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Growth Response of Oil Palm Plant Seeds After Administration of Vesicular Arbuscular Mycorrhiza (VAM) Fungus

Abstract

Objectives: This study aims to determine the growth response of oil palm seedlings to VAM and to determine the appropriate VAM dose for oil palm seedling growth.
Methods: This study took place in the screen house and plant protection laboratory and VAM isolate starters were gathered from the work area. The test used a factorial completely randomized design (CRD) with 2. The first treatment was VAM dose with 4 factors and the second was NPK fertilizer with two factors. The VAM was applied for one month after germination and plant maintenance for 16 weeks with seven parameters.
Findings: The results show that the VAM dose treatment and the application of NPK fertilizer did not have a significant effect on the growth of oil palm seedlings in the pre-nursery (16 weeks) so it took a long time to see the role of VAM in oil palm seedlings. The application of VAM 150 g/polybag on oil palm seedlings in pre-nursery tended to give the best response to the growth of seedling height, a number of leaves, fresh weight of the crown, dry weight of the crown, fresh weight of roots, dry weight of roots and root volume. The VAM inoculum supplied made symbiosis with oil palm seedling roots with a high infection rate. The number of VAM spores in the media and the percentage of colonization on the roots were influenced by the dose of VAM inoculum given.
Novelty: Vesicular Arbuscular Mycorrhiza (VAM) Fungus in oil palm seeds are important to increase the growth of the number of leaves, fresh weight of the crown, and dry weight of the crown. Efforts to increase the quality and quantity of oil palm production are appropriately necessary so that the desired target can be achieved.

Keywords: Growth Response, Oil Palm Seeds, VAM Fungus, Number of Leaves, Fresh Weight of the crown.

1. Introduction

The oil palm plantation sector has grown very rapidly in the last 10 years. In 1998 the area of oil palm plantations was 2.7 million ha with a CPO production volume of 5.6 million tons. In the same year, the export volume of palm oil was 852,843 tons with a value of US$ 333,866. In 2003 the area of oil palm plantations reached 5.06 million ha with a CPO production volume of 9.6 million tons or an average of 1.8 tons/hectare/year. The export volume of palm oil was 6.333 million tons with a value of US$ 2.092 billion. The composition of ownership of oil palm plantations consists of smallholder plantations 29.7%, PTPN 13.2%, and large private plantations 57.1% [1]. Root rot disease (BPB) in oil palm is caused by the fungus Ganoderma boninense. This fungus belongs to the class Basidiomycetes. Seeing the importance of oil palm plantations today and in the future and along with the increasing population's need for palm oil, it is necessary to think about efforts to increase the quality and quantity of oil palm production appropriately so that the desired target can be achieved.

Various problems faced in oil palm cultivation include infertile land and the presence of stem rot disease (BPB) in oil palm caused by the fungus G. boninense. In several oil palm plantations in Indonesia, this disease has caused the death of up to 80% or more of the entire population of oil palm plantations, resulting in a decrease in oil palm production per unit area. Various attempts have been made to reduce the incidence of BPB, but until now no effective control has been found. The improved growth to increase huge pressure in oil palm cultivation using cheaper alternatives such as combination of biofertilizers and organic substrates such as arbuscular mycorrhizal (AM) fungi and endophytic bacteria Pseudomonas aeruginosa[2]. The application of compost from OPEFB combined with Mycorrhiza arbuscular fungi (MAF) significantly improved soil fertility, which was indicated by improving soil chemical and biological properties. The application of MVA at various doses had a significant effect on the dry weight, root length of Calopogonium mucunoides and increase the number of MVA spores in the soil[3]. The AMF inoculation with treatments 30 grams of Trichoderma spp apllied at the start of pre-nursery planting had a significant impact on root colonization and spore number response. 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[4]. Application of Arbuscular mycorrhizal fungi (AMF) reduced 50% of compound fertilizer needed for oil palm seedlings, and the best dose for oil palm seedlings inoculated with AMF was 500 mg polybag-1 and the AMF inoculation without AMF inoculation was 1000 mg polybag-1[5]. Communities of arbuscular mycorrhizal fungi (AMF) in plant roots improve sago palm (Metroxylon sagu Rottb) in Sarawak, Malaysia. The higher number detected by amplicon sequencing of the partial small-subunit rRNA gene (MS, 78; SPS, 50)[6]. Arbuscular mycorrhizal fungi (AMF) impact the production of secondary metabolites directlyby indirectly by stimulating secondary metabolite biosynthetic pathways or directly by increasing plant biomass[7]. The inoculation of Arbuscular Mycorrhizal fungi (AMF) provides tolerance to host plants against various stressful situations like heat, salinity, drought, metals, and extreme temperatures. AMF, being natural root symbionts, provide essential plant inorganic nutrients to host plants, thereby improving growth and yield under unstressed and stressed regimes. AMF can potentially strengthen plants' adaptability to changing environment[8]. Date palm plantations and five native desert plants, respectively in the Southern Arabian Peninsula use and apply Arbuscular mycorrhizal fungi isolated from rhizosphere soils of agricultural and natural habitat. The results showed the growth of both plants potential for use in sustainable land region[9].

2. Methodology

2.1. Definition of *Mycorrhiza*

Currently G. boninense is a deadly disease in oil palm plantations in Indonesia and even in Southeast Asia[10]. The use of inoculation of several types of soil microbes such as Arbuscular Mycorrhizal Fungi is known to be able to increase the adaptability of plants to disease attacks[11]. One way to maximize agricultural yields without damaging the environment is to utilize vesicular arbuscular mycorrhizae (VAM). Mycorrhizae are biological fertilizers that can replace part or all of the function of artificial fertilizers. VAM diversity is the main factor that maintains plant diversity and its function in the ecosystem[11].

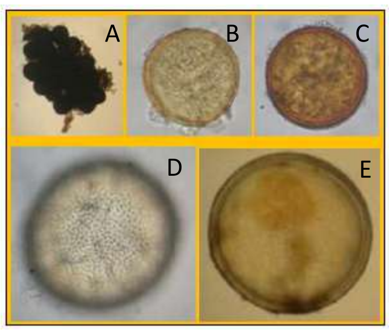

Figure 1. *Medan-based BBPPTP's VAM Spores Collection; Glomus sp – 2 (A), Glomus sp – 3 (B), Glomus sp – 5 (C), Acaulospora rehmii (D) dan Scutelospora sp (E).*

Fungal hyphae come out of plant roots to reach the soil and help absorb certain nutrients for further transmission to plants, especially immobilized nutrients such as phosphate (P), zinc (Zn), copper (Cu), and molybdate (Mo).

2.2. Role of *Vesicular Arbuscular Mycorrhiza (VAM)* Fungi

One of the causes of low productivity is that oil palm cultivation on a large scale is faced with the problem of limited water for watering, especially for oil palm development on marginal lands. Oil palm is a forest plant in the tropics that is humid and fresh, so this plant is vulnerable to water shortage conditions. VAM is known to improve plant growth and yield on soils with unfavorable conditions. VAM infects the root system of the host plant and produces an external hyphae network that grows expansively and penetrates the sub-soil layer thereby increasing the capacity of the roots to absorb nutrients and water[11]. The presence and diversity of VAM in an ecosystem can increase plant diversity. VAM diversity is the main factor that maintains plant diversity and its function in the ecosystem[11]. In addition, the interaction of VAM with other plant microbes can regulate ecosystem functions such as plant diversity, productivity and variability. This shows how important the role of VAM and its function in environmental management is.

The use of inoculation of several types of soil microbes such as Vesicular Arbuscular Mycorrhizae (VAM) is known to increase the adaptability of plants to disease attacks. Mycorrhizae are fungi that live in mutualistic symbiosis with the root system of higher plants. Both fungi and plants benefit from this association. Mushrooms get carbohydrates in the form of simple sugars (glucose) from plants and vice versa plants get water and nutrients from fungi that cause better growth. Mycorrhizal mechanisms in increasing plant resistance to control root pathogens can take place in the following ways: 1). the presence of a layer of hyphae (mantle) that can function as a physical barrier for the entry of pathogens, 2). mycorrhizal fungi utilize more carbohydrates from the roots before being excreted in the form of root exudates so that pathogens cannot develop, 3). the formation of substances that are antibiotics secreted to inhibit the development of pathogens, 4). stimulate the development of saprophytic microbes around the roots and 5). plant roots that have been infected with pathogenic fungi that show competition.

2.3. Prospects and Potential of *Mycorrhiza* for Plantation Plants

It is estimated that nursery management to produce quality seeds can be done by inoculation of mycorrhizal fungi early on at the time of sprouting. Mycorrhizal fungal infection was able to increase the growth of cacao seedlings, as indicated by most of the growth components (height, fresh weight of crown, length and number of roots, and ratio of shoot dry weight/root dry weight). Mycorrhizal infection is thought to be able to increase nutrient absorption by cocoa seedling roots. Mycorrhizal fungi are obligate fungi, where their survival is associated with plant roots with their spores. Spores germinate by forming apressoria as a means of infection, where infection usually occurs in the elongation zone.

Mycorrhizal fungi have enormous potential to increase plant growth and increase soil fertility. The use of mycorrhizae can be used as a biological tool to streamline the use of artificial fertilizers, especially phosphates, besides that it can streamline nutrient elements, especially on critical or marginal land. Soil environmental conditions suitable for seed germination are also suitable for the germination of mycorrhizal spores. Mycorrhizae also act as biocontrol for plants and increase plant resistance to drought, and conversely, mycorrhizae do not cause disease in plants.

In addition, the roots of mycorrhizal plants will grow faster and produce more harvest weight than those without mycorrhizal plants, and are more resistant to certain diseases. The presence of mycorrhizae in the soil in the form of associations between fungi and high plant root systems can affect the nature of soil fertility. The use of mycorrhizae is an ecological necessity, because in addition to being safe to use (not pathogenic), it does not cause environmental pollution, plays an active role in the nutrient cycle and can also improve soil fertility status because of its ability to extract bound nutrients.

Based on the test results, the application of mycorrhizae on cocoa seedling media aims to determine the ability of mycorrhizae in influencing the height and number of leaves of cocoa plants. The growth of cacao seedlings in nurseries is determined by the mechanism of mycorrhizal fungal infection which is symbiotic mutualism. Plants infected with mycorrhizal fungi will increase the volume and length of the roots, so that the nutrients absorbed by the roots will affect the height and number of plant leaves. Mycorrhizal roots have a higher auxin content which allows increased root growth.

2.4. Mycorrhizal Symbiosis in Oil Palm

Oil palms have different root growth patterns. At 0-1 years of age, the growth of oil palm roots downward (positive geotropism) while from the age of 11 years the growth of horizontal primary roots increased very sharply followed by the growth of secondary roots upward (negative geotropism)[12]. AMF (arbuscula mycorrhizal fungi) is a fungus that grows on the roots, thus the root growth pattern is thought to influence the dynamics of the AMF population and its diversity. AMF populations at several ages and depths of the oil palm rhizosphere have not been reported. Research on the interaction of AMF in oil palm was initiated in 1990 by Bal and Giovenetti who observed that there was no difference in P uptake of oil palms from in vitro cultures fertilized with superphosphate and rock phosphate. In the same year using in vitro cultured oil palm, it was shown that AMF colonization could increase the uptake of P and several other nutrients[13].

2.5. Methods

2.5.1. Place and Time

The study "Response of Oil Palm Plant Seed Growth Against VAM Mushrooms", was carried out at the screen house and integration laboratory of BBPPTP Medan, from July to December 2019.

2.5.2. Materials and tools

The materials used in this study were oil palm sprouts, NPK fertilizer, VAM starter, topsoil soil, polybags, aquades, filter paper, 5% KOH, 1% HCL, lactic acid, glycerol, trypan blue, melzer solution, Hyponex fertilizer, fertilizer. Terabuster organics, plastic bottles, plastic trays and other necessary materials. The tools used in this study were hoe, ponjo, plastic bag, permanent marker, graded filter, centrifuge, object glass, cover glass, compound microscope, stereo-binocular microscope, scissors, needle preparation, dropper and other necessary tools.

2.5.3. Implementation Method

2.5.3.1. Experimental Design

This experiment used a factorial Completely Randomized Design (CRD) with 2 treatments, namely the dose of VAM with 4 factors and the application of NPK fertilizer with 2 factors, so that there were 8 treatment combinations with each treatment repeated 3 times:

VAM dose treatment:

V_0 : 0 gr/seedling
V_1 : 50 gr/seedling
V_2 : 100 gr/seedling
V_3 : 150 gr/seedling

NPK fertilization treatment:

P_1 : 50 % suggested dose
P_2 : 100 % suggested dose

Linear model used:

$$Y_{ijk} = \mu + V_i + P_j + (MT)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} = The value of observations on the media treatment at i-level and j-level host plants on k-level replication
μ = Median
V_i = Effect of VAM dose treatment at i-level
P_j = Effect of NPK fertilization treatment at j-level
$(VP)_{ij}$ = Interaction between the VAM dose treatment at i-level and fertilization at j-level
ε_{ijk} = Experimental error in the media treatment at i-level and host plant at j-level for the replication at k-level

2.5.3.2. Technical Implementation

2.5.3.2.1. Seedling

The oil palm seeds used were DXP Lame and Yangambi which had germinated and were sown on sand media which had been sterilized until the age of 4 weeks before being transferred to the pre-nursery.

2.5.3.2.2. VAM Isolate Preparation and Spore Inoculation

VAM isolates obtained from oil palm VAM trapping results in the 2017 activity were examined for the number of spores and the percentage of VAM colonization on roots, with the requirement that VAM isolates that could be used were spore count 10 spores/gr soil and 70% VAM colonization on roots. The VAM inoculation process according to the treatment begins with removing the seeds from the seedling media. VAM inoculum (with zeolite carrier and sand) was sprinkled on the planting hole and partly sprinkled on the root surface. After the seedlings are 4 weeks old in the pre-nursery, urea fertilization is carried out at a dose of 2 g per liter for 100 seedlings by spraying on the leaves every week

2.5.3.2.3. Planting in Pre-Nursery

Oil palm seeds that have been germinated for 4 weeks are transferred to polybags measuring 18 cm x 25 cm (one seed per polybag) with planting media in the form of topsoil, sand, and organic matter (2:1:1) then the media is added with rock phosphate fertilizer as much as 100 gr for 60 pre-nursery polybags.

2.5.3.2.4. Raising

The maintenance carried out during the study included watering, weeding, pest and disease control, and NPK fertilization. The type and dose of fertilizer used are adjusted to the growth of the seeds and the treatment used.

2.5.4. Observation Parameter

2.5.4.1. Plant Height

Plant height was measured from the soil surface to the tip of the longest leaf. Measurements were made using a meter in centimeters (cm). Measurement of plant height begins after 1 month of oil palm seedlings from the pre-nursery with an interval of 1 month.

2.5.4.2. Number of Leaves

Observation of the number of leaves was carried out by counting the perfectly open leaves on each oil palm seedling. Measurement of the number of leaves begins after 1 month of oil palm seedlings from the pre-nursery with an interval of 1 month.

2.5.4.3. Header Fresh Weight

Measurement of root dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then the plant roots were put in an envelope and then in an oven at 105°C for 24 hours, then each sample was weighed using an analytical balance.

2.5.4.4. Head Dry Weight

Measurement of canopy dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and the plant crown was put in an envelope and then oven-dried at 105°C for 24 hours. Then each sample was weighed using an analytical balance.

2.5.4.5. Root Fresh Weight

Measurement of root fresh weight was carried out at 16 weeks DAP by disassembling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then each sample was weighed using an analytical balance.

2.5.4.6. Root Dry Weight

Measurement of root dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then the plant roots were put in an envelope and then in an oven at 105°C for 24 hours, then each sample was weighed using an analytical balance.

2.5.4.7. Root Volume

All the roots of the chopped oil palm seeds were put into a measuring cup filled with 100 ml of water. Root volume is the addition of the volume of water in the measuring cup in ml.

2.5.4.8. Percentage of VAM Colonization in Roots

The degree/percentage of root colonization was calculated using the formula:

$$\%\ \text{Root colonization} = \frac{\text{Number of fields of view marked} +}{\text{Overall number of fields of view}} \text{x } 100\%$$

The level of colonization is further categorized according to the Institute of Mycorrhizal Research and Development, USDA Forest Service, Athens Georgia[14] as follows:

1. Class 1 is very low when infection ranges between 0% – 5%
2. Class 2 is low when infection ranges between 6% – 25%
3. Class 3 is modest when infection ranges between 26% – 50%
4. Class 4 is high when infection ranges between 51% – 75%
5. Class 5 is very high when infection ranges between 76% – 100%

2.5.4.9. Number of Spores

The number of spores was observed at the end of the study (12 weeks DAP). The technique used to determine the number of VAM spores is the pour-filter technique[15] and will be followed by the centrifugation technique[16].

3. Results and Discussion

3.1. Plant Height

The results of analysis of variance on plant height parameters can be seen in Appendix 1 to 4. Based on the results of analysis of variance in plant height, it shows that VAM and fertilizer did not show a significant effect. The results of plant height observations in the 4th observation can be seen in Table 1.

Tabel 1. Average Plant Height (cm) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	35,08	38,45	33,93	39,95	36,85
P2	36,08	35,85	35,48	39,30	36,68
Average	35,58	37,15	34,70	39,63	36,76

Table 1 shows that the treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the increase in plant height. However, from the treatment with mycorrhizae, there was a tendency to give VAM in treatment V3 (150 g/seed) which had the highest plant height (39.63 cm) compared to other treatments.

3.2. Number of Leaves

The results of the analysis of variance on the number of leaves can be seen in Appendix 5 to 8. Based on the results of analysis of variance on the average number of leaves, it was shown that VAM and fertilizer application did not show a significant effect.

Table 3 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the increase in the number of leaves. However, from treatment with mycorrhizae, there was a tendency to give VAM to treatment V3 (150 g/seed) which had the highest mean number of leaves compared to other treatments, which was 4.88 strands.

3.3. Head Fresh Weight

The results of the analysis of variance on the fresh canopy weight parameter can be seen in Appendix 9. Based on the results of the analysis of the variance of the average fresh crown weight, it shows that the application of VAM and NPK fertilizer did not show a significant effect.

With reference to the treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant crown weight. However, there was a tendency that the highest number of VAM (150 g/plant) showed the heaviest crown weight, which was 6.60 g compared to other treatments. Meanwhile, the interaction treatment which showed the best fresh weight of the crown was the application of 50% fertilizer and the provision of VAM as much as 150 g/plant, which was 7.27 g.

3.4. Head Dry Weight

The results of the analysis of variance on the canopy dry weight parameter can be seen in Appendix 10. Based on the results of the analysis of variance on the average dry weight of the canopy, it was shown that VAM and NPK fertilizers did not show a significant effect. The results of the fresh weight of the crown can be seen in Table 2.

Table 2. Head Dry Weight (gr) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	1.35	1.53	1.44	1.55	1.47
P2	1.44	1.53	1.47	1.41	1.46
Average	1.39	1.53	1.45	1.48	

Table 5 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the dry weight of the plant crown. However, there was a tendency that the highest VAM (150 g/plant) and 50% NPK fertilizer application showed the heaviest canopy dry weight, which was 1.55 g compared to other treatments.

3.5. Root Fresh Weight

The results of the analysis of variance on the fresh root weight parameter. Based on the analysis of the variance of the average fresh root weight, it shows that VAM and NPK fertilizer did not show a significant effect. The results of observations of fresh root weight can be seen in Table 3.

Table 3. Fresh Weight of Roots (gr) at 4 Months of Age after Tranplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	1.50	1.53	1.54	1.73	1.57
P2	1.28	1.63	1.54	1.62	1.52
Average	1.39	1.58	1.54	1.67	

Table 6 shows that treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant fresh weight. However, there was a tendency that the highest amount of VAM (150 g/plant) would give the heaviest average fresh weight of crown compared to other treatments, which was 1.67 g.

3.6. Root Dry Weight

The results of the analysis of variance on the fresh root weight parameter. Based on the analysis of the variance of the average fresh root weight, it shows that VAM and NPK fertilizer did not show a significant effect. The results of observations of fresh root weight can be seen in Table 4.

Table 4. Root Dry Weight (gr) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	0.98	1.04	0.99	1.06	1.02
P2	0.91	1.02	1.01	1.01	0.99
Average	0.94	1.03	1.00	1.04	

Table 4 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant dry weight. However, there was a tendency with the highest VAM (150 g/plant) giving the heaviest average dry weight of roots compared to other treatments, which was 1.04 g.

3.7. Root Volume

The results of the analysis of variance on the root volume parameters. Based on the analysis of the variance of the mean root volume, it shows that VAM and NPK fertilizers did not show a significant effect. The results of observations of root volume can be seen in Table 5.

Table 5. Root Volume (ml) at 4 Months of Age after Transplanting

Fertlizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	2.38	3.55	3.20	3.80	3.23
P2	2.75	3.63	3.13	3.75	3.31
Average	2.56	3.59	3.16	3.78	

Table 8 shows that treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant dry weight. However, there was a tendency that with the highest VAM administration (150 g/plant) it showed the heaviest average fresh root weight of 3.78 ml compared to other treatments.

3.8. Number of Spores

The results of the analysis of variance on the parameters of the number of spores can be seen in Appendix 14. Based on the results of the analysis of variance on the average number of spores, it shows that the application of VAM and NPK fertilizer showed a significant effect. The results of observations of root volume can be seen in Table 6.

Table 6. Number of Spores Per 50 g Media at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	24G	290C	286D	316B	229.00

P2	17H	241E	118F	386A	190.50
Average	20.50	265.5	202	351	

Table 6 above shows that the number of VAM spores on the media was in line with the amount of VAM given. The highest mean number of spores in the VAM treatment was found in V3 treatment (150gr/plant) as much as 351 spores/50 gr of media. Meanwhile, the effect of NPK fertilizer on the average number of VAM spores was highest in treatment P1 (50% recommended dose) of 229 spores/50 g of media. So for the interaction effect, the highest average number of spores was found in the V3P1 treatment (giving 150 g VAM/plant and 100% recommended dose of NPK fertilizer) which was significantly different from other treatments.

3.9. Percentage of VAM Colonization in Roots

The results of the analysis of variance on the parameters of the number of spores. Based on the results of the analysis of variance on the average number of spores, it was shown that the application of VAM and NPK fertilizer showed a significant effect. The results of observations of root volume can be seen in Table 7.

Table 7. Percentage of VAM Colonization in Oil Palm Seed Roots at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	58D	60D	76B	86A	70.00
P2	56E	72C	83A	84A	73.75
Average	57	66	79,5	85	

Note: Numbers followed by unequal letters in the same column and row are very significantly different at the 1% level according to Duncan's Distance Test (DMRT).

Table 7 shows that the percentage of VAM colonization in oil palm seedlings was in line with the amount of VAM given. The highest mean percentage of VAM colonization was found in treatment V3 (150g VAM/plant) of 85%. Meanwhile, the effect of NPK fertilizer on the average percentage of VAM colonization was found in treatment P2 (100% recommended dose) of 73.75%. Meanwhile, the interaction effect of the mean percentage of VAM colonization on the roots of oil palm seedlings was highest in the V3P1 treatment (150 g VAM/plant and 100% of the recommended dose of NPK fertilizer) but it was not significantly different from the V3P1 treatment (150g VAM/plant and 100% recommended dose), application of NPK fertilizer) and V2P2 (100g VAM/plant and 100% recommended dose of NPK fertilizer) with colonization percentage values of 86%, 84% and 83% respectively.

Discussion

This study was conducted for 16 months on oil palm seedlings in the pre-nursery. VAM was given in the form of spore inoculum which was inoculated on the roots of the seedlings at the beginning of transplanting from the nursery. While the application of NPK fertilizer was carried out after the plants were 14 weeks old. So, in this study the response to the provision of NPK fertilizer for oil palm seeds is not a concern (discussion). Observational data that has been analyzed for variance shows that the combination of VAM dose and percentage of NPK fertilizer for oil palm seedlings in pre-nursery has no significant effect on plant growth parameters. This is presumably because the nutrient content of the growing media used in this study was quite high, so that the addition of fertilizer and VAM did not significantly affect plant growth. The planting medium used in this study was a mixture of topsoil, organic matter, and sand with a ratio of 2:1:1. Topsoil is soil that is in the top layer and contains all the chemical, physical, and biological components that plants need to grow properly.

The availability of high enough nutrients creates conditions where VAM cannot perform its function in facilitating the absorption of nutrients by plants. Mycorrhizae is a form of mutualism symbiosis between fungi and plant root systems. The role of mycorrhizae is to help the absorption of plant nutrients, increase growth and yield of plant products. On the other hand, fungi obtain assimilated energy from plants. VAM symbiosis with plants on fertile land does not have much positive effect, but in extreme conditions it can increase most plant growth[17]. Mycorrhizae promote plant growth in low soil fertility, degraded land and help expand the function of the root system in obtaining nutrients[18,19]. In particular, mycorrhizal fungi play an important role in increasing the absorption of ions with low mobility levels, such as phosphate (PO43-) and ammonium (NH4+) and other relatively immobilized soil nutrients such as sulfur (S), copper (Cu), zinc (Zn), and also Boron (B)[20]. Mycorrhizae also increase the surface area in contact with the soil, thereby increasing the root absorption area up to 47 times, which makes it easier to access nutrients in the soil. Mycorrhizae not only increase the rate of nutrient transfer in the roots of the host plant, but also increase resistance to biotic and abiotic stresses[17]. Mycorrhizae are able to help maintain the stability of plant growth in polluted conditions[21].

Observations of seedling growth carried out in this study were for 4 months after VAM spore inoculation. This time is thought to be insufficient for oil palm seedlings to show their growth response. The response of VAM inoculation to seedling growth and nutrient uptake can be seen at the age of 6 months after VAM spore inoculation [22]. VAM spores are a type of inoculum that can be used for plants in nurseries, but has drawbacks. i.e. spores take several days to germinate and some species have a period of dormancy before they can germinate. Especially for field applications, the inoculum in the form of spores has weaknesses, namely the slow initial development and slow absorption in the roots so that the inoculum is unable to compete with native VAM and other natural soil microbes [23,24]. fast and high through inoculation is a requirement to get an effective symbiosis of inoculation.

From Table 8 it is known that there is no significant difference in the number of seedling leaves due to VAM administration, this is because the increase in the number of leaf midribs of oil palm plants is determined by genetic factors of the plant itself, besides the age factor also affects it, causing the number of leaf midribs. each treatment showed numbers that were not significantly different. Genetic factors determine the number of leaves that will be formed, therefore it is very important in breeding using quality seeds[25/ 19].

Table 8. Data on Observation of Oil Palm Seed Growth and VAM Development at 4 Months of Age after Transplanting

Treatment	Observation Parameter								
	TT (cm)	JD	BST (g)	BKT (g)	BSA (g)	BKA (g)	VA (ml)	JSV	PKV (%)
V0P1	32,48	3,75	4,76	1,33	1,77	0,46	2,38	24G	58D
V0P2	34,05	3,75	4,68	1,63	1,14	0,33	2,75	17H	56E
V1P1	36,20	4,00	5,62	1,86	1,88	0,62	3,55	29F	60D
V1P2	33,83	4,00	6,28	1,85	2,18	0,54	3,63	241E	72C
V2P1	31,48	4,00	6,02	1,59	1,93	0,49	3,20	286D	76B
V2P2	31,58	4,00	4,15	1,67	2,00	0,53	3,13	118C	83A
V3P1	36,90	4,25	7,27	1,91	2,59	0,60	3,80	316B	86A
V3P2	36,45	4,00	5,93	1,53	2,18	0,54	3,75	386A	84A

Legend: Numbers followed by different letters in the same column were significantly different at the 1% level according to the Duncan Distance Test (DMRT). TT = plant height, JD = number of leaves, BBT = fresh weight of crown, BKT = dry weight of crown, BBA = fresh weight of roots, BKA = dry weight of roots, VA = root volume, JSV = number of VAM spores and PKV = percentage of VAM colonization

In this study, it can be seen from the 4 levels of VAM given that oil palm seeds given VAM gave the best results in response to seedling growth compared to the control treatment (without VAM) Table 11. This can be seen in the variables of plant height, number of leaves, Fresh and dry weight of crown, Fresh and dry weight of roots and root volume. It is suspected that the VAM given has helped increase the nutrient uptake of the seedlings. VAM can extend and expand the reach of roots for nutrient uptake, especially non-mobile nutrients in the soil such as phosphate (P) [26]. VAM hyphae that develop outside the roots can absorb nutrients and water from the soil to given to the host plant. VAM hyphae also have a higher affinity for phosphorus than root hairs. The phosphatase enzyme produced by VAM hyphae is also one of the mechanisms of this fungus in increasing P uptake by plants.

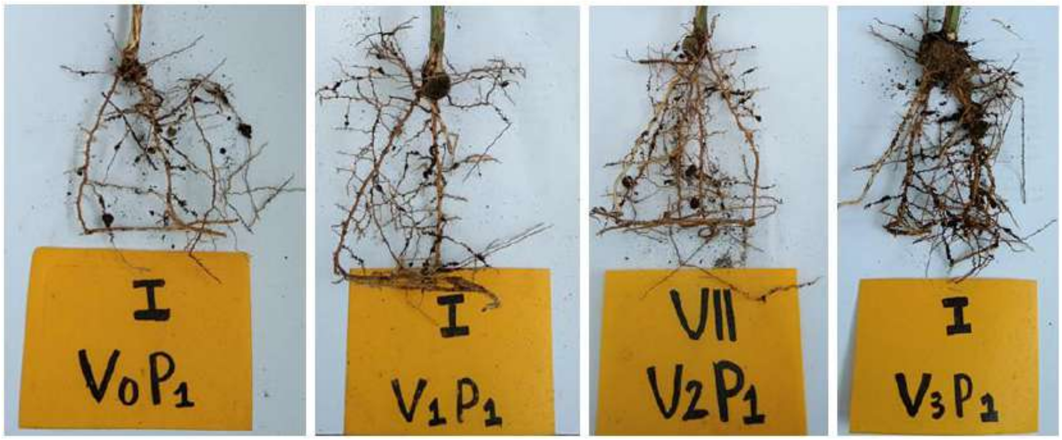

Figure 3. *Root morphology of oil palm seedlings at 16 weeks after VAM inoculation*

Table 8 and Figure 3 show that VAM inoculation affects the morphology of seedling roots. The root architecture of oil palm seeds inoculated with VAM is better than seeds that are not inoculated [27]. It is suspected that there was a higher uptake of P nutrients in the seeds given the VAM inoculum so that it affected root development because P is the main component of nucleic acids that play a role in root formation. This situation is related to the function of P in cell metabolism, it is also explained that when given P, it turns out that the growth of the root part is greater than the upper part of the plant. The growth of one part of the plant was followed by the growth of other parts of the plant. Good roots will affect the formation of a good plant crown, thus increasing seedling height, number of leaf curls, root crown ratio are interrelated and will also affect the dry weight of seedlings[28].

The results of observations on the number of spores and the percentage of VAM colonization on the roots (Table 11) showed that in the treatment without VAM inoculum, spores were also found and VAM colonization was seen on the roots of seedlings. This is suspected to be the presence of VAM inoculum carried by the media (top soil) because the media used was not sterilized.

The results of observations in this study indicate that the VAM inoculum given can be in symbiosis with the roots of the oil palm seeds used. In addition, the percentage of VAM colonization on the roots is also quite high. The percentage value of roots in the range of 0-25% belongs to the category of low infection, in the range of 26-50% belongs to the category of moderate infection, and in the range of 51-75% belongs to the category of high infection[14]. The high percentage of VAM in the roots of oil palm seedlings was in line with the high dose of VAM inoculum given. The high dose causes the opportunity to infect plant roots to be greater. Propagule density is one of the factors influencing primary infection in addition to spore germination, growth rate in the medium and root growth rate[29].

4. Conclusion and Recommendations

Conclusion

Application of VAM 150 g/polybag on oil palm seedlings in pre-nursery tended to give the best response to the growth of seedling height, number of leaves, crown fresh weight, shoot dry weight, root fresh weight, root dry weight and root volume. To be able to know the real potential of VAM, it takes a longer time (> 8 months) in testing the effectiveness of VAM in relation to fertilization efficiency in oil palm seedlings. The given VAM inoculum can be in symbiosis with the roots of oil palm seedlings with a high infection rate. The number of VAM spores in the media and the percentage of colonization on the roots were influenced by the dose of VAM inoculum given. Based on the results of the studies that have been carried out, it is necessary to test the effectiveness of VAM in relation to the nutrient content of the medium. In addition, it is necessary to test the effectiveness of VAM in relation to its potential in controlling stem rot disease of Ganoderma boninense.

This research is meant to save the use of chemical fertilizers because mycorrhizae is living things that live in the roots. It is hoped that mycorrhizae will continue to live there and thrive. The development of mycorrhizae is in line with the development of finished roots and, if planted in the field, mycorrhizae continues to multiply. In nurseries mycorrhizae is used to save fertilizer application. Due to the relatively high price of mycorrhizae, the mycorrhizae is applied to nurseries. Some of the limiting factors for mycorrhizae is that chemical fertilizers should not be used too high. These mycorrhizae live in depressed or critical soils, the application of mycorrhizae is used in marginal soils where nutrients are present but cannot be absorbed by plants with these mycorrhizae so that the nutrients are available to plants. If the soil is fertile there are no mycorrhizae.

Recommendations

The best application is given at germination at a dose of 5/10 g / plant, then repeated mycorrhizal applications during prenursery. If you have used mycorrhizae, the use of fertilizers, fungicides and insecticides should be reduced; so the function of mycorrhizae here is to improve plant health.

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5.

Growth Response of Oil Palm Plant Seeds After Administration of Vesicular Arbuscular Mycorrhiza (VAM) Fungus

Abstract

Objectives: This study aims to determine the growth response of oil palm seedlings to VAM and to determine the appropriate VAM dose for oil palm seedling growth.
Methods: This study took place in the screen house and plant protection laboratory and VAM isolate starters were gathered from the work area. The test used a factorial completely randomized design (CRD) with 2. The first treatment was VAM dose with 4 factors and the second was NPK fertilizer with two factors. The VAM was applied for one month after germination and plant maintenance for 16 weeks with seven parameters.
Findings: The results show that the VAM dose treatment and the application of NPK fertilizer did not have a significant effect on the growth of oil palm seedlings in the pre-nursery (16 weeks) so it took a long time to see the role of VAM in oil palm seedlings. The application of VAM 150 g/polybag on oil palm seedlings in pre-nursery tended to give the best response to the growth of seedling height, a number of leaves, fresh weight of the crown, dry weight of the crown, fresh weight of roots, dry weight of roots and root volume. The VAM inoculum supplied made symbiosis with oil palm seedling roots with a high infection rate. The number of VAM spores in the media and the percentage of colonization on the roots were influenced by the dose of VAM inoculum given.
Novelty: Vesicular Arbuscular Mycorrhiza (VAM) Fungus in oil palm seeds are important to increase the growth of the number of leaves, fresh weight of the crown, and dry weight of the crown. Efforts to increase the quality and quantity of oil palm production are appropriately necessary so that the desired target can be achieved.

Keywords: Growth Response, Oil Palm Seeds, VAM Fungus, Number of Leaves, Fresh Weight of the crown.

1. Introduction

The oil palm plantation sector has grown very rapidly in the last 10 years. In 1998 the area of oil palm plantations was 2.7 million ha with a CPO production volume of 5.6 million tons. In the same year, the export volume of palm oil was 852,843 tons with a value of US$ 333,866. In 2003 the area of oil palm plantations reached 5.06 million ha with a CPO production volume of 9.6 million tons or an average of 1.8 tons/hectare/year. The export volume of palm oil was 6.333 million tons with a value of US$ 2.092 billion. The composition of ownership of oil palm plantations consists of smallholder plantations 29.7%, PTPN 13.2%, and large private plantations 57.1% [1]. Root rot disease (BPB) in oil palm is caused by the fungus Ganoderma boninense. This fungus belongs to the class Basidiomycetes. Seeing the importance of oil palm plantations today and in the future and along with the increasing population's need for palm oil, it is necessary to think about efforts to increase the quality and quantity of oil palm production appropriately so that the desired target can be achieved.

Various problems faced in oil palm cultivation include infertile land and the presence of stem rot disease (BPB) in oil palm caused by the fungus G. boninense. In several oil palm plantations in Indonesia, this disease has caused the death of up to 80% or more of the entire population of oil palm plantations, resulting in a decrease in oil palm production per unit area. Various attempts have been made to reduce the incidence of BPB, but until now no effective control has been found. The improved growth to increase huge pressure in oil palm cultivation using cheaper alternatives such as combination of biofertilizers and organic substrates such as arbuscular mycorrhizal (AM) fungi and endophytic bacteria Pseudomonas aeruginosa[2]. The application of compost from OPEFB combined with Mycorrhiza arbuscular fungi (MAF) significantly improved soil fertility, which was indicated by improving soil chemical and biological properties. The application of MVA at various doses had a significant effect on the dry weight, root length of Calopogonium mucunoides and increase the number of MVA spores in the soil[3]. The AMF inoculation with treatments 30 grams of Trichoderma spp apllied at the start of pre-nursery planting had a significant impact on root colonization and spore number response. 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[4]. Application of Arbuscular mycorrhizal fungi (AMF) reduced 50% of compound fertilizer needed for oil palm seedlings, and the best dose for oil palm seedlings inoculated with AMF was 500 mg polybag-1 and the AMF inoculation without AMF inoculation was 1000 mg polybag-1[5]. Communities of arbuscular mycorrhizal fungi (AMF) in plant roots improve sago palm (Metroxylon sagu Rottb) in Sarawak, Malaysia. The higher number detected by amplicon sequencing of the partial small-subunit rRNA gene (MS, 78; SPS, 50)[6]. Arbuscular mycorrhizal fungi (AMF) impact the production of secondary metabolites directlyby indirectly by stimulating secondary metabolite biosynthetic pathways or directly by increasing plant biomass[7]. The inoculation of Arbuscular Mycorrhizal fungi (AMF) provides tolerance to host plants against various stressful situations like heat, salinity, drought, metals, and extreme temperatures. AMF, being natural root symbionts, provide essential plant inorganic nutrients to host plants, thereby improving growth and yield under unstressed and stressed regimes. AMF can potentially strengthen plants' adaptability to changing environment[8]. Date palm plantations and five native desert plants, respectively in the Southern Arabian Peninsula use and apply Arbuscular mycorrhizal fungi isolated from rhizosphere soils of agricultural and natural habitat. The results showed the growth of both plants potential for use in sustainable land region[9].

2. Methodology

2.1. Definition of *Mycorrhiza*

Currently G. boninense is a deadly disease in oil palm plantations in Indonesia and even in Southeast Asia[10]. The use of inoculation of several types of soil microbes such as Arbuscular Mycorrhizal Fungi is known to be able to increase the adaptability of plants to disease attacks[11]. One way to maximize agricultural yields without damaging the environment is to utilize vesicular arbuscular mycorrhizae (VAM). Mycorrhizae are biological fertilizers that can replace part or all of the function of artificial fertilizers. VAM diversity is the main factor that maintains plant diversity and its function in the ecosystem[11].

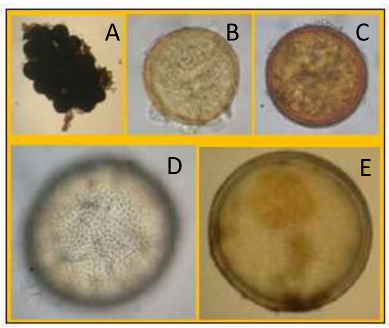

Figure 1. *Medan-based BBPPTP's VAM Spores Collection; Glomus sp – 2 (A), Glomus sp – 3 (B), Glomus sp – 5 (C), Acaulospora rehmii (D) dan Scutelospora sp (E).*

Fungal hyphae come out of plant roots to reach the soil and help absorb certain nutrients for further transmission to plants, especially immobilized nutrients such as phosphate (P), zinc (Zn), copper (Cu), and molybdate (Mo).

2.2. Role of *Vesicular Arbuscular Mycorrhiza (VAM)* Fungi

One of the causes of low productivity is that oil palm cultivation on a large scale is faced with the problem of limited water for watering, especially for oil palm development on marginal lands. Oil palm is a forest plant in the tropics that is humid and fresh, so this plant is vulnerable to water shortage conditions. VAM is known to improve plant growth and yield on soils with unfavorable conditions. VAM infects the root system of the host plant and produces an external hyphae network that grows expansively and penetrates the sub-soil layer thereby increasing the capacity of the roots to absorb nutrients and water[11]. The presence and diversity of VAM in an ecosystem can increase plant diversity. VAM diversity is the main factor that maintains plant diversity and its function in the ecosystem[11]. In addition, the interaction of VAM with other plant microbes can regulate ecosystem functions such as plant diversity, productivity and variability. This shows how important the role of VAM and its function in environmental management is.

The use of inoculation of several types of soil microbes such as Vesicular Arbuscular Mycorrhizae (VAM) is known to increase the adaptability of plants to disease attacks. Mycorrhizae are fungi that live in mutualistic symbiosis with the root system of higher plants. Both fungi and plants benefit from this association. Mushrooms get carbohydrates in the form of simple sugars (glucose) from plants and vice versa plants get water and nutrients from fungi that cause better growth. Mycorrhizal mechanisms in increasing plant resistance to control root pathogens can take place in the following ways: 1). the presence of a layer of hyphae (mantle) that can function as a physical barrier for the entry of pathogens, 2). mycorrhizal fungi utilize more carbohydrates from the roots before being excreted in the form of root exudates so that pathogens cannot develop, 3). the formation of substances that are antibiotics secreted to inhibit the development of pathogens, 4). stimulate the development of saprophytic microbes around the roots and 5). plant roots that have been infected with pathogenic fungi that show competition.

2.3. Prospects and Potential of *Mycorrhiza* for Plantation Plants

It is estimated that nursery management to produce quality seeds can be done by inoculation of mycorrhizal fungi early on at the time of sprouting. Mycorrhizal fungal infection was able to increase the growth of cacao seedlings, as indicated by most of the growth components (height, fresh weight of crown, length and number of roots, and ratio of shoot dry weight/root dry weight). Mycorrhizal infection is thought to be able to increase nutrient absorption by cocoa seedling roots. Mycorrhizal fungi are obligate fungi, where their survival is associated with plant roots with their spores. Spores germinate by forming apressoria as a means of infection, where infection usually occurs in the elongation zone.

Mycorrhizal fungi have enormous potential to increase plant growth and increase soil fertility. The use of mycorrhizae can be used as a biological tool to streamline the use of artificial fertilizers, especially phosphates, besides that it can streamline nutrient elements, especially on critical or marginal land. Soil environmental conditions suitable for seed germination are also suitable for the germination of mycorrhizal spores. Mycorrhizae also act as biocontrol for plants and increase plant resistance to drought, and conversely, mycorrhizae do not cause disease in plants.

In addition, the roots of mycorrhizal plants will grow faster and produce more harvest weight than those without mycorrhizal plants, and are more resistant to certain diseases. The presence of mycorrhizae in the soil in the form of associations between fungi and high plant root systems can affect the nature of soil fertility. The use of mycorrhizae is an ecological necessity, because in addition to being safe to use (not pathogenic), it does not cause environmental pollution, plays an active role in the nutrient cycle and can also improve soil fertility status because of its ability to extract bound nutrients.

Based on the test results, the application of mycorrhizae on cocoa seedling media aims to determine the ability of mycorrhizae in influencing the height and number of leaves of cocoa plants. The growth of cacao seedlings in nurseries is determined by the mechanism of mycorrhizal fungal infection which is symbiotic mutualism. Plants infected with mycorrhizal fungi will increase the volume and length of the roots, so that the nutrients absorbed by the roots will affect the height and number of plant leaves. Mycorrhizal roots have a higher auxin content which allows increased root growth.

2.4. Mycorrhizal Symbiosis in Oil Palm

Oil palms have different root growth patterns. At 0-1 years of age, the growth of oil palm roots downward (positive geotropism) while from the age of 11 years the growth of horizontal primary roots increased very sharply followed by the growth of secondary roots upward (negative geotropism)[12]. AMF (arbuscula mycorrhizal fungi) is a fungus that grows on the roots, thus the root growth pattern is thought to influence the dynamics of the AMF population and its diversity. AMF populations at several ages and depths of the oil palm rhizosphere have not been reported. Research on the interaction of AMF in oil palm was initiated in 1990 by Bal and Giovenetti who observed that there was no difference in P uptake of oil palms from in vitro cultures fertilized with superphosphate and rock phosphate. In the same year using in vitro cultured oil palm, it was shown that AMF colonization could increase the uptake of P and several other nutrients[13].

2.5. Methods

2.5.1. Place and Time

The study "Response of Oil Palm Plant Seed Growth Against VAM Mushrooms", was carried out at the screen house and integration laboratory of BBPPTP Medan, from July to December 2019.

2.5.2. Materials and tools

The materials used in this study were oil palm sprouts, NPK fertilizer, VAM starter, topsoil soil, polybags, aquades, filter paper, 5% KOH, 1% HCL, lactic acid, glycerol, trypan blue, melzer solution, Hyponex fertilizer, fertilizer. Terabuster organics, plastic bottles, plastic trays and other necessary materials. The tools used in this study were hoe, ponjo, plastic bag, permanent marker, graded filter, centrifuge, object glass, cover glass, compound microscope, stereo-binocular microscope, scissors, needle preparation, dropper and other necessary tools.

2.5.3. Implementation Method

2.5.3.1. Experimental Design

This experiment used a factorial Completely Randomized Design (CRD) with 2 treatments, namely the dose of VAM with 4 factors and the application of NPK fertilizer with 2 factors, so that there were 8 treatment combinations with each treatment repeated 3 times:

VAM dose treatment:
V_0 : 0 gr/seedling
V_1 : 50 gr/seedling
V_2 : 100 gr/seedling
V_3 : 150 gr/seedling

NPK fertilization treatment:
P_1 : 50 % suggested dose
P_2 : 100 % suggested dose

Linear model used:
$Y_{ijk} = \mu + V_i + P_j + (MT)_{ij} + \varepsilon_{ijk}$
where:

Y_{ijk} = The value of observations on the media treatment at i-level and j-level host plants on k-level replication
μ = Median
V_i = Effect of VAM dose treatment at i-level
P_j = Effect of NPK fertilization treatment at j-level
$(VP)_{ij}$ = Interaction between the VAM dose treatment at i-level and fertilization at j-level
ε_{ijk} = Experimental error in the media treatment at i-level and host plant at j-level for the replication at k-level

2.5.3.2. Technical Implementation

2.5.3.2.1. Seedling

The oil palm seeds used were DXP Lame and Yangambi which had germinated and were sown on sand media which had been sterilized until the age of 4 weeks before being transferred to the pre-nursery.

2.5.3.2.2. VAM Isolate Preparation and Spore Inoculation

VAM isolates obtained from oil palm VAM trapping results in the 2017 activity were examined for the number of spores and the percentage of VAM colonization on roots, with the requirement that VAM isolates that could be used were spore count 10 spores/gr soil and 70% VAM colonization on roots. The VAM inoculation process according to the treatment begins with removing the seeds from the seedling media. VAM inoculum (with zeolite carrier and sand) was sprinkled on the planting hole and partly sprinkled on the root surface. After the seedlings are 4 weeks old in the pre-nursery, urea fertilization is carried out at a dose of 2 g per liter for 100 seedlings by spraying on the leaves every week

2.5.3.2.3. Planting in Pre-Nursery

Oil palm seeds that have been germinated for 4 weeks are transferred to polybags measuring 18 cm x 25 cm (one seed per polybag) with planting media in the form of topsoil, sand, and organic matter (2:1:1) then the media is added with rock phosphate fertilizer as much as 100 gr for 60 pre-nursery polybags.

2.5.3.2.4. Raising

The maintenance carried out during the study included watering, weeding, pest and disease control, and NPK fertilization. The type and dose of fertilizer used are adjusted to the growth of the seeds and the treatment used.

2.5.4. Observation Parameter

2.5.4.1. Plant Height

Plant height was measured from the soil surface to the tip of the longest leaf. Measurements were made using a meter in centimeters (cm). Measurement of plant height begins after 1 month of oil palm seedlings from the pre-nursery with an interval of 1 month.

2.5.4.2. Number of Leaves

Observation of the number of leaves was carried out by counting the perfectly open leaves on each oil palm seedling. Measurement of the number of leaves begins after 1 month of oil palm seedlings from the pre-nursery with an interval of 1 month.

2.5.4.3. Header Fresh Weight

Measurement of root dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then the plant roots were put in an envelope and then in an oven at 105°C for 24 hours, then each sample was weighed using an analytical balance.

2.5.4.4. Head Dry Weight

Measurement of canopy dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and the plant crown was put in an envelope and then oven-dried at 105°C for 24 hours. Then each sample was weighed using an analytical balance.

2.5.4.5. Root Fresh Weight

Measurement of root fresh weight was carried out at 16 weeks DAP by disassembling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then each sample was weighed using an analytical balance.

2.5.4.6. Root Dry Weight

Measurement of root dry weight was carried out at 16 weeks DAP by dismantling the sample plants and then cleaning them from soil and other impurities by washing them with water in a container. Separated between the roots and plant crowns by cutting. After that, it was air-dried and then the plant roots were put in an envelope and then in an oven at 105°C for 24 hours, then each sample was weighed using an analytical balance.

2.5.4.7. Root Volume

All the roots of the chopped oil palm seeds were put into a measuring cup filled with 100 ml of water. Root volume is the addition of the volume of water in the measuring cup in ml.

2.5.4.8. Percentage of VAM Colonization in Roots

The degree/percentage of root colonization was calculated using the formula:

$$\% \text{ Root colonization} = \frac{\text{Number of fields of view marked} +}{\text{Overall number of fields of view}} \text{x } 100\%$$

The level of colonization is further categorized according to the Institute of Mycorrhizal Research and Development, USDA Forest Service, Athens Georgia[14] as follows:

1. Class 1 is very low when infection ranges between 0% – 5%
2. Class 2 is low when infection ranges between 6% – 25%
3. Class 3 is modest when infection ranges between 26% – 50%
4. Class 4 is high when infection ranges between 51% – 75%
5. Class 5 is very high when infection ranges between 76% – 100%

2.5.4.9. Number of Spores

The number of spores was observed at the end of the study (12 weeks DAP). The technique used to determine the number of VAM spores is the pour-filter technique[15] and will be followed by the centrifugation technique[16].

3. Results and Discussion

3.1. Plant Height

The results of analysis of variance on plant height parameters can be seen in Appendix 1 to 4. Based on the results of analysis of variance in plant height, it shows that VAM and fertilizer did not show a significant effect. The results of plant height observations in the 4th observation can be seen in Table 1.

Tabel 1. Average Plant Height (cm) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	35,08	38,45	33,93	39,95	36,85
P2	36,08	35,85	35,48	39,30	36,68
Average	35,58	37,15	34,70	39,63	36,76

Table 1 shows that the treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the increase in plant height. However, from the treatment with mycorrhizae, there was a tendency to give VAM in treatment V3 (150 g/seed) which had the highest plant height (39.63 cm) compared to other treatments.

3.2. Number of Leaves

The results of the analysis of variance on the number of leaves can be seen in Appendix 5 to 8. Based on the results of analysis of variance on the average number of leaves, it was shown that VAM and fertilizer application did not show a significant effect.

Table 3 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the increase in the number of leaves. However, from treatment with mycorrhizae, there was a tendency to give VAM to treatment V3 (150 g/seed) which had the highest mean number of leaves compared to other treatments, which was 4.88 strands.

3.3. Head Fresh Weight

The results of the analysis of variance on the fresh canopy weight parameter can be seen in Appendix 9. Based on the results of the analysis of the variance of the average fresh crown weight, it shows that the application of VAM and NPK fertilizer did not show a significant effect.

With reference to the treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant crown weight. However, there was a tendency that the highest number of VAM (150 g/plant) showed the heaviest crown weight, which was 6.60 g compared to other treatments. Meanwhile, the interaction treatment which showed the best fresh weight of the crown was the application of 50% fertilizer and the provision of VAM as much as 150 g/plant, which was 7.27 g.

3.4. Head Dry Weight

The results of the analysis of variance on the canopy dry weight parameter can be seen in Appendix 10. Based on the results of the analysis of variance on the average dry weight of the canopy, it was shown that VAM and NPK fertilizers did not show a significant effect. The results of the fresh weight of the crown can be seen in Table 2.

Table 2. Head Dry Weight (gr) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	1.35	1.53	1.44	1.55	1.47
P2	1.44	1.53	1.47	1.41	1.46
Average	1.39	1.53	1.45	1.48	

Table 5 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on the dry weight of the plant crown. However, there was a tendency that the highest VAM (150 g/plant) and 50% NPK fertilizer application showed the heaviest canopy dry weight, which was 1.55 g compared to other treatments.

3.5. Root Fresh Weight

The results of the analysis of variance on the fresh root weight parameter. Based on the analysis of the variance of the average fresh root weight, it shows that VAM and NPK fertilizer did not show a significant effect. The results of observations of fresh root weight can be seen in Table 3.

Table 3. Fresh Weight of Roots (gr) at 4 Months of Age after Tranplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	1.50	1.53	1.54	1.73	1.57
P2	1.28	1.63	1.54	1.62	1.52
Average	1.39	1.58	1.54	1.67	

Table 6 shows that treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant fresh weight. However, there was a tendency that the highest amount of VAM (150 g/plant) would give the heaviest average fresh weight of crown compared to other treatments, which was 1.67 g.

3.6. Root Dry Weight

The results of the analysis of variance on the fresh root weight parameter. Based on the analysis of the variance of the average fresh root weight, it shows that VAM and NPK fertilizer did not show a significant effect. The results of observations of fresh root weight can be seen in Table 4.

Table 4. Root Dry Weight (gr) at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	0.98	1.04	0.99	1.06	1.02
P2	0.91	1.02	1.01	1.01	0.99
Average	0.94	1.03	1.00	1.04	

Table 4 shows that the treatment of 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant dry weight. However, there was a tendency with the highest VAM (150 g/plant) giving the heaviest average dry weight of roots compared to other treatments, which was 1.04 g.

3.7. Root Volume

The results of the analysis of variance on the root volume parameters. Based on the analysis of the variance of the mean root volume, it shows that VAM and NPK fertilizers did not show a significant effect. The results of observations of root volume can be seen in Table 5.

Table 5. Root Volume (ml) at 4 Months of Age after Transplanting

Fertlizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	2.38	3.55	3.20	3.80	3.23
P2	2.75	3.63	3.13	3.75	3.31
Average	2.56	3.59	3.16	3.78	

Table 8 shows that treatment with 4 levels of VAM and 2 levels of NPK fertilizer and their interactions did not have a significant effect on plant dry weight. However, there was a tendency that with the highest VAM administration (150 g/plant) it showed the heaviest average fresh root weight of 3.78 ml compared to other treatments.

3.8. Number of Spores

The results of the analysis of variance on the parameters of the number of spores can be seen in Appendix 14. Based on the results of the analysis of variance on the average number of spores, it shows that the application of VAM and NPK fertilizer showed a significant effect. The results of observations of root volume can be seen in Table 6.

Table 6. Number of Spores Per 50 g Media at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	24G	290C	286D	316B	229.00

P2	17H	241E	118F	386A	190.50
Average	20.50	265.5	202	351	

Table 6 above shows that the number of VAM spores on the media was in line with the amount of VAM given. The highest mean number of spores in the VAM treatment was found in V3 treatment (150gr/plant) as much as 351 spores/50 gr of media. Meanwhile, the effect of NPK fertilizer on the average number of VAM spores was highest in treatment P1 (50% recommended dose) of 229 spores/50 g of media. So for the interaction effect, the highest average number of spores was found in the V3P1 treatment (giving 150 g VAM/plant and 100% recommended dose of NPK fertilizer) which was significantly different from other treatments.

3.9. Percentage of VAM Colonization in Roots

The results of the analysis of variance on the parameters of the number of spores. Based on the results of the analysis of variance on the average number of spores, it was shown that the application of VAM and NPK fertilizer showed a significant effect. The results of observations of root volume can be seen in Table 7.

Table 7. Percentage of VAM Colonization in Oil Palm Seed Roots at 4 Months of Age after Transplanting

Fertilizer (NPK)	*Vesicular Arbuscular Mychorhiza*				
	V0	V1	V2	V3	Average
P1	58D	60D	76B	86A	70.00
P2	56E	72C	83A	84A	73.75
Average	57	66	79,5	85	

Note: Numbers followed by unequal letters in the same column and row are very significantly different at the 1% level according to Duncan's Distance Test (DMRT).

Table 7 shows that the percentage of VAM colonization in oil palm seedlings was in line with the amount of VAM given. The highest mean percentage of VAM colonization was found in treatment V3 (150g VAM/plant) of 85%. Meanwhile, the effect of NPK fertilizer on the average percentage of VAM colonization was found in treatment P2 (100% recommended dose) of 73.75%. Meanwhile, the interaction effect of the mean percentage of VAM colonization on the roots of oil palm seedlings was highest in the V3P1 treatment (150 g VAM/plant and 100% of the recommended dose of NPK fertilizer) but it was not significantly different from the V3P1 treatment (150g VAM/plant and 100% recommended dose), application of NPK fertilizer) and V2P2 (100g VAM/plant and 100% recommended dose of NPK fertilizer) with colonization percentage values of 86%, 84% and 83% respectively.

Discussion

This study was conducted for 16 months on oil palm seedlings in the pre-nursery. VAM was given in the form of spore inoculum which was inoculated on the roots of the seedlings at the beginning of transplanting from the nursery. While the application of NPK fertilizer was carried out after the plants were 14 weeks old. So, in this study the response to the provision of NPK fertilizer for oil palm seeds is not a concern (discussion). Observational data that has been analyzed for variance shows that the combination of VAM dose and percentage of NPK fertilizer for oil palm seedlings in pre-nursery has no significant effect on plant growth parameters. This is presumably because the nutrient content of the growing media used in this study was quite high, so that the addition of fertilizer and VAM did not significantly affect plant growth. The planting medium used in this study was a mixture of topsoil, organic matter, and sand with a ratio of 2:1:1. Topsoil is soil that is in the top layer and contains all the chemical, physical, and biological components that plants need to grow properly.

The availability of high enough nutrients creates conditions where VAM cannot perform its function in facilitating the absorption of nutrients by plants. Mycorrhizae is a form of mutualism symbiosis between fungi and plant root systems. The role of mycorrhizae is to help the absorption of plant nutrients, increase growth and yield of plant products. On the other hand, fungi obtain assimilated energy from plants. VAM symbiosis with plants on fertile land does not have much positive effect, but in extreme conditions it can increase most plant growth[17]. Mycorrhizae promote plant growth in low soil fertility, degraded land and help expand the function of the root system in obtaining nutrients[18,19]. In particular, mycorrhizal fungi play an important role in increasing the absorption of ions with low mobility levels, such as phosphate (PO43-) and ammonium (NH4+) and other relatively immobilized soil nutrients such as sulfur (S), copper (Cu), zinc (Zn), and also Boron (B)[20]. Mycorrhizae also increase the surface area in contact with the soil, thereby increasing the root absorption area up to 47 times, which makes it easier to access nutrients in the soil. Mycorrhizae not only increase the rate of nutrient transfer in the roots of the host plant, but also increase resistance to biotic and abiotic stresses[17]. Mycorrhizae are able to help maintain the stability of plant growth in polluted conditions[21].

Observations of seedling growth carried out in this study were for 4 months after VAM spore inoculation. This time is thought to be insufficient for oil palm seedlings to show their growth response. The response of VAM inoculation to seedling growth and nutrient uptake can be seen at the age of 6 months after VAM spore inoculation [22]. VAM spores are a type of inoculum that can be used for plants in nurseries, but has drawbacks. i.e. spores take several days to germinate and some species have a period of dormancy before they can germinate. Especially for field applications, the inoculum in the form of spores has weaknesses, namely the slow initial development and slow absorption in the roots so that the inoculum is unable to compete with native VAM and other natural soil microbes [23,24]. fast and high through inoculation is a requirement to get an effective symbiosis of inoculation.

From Table 8 it is known that there is no significant difference in the number of seedling leaves due to VAM administration, this is because the increase in the number of leaf midribs of oil palm plants is determined by genetic factors of the plant itself, besides the age factor also affects it, causing the number of leaf midribs. each treatment showed numbers that were not significantly different. Genetic factors determine the number of leaves that will be formed, therefore it is very important in breeding using quality seeds[25/ 19].

Table 8. Data on Observation of Oil Palm Seed Growth and VAM Development at 4 Months of Age after Transplanting

Treatment	Observation Parameter								
	TT (cm)	JD	BST (g)	BKT (g)	BSA (g)	BKA (g)	VA (ml)	JSV	PKV (%)
V0P1	32,48	3,75	4,76	1,33	1,77	0,46	2,38	24G	58D
V0P2	34,05	3,75	4,68	1,63	1,14	0,33	2,75	17H	56E
V1P1	36,20	4,00	5,62	1,86	1,88	0,62	3,55	29F	60D
V1P2	33,83	4,00	6,28	1,85	2,18	0,54	3,63	241E	72C
V2P1	31,48	4,00	6,02	1,59	1,93	0,49	3,20	286D	76B
V2P2	31,58	4,00	4,15	1,67	2,00	0,53	3,13	118C	83A
V3P1	36,90	4,25	7,27	1,91	2,59	0,60	3,80	316B	86A
V3P2	36,45	4,00	5,93	1,53	2,18	0,54	3,75	386A	84A

Legend: Numbers followed by different letters in the same column were significantly different at the 1% level according to the Duncan Distance Test (DMRT). TT = plant height, JD = number of leaves, BBT = fresh weight of crown, BKT = dry weight of crown, BBA = fresh weight of roots, BKA = dry weight of roots, VA = root volume, JSV = number of VAM spores and PKV = percentage of VAM colonization

In this study, it can be seen from the 4 levels of VAM given that oil palm seeds given VAM gave the best results in response to seedling growth compared to the control treatment (without VAM) Table 11. This can be seen in the variables of plant height, number of leaves, Fresh and dry weight of crown, Fresh and dry weight of roots and root volume. It is suspected that the VAM given has helped increase the nutrient uptake of the seedlings. VAM can extend and expand the reach of roots for nutrient uptake, especially non-mobile nutrients in the soil such as phosphate (P) [26]. VAM hyphae that develop outside the roots can absorb nutrients and water from the soil to given to the host plant. VAM hyphae also have a higher affinity for phosphorus than root hairs. The phosphatase enzyme produced by VAM hyphae is also one of the mechanisms of this fungus in increasing P uptake by plants.

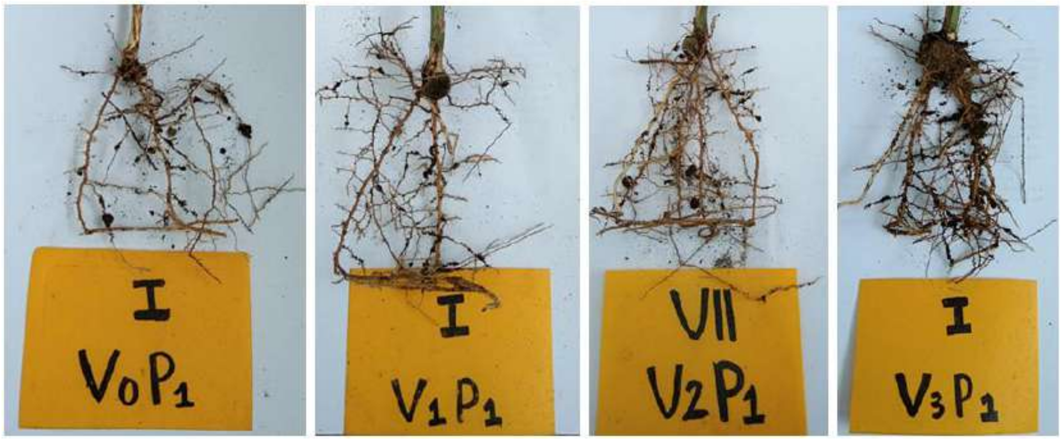

Figure 3. *Root morphology of oil palm seedlings at 16 weeks after VAM inoculation*

Table 8 and Figure 3 show that VAM inoculation affects the morphology of seedling roots. The root architecture of oil palm seeds inoculated with VAM is better than seeds that are not inoculated [27]. It is suspected that there was a higher uptake of P nutrients in the seeds given the VAM inoculum so that it affected root development because P is the main component of nucleic acids that play a role in root formation. This situation is related to the function of P in cell metabolism, it is also explained that when given P, it turns out that the growth of the root part is greater than the upper part of the plant. The growth of one part of the plant was followed by the growth of other parts of the plant. Good roots will affect the formation of a good plant crown, thus increasing seedling height, number of leaf curls, root crown ratio are interrelated and will also affect the dry weight of seedlings[28].

The results of observations on the number of spores and the percentage of VAM colonization on the roots (Table 11) showed that in the treatment without VAM inoculum, spores were also found and VAM colonization was seen on the roots of seedlings. This is suspected to be the presence of VAM inoculum carried by the media (top soil) because the media used was not sterilized.

The results of observations in this study indicate that the VAM inoculum given can be in symbiosis with the roots of the oil palm seeds used. In addition, the percentage of VAM colonization on the roots is also quite high. The percentage value of roots in the range of 0-25% belongs to the category of low infection, in the range of 26-50% belongs to the category of moderate infection, and in the range of 51-75% belongs to the category of high infection[14]. The high percentage of VAM in the roots of oil palm seedlings was in line with the high dose of VAM inoculum given. The high dose causes the opportunity to infect plant roots to be greater. Propagule density is one of the factors influencing primary infection in addition to spore germination, growth rate in the medium and root growth rate[29].

4. Conclusion and Recommendations

Conclusion

Application of VAM 150 g/polybag on oil palm seedlings in pre-nursery tended to give the best response to the growth of seedling height, number of leaves, crown fresh weight, shoot dry weight, root fresh weight, root dry weight and root volume. To be able to know the real potential of VAM, it takes a longer time (> 8 months) in testing the effectiveness of VAM in relation to fertilization efficiency in oil palm seedlings. The given VAM inoculum can be in symbiosis with the roots of oil palm seedlings with a high infection rate. The number of VAM spores in the media and the percentage of colonization on the roots were influenced by the dose of VAM inoculum given. Based on the results of the studies that have been carried out, it is necessary to test the effectiveness of VAM in relation to the nutrient content of the medium. In addition, it is necessary to test the effectiveness of VAM in relation to its potential in controlling stem rot disease of Ganoderma boninense.

This research is meant to save the use of chemical fertilizers because mycorrhizae is living things that live in the roots. It is hoped that mycorrhizae will continue to live there and thrive. The development of mycorrhizae is in line with the development of finished roots and, if planted in the field, mycorrhizae continues to multiply. In nurseries mycorrhizae is used to save fertilizer application. Due to the relatively high price of mycorrhizae, the mycorrhizae is applied to nurseries. Some of the limiting factors for mycorrhizae is that chemical fertilizers should not be used too high. These mycorrhizae live in depressed or critical soils, the application of mycorrhizae is used in marginal soils where nutrients are present but cannot be absorbed by plants with these mycorrhizae so that the nutrients are available to plants. If the soil is fertile there are no mycorrhizae.

Recommendations

The best application is given at germination at a dose of 5/10 g / plant, then repeated mycorrhizal applications during prenursery. If you have used mycorrhizae, the use of fertilizers, fungicides and insecticides should be reduced; so the function of mycorrhizae here is to improve plant health.

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IJST-2022-1190 - Recommendation for minor revisions

Indian Journal of Science & Technology <indjst-journal@manuscriptcommunicator.com> 11 Oktober 2022 pukul 17.23

Kepada: litanasution@umsu.ac.id

Dear Dr. Lita Nasution,

Manuscript Id: IJST-2022-1190
Manuscript Title: Growth Response of Oil Palm Plant Seeds After Administration of Vesicular Arbuscular Mycorrhiza (VAM) Fungus
Manuscript Type: Research Article
Submitted Date: 05 August 2022

We wish to confirm that we have received your manuscript on the aforementioned date. Shortly after submission, the manuscript was sent to the reviewers who are experts in the field. The manuscript has received positive response from the reviewers. Meanwhile, they have recommended for minor revisions. The reviewed manuscript with comments and suggestions have been attached with this email for your reference. The reviewers seek your attention on to improve by incorporating the suggestions and comments. Further, we request you to submit the manuscript with the requested changes and additions to be accepted for the next phase of publication. We will be awaiting your response with an improved version of your manuscript.

Reviewer 1 comments:

Thorough proof reading is needed; very old references may be omitted; instead consider recent ones. As per the journal style include publication URL or DOI for each reference.

Reviewer 4 comments:

1. The manuscript need to be proofread and grammar 2. In introduction, many sentences need citation 3. Latin name should be italic 4. Some appendixs should be moved to results part 5. Some tables and figure in discussion should be moved to results part

The below link may be useful to know how to complete online the revised article resubmission: https://sciresol.s3.us-east-2.amazonaws.com/Knowledge_base/Author/Author_Resubmission.pdf (or) watch the video: https://www.youtube.com/watch?v=sYVkUskhleA During revision, please address to all comments made by the reviewers; Attach a rebuttal table with one side row contain different comments raised by reviewers and the other corresponding side should indicate your response to each comment. Also incorporate suitable changes in the manuscript, accordingly, as part of revision.

Regards
Prof. Natarajan Gajendran (indjst@gmail.com)
Editor-in-Chief
Indian Journal of Science and Technology

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IJST-2022-1190-R1 - Manuscript withheld

Indian Journal of Science & Technology <indjst-journal@manuscriptcommunicator.com> 28 November 2022 pukul 10.21
Kepada: litanasution@umsu.ac.id

Dear Dr. Lita Nasution,

Manuscript Id: IJST-2022-1190-R1
Manuscript Title: Growth Response of Oil Palm Plant Seeds after Application of Vesicular Arbuscular Mycorrhiza (VAM) Fungus
Manuscript Type: Research Article
Submitted Date: 25 November 2022

The manuscript submitted by you has been scrutinized by our editorial staff. As it has been found to be deviating from the style guidelines and upload instructions, the decision to publish has been withheld for a brief period of time. So we suggest you ponder over the recommendations from our editorial team as given. If these recommendations can be accepted and incorporated by you to your manuscript, then your manuscript can be forwarded for further review. We will be awaiting your response along with an improved version of your manuscript. For any information please contact: indjst2016@gmail.com

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Complete proof reading is suggested

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