Research article

Controlling Basal Stem Rot in Oil Palm Plantations by Applying Arbuscular Mycorrhizal Fungi and Trichoderma spp.

Henny Hendarjanti[1]* and Henik Sukorini[2]

[1]PT. Astra Agro Lestari Tbk. Jl.Pulo Ayang Raya Blok OR/I Pulogadung Industrial Area, Indonesia
[2]Faculty of Agriculture and Animal Science, Universitas Muhammadiyah Malang, Indonesia

ORCID
Henny Hendarjanti https://orcid.org/0000-0003-2683-2860

Abstract.
Biological agents are critical in the management of major diseases in oil palm plantations. The potential of arbuscular mycorrhiza fungi (AMF) and *Trichoderma* spp. to control basal stem rot disease was investigated in this study. Three-month-old seedlings treated with AMF since the beginning of planting demonstrated AMF colonization of the roots. With a 25 gram AMF treatment in the main nursery, AMF colonization increased by 85.99% in TBM-1 (the 1-year immature plant). In TBM-2, AMF colonization increased by 86.00-97.33% (2-year immature plant). All of the treatments started with 30 grams of *Trichoderma* spp. applied at the start of pre-nursery planting. Root colonization and the number of spores in the root rhizosphere in TBM-2 had a strong relationship (2-year immature plant). AMF inoculation had a significant impact on root colonization and spore number response. According to this study, the application of 25 grams of AMF in the pre-nursery and main nursery, as well as repeated additions at planting, were found to be effective in controlling attacks of basal stem rot disease through early prevention strategies.

Keywords: biological control agent, arbuscular mycorrhiza fungi (AMF), *Trichoderma* spp. oil palm

Corresponding Author: Henny Hendarjanti; email: henny.hendarjanti@gmail.com

Published 07 June 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the PGPR 2021 Conference Committee.

1. Introduction

The Oil palm (Elaeis guineensis) is one of the world's most important oil crops. It is widely cultivated in Asia, Africa, and Latin America and is traded internationally [1]. Palm oil is essential as a substantial global industry on market size & share was valued at USD 65.73 billion in 2015, is expected to reach USD 92.84 billion in 2021 [2]. Malaysia and Indonesia play an important role in the future production of palm oil, as these two countries account for 85% of global palm oil production [3], [4]. In 2020, the highest production of Palm oil, 58.8% (42.5 Mt), was from Indonesia, followed by Malaysia at 25.6% (18.5 Mt) [5]. Based on the data for the world supply and distribution of palm oil in 2021, Indonesia was the largest producer of palm oil, Malaysia was the second. In

Indonesia and Thailand, world palm oil production has increased, especially between 2017 and June 2021, increasing 12% annually [2]. Basal stem rot (BSR) disease is caused by the fungus Ganoderma boninense, one primary disease in oil palm plantations. Mentioned that BSR increases in this 2nd generation planting area in Bukit Sentang estate, North Sumatra. BSR is a severe disease of oil palm, which can reduce yields by 50–80% [6]. Over the past two or three decades, it has increased due to its spread from infection foci at a greater rate following repeated cycles of crop planting on infested sites [5]. The estimated economic loss was based on 216 infected palms, which is 43.32% (7,485.23 Fresh Fruit Bunch in kg) in six months or equivalent RM 3,293.50 of the potential yields Tawau district of Sabah, Malaysia. BSR disease could cause a significant economic loss to the planters if no treatment or control [7].

Various efforts have been made to reduce BSR disease in the field. The methods taken include modification of replanting, culture techniques, biocontrol agents, chemical fungicides, surgery, and treatment of mounds on plants infected with Ganoderma. Utilization of Ganoderma tolerant cultivars [8],[6], [9],[10] and surgery and mound treatment only prolongs the life of infected palms [6]. Chemical control with fungicides extends the economic life of oil palm plantations by up to five years. Control of Ganoderma-infected palm plants using Hexaconazole showed 95.64% of plants still standing and producing bunches after one year of treatment [9].

So far, biological control of BSR research has not been carried out on planting areas. One of the obstacles to developing BSR biological control in the planting area is the limited information about the influence of environmental factors and their interactions, especially the physical and biological environment of soil rhizosphere and AMF colonization on plant roots on BSR biological control. The relationship of all these factors in influencing BSR disease caused by oil palm *Ganoderma* in oil palm planting areas has not been widely reported. This study aims to determine the relationship and effect of AMF colonization on roots rhizosphere and *Trichoderma* sp on the intensity of *Ganoderma* attack on oil palm.

2. Methodology

2.1. Time and place of research

The research was conducted from August 2017 to July 2021 in an oil palm plantation located in Air Molek Village, Indragiri Hulu Distric Riau. The oil palm area is endemic of

basal stem rot disease caused by the *Ganoderma boninense*, with an infection more or less 0.32 – 0.93 % before replanting

2.2. Experimental design

The research was set up using a complete randomized block design with eighteen treatments replicated three times. The *Arbuscular Mycorrhiza Fungi* (AMF) and *Trichoderma* spp. Management Practices tested with the regular germinated seed and moderate tolerant *Ganoderma* (control) from Socfindo included:

Table 1: The Following Eighteen Treatments were The AMF and *Trichoderma* spp. Management Practices.

No.	Code	Treatments
1	Pt30-M0-T0	30 gr./plant *Trichoderma* in PN
2	P0-Mt30-T0	30 gr./plant *Trichoderma* in MN
3	Pt30-M0-Tt200	30 gr./plant *Trichoderma* in PN + 200 gr./plant *Trichoderma* in planting area
4	P0-Mt30-Tt200	30 gr./plant *Trichoderma* in MN + 200 gr./plant *Trichoderma* in the planting area
5	Pm25-M0-T0	25 gr. *AMF*/plant in PN
6	Pm25-M0-Tm100	25 gr. *AMF*/plant in PN + 100 gr./plant *AMF* in planting area
7	Pm25-M0-Tm200	25 gr. *AMF*/plant in PN + 200 gr./plant *AMF* in the planting area
8	P0-Mm25-T0	25 gr./plant *AMF* in MN
9	P0-Mm25-Tm100	25 gr. *AMF*/plant in MN + 100 gr./plant *AMF* in planting area
10	P0-Mm25-Tm200	25 gr. *AMF*/plant in MN + 200 gr./plant *AMF* in the planting area
11	Pt30-Mm25-Tt100m100	30 gr./plant *Trichoderma* in PN + 25 gr. *AMF*/plant in MN + 100 gr/plant *Trichoderma* and 100 gr./plant *AMF* in planting area
12	Pt30-Mm25-Tt100m200	30 gr./plant *Trichoderma* in PN + 25 gr. *AMF*/plant in MN + 100 gr/plant *Trichoderma* and 200 gr./plant *AMF* in planting area
13	P0-Mt30m25-Tm100	30 gr./plant *Trichoderma* and 25 gr. *AMF*/plant in MN + 100 gr./plant *AMF* in the planting area
14	P0-Mt30m25-Tm200	30 gr./plant *Trichoderma* and 25 gr. *AMF*/plant in MN + 200 gr./plant *AMF* in the planting area
15	P0-Mm25-Tt200m100	25 gr. *AMF*/plant in MN + 200 gr/plant *Trichoderma* and 100 gr./plant *AMF* in planting area
16	P0-Mm25-Tt200m200	25 gr. *AMF*/plant in MN + 200 gr/plant *Trichoderma* and 200 gr./plant *AMF* in the planting area
17	Pt30-Mm25-Tt400m250	30 gr./plant *Trichoderma* in PN + 25 gr. *AMF*/plant in MN + 400 gr/plant *Trichoderma* and 250 gr./plant *AMF* in planting area
18	MT-Gano	The Germinated Seed of Moderate Tolerant Ganoderma (Control)

The research was conducted in the 1st generation planting area in Air Molek Village, District, Indragiri Hulu, Riau. Replanting methods consisted of standard replanting method to palm infected with hole (2 x 2 x 1 m) and root sanitation, collection of root masses, uprooting root masses of oil palm, oil palm seedling planted in the center of

hole, hole planting point made on sanitized area. *Trichoderma* spp. and AMF biological agents were applied in pre-nursery (PN), main nursery (MN), and planting. At the time of application of *Trichoderma* spp. added organic material called *solid-state fermentation* derived from *Palm Oil Mill Effluent* (POME) compressed in a ratio of 1: 10 (*Trichoderma* : organic matter). After the seedlings are 12 months old, planting is carried out in the area.

The assessed of Palm's Vegetative Growth

Observation of vegetative growth was used to determine several parameters during the nursery. Vegetative growth was assessed at 3, 6, and 12 months after the application of biocontrol agents. Characters evaluated included: (i) plant height, obtained by attaching a measuring tape to the base of the plant up to the tip of the first midrib, (ii) number of leaves, assessed only by counting completely open leaves, and (iii) stem diameter, measured using a caliper. In each observation of vegetative growth, ten plant samples were taken in each treatment and group

2.3. Soil and root sampling

Soil and root samples were taken at three-block locations in Air Molek District. The soil sampling method was done by taking 3 sample points. Each sample point was taken as much as 100 g from around the roots, with a 0-60 cm depth. The distance of 10-50 cm from the base of the stem, the sample was composited with other sample points, then put in a plastic bag. Root samples were taken by cutting the tips of the young roots as much as 100 grams.

2.4. Counting of Trichoderma spores

The calculation of the density of *Trichoderma* spp. spores from the soil is by taking soil from the rhizosphere of oil palm nurseries aged 3, 6, and 12 months as a sample of 100 grams. First, observations were made using the serial dilution method, then ten microliters of the solution were grown on 15 ml of potato dextrose agar (PDA) media. Furthermore, the Petri dish was stored at room temperature for seven days. Finally, spore density was calculated by counting the *Trichoderma* colonies that grew on a petri dish [11].

2.5. Observation of root colonization/infection

The colonization/root infection rate of oil palm plants was observed by AMF through the root staining technique using the Giovannetti and Mosse method [12]. The calculation of the percentage of root colonization by AMF uses the following formula :

$$Mycorrhizal\,Infection\,Percentage(MIP) := \frac{total\ of\ root\ segments\ colonized}{total\ of\ root\ segments\ studied} \times 100$$

The level of root infection is classified into 5 classes [13], namely: very low (0-5%), low (6-25%), moderate (26-50%), high (51-75%) and very high (76-100%). The staining process was started by washing the roots, then cutting the clean roots into 1 cm, after which 100 pieces of roots were put into a test tube, soaked in 10% KOH, and heated in a microwave at 250˚C for 10 minutes. The heated roots were left for ± 12 hours at room temperature. After that, the 10% KOH solution was removed, and the roots were washed three times with distilled water. The roots were soaked in 3% H_2O_2 and stored for ±12 hours at room temperature. After storage completion, the H_2O_2 was removed, and the roots were washed three times with distilled water, soaked in 1% HCL, and stored for ±12 hours at room temperature. The HCl was removed, then the roots were soaked in trypan blue, then heated at 250˚C for 5 minutes in the microwave and stored for ± 12 hours at room temperature. The trypan blue was removed, and the roots were soaked in Lactoglycerol and heated at 250^{o}C for 5 minutes. The roots were then stored for ± 12 hours at room temperature. The roots were then stored for ± 12 hours at room temperature. After giving Lactoglyserol was completed, the root was taken with tweezers and placed on a glass object. The root infection by AMF and mycorrhizal structures (vesicles, arbuscular, hyphae) was observed under a microscope.

2.6. Counting and identification of AMF spores

Isolation of spores was carried out using the wet filtration technique from Pacioni [14], followed by the centrifugation technique from Brunndret [15], which was modified. Soil sample as much as 100 g was dissolved in a 1000 ml beaker glass by adding 1 l of water and stirred evenly for ± 10 minutes until homogeneous. The solution is allowed to stand for ± 1 minute until the large particles settle, filtered in a set of filters with hole diameters of 1 mm, 500 µm, 212 µm, 106 µm, and 53 µm, respectively, from large to small hole diameters. This procedure is repeated 4-5 times). The soil remaining in the 500, µm 212 µm, 106 µm, and 53 µm sieves was transferred to a centrifuge tube, added 25-40 ml of distilled water, then centrifuged at 2000 rpm for 5 minutes. The

results of the centrifuged supernatant were discarded, then 60% glucose was added and centrifuged at 2000 rpm for 1 minute. Finally, each centrifuge tube was rinsed using water on a sieve with a hole diameter of 53 m. The identification of spores was carried out microscopically using the guidelines according to INVAM [16] to determine the genus of AMF found, based on observations of spore arrangement, hyphal shape, size, color, and spore shape.

2.7. Disease Incidence Assessment

Basal stem rot disease development was monitored based on a quantitative assessment measured as Disease Incidence (DI), expressed as a percentage at monthly intervals. DI was assessed based on symptoms of *Ganoderma boninense* infection in oil palm plants (leaves (chlorosis and leaf necrosis, with or without Ganoderma basidiocarp and dead plants). DI refers to the number of plants exhibiting the symptoms mentioned above with the total number of plants assessed :

$$\% \text{ Disease Incidence (DI)} = \frac{\textit{Number of plants infecteddd}}{\textit{Total number of plants assessed}} \times 100$$

2.8. Data analysis

All data in this experiment were reported as the mean value of three replications from each soil and root sample. Data of the percentage of arbuscular mycorrhizal fungi root colonization and numbers of infective AMF propagules were analyzed by two-way ANOVA. The significance of differences between AMF spore propagule and % colonization AMF from different the sample root plants and soil were tested using Fisher's LSD *test* (Fisher's Least Significant Difference. *Test*) $p < 0.05$ and Tukey Test ($p < 0.05$)

3. Result and Discussion

3.1. Mycorrhizal Root Colonization

AMF colonization on oil palm roots that had been applied at the beginning of planting seedlings (pre-nursery) was seen in the observation of 3-month-old nurseries that the colonization rate was still low, ranging from 18.11% - 20.55%. Furthermore, plants that have been treated with AMF, both at the pre-nursery and in the main nursery, have

shown low to moderate colonization rates at the observation of roots at six months of age. Colonization ranged from 24.00 % to 33.33 %. There was a significant difference in the level of root colonization ($p < 0.05$) between treatments 9 (Pm25-M0-T0) and 17 (Pt30-Mm25-Tt400m250). Oil palm plants given *Trichoderma* spp. from the pre-nursery, the AMF application was given during the main nursery followed by repeated administration of *Trichoderma* 400 grams/plant and AMF 250 grams/plant at the time of planting had a higher percentage of root colonization compared to another treatment. Giving *Trichoderma* spp at the nursery, AMF in the main nursery, and giving both microorganisms at planting area gave good AMF colonization results in the roots, as long as the plant reproduces roots. It will allow AMF to continue to extend hyphae and colonize in plant roots. Types of plants with fibrous root systems are highly dependent on AMF. Plant signals and root geometry influences the degree of infection and the development of the dominant symbiotic agent [17].

One year's observation in the field (TBM-1) showed a significant difference between AMF colonization in roots, namely high - very high ranging from 55.11% - 85.99% (Figure ??). High AMF colonization at the time of the main nursery (observation of seedlings aged 6 and 12 months) will significantly affect the colonization of AMF in the field, so that the treatment with the highest % colonization in the main nursery as in treatment,11(Pt30-Mm25-Tt100m100),12(Pt30-Mm25-Tt100m200), and 17 (Pt30-Mm25-Tt400m250) also had high root colonization at TBM-2 plant age. In treatments 11 and 12, respectively, the application of *Trichoderma* 30 grams/plant in the pre-nursery and AMF 25 grams/plant in the main nursery was followed by the application of *Trichoderma* and AMF at the time of planting. Research on the application of AMF in nurseries that are continued to planting areas (Field) has never been reported, so support from other studies cannot be conveyed. However, suppose the plant produces or reproduces roots. In that case, it will allow the mycorrhizae to extend themselves by hyphae and colonize in the roots of new plants and strengthen their presence in the old roots (because AMF has colonized the nursery). Thus, developing mycorrhizae is very likely to continue to colonize, although their existence is also strongly influenced by the environment.

The application of *Trichoderma* spp. in the nursery is needed to accelerate AMF colonization in oil palm roots. Likewise, the application of mycorrhizae in the main nursery is necessary for preparation to deal with various kinds of harmful microorganisms in the soil. The results showed that the administration of mycorrhizae both in the main nursery and in the field would increase the mycorrhizal colonization rate. The observation of root colonization showed if mycorrhizae were not given during the main nursery, the colonization phase that occurred was in the extension phase of colonization to new roots

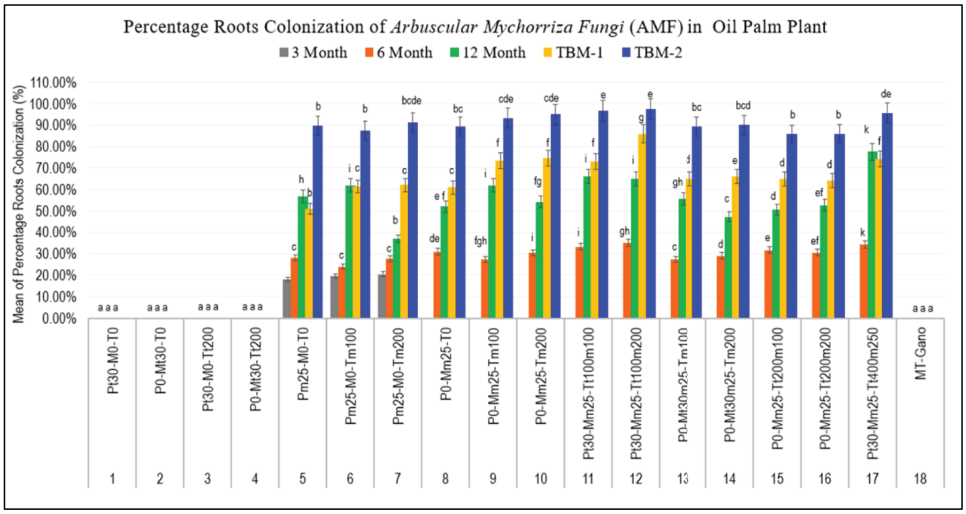

Figure 1: Root colonization (%) of *Arbuscular Mycorrhiza Fungi* (AMF) in Oil Plam Plant *Note:* Bars represent mean; different letters showed significant difference at $p<0.05$ by Tukey multiple comparison test.

as indicated by the presence of hyphae between root cells (Figure 3a), but if the main nursery had been given the colonization of the new roots was already at the stage of vesicular-arbuscular formation (Figures 3b and 3c). In oil palm roots colonized with AMF, internal hyphae and arbuscular structures and vesicles were found. This indicates that the plant can produce symbiosis with AMF, and AMF infection has occurred in the roots of oil palm plants (Figure 3). The development of AMF infection or colonization begins with forming an appressorium on the root surface by external hyphae derived from germinated spores [18]. The appressorium enters the root through the inter-epidermal gap, forming intracellular hyphae along the epidermis root, arbuscular, and vesicles. *Trichoderma* spp. application. At the time of the initial nursery (pre-nursery), it turned out to show its role as a biofertilizer that induces resistance of oil palm seedlings to soil pathogen infection and increased AMF colonization in roots, as shown in treatments 11, 12, and 17. *Trichoderma*'s increased plant resistance was also demonstrated in several horticultural crops [19],[20],[21].

Preventive measures to prevent *G. boninense* infection through the application of biological agents are carried out early. Since seeding and at the time of planting (in the field) affect AMF colonization in oil palm roots. Environmental and plant conditions strongly influence the AMF colonization rate. AMF works well in a minimal environment, especially since the fertilizer provided is insufficient for the plant's needs. Still, in an environment where all of them are fulfilled, AMF does not work optimally. The frequency and intensity of root colonization by mycorrhizae applied to sandy soil would increase significantly compared to soil given compost [22]. The degree of AMF colonization in roots provides information about the activity of mycorrhizal communities in specific

ecosystems [23]. Until this observation was carried out, namely oil palm aged TBM-3, no symptoms of stem rot disease were found. Study reported by [24] found a positive correlation between AMF biodiversity and soil pH. Although this correlation may be influenced by the history of land use (fertilizer and herbicide application) in oil palm plantations, differences in soil characteristics can determine differences in AMF communities.

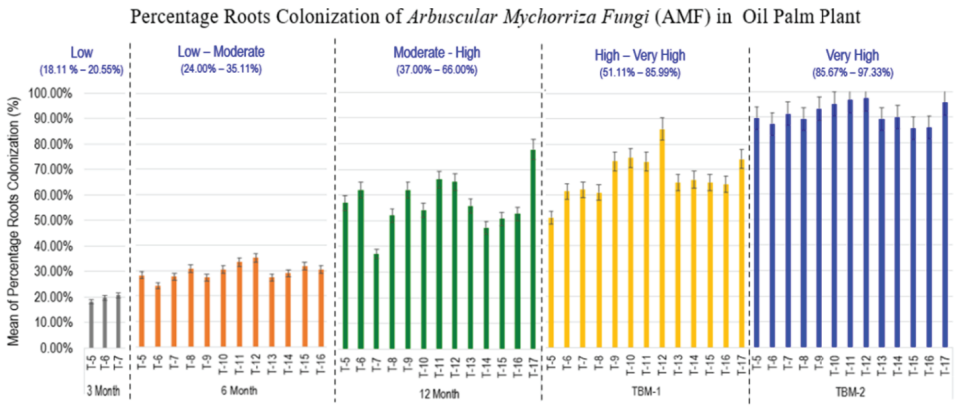

Figure 2: The Level of Root Colonization of Oil Palm Seedlings and Immature plant aged 3 Month, 6 Month, 12 Month, TBM-1 (1 Year-Immature Plant) and TBM-2 (2 Year-Immature Plant). Root infection rates are classified into 5 classes [13]; which are very low (0-5%), low (6-25%), moderate (26-50%), high (51-75%) and very high (76-100%).

Nevertheless, the results of this study support the potential use of AMF in plant growth promoters and pathogen resistance programs to achieve sustainability in oil palm plantations. This is also following the opinion by [25] which stated that AMF colonization on oil palm roots could inhibit the occurrence of *G. boninense* infection. Furthermore, the interaction between AMF and endosymbiotic bacteria *B. subtilis* B10 in oil palm increase phosphorus absorption and plant growth and protects the host from the *G. boninense* [26]

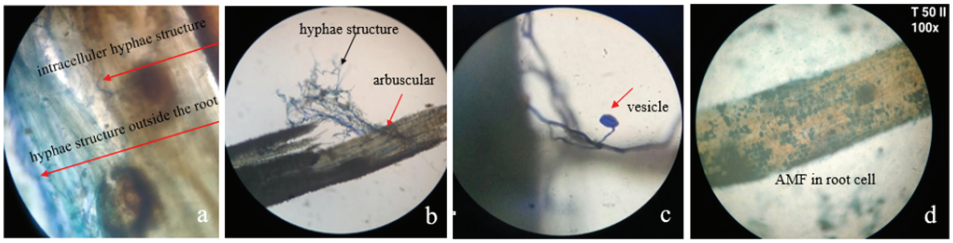

Figure 3: Arbuscular Mycorrhizal Fungi (AMF) Colonization on the Roots of Palm Oil Plant (a) Hyphae Structure, (b) Arbuscular, (c) Vesicle and (d) AMF in root cell, with Magnification of 100 times

This study showed that the structure of AMF isolated from oil palm cortical root consisted of hyphae, spores, arbuscular, vesicles, and chlamydospores (Figure ??). Hyphae are found along the root cortex. In the root cortex also found arbuscular, vesicular, internal hyphae, and external hyphae. The tips of the bubbly hyphae turn

into spores; therefore, spores are formed from the swelling of the hyphae. The spores originating from hyphae development are called chlamydospores [27]. In addition, each branching hyphae forms chlamydospores called sporocarps. [28] reported that endomycorrhizal fungi always form vesicular and arbuscular in cortical cells.

According to [28], External hyphae absorb phosphorus and water from the soil and are carried into plants. Mycorrhizae grow from outside the roots and then enter the root tissue. [29] reported that inside the cell, vesicles, small bubbles in the cytoplasm shaped granular vesicles that contain lipids become vegetative mycorrhizal reproductive cells. Arbuscular are hyphae that penetrate the plasmalemma and help transport nutrients to plant cells. In addition to vesicles and arbuscules, external hyphae are formed to help expand the absorption of nutrients by the roots. In certain plants, the length of the outer hyphae usually reaches 80 cm per 1 cm of root length. Outside the roots, hyphae can form sporangium, which produces spores as a means of reproduction. The comprehensive network of hyphae in the soil helps the roots to absorb nutrients and water. In this study, the formation of vesicles and arbuscules in oil palm root cells showed a perfect symbiosis so that plants increased the availability of nutrients absorbed from the soil. This is also supported by the [30] report that the first stage of AMF growth took more than three months. The first month after inoculation, new intraradical appressorium and hyphae were found in the root cortex tissue. At the end of the second month after inoculation, a new intraradical hyphal network begins. In the third month, a new arbuscular formation begins. Spores have been observed before the seedlings are transferred to the main nursery.

3.2. The density of Mycorrhizal in Roots Rhizhosphere

The number of AMF spores was found in the rhizosphere of oil palm roots starting at 12 months. The number is between 41.32 – 187.04 spores/100 grams of soil sample. The highest number of spores (187.04 propagules) was found in plants with treatment code 13 (P0-Mt30m25-Tm100), i.e., administration of *Trichoderma* 30 grams/plant and AMF 25 grams/plant to Main Nursery seedlings, followed by administration of AMF 100 g/plant at the time of planting. The number of spores increased in oil palm plantations in the area of TBM-2 age (2 years immature plant) which was 113.33 – 220.00 spores/100 g of soil sample. The highest number of AMF spores was 220 propagule spores/ 100 grams of soil sample in treatments 10 (P0-Mm25-Tm200) and 17 (Pt30-Mm25-Tt400m250), which were significantly different at ($p < 0.05$) compared to other treatments.

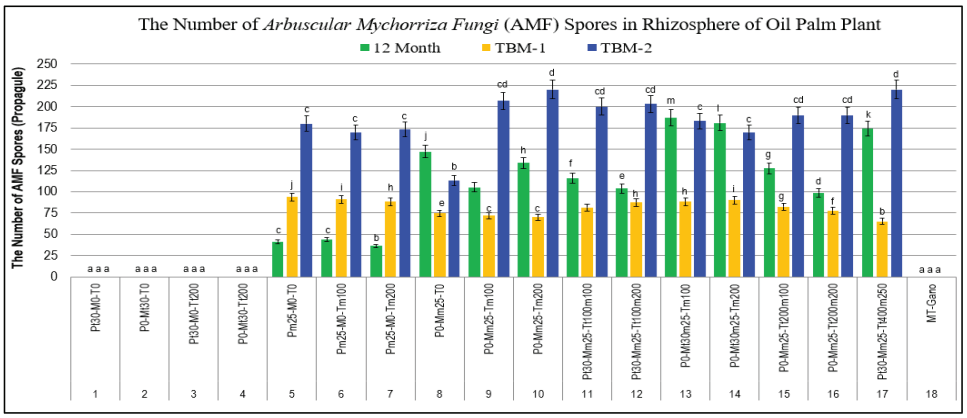

Figure 4: The Number of *Arbuscular Mycorrhiza Fungi* (AMF) in Rhizosphere of Oil Plam Plant (100 g soil sample) *Note:* Bars represent mean; different letters showed significant difference at $p<0.05$ by Tukey multiple comparison test

Based on the results of AMF identification from oil palm rhizospheric soil samples at Tunggal Perkasa Plantation co. ltd., Air Molek, Riau, found the genus Glomus. Similar to [31] with the object of oil palm plants at Socfindo North Sumatra found the genus Glomus and Acaulospora. [32] at PP London Sumatra Indonesia Arta Kencana Estate, North Sumatra, where the genus Glomus, Acaulospora, and Gigaspora were found. [33] at Lancang Kuning University Land, Riau found the genera Acaulospora, Gigaspora, Glomus, and Sclerocystis. [13] stated that there were two genera of endo mycorrhizae, Glomus and Entrophospora, isolated from salak roots. Glomus is a genus that has a wide distribution because it is found in all locations.

The results of this study are by [34] that the genus Glomus is present in all sampling locations. Glomus is a type of endomycorrhizal fungus that can adapt well in the soil rhizosphere. This study indicates that AMF genus Glomus found in Air Molek, Indragiri Hulu Riau has essential values such as increasing plant nutrient uptake and protecting oil palm from pathogens. [34] reported that the genus Glomusha has high adaptability to environmental conditions so that it is often found in symbiosis with the roots of various plant species. Naturally, in the oil palm rhizosphere, AMF isolates consist of Glomus and Gigaspora; the number of AMF spores in the oil palm rhizosphere is different for each variety, type, age of oil palm plants, and also season. While sampling also affected the number of AMF spores, the density of spores isolated from oil palm soil samples ranged from 7 – 127 spores/ 200 g of soil [35]; 306 spores/50 g soil [33]; 61 – 65 spores/ 50 g soil at the age of 5 – 15 years of oil palm plantations [36]; spores density varied from low: 0.81/50 g soil to moderately: 1.33 – 1.51/50 g soil [24].

3.3. Spores Density of Trichoderma spp. in rhizosphere

Trichoderma spp. reported to act as biocontrol agents through mechanisms of antibiosis, mycoparasites, space competition, and nutrient competition. The mechanism of the biocontrol agent for the fungus *Trichoderma* spp. This allows the prevention and suppression of basal stem rot disease in oil palm seedlings. *Trichoderma* spp. is one of the endophytic fungi that acts as a biological agent to control soilborne pathogens. *Trichoderma* spp. is one of the soil saprophytic fungi which is naturally a parasite that attacks many types of fungi that cause plant diseases [37]. Spore density is one factor that influences the success of *Trichoderma* spp. to develop and act as an antagonist fungus in controlling soilborne pathogens. Observation of the spore density of *Trichoderma* spp. (Table-1) from isolates of oil palm nursery soil at the age of 6 months showed that the treatment of *Trichoderma* spp. from the beginning of seedling planting (pre-nursery) had higher spore density, namely: 466.67 x 10^6, 241.67 x 10^6, 366.67 x 10^6, 366.67 x 10^6 and 300.00 x 10^6 in treatments 1 (Pt30-M0-T0), 3 (Pt30-M0-Tt200), 11 (Pt30-Mm25-Tt100m100), 12 (Pt30-Mm25-Tt100m200), and 17(Pt30-Mm25-Tt400m250) compared to *Trichoderma* spp given at the main nursery and without *Trichoderma* spp. Naturally, *Trichoderma* spp. is already in the root rhizosphere, as can be seen from the results of the calculation of spore density without *Trichoderma* treatment (Table 1). Still, it is necessary to apply it as an addition to *Trichoderma* in oil palm seedlings in the interest of being a biocontrol agent for basal stem rot disease. Furthermore, it can be seen that in the observation of oil palm nurseries aged 12 months, there was no development of spore density in treatments 1,3,11,12, and 17. On the contrary, spore density began to increase in treating *Trichoderma* spp. in the main nursery, namely 400.00 x 10^6 and 450.00 x 10^6 at treatments 4 (P0-Mt30-Tt200), 13 (P0-Mt30m25-Tm100) and 14 (P0-Mt30m25-Tm200). According to the standard for the biocontrol agent *Trichoderma* spp. The spore density has a value of 1 x 10^6 conidia/ml. and spore viability 60% [11]. The abundance of biocontrol agents less than 10^5 cfu g-1 in the soil will cause a high incidence of basal stem rot disease [38]. [39] reported that *Trichoderma* spp. isolates from soil that had a reasonably high spore density (1.26x10^8) and had high spore viability (100%), had potential as a biocontrol agent in controlling soilborne pathogens *Fusarium* sp.

Trichoderma as a biocontrol agent is also helpful as plant growth-promoting because it can produce phytohormones, decompose organic matter, and protect plants from biotic and abiotic stresses [40], [41]. However, success as a successful plant growth-promoting requires applying abundant *Trichoderma* spores in the soil [42]. Thus, it is

crucial to obtain a high density of *Trichoderma* spores in the soil rhizosphere. In this study, the application of *Trichoderma* spp. It added organic material Palm Oil Mill Effluent (POME) in solid-state fermentation (solid). It was reported that organic matter contained in compost can have dual benefits as a source of nutrition for biocontrol agents and can suppress diseases caused by soilborne pathogens [43]. Compared to liquid fermentation, solid-state fermentation is closer to natural habitat conditions, and many species have succeeded in producing spores through solid-state fermentation [44]. Therefore, it is essential to optimize the spore production conditions by *Trichoderma* spp. in the POME solid-state fermentation. POME contains high concentrations of protein, nitrogen compounds, carbohydrates, lipids, and minerals that serve as a food source for microorganisms. Changes in POME can be through an anaerobic process in which microorganisms break down biodegradable materials without oxygen [45]. The addition of organic matter to biocontrol agents is the management of growing habitats. Application of antagonistic fungi such as *Trichoderma* spp. it will be more effective when accompanied by the management of growing habitats by providing compost as a source of organic matter, while providing an inoculum of saprophytic microbes that can enrich the diversity of pathogenic antagonist microbes [38]. The research results by [46] stated that the organic solid-state fermentation *Stevia rebaudiana* was successfully used to produce *Trichoderma guizhouense* spores, with a maximum yield of up to 7 x 10^9cfu g-1. The application of *T. guizhouense* could induce some common microbes. The abundance and evenness of the fungus increased with the *Trichoderma* bioorganic fertilizer treatment compared to the organic fertilizer treatment [47]. There is a relationship between diversity and emergent traits of microbial communities [48].

Note : Means with the same letter are not significantly different by by Tukey multiple comparison test at 5% level of significance

3.4. Effect of AMF) and Trichoderma spp. on the Palm's Vegetative Growth

Table 2 shows that the parameters evaluated for estimating vegetative growth and development include the increase in average plant height, number of leaves, and stem girth of oil palm seedlings. Combination of AMF and *Trichoderma* spp. significant effect on these parameters, especially in the application treatment of *Trichoderma* spp. and AMF in pre and main nursery, compared to treatment with moderate tolerant Ganoderma oil palm seedlings as control (without *Trichoderma* spp and/or AMF). *Trichoderma* spp. and AMF can influence and promote seedling growth positively. The

Number	Treatmeant	Spores Density of *Trichoderma* spp. in Oil Palm Seedling (Cfu g^{-1})		
		3 Month	6 Month	12 Month
1	Pt30-M0-T0	867.00 x10^6	466.67 x 10^6 j	466.67 x 10^6 g
2	P0-Mt30-T0		88.33 x 10^6 f	88.33 x 10^6 b
3	Pt30-M0-Tt200	1,133.00 x10^6	241.67 x 10^6 g	241.67 x 10^6 c
4	P0-Mt30-Tt200		31.67 x 10^6 d	400.00 x 10^6 g
5	Pm25-M0-T0		1.5 x 10^5 a	1.0 x 10^5 a
6	Pm25-M0-Tm100		17.5 x 10^5 b	0.7 x 10^5 a
7	Pm25-M0-Tm200		4.5 x 10^5 a	0.7 x 10^5 a
8	P0-Mm25-T0		0.8 x 10^5 a	0.8 x 10^5 a
9	P0-Mm25-Tm100		0.5 x 10^5 a	0.5 x 10^5 a
10	P0-Mm25-Tm200		0.2 x 10^5 a	0.00 a
11	Pt30-Mm25-Tt100m100	833.00 x10^6	366.67 x 10^6 i	366.67 x 10^6 d
12	Pt30-Mm25-Tt100m200	1,133.00 x10^6	366.67 x 10^6 i	366.67 x 10^6 d
13	P0-Mt30m25-Tm100		40.00 x 10^6 d	400.00 x 10^6 e
14	P0-Mt30m25-Tm200		45.00 x 10^6 e	450.00 x 10^6 f
15	P0-Mm25-Tt200m100		1.8 x 10^5 a	1.3 x 10^5 a
16	P0-Mm25-Tt200m200		3.8 x 10^5 a	0.00 a
17	Pt30-Mm25-Tt400m250	1,267.00 x10^6	300.00 x 10^6 h	550.00 x 10^6 h
18	MT-Gano	1 x 10^3	23.3 x 10^5 c	3.3 x 10^5 a

no observations

Figure 5: Spores Density of *Trichoderma* spp. in Oil Palm Seedling.

combined application of these two biocontrol agents facilitates plant growth. Many studies have shown the potential use of *Trichoderma* sp. as a booster or enhancer of growth in oil palm plants. There are also reports on the intrinsic capacity to expand the biomass of plants' shoots, roots, and stems [49], [50]. A significant increase in mean plant height after treatment with a combination of *T. viride* and *T. harzianum*, as well as a mixture of *Trichoderma* sp. and *Stenotrophomonas* sp. In oil palm nurseries, this is different from a single application [51].

Trichoderma is an essential and widely used plant growth promoter fungus. The ability of *Trichoderma* spp. isolates as Plant Growth Promoting Fungi (PGPF) is determined from measurements of several growth parameters. These parameters include plant height, root length, shoot, root wet weight, shoot and root dry weight [52]. Treatment of *Trichoderma* spp. in nurseries promotes the growth of oil palm seedlings. [53] stated that the application of Trichoderma to the growth of plantation crops takes a long time compared to seasonal crops. Effects of *Trichoderma* spp. and AMF provided synergism in the growth of oil palm seedlings which was assessed by measuring the number of plant growth parameters during the post-inoculation nursery phase. In this study, the application of *Trichoderma* was added with organic matter from compacted POME. POME has the opportunity to be used as a nutrient source for oil palm plants. In addition to providing soil moisture, it can also improve soil physical-chemical properties

and can improve soil nutrient status [54]. Application of POME organic matter affects the growth of oil palm [55].

Number	Treatmeant	Seedling Growth Parameters								
		3 Month			6 Month			12 Month		
		Plant height (cm)	Number of leaves	Stem girt (cm)	Plant height (cm)	Number of leaves	Stem girt (cm)	Plant height (cm)	Number of leaves	Stem girt (cm)
1	Pt30-M0-T0	37.09 b	5.00 c	0.77 abc	52.80 c	8.27 bcd	1.62 a	143.17 c	18.57 ab	8.20 ab
2	P0-Mt30-T0	36.15 b	4.63 abc	0.80 bc	50.70 c	8.23 bcd	1.75 a	138.17 bc	18.33 ab	7.91 ab
3	Pt30-M0-Tt200	32.07 ab	4.43 abc	0.86 c	44.57 bc	8.00 bcd	1.79 a	132.70 abc	19.13 ab	7.88 ab
4	P0-Mt30-Tt200	35.90 b	4.60 abc	0.86 c	53.23 c	9.67 f	2.14 a	144.60 c	19.13 ab	8.78 b
5	Pm25-M0-T0	36.88 b	4.67 abc	0.77 abc	51.10 c	8.67 de	1.79 a	144.20 c	18.77 ab	8.24 ab
6	Pm25-M0-Tm100	33.18 b	4.97 c	0.80 bc	50.23 c	8.67 de	1.80 a	139.17 bc	18.63 ab	8.59 ab
7	Pm25-M0-Tm200	33.24 b	4.67 abc	0.79 bc	49.27 bc	7.87 bcd	1.73 a	134.90 bc	18.83 ab	8.06 ab
8	P0-Mm25-T0	33.75 b	4.67 abc	0.79 bc	47.50 bc	8.13 bcd	1.77 a	137.80 bc	19.47 b	8.11 ab
9	P0-Mm25-Tm100	29.42 ab	4.13 a	0.78 bc	45.57 bc	5.67 a	1.97 a	135.20 bc	19.33 ab	8.56 ab
10	P0-Mm25-Tm200	24.51 a	4.93 bc	0.59 ab	39.00 ab	7.80 bc	1.52 a	127.23 ab	18.63 ab	7.98 ab
11	Pt30-Mm25-Tt100m100	32.52 b	4.40 abc	0.75 abc	45.13 bc	9.13 e	1.84 a	137.60 bc	19.43 b	8.66 ab
12	Pt30-Mm25-Tt100m200	37.08 b	4.33 ab	0.90 c	52.33 c	8.50 cde	1.86 a	135.33 bc	19.50 b	8.18 ab
13	P0-Mt30m25-Tm100	21.43 a	4.37 ab	0.53 a	35.70 a	7.67 b	1.53 a	118.97 a	18.57 ab	7.79 a
14	P0-Mt30m25-Tm200	21.59 a	4.37 ab	0.60 ab	35.99 ab	7.70 bc	1.69 a	124.57 ab	18.20 a	7.87 a
15	P0-Mm25-Tt200m100	20.76 a	4.60 abc	0.67 abc	34.53 a	7.67 b	1.60 a	124.03 ab	18.20 a	7.79 a
16	P0-Mm25-Tt200m200	30.30 ab	4.60 abc	0.64 abc	40.87 ab	7.80 bc	1.96 a	131.30 abc	19.23 ab	7.92 ab
17	Pt30-Mm25-Tt400m250	32.63 b	4.90 bc	0.68 abc	47.50 bc	8.20 bcd	1.71 a	138.03 bc	18.77 ab	8.09 ab
18	MT-Gano	20.23 a	4.90 bc	0.61 ab	39.90 ab	8.20 bcd	1.69 a	132.07 abc	18.83 ab	8.10 ab

Figure 6: Effects of *Trichoderma* spp and AMF Treatments Mixture on Palm's Vegetative Growth Parameter.

Note : Means with the same letter are not significantly different by LSD (Least Significance Different) test at 5% level of significance.

3.5. Mitigation and Disease Incidence of Basal Stem Rot in Oil palm Plantation

Trichoderma spp. application provides a significant preventive effect in reducing disease symptoms in oil palm seedlings. Furthermore, the mixed application of *Trichoderma* spp. and AMF describes a synergistic effect that enhances AMF colonization in the biological control of *G. boninense* in oil palm nurseries and young plants. The control mechanism is that the competition for space in the root rhizosphere will cause AMF to accelerate colonization in the roots. In this study, the competition mechanism of *Trichoderma* spp. Biocontrol Agents mainly in the form of competition as indicated by the speed of growth of *Trichoderma* spp. it was resulting in less availability of nutrients and space for pathogens. This is in line with the opinion of [56], which states that the fungus *Trichoderma* spp. has many mechanisms in the process of controlling pathogens. The fungus *Trichoderma* spp. colonize roots, plant rhizosphere, and suppress pathogens by various mechanisms. Competition, mycoparasites, producing antibiotics, and induction of resistance were mechanisms. Another mechanism is mycoparasites, where the fungus *Trichoderma* spp. able to recognize and parasitize pathogenic hyphae. Mycorrhiza used haustoria to absorb

nutrients and penetrate the cell wall of pathogens. It used enzymes such as chitinase, glucanase, and protease [57].

Seedlings treated with *Trichoderma* spp in the early pre-nursery showed a high AMF colonization rate (Figure 6). This will be an opportunity to suppress diseases caused by soilborne pathogens. Until the observation of plants aged TBM-3 (July 2021 period), basal stem rot disease symptoms and incidence have not been found in the area planted with seeds with *Trichoderma* spp and AMF treatment or control (moderate tolerant Ganoderma). [37] stated that *Trichoderma* spp. (*T. harzianum* and *T. hamatum*) were reported to be effective in controlling the soilborne pathogen *Rigidoporus* micropores with an inhibition percentage of 81.85%. Mechanism of *Trichoderma* spp. in controlling soilborne pathogens does not directly kill the pathogen but only suppresses its development by applying the biocontrol agent *Trichoderma* spp. Usually, the incidence of the disease is still found but will be lower than the control treatment [58]. AMF is a biocontrol agent for plant pathogens because it is closely associated with roots forming a barrier for pathogen penetration, while helping roots to absorb nutrients and can produce toxic compounds for pathogens, forming a barrier on root cell walls against pathogen penetration or inducing plant resistance to disease [38]. The interaction between *Trichoderma* spp and AMF acts as a biocontrol agent that provides a synergistic effect in preventing infection with soilborne pathogens. This synergism reported by [59] is that *T. harzianum* perforates the AMF septa, then comes out and lives freely in the roots. Trichoderma is essentially a free-living fungus without being attached to plants. *Trichoderma* is a soil fungus but can colonize in plant roots on the root surface and into the roots [60],[25]. Furthermore, [61] stated that the mechanism of *Trichoderma* in the soil is more complex, namely competition for nutrients, space, and root exudates, which play a role in stimulating the germination of pathogenic propagules in the soil; degrade pectinase enzymes, and other enzymes that are essential for pathogens to penetrate; and also induce plant resistance to pathogens. The combination of Biological agents Arbuscular Mychorrizza Fungi (AMF) and *Trichoderma* spp. as a biocontrol with bio fertilization can improve sustainable agriculture, making the environment cleaner f free from pesticide residues and chemical fertilizers.

4. Conclusion

From this study, it can be concluded that Arbuscular Mycorrhizae Fungi (AMF) is a biocontrol agent to control basal stem rot disease in oil palm plantations, nurseries, and immature plants in plantation areas. The application of AMF in nurseries can

increase plant resistance to disease. This study showed that the application of AMF 25 grams both in pre-nursery and in the main nursery and repeated additions at planting in the field effectively controlled stem rot disease through early prevention strategies, evidenced by the highest percentage of AMF colonization.

References

[1] Qaim M, Sibhatu KT, Siregar H, Grass I. Environmental, economic, and social consequences of the oil palm boom. Annual Review of Resource Economics. 2020; 12:321-344

[2] United States Department of Agriculture (USDA). Oil seeds - World markets and trade, foreign agricultural service. United States: Department of Agriculture; 2021.

[3] Bentivoglio D, Finco A, Bucci G. Factors affecting the indonesian palm oil market in food and fuel industry: Evidence from a time series analysis. International Journal of Energy Economics and Policy. 2018;8(5):49-57.

[4] Fathana H. Palm oil poltics in Malaysia and Indonesia: Competition or Collaboration? Jati-Journal of SouthEast Asian studies. 2018; 23:47–64.

[5] Murphy DJ, Goggin K, Paterson RRM. Oil palm in the 2020s and beyond: Challenges and solutions. CABI Agric Biosci 2**,** 39 (2021). https://doi.org/10.1186/s43170-021-00058-3.

[6] Priwiratama H, Prasetyo AE, Susanto A. Incidence of basal stem rot disease of oil palm in converted planting areas and control treatments. IOP Conference Series: Earth and Environmental Science. 2020; 468:1–7.

[7] Assis K, Chong KP, Idris AS, Ho CM. Economic loss due to ganoderma disease in oil palm. World Academy of Science, Engineering and Technology. International Journal of Economics and Management Engineering 2016; 10(2):631-635.

[8] Siddiqui Y, Surendran A, Paterson RRM, Ali A, Ahmad K. Current strategies and perspectives in detection and control of basal stem rot of oil palm. Saudi Journal of Biological Sciences. 2021; 28: 2840–2849

[9] Jelani AR, Azaman MKH, Mohamed AR et al. Development and field evaluation of a tractor mounted oil palm trunk injector. Journal of Oil Palm Research. 2018;30(1):101-110.

[10] Tisné S, Pomiès V, Riou V, Syahputra I, Cochard B, Denis M. Identification of ganoderma disease resistance loci using natural field infection of an oil palm multiparental population. G3 Genes, Genomes, Genetics. 2017; 7:1683–1692.

[11] Standart Nasional Indonesia (SNI), Agent Bio Control (ABK). *Trichoderma* spp. Badan Standarisasi Nasional, Jakarta: Gedung Manggala Wanabakti; 2014.

[12] Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist. 1980; 84:489-500.

[13] Rai IN, Suada IK, Proborini MW, Wiraatmaja IW, Semenov M, Krasnov G. Indigenous endomycorrhizal fungi at Salak (*Salacca zalacca*) plantations in Bali, Indonesia and their colonization of the roots. Biodiversitas. 2019; 20(8):2410-2416.

[14] Pacioni G. Wet sieving and decanting techniques for the extraction of spores of Vesicular-arbuscular fungi: Methods in microbiology. Academic Press Limited. San Diego. 1992; 24:317-322.

[15] Brundrett MC, Bougher N, Dells B, Grove T, Malajozuk N. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research (ACIAR). Monograph 32, Canberra, Australia. 1996.

[16] International Culture Collection of (Vesicular) Arbuscular Mychorrhizal Fungi (INVAM). Key to fungi in Glomus. Morgantown, USA: West Virginia University. 2017. http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html.

[17] Tahira JJ, Khan SN, Anwar W, Suliman R. Mycorrhizal association in some weeds of *Ciercuma longa* fields of district Kasur, Pakistan. Pakistan Journal of Weed Science Research. 2012;18(3): 331-335.

[18] Wirawan G. Microscopic identification of arbuscular mycorrhizal fungi in reed plant rhizosphere [Skripsi]. Bali: Universitas Udayana; 2014.

[19] Buysens C, César V, Ferrais F, De Boulois HD, Declerck S. Inoculation of *Medicago sativa* cover crop with *Rhizophagus irregularis* and *Trichoderma harzianum* increases the yield of subsequently-grown potato under low nutrient conditions. Applied Soil Ecology. 2016; 105:137-143.

[20] Hu X, Roberts DP, Xie L et al. Use of formulated *Trichoderma* sp. Tri-1 in combination with reducedrates of chemical pesticide for control of *Sclerotinia sclerotiorium* on oil seed rape. Crop Protection. 2016; 79:124-127.

[21] Youssef SA, Tartoura KA, Abdelraouf GA. Evaluation of *Trichoderma harzianum* and *Serratia proteamaculans* effect on disease suppression, stimulation of ROS-scavenging enzymes and improving tomato growth infected by *Rhizoctonia solani* Biological Control. 2016; 100:79-86.

[22] El. Kinany S, Achbani E, Faggroud M et al. Effect of organic fertilizer and commercial arbuscular mycorrhizal fungi on the growth of micropropagated date palm cv. *Feggouss* Journal of the Saudi Society of Agricultural Sciences. 2019;18(4):411-417.

[23] Sturmer SL, Sturmer R, Pasqualini D. Taxonomic diversity and community structure of arbuscular mycorrhizal fungi (*Phylum glomeromycota*) in three maritime sand dunes in Santa Catarina State, South Brazil. Fungal Ecology. 2013; 6(1):27-36.

[24] Auliana, Kaonongbua W. Preliminary study on biodiversity of arbuscular mycorrhizal fungi (AMF) in oil palm (*Elaeis guineensis Jacq*) plantations in Thailand. IOP Conference Series: Earth and Environmental Science. 2018; 144: 1-8.

[25] Sundram S, Meon S, Seman IA, Othman R. Application of arbuscular mycorrhizal fungi with *Pseudomonas aeruginosa* UPMP3 reduces the development of ganoderma basal stem rot disease in oil palm seedlings. Mycorrhiza. 2014; 25(5):387–397.

[26] Bakhtiar Y, Yahya S, Sumaryono W, Sinaga MS, Budi SW. Adaptation of oil palm seedlings inoculated with arbuscular mycorrhizal fungi and mycorrhizal endosymbiotic bacteria *Bacillus subtilis* B10 towards biotic stress of pathogen ganoderma boninense pat. Microbiology. 2012;6(4):157-164.

[27] Navarro AM, Moragues JGS, Banuet AV, Verdu M. The network structure of plant-arbuscular mycorrhizal fungi. New Phytologist. 2012; 194:536-547.

[28] Kehri HK, Akhtar O, Zoomi I, Pandey D. Arbuscular mycorrhizal fungi: Taxonomy and its systematics. International Journal of Life Sciences Research. 2018; 6(4):58-71.

[29] Kavitha T, Nelson R. Diversity of arbuskular mycorrhizal fungi (AMF) in the rhizophere of *Helianthus annuus* L. American-Eurasian. Journal of Agriculture and Environmental Sciences (JAES). 2013; 13(7):982-987.

[30] Castaneda TG, Romero HM. Mycorrhization in oil palm (*Elaeis guineensis* and *E. oleifera* x *E. guineensis*) in the pre-nursery stage. Agronomia Colombiana. 2013; 31(1):95-102.

[31] Saputra ID, Kesumadewi AAI, Atmaja IWD. Total amount of vesicular arbuskular mycorrhiza fungi spores in the rhizosphere of oil palm (*Elaeis guineensis Jacq*). International Journal of Current Microbiology and Applied Sciences (IJCMAS). 2019;8(10):2001-2008

[32] Lestari E. Identification of fungi mycorrhizal arbuzcular at oil palm rhizosphere [Skripsi]. Program Studi Agroteknologi Fakultas Pertanian Universitas Sriwijaya. Palembang; 2018.

[33] Ervayenri. Mychorriza function diversity of arbuskua (AMF) Land University Lancang Yellow Campus (UNILAK). Wahana Forestra: Forestry Journal. 2020;15(2): 80–92

[34] Puspitasari D, Purwani KI, Muhibuddin A. Exploration of indigenous vesicular arbuscular mycorrhiza (VAM) in corn in Torjun Village, Sampang Madura. Jurnal Sains dan Seni Institut Teknologi Surabaya (ITS). 2012; 1:19-22.

[35] Bakhtiar Y. The role of asbucular mychorriza fungi and mycorrhizal endosymbiotic bacteria in increasing the adaptability of oil palm (*Elaeis quineensis Jacq*) seedlings to biotic stress of ganoderma binense pat [Post graduate dissertation]. Bogor: Bogor Agricultural Institute; 2011.

[36] Safran, Anhar A, Fikrinda. Exploration of arbuscular mycorrhizal fungi on oil palm plantation PT. Lembah Bhakti at Singkil swamp with trapping culture. Unsyiah Agricultural Student Scientific Journal. 2020;2(3):34-38.

[37] Berlian I, Setyawan B, Hadi H. Mechanism of antagonism of *Trichoderma* spp. against some soilborne pathogens. Warta Perkaretan, Sungei Putih. 2013;32(2):74-82.

[38] Sinaga MS. Plant disease biological control agents: A neglected spectacular bounty [scientific oration]. Bogor: Bogor Agricultural Institute; 2015.

[39] Matfuhah AN, Susanti A, Febrianti R. Test the effectiveness of the attitde of antagonism five local isolates of *Trichoderma* spp. againts *Fusarium* sp. Agrosaintifika: Jurnal Ilmu-Ilmu Pertanian. 2019;1(1):1-5.

[40] Cai F, Chen W, Wei Z, Pang G, Li R X, Ran W, Shen Q R. Colonization of Trichoderma harzianum strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. Plant and Soil. 2015. 338: 337–350.

[41] Druzhinina IS, Chenthamar K, Zhang J et al. Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus *Trichoderma* from its plant-associated hosts. PLOS Genetics. 2018; 14(4). https://doi.org/10.1371/journal.pgen.1007322

[42] Zhang F, Meng X, Feng C et al. Hydrolytic amino acids employed as a novel organic nitrogen source for the preparation of PGPF-containing bio-organic fertilizer for plant growth promotion and characterization of substance transformation during bof production. PLOS ONE. 2016;11(3):1-17.

[43] Noble R, Conventury E. Suppression of soilborne plant disease with compost: A review. Biocontrol Science and Technology. 2005; 15:3-20.

[44] Singhania RR, Patel AK, Soccol CR, Pandey A. Recent advances in solid-state fermentation. Biochemical Engineering Journal. 2009; 44(1):13-18.

[45] Adam S, Ahmad SSN, Hamzah NM, Darus NAB. Composting of empty fruit bunch treated with palm oil mill effluent and decanter cake. N.A. Yacob et al. (*eds*), Regional Conference on Science, Technology and Social Sciences (RCSTSS 2014), Springer Science+Business Media Singapore; 2016: 437-445. DOI 10.1007/978-981-10-0534-3_43

[46] Hong-jun L, Wan-dong D, Chao L et al. Spore production in the solid-state fermentation of stevia residue by *Trichoderma guizhouense* and its effects on corn growth. Journal of Integrative Agriculture. 2021;20(5):1147-1156.

[47] Xiong W, Guo S, Jousset A et al. Bio-fertilizer application induces soil suppressiveness against *Fusarium* wilt disease by reshaping the soil microbiome. Soil Biology and Biochemistry. 2017; 114:238–247.

[48] Shade A. Diversity is the question, not the answer. The International Society for Microbial Ecology Journal. 2017; 11:1-6.

[49] Stewart A, Hill R. Applications of *Trichoderma* in plant growth promotion. Bio-protection Research Center, Lincoln University, Canterbury, New Zealand. 2014.

[50] Junior AFC, Chagas LFB, Colonia BSO, de O. Miller L, de Oliveira JC. *Trichoderma asperellum* (UFT201) functions as a growth promoter for soybean plant. African Journal of Agricultural Research. 2019;14(33):1772-1777

[51] Fadly F, Lisnawita, Safni I, Lubis K, Nurliana. The potency of antagonistic microbes as plant growth promoting on oil palm seedling infected with basal stem rot disease (*Ganoderma boninense*). IOP Conf. Series: Earth and Environmental Science. 2021; 782: 1-7.

[52] Yogaswara Y, Suharjo R, Ratih S, Ginting C. Ability test of *Trichoderma* spp. isolate as antagonist of *Ganoderma boninse* and plant growth promoting fungi (PGPF). Jurnal Agrotek Tropika (JAT). 2020; 8(2):235-246.

[53] Juariyah S, Tondok ET, Sinaga MS. *Trichoderma* and *Gliocladium* for controling fusarium root rot disease of oil palm seedlings. Journal Phytopatologi Indonesia. 2019;14(6):196-204.

[54] Fitria A, Gunawan VS, Mardiah. Study of the utilization of palm oil industry liquid waste. Konversi. 2021;10(1): 31-40.

[55] Tambunan DS, Nelvia, Amri AI. Application of oil palm factory liquid waste with biopory methods on plant growth of immature palm oil (*Elaeis queninsis*). Journal of Soil and Land Utilization Management. 2019; 16(1):19-28.

[56] Belete E, Ayalew A, Ahmed S. Evaluation of local isolates of *Trichoderma* spp. against black root rot (*Fusarium solani*) on faba bean. Journal of Plant Pathology & Microbiology (JPPM). 2015;6(6): 1-5.

[57] Gajera H, Domadiya R, Patel S, Kapopara M, Golakiya B. Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system-A review. Current Research in Microbiology and Biotechnology. 2013;1(4):133-142.

[58] Soenartiningsih, Djaenuddin N, Saenong MS. The effectiveness of *Trichoderma* sp. and *Gliocladium* sp. as a biocontrol agent of late midrib rot in maize. Penelitian Pertanian Tanaman Pangan. 2014;33(2):129-135.

[59] Samolski I, Rincon AM, Pinzon LM, Viterbo A, Monte E. The qid74 gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. Microbiology. 2012; 158:129-138.

[60] Chacon MR, Rodriguez-Galan O, Benitez T, Sousa S, Rey M, Llobell A, Delgado-Jarana J. Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum* International Microbiology. 2007; 10:19-27.

[61] Chen SC, Zhao HJ, Wang ZH et al. *Trichoderma harzianum* induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content, cell viability and cell cycle-related genes expression in cucumber roots. European Journal of Plant Pathology. 2016; 147(1):3-53.