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APPLICATIONS OF ARBUSCULAR MYCORRHIZA FUNGI TO IMPROVE GROWTH OF OIL PALM SEEDLINGS AND DISEASE RESISTANCE AGAINST *Ganoderma* sp.

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Abstract. Currently, basal stem rot disease incident in oil palm caused by the *Ganoderma* sp. increasing rapidly. Arbuscular mycorrhizal fungi (AMF) are one of the types of mycorrhizae that are useful for plants as they can increase plant growth and resistance to disease. Therefore, this study aimed to determine whether the application of AMF to oil palm seedlings was able to increase the growth and resistance of oil palm seedlings against *Ganoderma* sp. The study used a factorial treatment design (4x2) with 5 replications arranged according to a completely randomized block design. The first factor was the type of AMF, namely without AMF (m0), *Glomus* sp. (m1), *Entrophospora* sp. (m2), and a mixture of *Glomus* sp. with *Entrophospora* sp. (m3). The second factor was soil from the oil palm rhizosphere that was attacked by *Ganoderma* sp., namely sterilized soil (g0) and unsterilized soil (g1). The data obtained were analyzed using analysis of variance and the mean separation was tested using the Least Significant Difference test at the 5% level. The results showed that all AMF treatments (m1, m2, and m3) increased the growth of oil palm seedlings compared to controls. *Ganoderma* sp. present in the planting medium has not inhibited the growth of oil palm seedlings (both control and those applied with AMF) as there is no *Ganoderma* sp. infection in the roots of oil palm seedlings observed at the end of the study (5 months after planting).

Keywords: arbuscular mycorrhizal fungi; oil palm seedling; *ganoderma* sp.; basal stem rot

1. Introduction

1 Oil palm is the main plantation commodity in Indonesia which land area has been increasing annually. According to Badan Pusat Statistik (Central Bureau Statistics) (2020), the land area of oil palm plantations in Indonesia is increased from 11.26 million ha in 2015 to 14.60 million ha in 2019. This land area is spread through 26 provinces in Indonesia with the largest land area found in Riau province (2,82 million ha) followed by West Kalimantan (1.89 million ha) and Central Kalimantan (1.72 million ha) in 2019.

The expanding land area of oil palm was coupled with the increasing crude palm oil (CPO) production and palm kernel oil (PKO). Since 2006, Indonesia has been the first palm oil producer in the world followed by Malaysia. Today, 61% of world palm oil production is from Indonesia (Lam *et al.*, 2019). However, the rapid development of oil palm is facing a threat from the massive emerging basal stem rot (BSR) diseases caused by *Ganoderma boninense* fungi. BSR diseases affect the disturbance of growth and production of oil palm. In fact, BSR can cause a severe disease that leads to the death of oil palm (Siddiqui *et al.*, 2021).

Hendarjanti (2020) stated that the BSR attack has been spread thoroughly in an oil palm plantation in Indonesia, including Sumatera, Java, Kalimantan, Sulawesi, and Irian Jaya with high pathogen attacks occurring in Sumatra. Furthermore, Paterson, (2019) reported that there is 45% of BSR diseases attack occurred in Sumatera's oil palm plantation. The attack of an oil palm tree by BSR showed several symptoms, including unopen young leaves which create more than one spear leaves in one tree, the color of mature leaves changing into pale green and wilting leaves, the fruiting body of *Ganoderma* sp. appearing on the basal stem when a major part of the basal stem cell has already damaged and decayed (Sujarit *et al.*, 2020; Siddiqui *et al.*, 2021). Despite that, those disease symptoms can only be observed after the plant has been severely infected by BSR. The initial attack occurred in the root, but the symptoms have not appeared in the stem part of the plant (Siddiqui *et al.*, 2021).

Nowdays, many face difficulties in controlling BSR diseases. Controlling pesticides using chemical fungicides still does not show a satisfying result, due to the symptoms on the plant only showed when the root and stem are severely damaged. One of the alternative to control and suppress BSR diseases is using a biocontrol agent, including arbuscular mycorrhizal fungi (AMF). Previous studies have shown the ability of AMF as a biocontrol agent which is enhancing the resilience of plants against fungal pathogen attacks. Olowe *et al.* (2018) stated that the application of *Glomus clarum* and *Glomus deserticola* significantly reduce the damage level attack of the fungal pathogen, *Fusarium verticillioides*, on the maize plant. On peanut, Swandi *et al.* (2020) reported that some isolates of arbuscular mycorrhizal fungi effectively reduced disease severity caused by *Sclerotium rolfsii*. Therefore, this study aimed to investigate whether the application of AMF on oil palm seedlings in pre-nursery could significantly increase the growth and resistance of oil palm seedlings against the attack of *Ganoderma boninense* (fungal pathogen) which caused basal stem rot diseases.

2. Methods

The study was conducted in the greenhouse at the Faculty of Agriculture, University of Lampung, Bandar Lampung. The oil palm germination seeds of Tenera type (DxP) used in this study were obtained from Indonesian Oil Palm Research Institute Medan and AMF inoculum which consists of *Glomus* sp. and *Entrophosphora* sp. were from Collection of Laboratorium of Estate Crop Production, Faculty of Agriculture, University of Lampung. The soil used for the main nursery was taken from the basal of oil palm tree which has been infected severely by *Ganoderma* sp. (more than 50% of the basal stem of oil palm was damaged).

This study used a factorial treatment design (4x2) with five replications and organized according to a randomized completely block design. The first factor was the AMF species which

consist as follows: control without AMF (m_0), *Glomus* sp. (m_1), *Entrophospora* sp. (m_2), and a mixture of *Glomus* sp. and *Entrophospora* sp. (m_3). The second factor was soil media derived from basal of oil palm tree which has been infected severely by *Ganoderma* sp. which consist as follows: sterilized soil (g_0) and unsterilized soil + fruiting bodies of *Ganoderma* sp. (g_1). The data obtained were analyzed using analysis of variance and continue with means separation using the least significant difference test at significance level of 5%. The growing media for pre-nursery were a mixture of topsoil, sand, and organic matter with a ratio of 4:2:1. The media was sterilized using autoclave at 121°C for \pm 1 hour. After cooling, the growing media was put into a baby polybag (\pm 1.5 kg media/polybag). The Tenera germinated seeds were then planted in the center of the polybag with 1 cm depth (one seed/polybag) and maintained for one month in the greenhouse under the shade of the net. The germinated seeds were watered regularly every morning and evening (two times a day). After one month, forty uniform seedlings (based on seedling height and number of leaf) were selected to be used in this study.

The one-month-old oil palm seedling was taken out carefully from the polybag, then the planting hole was made in the same polybag and AMF inoculum (using sand as a carrier) according to treatment was put into the planting hole. AMF inoculum consists of 500 spores per seedling. Spore counting was done using the wet sieving method (Brundrett *et al.*, 1996). After that, the oil palm seedling was put into the planting hole and covered by sand. For the control treatment, sterilized sand was used instead of AMF. The planted seedlings were then maintained in the greenhouse for 2 months and urea fertilizer (at 2g/L concentration) was given 10 ml/seedlings every week. After two months, the seedlings were moved into the main nursery using a bigger polybag size (30 x 30 cm), and *Ganoderma* sp. treatment was applied.

The growing media for the main nursery were taken from the area of the basal stem of the oil palm tree that has been infected with *Ganoderma* sp. The characteristics of the infected plant were more than 50% of the basal stem is damaged and the cells were decayed as well as fruiting bodies were observed around the basal stem. The soil was taken from several infected oil palm trees and mixed thoroughly. Half of the soil was sterilized using autoclave at 121°C for 1 hour. Sterilization was repeated with the same procedure the next day. The sterilized soil was used for g_0 treatment and unsterilized soil was used as g_1 . For the g_1 treatment, the chopped *Ganoderma* sp. fruiting bodies were mixed thoroughly into the soil. After that, the soil was put into a polybag for \pm 5 kg soil/polybag. The palm oil seedlings from pre-nursery were then transplanted into polybags that contain 5 kg of growing media according to the treatment design (treatment combination of AMF and *Ganoderma* sp.). Transplanting was done by making the planting hole inside the 5kg media in the polybag with a hole digger, the baby polybag was released carefully, and the seedlings were then put into the planting hole. Later, the seedlings were maintained for 5

months in the open field without shading, thus the age of the seedlings at the end of the study was 8 months old (from sowing to the end of the study).

Table 1. Fertilizer doses of oil palm seedling at main nursery.

Age (weeks)	Doses of NPK fertilizer 15:15:15 (g/polybag)
14	1.25
16	1.25
18	2.50
20	2.50

The maintenance activities were watering two times a day (morning and evening), fertilizing (time and doses of fertilizer were shown in Table 1, and weeding was done manually. At the end of the study, the parameters observed were seedling height (from basal stem to highest leaves), shoot fresh and dry weight, root fresh and dry weight, % root colonization by AMF according to Brundrett *et al.* (1996), and % root infection by *Ganoderma* sp. (through macroscopic observation towards primary and secondary root that decayed due to *Ganoderma* sp. infection).

3. Results and Discussion

According to the analysis of variance, the interaction between AMF and *Ganoderma* sp. treatment was not significant to all parameters measured. The results of AMF root colonization and *Ganoderma* sp. infection on oil palm seedlings were shown in Table 2. The highest AMF colonization of 68.4% was shown on seedlings inoculated with *Entrophospora* sp. and significantly different with the treatments of *Glomus* sp. and control. The control treatment also gave AMF colonization of 44.4% and the value was lower than the other AMF treatment. This indicated that the growing media have indigenous AMF. Soil sterilization using autoclaves has proved to be unable to kill the indigenous AMF in the growing media. Based on growing media treatment, AMF colonization in sterilized soil (g_0) was lower than the colonization in unsterilized growing media. The data in Table 2 also showed that there was no *Ganoderma* sp. infection observed in all treatments at the end of the study.

Table 2. The effect of AMF type and *Ganoderma* sp. on AMF colonization and *Ganoderma* sp. infection on 8 months old oil palm seedling.

Treatments	AMF colonization (%)	<i>Ganoderma</i> sp. infection (%)
Control (m_0)	44.4 c	0
<i>Glomus</i> sp. (m_1)	51.9 bc	0
<i>Entrophospora</i> sp. (m_2)	68.4 a	0
<i>Glomus</i> sp. + <i>Entrophospora</i> sp. (m_3)	61.4 ab	0
LSD 5%	14.7	0
Sterilized soil (g_0)	51.1 b	0
Infected soil by <i>Ganoderma</i> sp. (g_1)	61.9 a	0
LSD 5%	10.4	0

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

The seedling height data that has been transformed with $\sqrt{(x+0.5)}$ and the initial data were presented in Table 3. All AMF treated seedlings gave plant height that was significantly higher than control. However, there were no significant differences between AMF treatments. In this table also can be seen that the growing media gave no effect on the height of oil palm seedlings.

Table 3. The effect of AMF species and *Ganoderma* sp. on plant height of 8 months old oil palm seedling.

Treatments	Plant height $\sqrt{(x+0.5)}$ transformed (cm)	Original plant height (cm)
Control (m ₀)	7.5 b	56.0
<i>Glomus</i> sp. (m ₁)	7.6 ab	58.8
<i>Entrophospora</i> sp. (m ₂)	7.7 ab	60.2
<i>Glomus</i> sp. + <i>Entrophospora</i> sp. (m ₃)	7.9 a	64.1
LSD 5%	0.3	7.0
Sterilized soil (g ₀)	7.6 a	57.1
Infected soil by <i>Ganoderma</i> sp. (g ₁)	7.7 a	61.9
LSD 5%	0.2	3.7

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

AMF treatment gave a significant effect on both shoot fresh and dry weight of oil palm seedling. All AMF treatments showed higher shoot fresh and dry weight than the control seedlings. Nevertheless, no difference was observed on shoot fresh and dry weight between AMF treatment (Table 4). For growing media treatment, seedlings planted in unsterilized media that contain *Ganoderma* sp. inoculum gave higher shoot fresh weight of oil palm seedling. However, no effect of growing media was observed on the shoot dry weight of the seedling (Table 4).

Table 4. The effect of AMF types and *Ganoderma* sp. on shoot fresh weight and shoot dry weight of 8 months old oil palm seedling.

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)
Control (m ₀)	43.3 b	13.5 b
<i>Glomus</i> sp. (m ₁)	66.4 a	19.3 a
<i>Entrophospora</i> sp. (m ₂)	64.0 a	17.6 a
<i>Glomus</i> sp. + <i>Entrophospora</i> sp. (m ₃)	66.7 a	19.0 a
LSD 5%	9.3	3.5
Sterilized soil (g ₀)	56.2 a	16.7 a
Infected soil by <i>Ganoderma</i> sp. (g ₁)	64.0 a	18.1 a
LSD 5%	6.5	2.5

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

Similar to shoot fresh and dry weight, data on root fresh weight shown in Table 5 indicated that all AMF treatments had significantly higher root fresh weight over root fresh weight of control seedlings. Even though all AMF treatments showed enhancing root fresh weight compared to control, but no difference in root fresh weight was observed among the three AMF treatments. In

addition, all AMF treatments did not affect the root dry weight of oil palm seedlings (Table 5). As for growing media treatment, unsterilized soil (consisting of *Ganoderma* sp. inoculum) showed a significantly higher root fresh and dry weight over sterilized soil (Table 5).

Table 5. The effect of AMF species and *Ganoderma* sp. on root fresh and dry weight of 8 months old oil palm seedling.

Treatments	Root fresh weight (g)	Root dry weight (g)
Control (m ₀)	9.7 b	3.3 a
<i>Glomus</i> sp. (m ₁)	13.1 a	4.2 a
<i>Entrophospora</i> sp. (m ₂)	13.9 a	3.8 a
<i>Glomus</i> sp. + <i>Entrophospora</i> sp. (m ₃)	13.5 a	4.0 a
LSD 5%	2.8	0.9
Sterilized soil (g ₀)	10.0 b	3.2 b
Infected soil by <i>Ganoderma</i> sp. (g ₁)	15.0 a	4.4 a
LSD 5%	2.0	0.6

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

Based on the results of this study, it can be concluded that all AMF treatments used were able to form a symbiosis with the root of oil palm seedling with the colonization was above 50%. The formation of symbiosis provided a positive impact on the growth of oil palm seedlings which was demonstrated by plant height, shoot fresh and dry weight, and root fresh weight. Although the control seedlings were also colonized by indigenous AMF, however, the growth of the seedling was still lower compared to the seedling treated with AMF treatment. This may be due to the low activity of indigenous AMF, thus inoculated AMF could work better and effectively (Owen *et al.*, 2015; Deguchi *et al.*, 2021). A study conducted by Zhang *et al.* (2019) also reported that the exogenous AMF treatment showed the more effective result in enhancing the plant growth over indigenous AMF, due to the introduced AMF could suppress the abundance of AMF indigenous species.

The study results also revealed that *Ganoderma* sp. inoculum which is present in the growing media was not successfully infected the oil palm seedling. The roots of oil palm seedling planted in the unsterilized growing media showed no activity of decayed or necrosis as a result of *Ganoderma* sp. attack, even though in the unsterilized soil (originated from the basal stem of damaged oil palm tree due to *Ganoderma* sp. attack) there were chopped of oil palm root which infected by *Ganoderma* sp. and chopped of fruiting bodies of *Ganoderma* sp. Based on this result, we believed that 5 months period of the study is not enough time for *Ganoderma* sp. in the growing media to develop, thus the existed population was not able to infect the oil palm seedling yet. A similar study also reported by Rees *et al.* (2012) that there was a huge amount of *Ganoderma* inoculum required to infect plant roots. Spores from *Ganoderma* sp. fruiting bodies or infected root which was presented in the soil were incapable to infect the root in 5 months period after the

application of *Ganoderma* sp. In addition, Rees *et al.* (2007) mentioned that the failure of *Ganoderma* inoculum to infect the root can be caused by the roots are not in direct contact with the inoculum. Furthermore, they also found in their study that temperature was probably inhibited *Ganoderma boninense*. where severe BSR⁵ disease occurred after 8 months on inoculated seedlings under shade, but not on seedlings exposed to the sun. In our study, at the main nursery the seedlings were kept in an open area without shade after the seedlings were exposed to growing media that contained *Ganoderma* inoculum. This condition might probably also be one of the reasons why all the seedlings were yet to get infected by *Ganoderma*.⁴⁴

According to Susanto and Sudharto (2003), BSR incidence was higher with increasing oil palm planting generation, for instance, BSR pathogen occurrence in the 1st, 2nd, and 3rd generation was 17%, 18%, and 75% respectively. The higher incident of BSR in the higher generation might be due to the higher *Ganoderma* sp. inoculum in the soil. The 3rd generation of oil palm means that the land had been cultivated with oil palm for more than 50 years. During this period, oil palm root always exists in the soil which can be a host and as an organic carbon source for *Ganoderma* sp. growth. Thus, the longer the oil palm stays at the same land, the longer the time for *Ganoderma* sp. inoculum to develop and propagate, and in the end, the more oil palm will be infected.³⁴

The AMF used in this study successfully colonized the root of oil palm seedlings, resulting in better oil palm seedling growth. The use of AMF, either singly or in combination, has been shown to increase the growth of oil palm seedlings by increasing plant height, shoot fresh weight, shoot dry weight, and root fresh weight.¹ These results prove that the two types of AMF used in this study are suitable for oil palm.¹⁸ The same result was also reported by Krisnarini *et al.*, (2018) using *Gigaspora* sp. isolate MV 16 and *Glomus* sp. isolate MV17. They found that both AMF isolates significantly increased oil palm seedling growth. In contrast, Sundram (2010)³⁵ reported that the application of *Glomus etunicatum* successfully increased the vegetative growth of the oil palm seedling. However, the application of mixed *G. etunicatum* with *Gigaspora rosea* or *G. etunicatum* with *Scutellospora heterogama* depressed the oil palm seedling growth. Furthermore, the study by Rias *et al.* (2015) using 2 isolates of *Entrophospora* sp. and 3 isolates of *Glomus* sp. on oil palm seedlings showed that all AMF isolates used successfully colonized oil palm root and improved oil palm seedling growth except for *Glomus* sp. isolate 11 which have growth not significantly different from the control seedling. Different types of AMF can produce different growth responses in oil palm that can be referred to as mycorrhizal dependence.³⁷

Several mechanisms may be involved in how AMF can enhance plant growth. After symbiosis with plant roots occurs, AMF hyphae develop inside and outside the roots. AMF hyphae that develop outside the root (external hyphae) can directly absorb nutrients from the soil and transfer these nutrients into the root cells of the host plant (Souza, 2015). In addition, AMF hyphae³³

also produce phosphatase enzymes that can release fixed phosphate in the soil so that the phosphate ion can be absorbed by AMF hyphae as well as by plant roots (Sato *et al.*, 2015). The meta-analysis study conducted by Qin *et al.* (2019) proved that introduced AMF can enhance various enzyme activities in the soil, including phosphatase. These enzymes play a role in releasing nutrients that are fixed in the soil. The increase in nutrient absorption sequentially gave positive feedback to the plant growth (Qin *et al.*, 2020; Rini *et al.*, 2020). In addition, another mechanism that might be involved in the presence of AMF in plant roots to promote the increase of the photosynthetic rate of the host plant, thus plant that are colonized by AMF generate higher photosynthate for better plant growth (Szczalba *et al.*, 2019).

4. Conclusions

The study concluded that all AMF treatments showed a growth improvement of oil palm seedlings. However, the ability of AMF in increasing the resistance of oil palm seedlings against *Ganoderma* sp. has not yet been identified due to the *Ganoderma* sp. inoculum which is present within the growing media was unable to infect the root of oil palm seedling during the 5 months of this study. It is suspected that it takes a longer time for *Ganoderma* sp. present in the soil to develop and propagate so that it can infect the root of oil palm seedlings. Based on the results of this study, we suggest to the oil palm planters at the time of replanting to remove as much of the suspect infected palm biomass as possible from the soil to reduce the risk of *Ganoderma* sp. attack.

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