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The Application of Arbuscular Mycorrhizal Fungi Reduced the Required Dose of Compound Fertilizer for Oil Palm (*Elaeis Guineensis* Jacq.) in Nursery

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Abstract. Arbuscular mycorrhizal fungi have the ability to increase nutrient availability for the plant as well as improve the soil quality. However, the effectiveness of AMF is affected by soil fertility. Therefore, this study was carried out to determine the best dose of compound fertilizer for oil palm seedlings with and without AMF application. The factorial experiment (2 x 5) with four replication was carried out with the first factor was AMF inoculation, consisting of 2 levels: without AMF inoculation (m_0) and with AMF inoculation (m_1). The second factor was compound fertilizer dose, consisting of 5 levels: 0 mg polybag^{-1} (f_0), 250mg polybag^{-1} (f_1), 500 mg polybag^{-1} (f_2), 750 mg polybag^{-1} (f_3), and 1000 mg polybag^{-1} (f_4). The treatments were arranged in a randomized complete block design (RCBD). The data obtained were tested using analysis of variance and means separation tested using Least Significant Difference (LSD) at α 5%. The result showed that oil palm seedlings' response towards AMF inoculation was influenced by the dose of compound fertilizer based on plant height, leaf surface area, shoot fresh weight, and shoot dry weight. The best dose of fertilizer compound for oil palm seedlings inoculated with AMF was 500 mg polybag^{-1}, whereas without AMF inoculation was 1000 mg polybag^{-1}. In other words, the AMF application reduced 50% of compound fertilizer needed for oil palm seedlings.

1. Introduction

Due to limited fertile soil available, oil palm in Indonesia currently focuses on developing and operating the plantation in marginal land, where the soil types are mainly Ultisol and Oxisol. Ultisol has low C organic and is characterized by its low pH which generates an accumulation of Fe and Al mineral and is responsible to fix phosphate P (immobile P) [1]. Meanwhile, Oxisol soil has similar characteristic with Ultisol, but tend to have fewer macro and micronutrients [2]. Therefore, there is an urgent need to improve soil nutrients and plants in the area. One of the most promising alternative technologies is arbuscular mycorrhizal fungi (AMF) application as a biofertilizer to enhance crop productivity [3,4].

Arbuscular mycorrhizal fungi (AMF) are a type of mycorrhiza in which symbiont fungus penetrates root cortex cells and forms arbuscular. AMF is ubiquitous which can form a symbiotic association with around 80% of vascular plants [3]. This mutualism symbiosis occurred in the rhizosphere where fungi live by absorbing photosynthate products from roots and plant received nutrients (P, N, and trace elements) and water through hyphae of the fungi. Mycorrhizal arbuscular fungi hyphae network in the soil significantly improved plant growth as its ability to enhance roots access to large soil surface regions [5]. Besides, AMF has several advantages including, improving photosynthesis and plant growth, protection against the pathogen, increase tolerance against toxicities of heavy metals, better mineral nutrition in plants, increase fruit yield and essential amino acids, as well as improved biosynthesis of important molecules involved in oxidative stress metabolism [3,6–9].

Although arbuscular mycorrhiza fungi can adopt a wide range of soil environment status, their development and colonization are influenced by the availability of nutrients (particularly P), host plant, and soil fertility and structure. Many stated that high P concentrations in soil eliminate mycorrhizal colonization suggesting that the fungi are no longer useful to nutrient uptake for plants and due to reduced permeability of root membrane, thereby decreasing loss of metabolites [3,10]. High P decreases the production of root exudates that promote branching of hyphae nearing the roots and growth of extraradical mycelium, which results in primary and secondary colonization [3]. High P level of roots turns out to have a direct effect on internal hyphae growth rate and extraradical mycelium development, perhaps fewer carbohydrate supplies available for the fungus [3]. Another finding stated that plants in rich P soil are poor carbohydrate content which affects reduce the process of colonization [6]. According to some studies, agricultural land with higher plant density caused exhausting soil nutrients, thereby rapidly stimulating AMF activities in soil [11]. In other words, soil with low fertility and poor nutrients can boost AMF colonization. On the other hand, nowadays, agricultural management practices can affect various degrees of the AMF population, for instance, excessive use of fertilizer and pesticides [4]. These practices may alter the soil's physical fertility and lead to a change in its nutrient composition where the worst case might result in low soil nutrients and disturb AMF colonization and community through the process as mentioned above [12].

Based on different research studies, AMF as a biofertilizer has been highlighted for decades due to its benefits on soil health and crop productivity. Therefore, AMF is considered to be a good replacement for inorganic or chemical fertilizer in the future as AMF application can reduce the use of chemical fertilizer, especially phosphorus input [13]s. Hence, it is fascinating to investigate how to extend do a combination of AMF inoculation and a variety of compound fertilizer doses applications affect AMF colonization and its efficiency. The goal of the study is to observe the effect of compound fertilizer doses towards AMF colonization in enhancing growth traits of oil palm seedlings as host plants using Ultisol soil.

2. Materials and Methods

The factorial experiment (2 x 5) was conducted with the first factor was AMF (collection of Produksi Perkebunan Laboratory, Universitas Lampung) application i.e., without AMF (m_0) and inoculated with AMF (m_1). The second factor was five doses of compound fertilizer i.e., 0 mg polybag^{-1} (f_0), 250mg polybag^{-1} (f_1), 500 mg polybag^{-1} (f_2), 750 mg polybag^{-1} (f_3), and 1000 mg polybag^{-1} (f_4). The randomized complete block design was used as an experimental design with four replications. Data obtained were analyzed with analysis of variance and continued with mean separation using least significant difference at α 5%.

2.1. Pre-Nursery

Seventy-five Tenera germinated seeds of oil palm (from Indonesian Oil Palm Research Institute, Medan) were germinated in a plastic box containing sterilized river sand for one month. No fertilizer was added during this time. After one month, forty uniform seedlings were selected. The selected seedlings were then transferred into baby polybags (8 x 19 cm size) containing 1 kg of unsterilized Ultisol topsoil per polybag. During transplanting, the seedlings that received AMF treatment (20 seedlings) were inoculated with AMF inoculum. Arbuscular mycorrhiza fungi inoculum used contains a mixture of AMF from *Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., and *Entrophospora* sp. AMF inoculum (sand-based containing 500 spores) was spread evenly on root surfaces of the seedling and partly placed at the bottom of the planting hole. The polybags were then kept at a prenursery bed and shaded with a net for two months. At the prenursery, the seedlings were watered twice a day and only urea fertilizer (2 g L^{-1}) was applied as much as 10 ml per seedling every week.

2.2. *Main Nursery*

Three months old seedlings from prenursery were then transplanted to the bigger polybag in the main nursery. The size of the polybag in the main nursery was 20 cm x 45 cm. About 15 kg unsterilized Ultisol topsoil was mixed thoroughly with 50 mg rock phosphate and then put inside one polybag. At the center of the polybag, a planting hole was made using *ponjo* (hole digger). The prenursery polybag was carefully removed and the seedling was then placed in the planting hole of the main nursery polybag. For AMF treatment, at the time of transplanting, AMF inoculum (500 spores/polybag) was again applied at the bottom of the planting hole. After all the seedlings were transplanted, the polybags were then kept at the main nursery without shading with the triangle planting distance of 70 cm x 70 cm x 70 cm for 5 months. After two weeks at the main nursery, compound fertilizer according to treatment was applied. The compound fertilizer used consisted of macro and micronutrients i.e., Mg: S: B: Cu: Fe: Mn: Mo: Zn (4:12:0,5:0,5:4:4:0,5:4).

The seedling at the main nursery was water daily manually. Weeding inside and outside polybags was also done manually. In addition to compound fertilizer, basic fertilizer for oil palm at the main nursery was also given with half the dose of the recommended dose every two weeks. From week 14th to 17th, NPK (16:16:16) fertilizer was added for 2.5 g $polybag^{-1}$. From week 18th to 20th, NPK fertilizer was added for 3.5 $gpolybag^{-1}$. Last, from week 22th to 30th, NPK fertilizer was added for 5g $polybag^{-1}$. At the end of the experiment (after five months at the main nursery), data on seedling height, the number of leaves, total leaf area, fresh and dry weight of shoot and root, and root colonization by AMF were recorded. Assessment of AMF colonization was done using Trypan Blue as root dye [14].

3. Results and Discussion

Arbuscular mycorrhiza fungi root colonization is one of the indicators that symbiotic association between AMF and the root of oil palm seedling has occurred. The colonized root is characterized by the formation of fungal structures within root cortex cells such as hyphae, vesicle, and arbuscular. Results obtained indicated that AMF inoculation to the root of oil palm seedlings was shown to have a higher percent root colonization compared to seedlings without AMF inoculation (Table 1). This suggests that oil palm is suitable as a host plant to form a symbiosis with AMF, which was also proved by AMF colonization (indigenous from the soil) that occurred in oil palm seedlings without treatment of AMF, despite low infection at 15.6% (Table 1).

Table 1. Effect of AMF inoculation on root colonization of oil palm seedlings.

Treatment	Root Colonization
	….. % per plant ……
Without AMF	15.6 a
With AMF	36.3 b
LSD 5%	1.8

Note: Means followed by the same letters are not significantly different according to the least significant difference (LSD) test at α = 0.05

The higher AMF root colonization in the root of AMF inoculated seedling resulted in the higher seedling height, total leaf area, and shoot and root fresh and dry weight. As for seedling height, the highest seedling height for uninoculated AMF seedling was 79.25 cm for fertilizer compound of 750 mg $polybag^{-1}$ and 1000 mg $polybag^{-1}$. While for AMF treated seedlings, the highest seedling height of 85.50 cm was from 500 mg $polybag^{-1}$ compound fertilizer treatment (Table 2). The same trend results are also shown for the total leaf area. The AMF treated seedlings received compound fertilizer 500 mg $polybag^{-1}$, giving the highest total leaf area (Table 3). These results suggested that inoculated AMF is more effective than AMF that already exists (indigenous) in the soil. The inoculated AMF gave the higher seedling height and total leaf area at a lower dose of compound fertilizer.

Table 2. Effect of AMF inoculation and compound fertilizer dose on the height of oil palm seedlings.

Treatment	Plant Height	
Fertilizer compound dose	Without AMF Inoculation	With AMF Inoculation
	…….. cm plant^{-1} …….	
0 mg polybag^{-1}	72.00 a A	77.75 b B
250 mg polybag^{-1}	77.25 b A	77.25 b A
500 mg polybag^{-1}	70.75 a A	85.50 c B
750 mg polybag^{-1}	79.25 b B	71.25 a A
1000 mg polybag^{-1}	79.25 b A	78.75 b A
LSD 5%	4.20	

Note: Means followed by the same letters are not significantly different according to the least significant difference (LSD) test at α = 0.05 (the capital letter read horizontally, the small letter read vertically).

Table 3. Effect of AMF inoculation and compound fertilizer dose on total leaf area of oil palm seedlings.

Treatment	Total Leaf area	
Fertilizer compound dose	Without AMF Inoculation	With AMF Inoculation
	…….. cm^{2} plant^{-1} …….	
0 mg polybag^{-1}	4564 a A	5195 ab B
250 mg polybag^{-1}	5473 b A	5578 bc A
500 mg polybag^{-1}	4370 a A	6046 c B
750 mg polybag^{-1}	5525 b B	4646 a A
1000 mg polybag^{-1}	5923 b A	4998 ab A
LSD 5%	607	

Note: Means followed by the same letters are not significantly different according to the least significant difference (LSD) test at α = 0.05 (the capital letter read horizontally, the small letter read vertically).

The application of AMF has also increased oil palm seedling growth as indicated by shoot dry weight and root dry weight (Table 4 and Table 5). The dose of compound fertilizer needed to get the highest shoot dry weight was 500 mg polybag^{-1} for AMF treated seedling and 750 mg polybag^{-1} and 1000 mg polybag^{-1} for the uninoculated seedling. This result again confirms that AMF inoculation can reduce the use of compound fertilizer.

Table 4. Effect of AMF inoculation and compound fertilizer dose on shoot dry weight of oil palm seedlings.

Treatment	Shoot dry weight	
Fertilizer compound dose	Without AMF Inoculation	With AMF Inoculation
	…….. g per plant^{-1} …….	
0 mg polybag^{-1}	79.1 a A	92.4 a B
250 mg polybag^{-1}	92.4 bc A	81.6 a A
500 mg polybag^{-1}	82.0 ab A	120.6 b B

750 mg polybag^{-1}	98.3 c B	84.4 a A
1000 mg polybag^{-1}	103.8 c B	90.5 a A
LSD 5%	11.8	

Note: Means followed by the same letters are not significantly different according to the least significant difference (LSD) test at α = 0.05 (the capital letter read horizontally, the small letter read vertically).

For the root dry weight variable, the results of the analysis of variance showed that there was no interaction between the AMF factor and fertilizer dose factor, therefore the data were presented per factor as shown in Table 6. The application of AMF significantly increases the root dry weight of oil palm seedlings, on the other hand, compound fertilizer dose did not. Root dry weight for all compound fertilizers did not significantly different.

Table 5. Effect of AMF inoculation and compound fertilizer dose on root dry weight of oil palm seedlings.

Treatment	Root dry weight
AMF dose	… g per plant …
Without AMF	27.5 a
With AMF	32.1 b
LSD 5%	3.9
Fertilizer dose (mg polybag^{-1})	
0	26.8 a
250	30.6 a
500	30.6 a
750	29.2 a
1000	31.6 a
LSD 5%	6.2

Note: Means followed by the same letters are not significantly different according to the least significant difference (LSD) test at α = 0.05

The development and effectivity of AMF symbiosis are affected by nutrient availability within the soil. The plant can normally uptake nutrients without support from AMF when its availability is high. In contrast, plants grown on soil with less or depleted available nutrients need AMF as a symbiont for more effective nutrient uptake [15]. This study shows that there is an interaction between mycorrhiza inoculation and the application of compound fertilizer in various doses. The results of this study confirm that soil fertility can affect AMF effectiveness. The best dose of compound fertilizer for the best growth of oil palm seedling in this study is 500 mg polybag^{-1} for AMF treatment and 1000 mg polybag^{-1} for non-AMF treatment. [16] reported that in no addition to the nitrogen plot, the addition of phosphorous significantly decreased AMF colonization. Decreased AMF colonization will affect the ability of AMF in increasing seedling growth.

Although the roots of non-AMF seedlings were colonized by indigenous AMF, the growth of the seedlings was lower than those inoculated AMF. This suggested that AMF used in this study is more effective than the indigenous one. The same result was also reported by [17].

Arbuscular mycorrhizal fungi that have been associated with the roots of AMF-treated oil palm seedlings have increased seedling growth as indicated by plant height, total leaf area, and dry weight of shoots and roots. Some mechanisms may be involved in this increment. The external hyphae of AMF that develop in the soil can absorb nutrients directly (especially immobile nutrients such as phosphorous) and transfer them to the root cell cortex [18]. The nutrient uptake efficiency on plant-associated with AMF occurs when macro and micronutrients have low mobility, thus nutrients are trapped within the soil. At this stage, the normal root is not capable of uptake nutrients in the depleted zone. A plant associated with AMF has an ability to access low nutrient mobility in the exhausted area as well as trapped nutrient through the extension of external hyphae [4].

The external hyphae of AMF can also produce phosphatase enzymes that can liberate fixed P in the soil so that it can be absorbed by both AMF hyphae and seedling roots [19]. An increase in nutrient uptake will positively increase oil palm seedling growth. Based on experimental trials on tomato seedlings inoculated with AMF by [20], they found that AMF could enhance plant growth by increasing leaf area and other minerals content (N, P, K, Ca). A study by [21] also found that plant height, shoot dry weight, photosynthetic characteristics, and nutrient contents in cucumber seedlings inoculated with AMF were significantly higher than those without AMF. In addition, [22] reported that the application of AMF *Entrophospora* sp. combined with 50% NPK fertilizer dose resulted in higher oil palm seedling growth compared to 100% dose recommendation. This proved that AMF improved plant growth and development and highly possible to reduce the use of fertilizer.

Study results indicated that AMF inoculation combined with 500mg polybag^{-1} produced the highest plant height, total leaf area, and shoot dry weight. The study discovered that there is a relation between those variables, plants with good height can promote shoot growth which was shown in large total leaf areas. According to research carried by [23], total leaf area gives a positive impact on the addition of plant biomass. The accumulation of biomass is mainly determined by the photosynthetic performance in the plant [21]. Another finding reports that AMF colonization can increase the number of chlorophyll and carotenoid in the leaves, enhance root absorption area and root activity, strengthen the water transport and other nutrients, thereby increasing photosynthesis and biomass accumulation [24].

4. Conclusion

The best dose of fertilizer compound for oil palm seedlings inoculated with AMF was 500 mg polybag^{-1}, whereas without AMF inoculation was 1000 mg polybag^{-1}. In other words, the AMF application reduced 50% of compound fertilizer needed for oil palm seedlings.

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