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Exploration and Identification of Arbuscular Mycorrhizal Fungi (FMA) in Sungkai Plants (*Peronema canescens* Jack) at Various Soil Depths

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Abstract: Sungkai (Peronema canescens Jack.) is one of the native species that can be developed in plantation forest development, where almost all soil types can be grown by sungkai because sungkai is one of the leading plant species. Therefore, it is necessary to carry out further sungkai cultivation activities to be developed and cultivated. The provision of FMA inoculation in plants to assist growth will be more optimal if using indigenous FMA spores (from the original plant stand). This research aims to explore and identify the types of Arbuscular Mycorrhizal Fungi found under Sungkai stands and analyze the effect of soil depth on the diversity and abundance of FMA spores under Sungkai stands. This research was conducted in sungkai stands with an area of 1.5 Ha by purposive sampling at a depth of 20-60 cm. Soil samples were taken from 5 points then composited and taken as much as 1 kg and inserted into an airtight plastic bag. The results of identification showed that there were 5 FMA genus in the Sungkai Planting Area, namely Glomus, Acaulospora, Entrospora, Gigaspora, and Scutellospora. The highest FMA spore density in each research location was found in the location at a depth of 20 cm with an average of 48 spores.

Keywords: Sungkai; Indigenous; FMA; Soil Depths

Introduction

Sungkai (Peronema canescens Jack.) is one of the native species that can be developed in plantation forest development, where almost all soil types can be grown by sungkai because sungkai is one of the leading plant species. Besides being utilized for its wood, sungkai leaves can also be used as a traditional medicinal plant. For this reason, it is necessary to carry out further sungkai cultivation activities to be developed and cultivated optimally in order to meet the needs of sungkai plants from the aspects of wood, herbal medicine, and other important uses.

The attempt has been made by the development of sungkai plantation forest. In the concession area of PT Mekar Agro Sawit, sungkai planting has been carried out in an area of ± 1.5 ha. The soil type at the sungkai planting site is Ultisol. Ultisol is a soil type that is known to be nutrient-poor due to its low P-availability and pH and high Fe and Al content. The low availability of P is due to the high fixation of P by Fe and Al minerals, so plants find it difficult to absorb (Sari et al., 2017).

Arbuscular mycorrhizal fungi have a very important role in the ecosystem and plants. The functional role of FMA in the ecosystem includes biofertilizer, bioprotector, bioregulator, and phytoremediation gene (Chahyadi et al., 2014). Biofertilizer helps plants in absorbing water and nutrients. FMA is one of the most widespread types of fungi and is associated with almost all types of plants (Khairuna et al., 2015). According to Hermawan et al. (2015), Suharno et al. (2020) more than 80% of plant species are associated with arbuscular mycorrhizal fungi. FMA can be found in mangrove ecosystems, coastal

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forest ecosystems, peat ecosystems, and lowland forest ecosystems. Population levels and species composition of FMA are very diverse and are influenced by plant characteristics and environmental factors such as temperature, soil pH, soil moisture, phosphorus and nitrogen content, and heavy metal concentrations (Manaroinsong & Lolong, 2015). Differences in soil depth also greatly influence the development of mycorrhiza. Based on research by (Manurung, 2018), the highest spore density is found at a depth of 0-20 cm with an average spore density of 127 spores per 50 g of soil, while the lowest spore density is found at a depth of 60-80 cm with an average spore density of 15 spores per 50 g under a stand of sea cypress (Casuarina equisetifolia) on alluvial soil. Purwati et al. (2019)stated that at a depth of 0-20 cm the number of spores was higher, namely 209.46 per g, while at a soil depth of 20-40 cm, it was 106.39 per g under the rhizosphere of jernang (Daemonorops draco) on clayey clay soil.

Inoculation of FMA in plants to assist growth will be more optimal if using indigenous FMA spores (from the original plant stand). To obtain FMA, several stages of activities need to be carried out, starting with exploration, identification, isolation, and application of FMA to the intended plant.

The purpose of this research was to explore and identify the types of Arbuscular Mycorrhizal Fungi found under sungkai stands (Peronema canescens Jack.) and see the effect of soil depth on the diversity and abundance of FMA in the soil under Sungkai stands based on differences in soil depth.

Method

This research was conducted for 8 months, starting from April to November 2022, at PT. Mekar Agro Sawit and Biotechnology Laboratory, Faculty of Agriculture, Jambi University.

The materials used in this research are soil samples from sungkai stands, granulated sugar, Melzer solution, and PVLG solution. The tools used included hoes, digital scales, sieves with sizes of 500 μm, 250 μm, 63 μm, and 38 μm, petri dishes, spore pipettes, stereo microscopes, binocular microscopes, preparation glass, spray bottles, cover glass, watch glasses, goblets, stirring rods, setrifuse tubes, refrigerators, centrifuges, label paper, plastic, cameras, markers, toothpicks, plastic buckets, pH meters, Thermo hygrometers.

FMA exploration was carried out in sungkai stands with an area of 1.5 ha by purposive sampling at various depths, including 0-20 cm, 20-40 cm, and 40-60 cm. Soil samples were taken from 5 points then composited and taken as much as 1 kg and inserted into an airtight plastic bag.

Spore extraction was carried out on soil samples with 3 replicates. Spore extraction serves to separate soil and spores so that it will be easier to identify FMA. The spore identification technique uses the wet filter pour technique and continues with centrifugation.

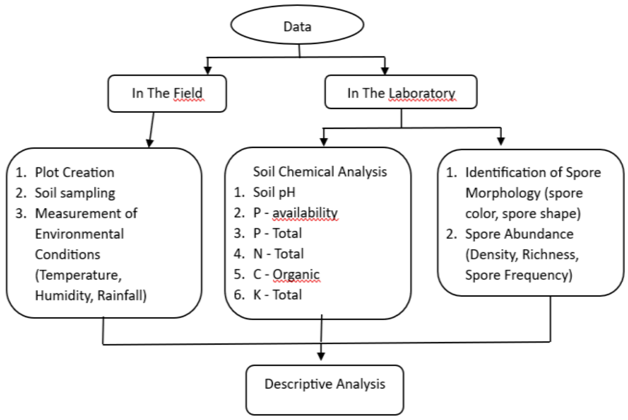

Figure 1. Study flowchart

Initial observations for spore identification were carried out using a stereo microscope by separating spores from the centrifuged waste, for spore counting and making preparations for the identification of existing FMA spores. The spores seen in the Petri dish were described as the initial shape seen then the spores were separated into the watch glass using a spore pipette, the spores that had been separated were then identified by placing them into a glass preparation that had been given Melzer and PVLG solutions, PVLG solution was dripped on the left and right sides for Melzer solution.

Further observation of spores using a binocular microscope, the spores that have been found are then carefully broken by pressing the glass cover of the preparation with the tip of a toothpick. The color change of spores in Melzer and PVLG is one way to determine the type of spores present, and then the observations are recorded and documented. Variables observed in spore identification include: (1) Character Identification of Spore Morphology; (2) Abundance of Spores.

Spore abundance is seen based on the results of the total number of spores that have been identified per 50 g soil sample, then grouped according to the samples taken based on the six locations where soil samples were taken, so that the abundance of spores in the six areas can be compared. Based on the formula, it can be calculated as follows.

- Spore Density $= \frac{Number\ of\ spores}{Soil\ weight\ (g)}$
- Spore Richness = Number of genus in 50 g of soil
- Frequency $= \frac{Number\ of\ samples\ with\ spores\ found}{Total\ samples} \times 100\%$
- Relative abundance $= \frac{Number\ of\ FMA\ genus}{Total\ spore} \times 100\%$

Result and Discussion

Soil Chemical Condition

The results of soil analysis from three soil depths conducted at the Integrated Testing Laboratory of Soil, Plant Fertilizer Water Research and Development Agency are presented in Table 1.

Table 1. Results of soil chemical analysis under Sungkai stands at various depths at PT. Mekar Agro Sawit

Parameters	Unit	Depth (cm)		
		0-20	20-40	40-60
pH		4.80	4.80	4.80
C-Organic	%	1.51	0.89	0.85
N-total	%	0.17	0.12	0.10
P- availability	ppm	5.30	10.60	64.30
P- total	mg/100g	4.41	2.78	2.31
K- total	mg/100g	9.91	5.93	3.61

The analysis showed that soil samples at various soil depths have the same pH, which is acidic. The content of C-organic material decreased in line with the increase in soil depth. at a depth of 0-20 cm is classified as low, while at a depth of 20-40 cm and 40-60 cm is classified as very low. Likewise, phosphorus and potassium contents decreased in line with the increase in soil depth. The content of phosphorus and potassium at various depths is classified as very low. Based on the results of the soil analysis in Table 1, it can be concluded that the soil used is classified as marginal and infertile soil. These results are in accordance with the characteristics of ultisol soils, which are nutrient-poor soils characterized by acidic soil pH. Soils that have an acidic pH will prevent the roots from absorbing macro elements (NPK) in the soil because acidic soils have a lot of Fe and Al content, where these elements can bind macronutrients in the soil so that they are not available to plants. The presence of P content in the soil can affect the growth of mycorrhiza in the soil. The higher the available P content or the better the soil fertility level, the mycorrhizal function becomes inactive (Yusriadi et al., 2017)

FMA Identification

The identification results found 5 FMA genus namely Glomus, Acaulospora, Entrospora, Gigaspora, and Scutellospora. The characteristics of each genus and the discovery of the genus can be seen in Table 2. From the identification results, the Glomus genus was found in all locations and soil depths under Sungkai stands, and each soil depth had different spore types and numbers. At each depth, the most common type of spore found was the Glomus spore type. This is thought to be because Glomus has properties that are easy to grow and develop in various environmental conditions, especially soil types.

The results of this study are in line with many previous studies which state that Glomus is the most dominant type of arbuscular mycorrhizal spores found in FMA diversity studies in various growing places (Hermawan et al., 2015; Widiatma et al., 2015;Rini et al., 2017). This shows that Glomus has a higher adaptability to environmental conditions and has more species (Sari, 2016). The results of research by Kurnia & Larekeng, (2019)showed that Glomus had the highest abundance in Ko'mara Village. The colonization percentage on-site was intermediate

Spore Abundance

Spore Density

The density of FMA spores obtained from each soil sampling sample point in the sungkai planting area can be seen in Table 2.

Table 2. FMA spore density at various soil depths under Sungkai plant stands.

Location	Depth	Repli cation	Total Spores	Number of Spores	Spores Average
		1	52		
MK	20	2	38	145	48
		3	55		
		1	30		
MK	40	2	44	103	34
		3	29		
		1	25		
MK	60	2	30	89	30
		3	34		
		1	44		
PK	20	2	43	118	39
		3	31		
		1	19		
PK	40	2	25	68	23
		3	24		
		1	25		
PK	60	2	23	67	22
		3	19		

Spore density in each research location has an average number that varies. From the results obtained, the higher the depth, the lower the spore density. The highest FMA spore density in each research location was found in the M.K location at a depth of 20 cm with an average of 48 spores found per 50 gram soil sample, followed by P.K location at a depth of 20 cm with an average of 39 spores found per 50 gram soil sample, M.K location at a depth of 40 cm with an average of 34 spores found per 50 gram soil sample, M.K location at a depth of 60 cm with an average of 30 spores found per 50 gram soil sample, P.K location at a depth of 40 cm with an average of 23 spores found per 50 gram soil sample, and P.K location at a depth of 60 cm with an average of 22 spores found per 50 gram soil sample.

The variation in FMA spore density that occurs is related to the distribution of spores at the soil sampling location and the different levels of soil sampling depth. The highest spore density difference was found at a depth of 20 cm. This happens because at that depth, decomposition is still actively working and soil conditions are not too dense. The results of research by (Khairuna et al., 2015) revealed that the provision or addition of organic matter creates favorable conditions for the development and infectious power of mycorrhiza. Organic material provides carbon for the development of mycorrhizae and other organisms. The highest spore abundance is generally found in the surface layer of the soil and decreases with the depth of the layer. Hermawan et al. (2015) stated in their research that soil conditions that are increasingly dense and have a lot of water content will cause the soil to lack oxygen so that the space for FMA spore growth is reduced. Decrease in oxygen can inhibit the development and colonization of FMA spores. Delvia & Rambey (2017) said that reduced oxygen levels in the soil with increasing soil depth can inhibit the spread of arbuscular mycorrhiza to deep soil layers. The arbuscular mycorrhizal spores found in the deepest soil layer (> 60 cm) are probably spores carried by water infiltrating into the lower layers.

FMA Genus Abundance

The abundance of FMA genus obtained from each PT Mekar Agro Sawit research site in the sungkai (*Peronema canescens* Jack) planting area can be seen in Table 3.

Table 3. Abundance of FMA genus at various soil depths under Sungkai plant stands.

Locatin	Number of genus	Genus	Number of species	Number of spores
MK 20	2	Glomus	27	132
		Acaulospora	6	13
MK 40	3	Glomus	14	91
		Acaulospora	4	9
		Entrospora	1	3
MK 60	2	Glomus	10	80
		Acaulospora	3	9
PK 20	4	Glomus	17	98
		Acaulospora	1	9
		Gigaspora	3	9
		Scutellospora	1	2
PK 40	3	Glomus	12	55
		Acaulospora	3	8
		Gigaspora	2	5
PK 60	2	Glomus	15	59
		Acaulospora	2	8

Table 3 above shows that the identified genus varies greatly in each research location. FMA spores of the genus Glomus and Acaulospora were the most common FMA genus found in each research location, Gigaspora in two research locations, Entrospora and Scutellospora were only found in one research location. Arbuscular mycorrhizal fungi (FMA) under sungkai (*Peronema canescens* Jack) stands were found 27 species of the genus Glomus, 6 species of the genus Acaulospora, 3 species of the genus Gigaspora, 1 species of the genus Scutelospora and Entrospora. The Glomus genus is the most common genus found in every soil sampling under sungkai (*Peronema canescens* Jack) then followed by the genus Acaulospora, Gigaspora, Scutellospora, and Entrospora.

The high number of glomus genus found in each research location is due to the adaptive nature of Glomus to various environmental conditions. This condition is in line with the results of research conducted by Miska et al. (2016) that the glomus genus dominates the most from soil samples in the rhizosphere of sugar palm plants. The glomus genus has a high level of adaptation and has a wide host range (Muryati et al., 2016). Kurnia & Larekeng, (2019) stated that the Glomus genus has the widest distribution, followed by the Gigaspora genus, while for the Acaulospora and Scutellospora genus the distribution is still limited.

FMA Spore Frequency

The frequency of FMA spores obtained from each PT Mekar Agro Sawit research site in the sungkai (*Peronema canescens* Jack) planting area can be seen in Table 4.

Table 4. Frequency of FMA spores at various soil depths under Sungkai plant stands.

Genus	Research location					
	M.K. 20	M.K. 40	M.K. 60	P.K. 20	P.K. 40	P.K. 60
						(%)
Glomus	100	100	100	100	100	100
Acaulospora	66	66	66	33	66	66
Entrospora	0	33	0	0	0	0
Gigaspora	0	0	0	66	66	0
scutellospora	0	0	0	33	0	0

The table above shows that each research location has a different frequency. The FMA genus that has a frequency of 100% found in each research location is from the glomus genus. Acaulospora genus with a frequency of 66% in five research locations, 33% in one research location. Entrospora genus was only found in one research location with a frequency of 33%. Gigaspora genus in two research locations with a frequency of 66%. The scutellospora genus was found only in one research location with a frequency of 33%.

The glomus and acaulospora genus are the most commonly found genus in each research location because these two genus can adapt to very acidic conditions. In the table above, it can be seen that in each location with a depth of 20 cm there are all genus, this is because the soil at a depth of 20 cm is rich in soil nutrients and at this depth there are many roots so there is a possibility that plant roots are infected with mycorrhiza, this is what affects the density of the number of spores and genus (Rasyid et al., 2016). The tendency to decrease the spore population occurs at depths above 30 cm due to a decrease in organic matter content (Yusril & Burhanuddin, 2018).

The frequency of FMA spores is related to the distribution of spores at the soil sampling location, the number of FMA spores is also influenced by temperature and humidity, low temperatures can affect the development of FMA spores. Temperature and humidity at each research location are presented in Table 5.

Table 5. Temperature and Humidity at Each Research Location

Location	Temperature (°C)	Humidity (%)
P.K	28.2	71.8
M.K	29.8	70

The temperature in the two research locations ranged between 28,2°C -29,8°C, at this temperature FMA spores are still able to develop well because the temperature is still above 5°C and below 35°C. FMA development decreases when the temperature is above 35°C and can kill FMA if it exceeds this temperature. Most mycorrhizal fungi require temperature optimum for the formation and survival of mycorrhiza. Glomus found in the root hairs of bitti, tanjung and kecrutan trees at air temperatures of 30-32.6°C, air humidity 58-78% and soil humidity 40-72% (Ura et al., 2015).The best air temperature for the development of arbuscular mycorrhiza is around 30°C, for mycelium colonization on the root surface 28°-34°C and for sporulation and vesicle development at 35°C

Conclusion

This research concludes that 5 genus of FMA were found in the location of Sungkai Planting Area (Peronema canescens Jack.). The 5 Genus consisted of Glomus, Acaulospora, Entrospora, Gigaspora and Scutellospora. The more dominant spore type found at all levels of soil depth (0-20 cm, 20-40 cm, and 40-60 cm) is Glomus sp, while the Entrospora and Scutellospora spore types are only found at one soil depth each.

Soil depth will reduce the number of FMA spores where the highest number of spores is obtained at a soil depth of 0-20 cm at 48 per 50 g of soil, while the lowest number of spores is found at a soil depth of 40-60 cm at 30 per 50 g of soil.

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Author Contributions

Each autor contributed in some way to the project's completion. All author decided on the study materials, fundamental ideas, and research methods. Subsequently, all autors share responsibility for data collection, data analysis, the review process and paper writing.

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Conflicts of Interest

No conflict interest.

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