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The association of four species of arbuscular mycorrhizal fungi with oil palm seedlings planted on inceptisol soil from Central Kalimantan Indonesia

Rini, M. V. [1*], Irvanto, D.[2] and Ardiyanto, A.[2]

[1]Department of Agronomy and Horticulture, University of Lampung, Lampung Indonesia; [2]Research and Development Department of PT Bumitama Gunajaya Agro, Central Kalimantan Indonesia.

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Abstract Results showed that all AMF-treated seedlings (except the combination of *Gigaspora* sp. and *Entrophospora* sp.) were successfully colonized by AMF, with a high root colonization rate of >50%. The control seedling was also colonized by indigenous AMF presented in the soil, with the lowest root colonization rate recorded at 40.8%. Although the oil palm roots were colonized by AMF with a high colonization rate, the improvement of oil palm seedling growth and nutrient uptake varied. The AMF treatments consistently showing better growth and nutrient uptake than the control seedlings were *Glomus* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp. These treatments increased plant growth in terms of shoot fresh weight by 32%, 41%, and 32%, and shoot dry weight by 45%, 48%, and 64%, respectively. Nutrient uptake also increased by 41-61% for phosphorus (P), 68-100% for Calcium (Ca), 26-38% for magnesium (Mg), and 34-58% for Boron (B).

Keywords: Selection, Marginal soil, Symbiosis, Nutrient uptake

Introduction

Most oil palm plantations in Indonesia are established on marginal land, including inceptisol soil. Inceptisol is a mineral soil with low nutrient availability (N, P, and K). These soils are commonly found and spread in Kalimantan and Sumatra (Safitri *et al.*, 2018). Subagyo *et al*. (2000) reported over 70 million hectares of land in Indonesia are inceptisol soils, with the largest area located on Sumatra Island, which accounted for 17.6 million hectares.

The inceptisol soil has a coarse texture at the topsoil, but a less coarse texture in the lower soil, resulting in higher permeability in the topsoil, and a notable presence of aluminum (Al) in the soil (Bortolanza and Kelin, 2016). Due to inceptisol soil characteristics, a chemical fertilizer needs to be added to

* **Corresponding Author**: Rini, M. V.; **Email:** maria.vivarini@fp.unila.ac.id

improve the growth and productivity of a plant (Zainuddin *et al.,* 2022). However, the excessive application of chemical fertilizer can lead to soil acidification and soil crust formation. These outcomes have the potential to diminish the abundance of beneficial soil microbes, reduce nutrient availability, induce drought condition, and contribute to pollution in the surrounding ecosystem. Moreover, such practices often elevate the incidence of plant diseases (Bisht and Chauhan, 2020; Meena *et al*., 2020; Rahman *et al*., 2020). Therefore, it is essential to explore alternatives that can minimize the reliance on chemical inputs in agricultural lands.

Arbuscular Mycorrhizal Fungi (AMF) is an environmentally friendly biofertilizer that has been successfully proven to improve plant growth and soil quality without adversely affecting the environment. AMF is ubiquitous and the most common type of mycorrhizal association, widely found in variety of host plants, including oil palm, through obligate symbiotic relationships (Smith and Read, 2008; Rini *et al*., 2021a; Ritaqwin *et al*., 2021).

The AMF enhance water uptake and solubilizes immobile nutrients in the soil, making them readily available for plant uptake. The plant, in turn, provides carbon nutrients from photosynthesis products to the fungi (Smith and Read, 2008; Souza, 2015). A study reported an improvement in the quality of potato tubers after AMF inoculation in potato seedlings grown in inceptisol soils (Nurbaity *et al.*, 2019). Studies by Trejo *et al*. (2021) on pineapple, revealed that the inoculation of AMF can reduce chemical fertilizer usage by 50% without a loss in yield and with improved fruit quality. Similar results were reported by Rini *et al.* (2022) for oil palm, indicating that the application of AMF can effectively reduce the use of chemical fertilizer by 50%.

The effectiveness of AMF inoculation is influenced by various environmental factors, including AMF species, soil type, and host plant (Melo *et al*., 2019; Damayanti *et al.,* 2015; Ndoye *et al*., 2012; Yang *et al*., 2012). Studies indicated that different AMF species, whether applied individually or in mixture, can have distinct effects on plants. Cao *et al*. (2020) reported that the inoculations of *Funneliformis mosseae*, *Rhizophagus intraradices*, and the mixture of five AMF species had more beneficial effects on enhancing the stress tolerance of *Leymus chinensis* under a saline-alkaline gradient compared to the inoculations of *Diversispora versiformis* and *Acaulospora scrobiculata.* Auliana and Kaonongbua (2018) conducted a study on the biodiversity of AMF in three oil palm plantations in Thailand. They found that the diversity of AMF in each plantation was slightly to moderately different, and they assumed that these differences may be attributed to varying soil characteristics in each plantation. These results highlight that the same host plant can associate with different AMF species under different soil condition. To achieve successful and optimum plant growth, it is essential to screen among AMF species that contribute the best beneficial effects to plants in specific conditions. Therefore, the objective was to investigate the best formulation of arbuscular mycorrhizal fungal species to promote the growth of oil palm seedlings grown in inceptisol soil.

Materials and methods

The research was conducted at the Research Department of PT Bumitama Gunajaya Agro, Central Kalimantan, Indonesia from October 2018 to September 2019. The study employed a single-factor treatment of arbuscular mycorrhizal fungi with the nine treatments, including a control without mycorrhizae, *Glomus* sp. (G), *Gigaspora* sp. (Gi), *Entrophospora* sp. (E), *Acaulospora* sp. (A), G + Gi, G + E, Gi + E, and G + Gi + E + A. Photos of these 4 AMF species are presented in Figure 1. Each treatment was repeated five times and each experimental unit was represented by one oil palm seedling. The experiment used a Randomized Completely Block Design (RCBD), with grouping based on the uniformity of the seedlings in the main nursery. The obtained data were analyzed using analysis of variance, followed by mean separation using the Least Significant Difference (LSD) test at a significance level of 5%.

The pre-nursery was conducted using inceptisol soil obtained from the Sungai Cempaga Estate of PT Bumitama Gunajaya Agro in Central Kalimantan, Indonesia. The characteristics of the inceptisol soil are detailed in Table 1. The soil sample was sieved using a 1 cm sieve and then placed into polybags measuring 22 x 14 cm, with each polybag containing 1 kg of soil. In the growing media, there were 157 indigenous AMF spores per 50 grams of soil. However, some of the spores were damaged or dead, as indicated by the absence of liquid inside these spores.

The germinated oil palm seeds used were of the Tenera type, with hybridization numbers F160801946 and F160802120, obtained from the Oil Palm Research Institute of Indonesia in Medan. Each polybag was planted with one germinated seed and then maintained in the pre-nursery for 14 weeks under net shade. Bayfolan fertilizer (N:P:K=11:8:6) was applied when the seedlings were 2-11 weeks old, with a dose of 2 g/L of water for 100 seedlings.

After 14 weeks in the pre-nursery, the seedlings were transplanted to the main nursery. The planting medium used in the main nursery was also inceptisol soil, with each polybag weighing approximately 16 kg. The polybags were arranged in an open field with a triangular planting distance of 75 cm x 75 cm x 75 cm.

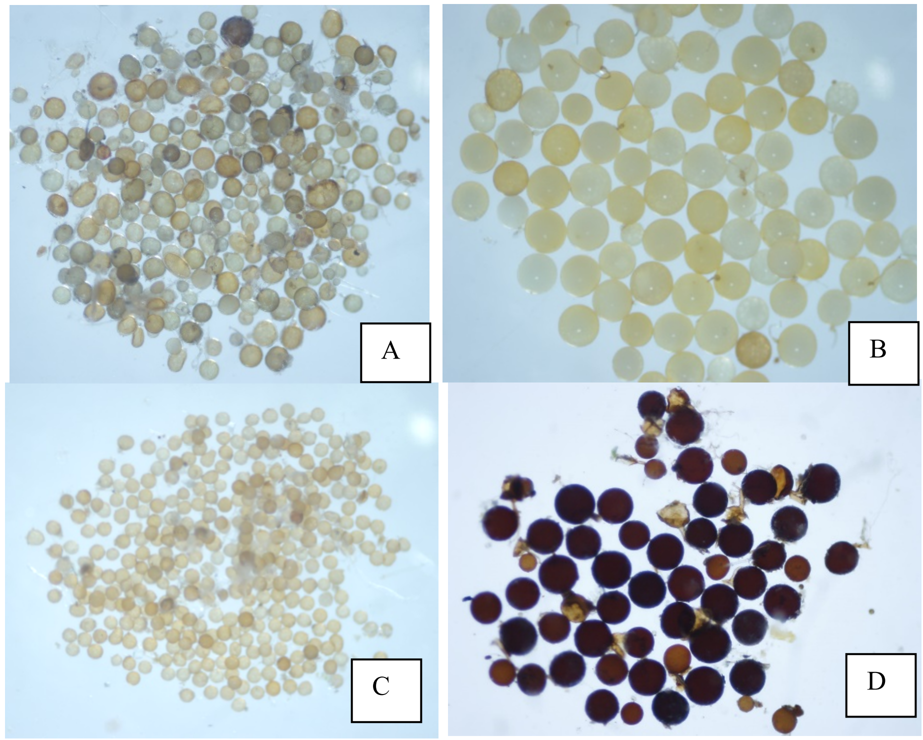

Figure 1. AMF's spores of *Glomus* sp. (A), *Gigaspora* sp. (B), *Entrophosphora* sp. (C), and *Acaulospora* sp. (D).

Table 1. Result of inceptisol soil analysis which used as planting media

Characteristics	Value	Characteristics	Value
pH	4.85	Total P	128.33 ppm
C-organic	0.75 %	K	0.220 Cmol (+)/kg
N	0.187 %	Ca	1.257 Cmol (+)/kg
Available P	27.04 ppm	Mg	0.413 Cmol (+)/kg

The AMF isolates included *Glomus* sp., *Gigaspora* sp., *Entrophospora* sp., and *Acaulospora* sp. These isolates were obtained from the Plantation Production Laboratory, Faculty of Agriculture, the University of Lampung, which were propagated using a mixture of river sand and zeolite as a growing medium. *Glomus* sp., *Entrophospora* sp., and *Acaulospora* sp. isolates were isolated from the oil palm rhizosphere, while the *Gigaspora* sp. isolate was isolated from Albizia rhizosphere.

The application of AMF on oil palm seedlings was carried out when the seedlings were transplanted from pre-nursery to main nursery. AMF inoculum, containing as many as 500 spores was applied based on the treatment by sprinkling the inoculum at the bottom of the planting hole. The polybags were then arranged in a randomized completely block design layout.

The seedlings were maintained for nine months in the main nursery. The seedlings were regularly watered twice a day, in the morning and evening. Weed control was performed manually, and pest control involved the use of insecticides with cypermethrin as the active ingredient. NPKMg fertilizer (12:12:17:2) was applied every 2 weeks, with doses of 5 g/seedling at 14-25 weeks after planting (WAP), 7.5 g/seedling at 27 and 29 WAP, 10 g/seedling at 31-37 WAP, and 15 g/seedling at 39-48 WAP (75% of recommended dose).

Data were collected at the end of the study, after the seedlings had been maintained for nine months (36 weeks) in the main nursery. The variables observed included % root colonization by AMF, plant height, stem diameter, fresh and dry weight of shoot, and analysis of N, P, K, Ca, Mg, and B nutrients in the leaves. For leaf nutrient analysis, two pairs of leaflets from the 3rd leaf were taken from each seedling. Nutrient analysis was conducted at the Analytical Laboratory of PT Bumitama Gunajaya Agro, Central Kalimantan.

Results

Oil palm seedling growth

All oil palm seedlings in this study were successfully colonized by AMF, including control seedlings. Generally, all mycorrhizal treated seedlings showed high mycorrhizae colonization rate (root colonization rate more than 50%), significantly higher than control. Only the treatment with a mixture of *Gigaspora* sp. and *Entrophospora* sp. had a moderate root colonization rate of 42,9%. AMF successfully colonized the oil palm seedling roots, the growth of the seedlings was affected by the AMF treatment. Seedlings treated with *Gigaspora* sp., *Acaulospora* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp. exhibited significantly greater height than those in the control treatment. However, concerning stem diameter, only the *Glomus* sp. + *Entrophospora* sp. treatment showed a higher value compared to the control seedlings (Table 2).

The application of AMF significantly influenced both fresh and dry shoot weight. Seedlings treated with *Glomus* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp. exhibited markedly higher shoot fresh and dry weight than the control group (Table 3).

Table 2. The effect of AMF on root colonization, seedling height, and stem diameter

Treatments	Root colonization (%)		Seedling Height (cm)		Stem Diameter (cm)	
Control	40.8	c	126.9	d	8.36	b
Glomus sp. (G)	70.0	a	135.1	bcd	8.96	ab
Gigaspora sp. (Gi)	76.6	a	**138.6**	**abc**	8.61	ab
Entrophospora sp. (E)	71.2	a	131.2	cd	9.32	ab
Acaulospora sp. (A)	69.2	a	**139.4**	**abc**	8.93	ab
G + Gi	71.3	a	**147.3**	**a**	**9.51**	**a**
G + E	61.9	abc	**145.5**	**ab**	9.20	ab
Gi + E	42.9	bc	128.4	cd	8.73	ab
G + Gi+ E + A	65.7	ab	135.0	bcd	8.76	ab
LSD 5%	24.12		11.6		1.01	

Note: The means followed by the same letter at the same column are not significantly different according to LSD (Least Significant Different) test at alfa 5%.

Table 3. The effect of AMF on shoot fresh and dry weight

Treatments	Shoot fresh weight (g)		Shoot dry weight (g)	
Control	1608.4	c	367.4	c
Glomus sp. (G)	**2114.8**	**ab**	**532.6**	**ab**
Gigaspora sp. (Gi)	1942.5	abc	471.2	bc
Entrophospora sp. (E)	1783.6	bc	440.3	bc
Acaulospora sp. (A)	1908.7	abc	444.8	bc
G + Gi	**2268.6**	**a**	**545.1**	**ab**
G + E	**2126.8**	**ab**	**603.1**	**a**
Gi + E	1593.9	c	405.2	c
G + Gi+ E + A	1874.3	bc	438.8	bc
LSD 5%	365.2		119.3	

Note: The means followed by the same letter at the same column are not significantly different according to LSD (Least Significant Different) test at alfa 5%.

Nutrient uptake

Association of AMF with the oil palm seedling roots improved nutrient uptake by the seedling except for nitrogen. The data presented in Table 4 indicated that nitrogen uptake in all AMF treatments is not significantly different when compared to the control. Among the AMF treatments, no significant differences in nitrogen uptake were observed, except for the *Glomus* sp. treatment, which exhibited a higher nitrogen uptake than *Glomus* sp. + *Entrophospora* sp. treatment.

P uptake by oil palm seedling was influenced by the AMF treatment, as shown in Table 4. Single AMF treatments, such as *Glomus* sp. and *Gigaspora* sp., as well as the mixtures of *Glomus* sp. + *Gigaspora* sp. and *Glomus* sp. +

Entrophospora sp. had higher P uptake compared to the control treatment. The remaining AMF treatments did not result in different P uptake than the control seedlings. Regarding K uptake, only the AMF treatment of *Glomus* sp. + *Entrophospora* sp. showed a higher value than the control.

Table 4. The effect of AMF on N, P, and K uptake by oil palm seedling

Treatment	Nutrient uptake (g)					
	N		P		K	
Control	13.87	ab	0.78	e	6.93	b
Glomus sp. (G)	18.69	a	**1.10**	**abc**	9.51	ab
Gigaspora sp. (Gi)	15.88	ab	**1.02**	**bcd**	8.59	b
Entrophospora sp. (E)	16.63	ab	0.90	de	8.99	ab
Acaulospora sp. (A)	17.16	a	0.95	cde	7.91	b
G + Gi	13.97	a	**1.15**	**ab**	9.39	ab
G + E	09.91	b	**1.26**	**a**	**11.45**	**a**
Gi + E	14.79	ab	0.87	de	7.53	b
G + Gi+ E + A	14.96	ab	0.93	cde	7.59	b
LSD 5%	6.41		0.20		2.84	

Note: The means followed by the same letter at the same column are not significantly different according to LSD (Least Significant Different) test at alfa 5%.

Table 5. The effect of AMF on Ca, Mg, and B uptake by oil palm seedling

Treatment	Nutrient uptake					
	Ca		Mg		B	
	(g)		(g)		(mg)	
Control	2.36	d	1.39	c	15.07	b
Glomus sp. (G)	**3.98**	**abc**	1.76	abc	**23.84**	**a**
Gigaspora sp. (Gi)	**3.82**	**abc**	1.67	abc	17.85	ab
Entrophospora sp. (E)	3.35	bcd	1.41	c	20.63	ab
Acaulospora sp. (A)	3.48	abcd	1.56	abc	16.66	b
G + Gi	**4.56**	**ab**	**1.89**	**ab**	**23.59**	**a**
G + E	**4.72**	**a**	**1.92**	**a**	20.23	ab
Gi + E	3.06	cd	1.36	c	16.27	b
G + Gi+ E + A	3.44	bcd	1.49	bc	17.91	ab
LSD 5%	1.25		0.41		6.50	

Note: The means followed by the same letter at the same column are not significantly different according to LSD (Least Significant Different) test at alfa 5%.

Ca uptake by oil palm seedlings was influenced by the AMF treatment, as indicated in Table 5. The Ca uptake showed a similar trend to P uptake. The single AMF treatments, such as *Glomus* sp. and *Gigaspora* sp. and mixture treatments like *Glomus* sp. + *Gigaspora* sp. and *Glomus* sp. + *Entrophospora* sp. resulted in higher Ca uptake compared to the control treatment. The uptake of Mg was also influenced by AMF treatment. *Glomus* sp. + *Gigaspora* sp. and *Glomus* sp. + *Entrophospora* sp. Treatments showed higher Mg uptake than the control treatment. The lowest uptake of Boron (B) by the oil palm seedling was observed in the control treatment. However, only the *Glomus* sp. and *Glomus* sp. + *Gigaspora* sp. treatments had significantly higher B uptake when compared to the control treatment.

Discussion

Arbuscular mycorrhizal fungi are believed to be in symbiosis mutualism with nearly 90% of plant species, including oil palm in various ecosystems (Smith and Read, 2008, Sieverding, 1991). In this study, using inceptisol soil, we observed that all AMFs applied, whether singly or in combination, successfully colonized oil palm seedling roots with a relatively high colonization rate (above 50%). The only exception was the mixture of *Gigaspora* sp. and *Entrophospora* sp., which exhibited a medium root colonization rate of 42,9%. A study conducted in an oil palm plantation nursery in Colombia by Galindo-Castañeda and Romero (2013) revealed a high root colonization of 80% by *Glomus intraradisces* at 80 days after inoculation. Phosri *et al*. (2010) suggested that the high root colonization of oil palm roots by AMF might be attributed to the limited development of the oil palm root system.

In this study, the control seedlings were also colonized by AMF, with the lowest colonization rate compared to the other treatments. Seedlings in the control treatment were likely colonized by indigenous mycorrhizae present in the soil, as the soil used contained indigenous AMF spores. The natural presence of AMF in the rhizosphere of oil palm plantations was also reported by Abd Rahim *et al*. (2016), who found that the total amount of propagules in the soil varied depending on the soil type. Additionally, Rini *et al.* (2017), using unsterilized ultisol soil, observed AMF root colonization of 51% in oil palm seedling without AMF inoculation. Similar results were reported by Galindo- Castañeda and Romero (2013), stating that the control (without AMF application) seedling roots of oil palm had a 50% root colonization rate in pre-nursery at 80 days after AMF inoculation. However, the root colonization rate was lower than the *Glomus intraradices* treatment (80%). They also reported that seedlings treated with *G. intraradices* exhibited significantly better seedling growth, as demonstrated by total dry weight. The conclusion drawn was that higher root colonization by AMF treatment indicated that the applied AMF were more effective and could compete with indigenous AMF. The high root colonization in this study might be also be attributed to the low nutrient content in the soil (Table 1). Some studies have reported that high nutrient content in the soil especially phosphorus (P), can inhibit AMF symbiosis (Grünfeld *et al*., 2022; Salim *et al*., 2020).

Although almost all AMF treatments resulted in a high root colonization rate, their effects on oil palm seedling growth varied. Regarding seedling height, four AMF treatments (*Gigaspora* sp., *Acaulospora* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp.) produce better results than the control. However, only one AMF treatment (*Glomus* sp, + *Gigaspora* sp.) led to a stem diameter significantly higher than that of the control. Based on our observation during the experiment, seedling height and stem diameter cannot be used as an appropriate criteria for assessing plant growth. Some seedlings appeared tall, but the leaflets were narrow. Similarly, with stem diameter, some seedlings had a wide diameter, but they were short. Therefore, we chose to use shoot fresh weight and shoot dry weight to examine the effect of AMF on seedling growth. Plant dry weight is an accurate indicator of growth since it measures the biomass formed during plant growth.

The AMF treatments, namely *Glomus* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp. exhibited higher shoot fresh and dry weights than the control. Our results align with findings from other researchers indicating that AMF application can enhance plant growth. Specifically, the application of *Glomus* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp. increased shoot fresh weight by 32%, 41%, and 32%, and shoot dry weight by 45%, 48%, and 64%, respectively, compared to the control. The improved plant growth in these treatments could be attributed to the development of AMF hyphae in the roots (root colonization rate 61-71%) and in the soil (external hyphae).

AMF external hyphae can extend several centimeters from the root surface into the soil (Smith and Read, 2008). These hyphae can access water and nutrients that are immobile in the soil and transfer them to their host. Additionally, external hyphae can bridge the nutrient-depletion zone that forms around the root surface. The length of the hyphae outside the root (external hyphae) can reach 111 m per cm^3 of soil in grasslands and 81 m per cm^3 of soil in pastureland (Miller *et al*., 1995). The increased absorption of water and nutrients in AMF-colonized plants can further improve plant water relations, such as transpiration and photosynthesis processes. Chandrasekaran *et al*. (2019) reported that the application of AMF increased the conductance rate of stomata and the transpiration rate of plants in saline soils, resulting in an increase in the rate of photosynthesis. They also found that, of the two types of AMF used in the saline soil, *Funnelliformis mosseae* species showed better effectiveness than *Rhizophagus* intraradices. The results of this study also confirm the fact that the effectiveness of AMF in certain soil conditions is influenced by the type of AMF.

It is believed that one of the ways in which AMF enhances the growth of its host plants is through increase nutrient uptake from the soil by AMF external hyphae (Begum *et al*., 2019; Wang *et al*., 2017). In our study, the improved growth of the seedlings in the AMF treatments (*Glomus* sp., *Glomus* sp. + *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp.) corresponds to enhanced nutrient uptake of P, Ca, Mg, and B by the seedlings. These treatments increased nutrient uptake by 41-61% for P, 68-100% for Ca, 26-38% for Mg, and 34-58% for B. An increase in the uptake of P, Fe and Zn by wheat plant was reported by Ingraffia *et al.* (2019). Yeasmin *et al.* (2019) demonstrated in their study that the uptake of N, P, and K was significantly higher in asparagus treated with AMF under normal condition, and even under mild and severe heat condition.

In this study, some of the AMF treatments also exhibited a higher percentage of root colonization; however, the growth and nutrient uptake by the seedlings were not significantly better than those of the control seedlings. Similar findings were reported by Simó-González *et al.* (2019), who observed that *Rhizoglomus intraradices* produced the highest biomass weight and nutrient content in *Canavalia ensiformis* L. The applications of *Glomus cubense* and *Claroideoglomus claroideum* also resulted in better biomass weight than the control, but lower than *R. Intraradices*. Meanwhile, the growth and nutrient uptake of the seedlings inoculated with *Funneliformis mossea* were not significantly different from the control. The lack of growth improvement in some AMF treatments in this study could be attributed to the carbon loss from the host plant, may not be compensated by AMF in the form of an increase in water and nutrient uptake (Campo *et al*., 2020).

In this study, we found that the application of AMF treatments, specifically *Glomus* sp., *Glomus* sp. + with *Gigaspora* sp., and *Glomus* sp. + *Entrophospora* sp., resulted in better growth and nutrient uptake of seedlings planted in inceptisol soil. Additionally, our previous study (Rini *et al*., 2021b) using the same AMF species in histosol soil, showed that the AMF treatments of *Glomus* sp. and Gigaspora sp. + *Entrophospora* sp. led to better oil palm seedling growth and nutrient uptake. A similar outcome was reported by Sundram (2010), using a growing medium consisting of a mixture of peat, clay, and sand with a ratio of 3:2:1. Therefore, it is necessary to screen which AMF species are suitable for specific soil conditions to maximize the benefits of this fungi.

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