

The Growth of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings with the Application of Different Arbuscular Mycorrhiza Fungi and Various Phosphorous Dosages

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ABSTRACT

The effectiveness of Arbuscular Mycorrhiza Fungi (AMF) colonization depends on the type of AMF, plant species and phosphorus fertilization. The aims of this study were to determine: the best type of AMF and the appropriate dosage of P fertilizer for the growth of oil palm seedlings, and whether the oil palm seedling responses to the application of AMF type is determined by the dosage of phosphorus applied. The experiment was arranged in a Randomized Complete Block Design (RCBD) with two factors and 5 replications. The first factor was the types of AMF (m), consisting of without mycorrhiza inoculation (m_0), *Gigaspora* sp. MV16 isolate (m_1), *Glomus* sp. MV7 isolate (m_2), *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 (m_3) isolate. The second factor was phosphorus fertilization (SP-36), consisting of 3 levels: 1/3 recommended dosage (p_1), 2/3 recommended dosage (p_2), full recommended dosage (p_3). The results showed that the application of all AMF types resulted in the same effects on the growth of oil palm seedlings, however the seedling growth in the inoculated plants were better than in uninoculated plants (control). The application of the recommended dosage of P fertilizer produced the highest seedling growth. The response of seedlings to the application of AMF type was not determined by doses of phosphorus fertilizer applied.

Keywords: *Gigaspora* sp., *Glomus* sp., phosphate fertilizer

ABSTRAK

Ketersediaan fosfor (P) di dalam tanah akan mempengaruhi Fungi Mikoriza Arbuskula (FMA) dalam melakukan aktivitasnya untuk melarutkan senyawa kompleks menjadi bentuk P-tersedia tanah. Tujuan penelitian ini yaitu menentukan jenis FMA terbaik untuk pertumbuhan bibit kelapa sawit; menentukan dosis P terbaik untuk pertumbuhan bibit kelapa sawit; dan mempelajari apakah respon bibitkelapa sawit terhadap pemberian jenis FMA ditentukan oleh dosis pupuk P yang diaplikasikan ke tanah. Percobaan disusun menggunakan Rancangan Acak Kelompok Lengkap (RAKL) secara faktorial (4x3) dengan 5 ulangan. Faktor pertama yaitu jenis FMA (m), yang terdiri dari tanpa inokulasi FMA (m_0), *Gigaspora* sp. isolat MV16 (m_1), *Glomus* sp. isolat MV7 (m_2), *Gigaspora* sp. isolat MV16 + *Glomus* sp. isolat MV7 (m_3). Faktor kedua yaitu pemberian pupuk P yang terdiri dari 3 taraf yaitu 1/3 dosis anjuran (p_1), 2/3 dosis anjuran (p_2), sesuai dosis anjuran (p_3). Semua jenis FMA yang diinokulasikan menghasilkan pertumbuhan bibit kelapa sawit yang sama, akan tetapi pertumbuhan bibit kelapa sawit yang diinokulasi dengan FMA lebih baik dibandingkan kontrol. Aplikasi pupuk P sesuai dosis anjuran (p_3) menghasilkan pertumbuhan bibit kelapa sawit tertinggi. Respon bibit kelapa sawit terhadap pemberian jenis FMA tidak ditentukan oleh dosis pupuk P yang diaplikasikan.

Kata kunci : *Gigaspora* sp., *Glomus* sp., pupuk fosfor

INTRODUCTION

In Indonesia, oil palm is generally planted on acid soils, and the constraints faced by acid soils is the low availability of phosphorus (P). Inoculation of Arbuscular Mycorrhiza Fungi (AMF) on the roots of oil palm will result in a mutualistic symbiosis between oil palm and AMF. The symbiosis between AMF and roots can increase the ability of plants to take up macronutrients, especially phosphorus (P), and micronutrients from soil. This is because the hyphae of AMF have access and a wider range to exploit nutrients in soil (Smith and Read 2008).

Agustin *et al.* (2010) reported that the inoculation of AMF can support the growth and yield of chili plants. Each type of AMF also has different effectiveness. Kartika *et al.* (2014) reported that the application of AMF *Glomus* sp-3 supported the growth of *Jathropa* seedlings better than *Glomus* sp-6, *Glomus* sp-15, and *Glomus* sp-16. The benefit of AMF for plants among others is to help the uptake of P. The rate of P uptake by the AMF infected roots is very significantly different from the uninfected roots. This is in accordance with Damayanti *et al.* (2014) who reported that the root architecture of oil palm seedlings inoculated with AMF showed better outcome than in control (without AMF inoculation). This indicates that there is an increase in P uptake and plant growth. Hadianur *et al.* (2016) reported that the inoculation of *Gigaspora* sp. resulted in better growth and yield of tomato plants (*Lycopersicon esculentum* Mill) than the inoculation of *Glomus* sp., and a mixture of *Glomus* sp. and *Gigaspora* sp.

However, a high soil P content causes AMF to inactivate its activity to dissolve the bound-P into available-P in soil. This is because the plants that receive high P fertilization will have roots with low membrane permeability. While the AMF colonization process will be easily occurred on the roots with high membrane permeability. Tripsilawati and Yusron (2008) reported that fertilization of P with the level of 0, 2, 4, 6, and 8 g plant⁻¹ resulted in the highest Patchouli (*Pogostemon cablin*) yield at a dose of 2-4 g plant⁻¹ compared to other P fertilizer doses. Indriani *et al.* (2006) also reported that the application of AMF together with phosphate rock fertilizer at the level of 0, 100, 200, 300 kg P₂O₅ ha⁻¹ resulted in the highest dry matter content of tropical kudzu at a dose of 200 kg P₂O₅ ha⁻¹. Chairunnisa *et al.* (2013) also reported that the excessive P fertilization can disrupt the balance of other nutrients such as N so that the plant growth can be inhibited.

Based on the description mentioned above, it is necessary to test the type of AMF, and the appropriate dose of P fertilization on oil palm seedlings. This study aimed to determine 1) the best type of AMF for seedling growth; 2) the best P dose for seedling growth; 3) whether the seedling response to AMF type is determined by P fertilizer dose.

MATERIALS AND METHODS

Study Site

This study was conducted at Laboratory of Plant Production and in the greenhouse of Faculty of Agriculture, University of Lampung from November 2013 to May 2014. The soil analysis was conducted in the Laboratory of Politeknik Negeri Lampung.

Experimental Design

The experiment was arranged in a Randomized Complete Block Design (RCBD) with two factors and five replications. The first factor was the type of AMF (m) consisting of 4 levels, *i.e.* without AMF inoculation (m0), *Gigaspora* sp. MV16 isolate (m1), *Glomus* sp. MV7 isolate (m2), *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate (m3). The second factor was the levels of P fertilization, namely 1/3 recommended dosage of SP-36 (p1), 2/3 recommended dosage of SP-36 (p2), 3/3 recommended dosage of SP-36 (p3). Except for the treatments of P fertilization, other dosage of fertilization followed the advice of fertilization for oil palm seedlings from the Center for Research of Oil Palm (PPKS), Medan, Indonesia (Table 1).

Preparation of Oil Palm Seedlings and Planting Media

The seeds of oil palm were planted in small polybags using sterile sand media for 1 month. The seeds of oil palm that have germinated were sown by immersing the seeds into the media of approximately 1 cm depth with the radicle facing down and plumula upward.

The media used for planting oil palm seedlings was subsoil from Tulang Bawang, Lampung, Indonesia. The soil has been sieved using 7 mesh (2.83 mm) sieve, and then fumigated using Dazomet 98% (3.5 g m⁻³). The soil was put into polybags with the capacity of ± 6 kg. In total there were 60 polybags. The polybags were arranged and covered with plastic for 1 month, then after 1 month the soil

Table 1. Fertilization schedule and dosages applied in the study.

Age of oil palm seedlings (weeks)	Type and dosage of fertilizers (g per seed or seedling)		
	Pre nursery		
		NPK 15:15:7	
5	-	2.5	-
	Main Nursery		
	Urea	SP-36	KCl
14 (1 st fertilization)	0.83	p ₁ =0.35 p ₂ =0.69 p ₃ =1.04	0.25
16 (2 nd fertilization)	1.67	p ₁ =0.69 p ₂ =1.38 p ₃ =2.08	0.5
18 (3 rd fertilization)	2.49	p ₁ =1.04 p ₂ =2.08 p ₃ =3.12	0.75
22 (4 th fertilization)	3.33	p ₁ =1.39 p ₂ =2.78 p ₃ =4.2	1
26 (5 th fertilization)	2.67	p ₁ =1.11 p ₂ =2.22 p ₃ =3.33	2.83
28 (6 th fertilization)	2.67	p ₁ =1.11 p ₂ =2.22 p ₃ =3.33	2.83
30 (7 th fertilization)	2.67	p ₁ =1.11 p ₂ =2.22 p ₃ =3.33	2.83
32 (8 th fertilization)	2.67	p ₁ =1.11 p ₂ =2.22 p ₃ =3.33	2.83
33 (Observation period)			

Note : When the seeds of oil palm were 1-3 weeks old, the seeds were fertilized with Urea 2 g L⁻¹ water per 10 seeds. p₁ = 1/3 recommended dosage of SP-36; p₂ = 2/3 recommended dosage of SP-36; p₃ = recommended dosage of SP-36.

in polybags was watered until reaching saturated condition. Two weeks after that the soil in polybags was used for planting oil palm seedlings.

Application of AMF, Plant Nurturing and Plant Growth Measurements

The AMF application was done shortly before planting oil palm seedlings into the polybags. A hole with a diameter of ± 6 cm and a depth of ± 10 cm was made in the middle part of the soil planting media in the polybags. The AMF inoculant (with a sand carrier) containing about 500 spores according to the treatment was placed at the bottom of the hole, then the oil palm seedling was planted into the hole.

Fertilization was done using Urea, SP-36, and KCl fertilizers. Fertilizers were applied according to the treatments by following the standard doses for oil palm seedlings recommended by the Center for Research of Oil Palm (PPKS). Fertilization schedules and dosages applied in this study are presented in Table 1. In this study, Mg and Kieserit fertilizers were not applied as recommended by the PPKS, Medan, Indonesia.

Plant nurturing was done by watering the oil palm seedlings every day. Disease control was done manually by cleaning the fungi in the planting media. The measurements of plant growth parameters were conducted 6 months after planting. The parameters

Table 2. The effect of application of AMF and P fertilizer on the plant height, dry weight of canopy, dry weight of roots, and total dry weight of oil palm seedlings.

Treatment	Plant Height (cm)	Dry Weight of Canopy (g)	Dry Weight of roots (g)	Total Dry Weight (g)
AMF type :				
Without AMF inoculation	56.5 b	19.43 b	7.33 b	26.75 b
<i>Gigaspora</i> sp. MV16 isolate	67.2 a	35.33 a	12.21 a	47.56 a
<i>Glomus</i> sp. MV7 isolate	66.1 a	31.93 a	11.61 a	43.52 a
<i>Gigaspora</i> sp. MV16 isolate + <i>Glomus</i> sp. MV7 isolate	67.9 a	34.63 a	12.68 a	47.31 a
LSD 5%	7.7	5.6	1.78	7.05
Phosphorus fertilizer :				
1/3 recommended dosage	57.3 b	25.43 b	10.65 a	36.04 b
2/3 recommended dosage	62.9 b	30.43 b	10.99 a	41.42 ab
3/3 Recommended dosage	70.0 a	35.15 a	11.24 a	46.39 a
LSD 5%	6.7	4.9	1.54	6.11

Note: The numbers followed by the same letters in the same column are not significantly different according to LSD test at 5% level. The data of plant height and dry weight of canopy are transformed into \sqrt{x} .

measured included (1) plant height, (2) dry weight of the canopy, (3) dry weight of roots, (4) total dry weight of plant, and (5) AMF root infection.

Data Analysis

Homogeneity of variance was tested using Bartlett's test and the additivity of data was tested using Tukey's test. All data were subjected to analysis of variance, and further the difference of the means was tested using Least Significant Difference (LSD) at 5% level.

RESULTS AND DISCUSSION

Inoculation of various AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate) on oil palm seedlings resulted in similar effects on the height of oil palm seedlings, however, the height of oil palm seedlings of the inoculated plants increased by 9.45%; 8.29%; and 10%, respectively compared to that of uninoculated plants (control). Application of P fertilizer according to the recommended dosage resulted in the highest plants compared to 1/3 and 2/3 recommended dosages. Application of P at 1/3 and 2/3 recommended doses resulted in the same plant height (Table 2).

Inoculation of various AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate) on oil palm seedlings resulted in the same dry weight of the canopy, however, there were an increase in dry weight of the canopy of inoculated plants by 37.48%; 29.06%; and 36.11%, respectively

compared to that of uninoculated plants (control). Application of P fertilizer according to the recommended dosage gave the highest dry weight of the canopy, while 1/3 and 2/3 recommended dosages gave the same dry weight of the canopy (Table 2).

Inoculation of various AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate) on oil palm seedlings resulted in similar effects on dry weight of roots, but there were an increase of dry weight of roots by 66.55%; 58.36%; and 72.91%, respectively compared to that of uninoculated plants (control). The application of P fertilizer at 1/3, 2/3, and full recommended doses gave the same dry weight of roots (Table 2).

Application of various types of AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate) on oil palm seedlings resulted in the same effects on the total dry weight of plants, however, there were an increase in total dry weight of plants by 77.82%; 62.71%; and 76.89%, respectively compared to that in control treatment. The application of P fertilizer at 2/3 of recommended dose and full recommended dose resulted in the same total dry weight of plants, but the application of the recommended P fertilizer increased the total dry weight dry of plants by 28.72% compared to 1/3 recommended dosage (Table 2).

Inoculation of various types of AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus*

Table 3. The effect of application of AMF and P fertilizer on root infections of oil palm seedlings.

Treatment	Root Infection (%)
AMF type :	
Without AMF inoculation	7.70 b
<i>Gigaspora</i> sp. MV16 isolate	29.00 a
<i>Glomus</i> sp. MV7 isolate	25.63 a
<i>Gigaspora</i> sp. MV16 isolate + <i>Glomus</i> sp. MV7 isolate	30.47 a
LSD 5%	7.7
Phosphorus fertilizer :	
1/3 recommended dosage	25.78 a
2/3 recommended dosage	24.88 a
3/3 Recommended dosage	18.95 a
LSD 5%	6.7

Note: The numbers followed by the same letters in the same column are not significantly different according to LSD test at 5% level.

sp. MV7 isolate) on oil palm seedlings showed no significant effects on root infection, however, there were an increase in root infections by 121.42%; 110.56%; and 133.37%, respectively compared to that in control treatment. Application of P fertilizer at 1/3, 2/3, and full recommended dosages gave the same results in root infections (Table 3).

All the AMF types applied in this study provide similar response to the growth of oil palm seedlings, however, the responses on inoculated plants are better than on uninoculated plants (control/without AMF). This is allegedly due to the compatibility between AMF and plant roots. Exudates released by plant roots are suitable for the applied AMF, so the AMF utilize the root exudates for the growth of the hyphae at the beginning of the infection process.

Interwoven hyphae will be formed intensively, so that the mycorrhizal inoculated plants will be able to increase their capacity on nutrient uptake, especially P. The AMF are able to increase the uptake of nutrients and plant growth. This indicates that all types of AMF applied in this study match with oil palm plants resulting in a mutualistic symbiosis. According to Smith and Read (2008), mycorrhiza is a symbiotic association between fungi and roots of host plant. Mycorrhiza is able to improve nutrient uptake and increase plant growth and yield. Conversely, the fungi will take up substrates, such as glucose and sucrose from plant roots. The results of current study are in line with Purlasyanko's research (2012) which reported that the type of AMF used (*Entrophospora* sp., *Glomus* sp. MV10,

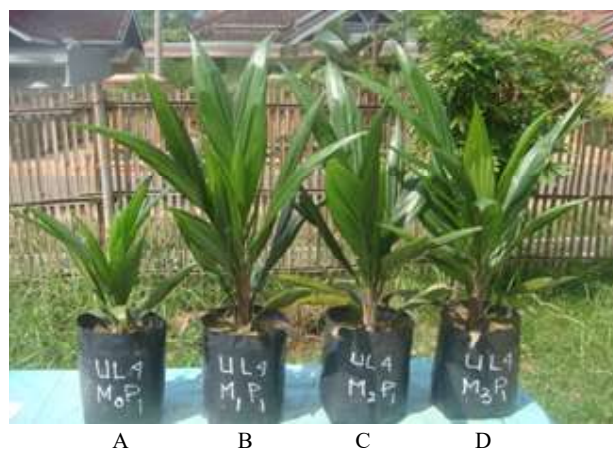


Figure 1. The growth of oil palm seedlings without AMF inoculation (A), *Gigaspora* sp. MV16 isolate (B), *Glomus* sp. MV7 isolate (C), *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate (D) at a dose of 1/3 recommended dosage of P fertilizer.

Gigaspora sp. MV8) produced similar response to the growth of oil palm seedlings.

The increase of root system is one of the mechanisms of oil palm seedlings in improving nutrient uptake and plant growth. A good root system will cause higher volume of soil that can be explored by the roots to absorb water and nutrients. Adequate water and nutrients will increase plant growth, which is indicated by the increase of plant height and dry weight of canopy in all AMF treatments (Table 2). This result is in line with the study of Pamuna *et al.* (2013), which showed that the inoculation of AMF increased dry weight of shoots of corn plants in comparison to that without AMF inoculation.

The results showed that the percentage of root infections on oil palm seedlings inoculated with AMF was not significantly different (25-31%), but the percentage of root infections on inoculated plants were significantly higher than on uninoculated plants (control) (7.7%). This result shows that there has been a symbiosis between AMF and roots of oil palm seedlings, which further lead to better nutrient uptake by the plants. This fact explains that the increased growth of mycorrhizal inoculated plants is a positive contribution of the symbiosis that occurs between AMF and the roots of oil palm seedlings. The growth of oil palm seedlings that are inoculated with mycorrhiza are much better than non-inoculated plants as shown in Figure 1. The insignificant difference on root infections among various AMF that have been applied is in line with the insignificant difference of plant growth that have been inoculated by various AMF.

The results of this study is in line with the study of Rumondang and Setiadi (2011) that reported that the types of *Glomus aggregatum* and *Glomus manihotis* AMF provide positive growth response and effectiveness of mycorrhizal inoculation on teak growth. The results of Amina *et al.* (2014) also showed that the inoculation of AMF species (*Gigaspora margarita*, and *Glomus mosseae*) resulted in good hazelnut growth, and better than

the growth of hazelnut plants without AMF application. This was indicated by the growth parameters of plant height, stem diameter, number of leaves and fresh weight and dry weight of roots and canopy. Budi *et al.* (2013) also reported that the inoculation of *Glomus* sp. and *Gigaspora* sp. showed the same effects on the height and diameter of *Falcataria moluccana* seedlings. Both types of AMF also resulted in the same effects on the shoot:root ratio of *Samanea saman* seedlings. Prayudyarningsih *et al.* (2013) reported that all types of tuber plants grown under stands of forest in South Sulawesi are associated with AMF including 3 genera of AMF, namely *Glomus* sp., *Acaulospora* sp., and *Gigaspora* sp.

The application of the recommended dose of P provides the highest oil palm seedlings and the highest dry weight of canopy (Table 2). The increase of plant growth can be caused by the adequate amount of nutrients needed by plants, one of them is P, which is needed by plants to stimulate root growth. Phosphorus is required by plants for the growth of cells in root tissues and buds that strengthen the stem (Liferdi 2010).

The response of the growth of oil palm seedlings to the various types of AMF is not determined by the applied phosphate fertilizer dosages. This is presumably because the amount of phosphate applied is still low, so that the root permeability is high enough that it can excrete the root exudates required by the AMF, consequently the AMF will infect the plant roots. The soil used to grow the oil palm seedlings is subsoil which contains low amount of available P, *i.e.* 9.264 mg kg⁻¹ (Table 4). Sudomo *et al.* (2007) indicated that subsoil is a nutrient-poor soil with a dense of soil structure, and unable to hold nutrients and water so that plant roots can not grow optimally.

In addition, the subsoil used contains low organic materials, while the number of AMF spores is closely related to organic matter content in soil. The maximum number of spores can be found in

Table 4. Chemical characteristics of the soil used in the experiment.

No.	Parameter	Unit	Value	Criteria	Method
1	pH	-	5.89	Rather sour	Potensiometri
2	Nitrogen	%	0.157	Low	Kjehdahl
3	Phosfor (P-available)	Mg kg ⁻¹	9.264	Medium	Spektrofotometri
4	Kalium (K-dd)	me/100 gr	0.382	Medium	Flamefotometri
5	C Organic	%	1.206	Low	Walkley-black

soils containing 1-2% organic matter, while the number of spores in soil with organic matter content < 0.5% is very low.

Subsoil has poor chemical properties. High retention of P on subsoil is the result of binding of P from the soil solution so that the amount of P that can be absorbed by plant is reduced. It was expected that P application at higher dose will increase the availability of P in the soil that will affect the success of symbiosis by AMF.

Carrenho *et al.* (2007) reported that application of P fertilizer showed no effect on root colonization (RC) of peanuts, sorghum and maize. Ghulamahdi *et al.* (2008) reported that there was no interaction effect between P fertilization and AMF inoculation on all observed variables (length, diameter, number of tuber per plant, weight, and dry weight of sweet potato (*Ipomoea batatas*). Higher doses of phosphate supplementation are expected to increase the availability of soil P that will affect the symbiosis of AMF and plant roots. Naher *et al.* (2013) reported that high P-supply reduced root colonization, while the optimum-P application tended to increase colonization and fungal richness on all sampling occasions.

This is consistent with the study of Djazuli (2011) that reported that high dose of P fertilization is seen to suppress the population and percentage of AMF infections at purwoceng (*Pimpinella pruatjan*) roots. Smith and Read (2008) showed that in low nutrient availability condition, hyphae can absorb nutrients from the soils. However, in high available P soil, plant roots may act as nutrient-absorbing organs so that plants can accumulate P in high quantities from the soil. In these circumstances the AMF keeps the C compounds from the plants resulting in a negative response to colonization that can further affect plant metabolism. Therefore, to increase the population and effectiveness of AMF on plant roots it is necessary to have an optimum amount of available P in soil for AMF to grow.

CONCLUSIONS

The inoculation of all types of AMF (*Gigaspora* sp. MV16 isolate, *Glomus* sp. MV7 isolate, and *Gigaspora* sp. MV16 isolate + *Glomus* sp. MV7 isolate) on oil palm seedlings resulted in the same effects on the growth of oil palm seedlings, but the effects on the inoculated plants were better than on uninoculated plants (control). The application of the recommended dosage of P fertilizer resulted in the highest growth of oil palm seedlings. The response

of oil palm seedlings to the application of AMF was not determined by the dose of P applied (no interaction effect), so that good interaction effects between AMF inoculation and P fertilization on the growth of oil palm seedlings were not observed.

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