with standard NPK fertilizer (B) produced the highest canopy wet weight, namely 212.68 grams. However, it was not statistically significantly different from the combination treatments of $\frac{3}{4}$ NPK + mycorrhiza + humic acid (F), $\frac{1}{2}$ NPK + mycorrhiza + humic acid (E), and 1 dose of NPK + mycorrhiza + humic acid (G). Compared to the untreated control, the application of standard NPK increased canopy wet weight by up to 275.56%. However, the full combination of NPK + mycorrhiza + humic acid resulted in a wet weight 17.09% lower than single NPK.

In terms of shoot dry weight, NPK treatments, both

alone and in combination with biofertilizer and humic acid, showed higher results compared to the control and the separate treatments of mycorrhizae and humic acid. Treatment B produced the highest dry weight (72.85 grams), which was not significantly different from the combination treatments E, F, and G. Interestingly, combination E was only 2.86% lower than the single NPK treatment. The difference between the standard and combined NPK treatments showed that reducing the NPK dose by $\frac{1}{2}$ or $\frac{3}{4}$ could still provide equivalent results when combined with mycorrhizae and humic acid. This result proves that the combination can increase fertilization efficiency by reducing the use of chemical fertilizers. Biofertilizer and humic acid help provide nutrients through microbial activities such as phosphate solubilization and nitrogen fixation, as well as increasing cation exchange capacity and soil nutrient retention (Wicaksono et al., 2014).

The support of key nutrients in NPK fertilizers, such as N, P, and K, is very important in supporting plant physiological activities, including cell division. Research by Saputra et al. (2024) strengthened this finding, showing that a dose of 4.5 g NPK per plant significantly increased shoot weight.

3.3. Wet and Dry Weight of Plant Roots

Based on the research results, it was found that the application of biofertilizer and humic acid significantly increased the wet and dry weight of plant roots. The average wet and dry weight of plant roots resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer is shown in Table 4.

Table 4. Wet and dry weight of plant roots using mycorrhizal and humic acid biofertilizers

Treatment	Wet Weight Root	Dry Weight Root
A (Control)	30.63 ± 2.82b	6.29 ± 0.37b
B (NPK Standard)	61.08 ± 11.37a	14.22 ± 3.69a
C (Biofertilizer 20 g/polybag)	25.58 ± 4.47b	4.74 ± 1.46b
D (Humic Acid 25 g/polybag)	25.10 ± 2.00b	4.59 ± 1.01b
E (1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	62.83 ± 6.81 a	15.02 ± 2.41 a
F (3/4 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	59.20 ± 13.41a	12.02 ± 3.48a
G 1 (1 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	63.38 ± 11.57a	12.23 ± 2.57a

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$.

Root wet weight is an important parameter in assessing the quality of the root system, because larger and healthier roots reflect the plant's ability to absorb water and nutrients more effectively. The results showed that the application of NPK fertilizer, either alone or in combination with mycorrhizal and humic acid biofertilizers, resulted in higher root wet weight compared to the control treatment or the single mycorrhizal or humic acid treatments. The combination treatment of standard NPK with 20 g of mycorrhizal and 25 g of humic acid per polybag (G) showed the highest root wet weight, with an average of 63.38 grams. This result was not significantly different from treatments E (½ dose of standard NPK + mycorrhizal + humic acid), B (standard NPK), and F (¾ dose of standard NPK + mycorrhizal + humic acid). When compared to the control, treatment G increased root wet weight by 106.92%, and compared to treatment B, the increase was 3.76%. This increase is thought to originate from the role of mycorrhizal and humic acid in increasing the efficiency of nutrient absorption and supporting root development. Humic acid improves soil structure and increases water retention capacity, making it easier for roots to absorb nutrients for growth. According to Rahmandhias et al. (2020), humic acid not only acts as a biostimulant but also as a nitrogen source, with a content of up to 12%, significantly increasing nitrogen uptake by plants.

Root dry weight reflects the accumulation of biomass in the root system (Ariyanti et al., 2019). The results showed that treatment E (½ NPK + mycorrhiza + humic acid) provided the highest root dry weight, namely 15.02 grams, which was not significantly different from treatments B, F, and G. When compared to the control, treatment E increased root dry weight by 138.7%, while treatment B increased by 126.1%. In fact, treatment E

recorded 5.63% higher than single NPK, indicating that the combination of lower doses of NPK with biofertilizer can still produce equivalent, or even better, root growth. The correct fertilizer dosage plays an important role in encouraging root formation. Potassium (K) supports the process of cell division and photosynthesis, while phosphorus (P) significantly influences root formation, especially in the early phase of growth (Idan and Olewi, 2024).

The combination of NPK fertilizer, mycorrhizae, and humic acid has been shown to optimize root growth. This is supported by research by Hazra et al. (2023), which found that this combination increased the dry weight of oil palm seedling roots. Mycorrhizae can expand and multiply roots through hyphal formation and accelerate plant metabolism, thus supporting new root formation. Increased root dry weight indicates efficient allocation of plant resources to root system growth, which ultimately has a positive impact on overall plant growth and productivity.

3.4. Root Crown Ratio

Based on the research results, it was found that the application of biofertilizer and humic acid significantly increased the root crown ratio. The average root crown ratio resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer is shown in Table 5.

The ratio between shoots and roots reflects the balance of plant growth, where the growth of other parts generally accompanies the development of one part of the plant. Based on the results of the study, the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer was proven to increase the shoot-root ratio significantly compared to plants that were not treated or those that were only given mycorrhizal biofertilizer or humic acid alone.

The treatment with $\frac{3}{4}$ dose of standard NPK combined with 20 g of mycorrhizal and 25 g of humic acid per polybag (F) showed the highest shoot-root ratio with an average of 6.03. However, this result was not statistically significantly different from treatment B (standard NPK), treatment E ($\frac{1}{2}$

NPK + mycorrhizal + humic acid), or treatment G (standard NPK + mycorrhizal + humic acid). Compared with the untreated control, the F combination increased the shoot-root ratio by 121.17%, while the standard NPK treatment increased it by 92.83%.

Table 5. Root crown ratio in the use of mycorrhizal and humic acid biofertilizers

Treatment	Heading Ratio Root
A (Control)	2.65 ± 0.60bc
B (NPK Standard)	5.11 ± 1.26a
C (Biofertilizer 20 g/polybag)	2.30 ± 0.90c
D (Humic Acid 25 g/polybag)	2.26 ± 0.32c
E ($\frac{1}{2}$ standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	4.79 ± 1.53ab
F ($\frac{3}{4}$ standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	6.03 ± 3.17a
G 1 (1 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	4.07 ± 0.26abc

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$.

These findings align with research by Putra and Pamungkas (2022), who reported that applying humic acid in combination with a single fertilizer, at doses of 50%, 75%, or 100%, resulted in higher plant fresh and dry weights compared to the unfertilized treatment and were statistically similar to the full NPK treatment. This indicates that humic acid can increase the efficiency of inorganic fertilizer use, allowing dose reductions of up to 50% without compromising plant and root growth effectiveness. Furthermore, humic acid is known to increase nutrient availability in the soil, thus optimally meeting plant nutritional needs and increasing biomass

production (Stevanus and Cahyo, 2021). Furthermore, humic acid plays a crucial role in maintaining soil fertility by improving soil physical structure, increasing porosity, aiding oxygen absorption, and retaining soil moisture.

3.5. Plant P Uptake

Based on the research results, it was found that the application of biofertilizer and humic acid significantly affected plant P uptake. The average plant P uptake resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer can be seen in Table 6.

Table 6. Plant P uptake in the use of mycorrhizal and humic acid biofertilizers

Treatment	Plant P Uptake -----mg-----
A (Control)	5.78 ± 1.07bc
B (NPK Standard)	22.26 ± 7.79a
C (Biofertilizer 20 g/polybag)	2.61 ± 0.60c
D (Humic Acid 25 g/polybag)	2.35 ± 0.43c
E ($\frac{1}{2}$ standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	18.13 ± 6.43a
F ($\frac{3}{4}$ standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	19.58 ± 12.3a
G 1 (standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	13.25 ± 6.34ab

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$.

Based on the research results, the highest P uptake was obtained in treatment B (standard NPK) at 22.26 mg, followed by treatments F (19.58 mg) and E (18.13 mg). Despite using lower NPK doses, treatments E and F achieved approximately 81–88% of the P uptake in the full NPK treatment. In fact, compared to the control (5.78 mg), treatments E and F showed an increase in P uptake of approximately 213% and 239%, respectively. Meanwhile, the single application of biofertilizer (2.61 mg) and humic acid (2.35 mg) was lower than the control, decreasing by approximately 55–59%, indicating that without the support of inorganic fertilizers, their effectiveness is limited.

This study shows that the combination of NPK fertilizer, mycorrhizae, and humic acid improves

phosphorus absorption better than single applications. Adequate phosphorus is essential for early root development before shoot growth, and this impacts the increase in root fresh weight. The role of mycorrhizae in symbiosis with roots expands nutrient absorption through hyphae, especially phosphorus, which is difficult to move in the soil. Mycorrhizae also stimulate the production of enzymes and organic acids that help mobilize nutrients. In addition to phosphorus, mycorrhizae facilitate the absorption of other nutrients essential for plant growth. Phosphorus is absorbed in the form of orthophosphate and used in root system development. The application of mycorrhizae has been shown to increase the efficiency of P absorption and support plant growth, as demonstrated in

sorghum (Sowmen et al., 2019). The results of this study show a consistent pattern. However, the combination of mycorrhizae and humic acid can compensate for a reduction in NPK fertilizer by up to 50% without a significant decrease in P absorption. This result confirms that the synergy between mycorrhizae and humic acid not only increases P absorption efficiency but also has the potential to reduce dependence on inorganic fertilizers.

3.6. Root Colonization Infection

Based on the research results, it was found that the application of biofertilizer and humic acid significantly affected root colonization infections. The average root colonization infection rate resulting from the combination of mycorrhizal biofertilizer, humic acid, and NPK fertilizer is shown in Table 7.

Table 7. Root colonization infection in the use of mycorrhizal and humic acid biofertilizers

Treatment	Root Colonization
	-----%-----
A (Control)	5 ± 5.77 b
B (NPK Standard)	3 ± 5.00 b
C (Biofertilizer 20 g/polybag)	20 ± 14.14 ab
D (Humic Acid 25 g/polybag)	2.5 ± 5.00 b
E (1/2 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	37.5 ± 5.00 a
F (3/4 standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	37.5 ± 20.62 a
G 1 (standard NPK + 20 g mycorrhizal biofertilizer + 25 g Humic Acid/polybag)	32.5 ± 15.00 a

Description: Numbers followed by the same letter in the same column indicate values that are not significantly different from the DMRT test at $\alpha = 5\%$.

The results of this study indicate that the highest levels of root colonization were found in treatments E and F, which used lower NPK doses than the standard ($\frac{1}{2}$ and $\frac{3}{4}$ NPK). This indicates that the use of lower NPK fertilizers, when combined with mycorrhizal biofertilizers and humic acid, can create more optimal conditions for the development of mycorrhizal fungi. Conversely, the application of NPK in full doses can inhibit mycorrhizal colonization, because high phosphorus availability can reduce the need for plants to establish symbiotic relationships with mycorrhizal fungi (Smith and Read, 2008).

According to Istiqomah et al. (2023), the age of mycorrhizal isolates also influences the percentage of root infection in oil palm seedlings. The longer a mycorrhizal isolate is stored, the more its infectivity in

colonizing the host plant will decrease, ultimately resulting in a lower percentage of root infection. Furthermore, research by Kafrawi et al. (2022) revealed that several factors, including mycorrhizal spore germination, hyphal growth in the soil, and the location of the entry point on the plant roots, influence the percentage of mycorrhizal infection. Another factor that also influences root infection is water content or watering levels, where drier soil conditions can actually increase root colonization by mycorrhizae.

The results of the study show a cross-section of the roots of oil palm seedlings that have been infected by mycorrhiza, with the structure of the colonized mycorrhizal tissue, namely spores, vesicles, and hyphae shown in Figure 2.

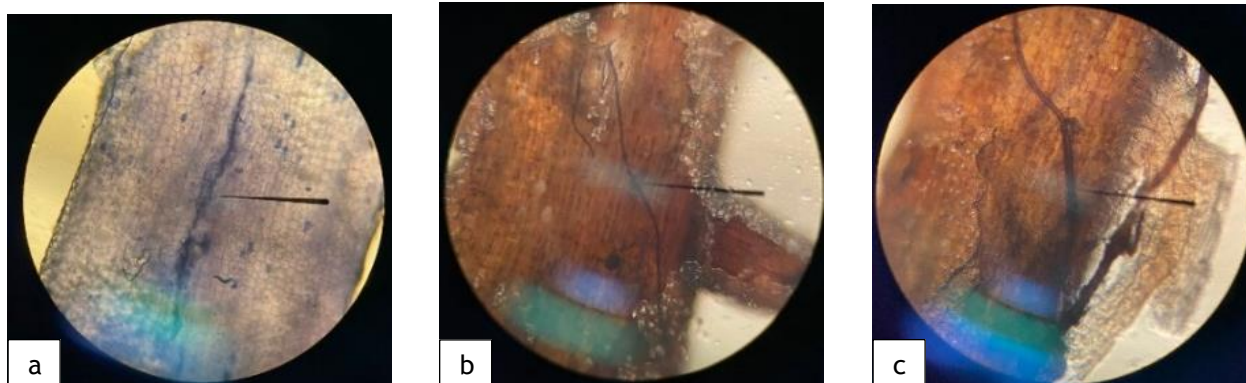


Figure 1Hyphal structure of AMF in oil palm roots. (a) spores, (b) vesicles, (c) hyphae.

4. Conclusion

The application of mycorrhizal and humic acid biofertilizer significantly increased oil palm seedling growth, as well as phosphorus uptake and efficiency. The $\frac{1}{2}$ NPK + mycorrhizal + humic acid (E) treatment provided

the best growth, while $\frac{3}{4}$ NPK + mycorrhizal + humic acid (F) showed P uptake efficiency equivalent to standard NPK. Furthermore, both treatments also increased root colonization. Therefore, the combination of mycorrhizal and humic acid is recommended to reduce the NPK dose by

up to 50% without reducing growth and nutrient uptake, thus being more efficient and sustainable.

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