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Arbuscula Mycorrhizal Fungi on The Rhizosphere Three in The Ex-Sand Mine at Cipancur-Kuningan West Java

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Abstract: *Sand and stone mining activities in Kuningan Regency cause damage to soil and environmental ecosystems, so reclamation and revegetation are carried out. The purpose of this study is to determine the existence of indigenous / local Arbuscular Mycorrhizal Fungi (FMA) found in the rhizozofer stands of Sengon (Paraserianthes falcataria) and Acacia (Acacia mangium) which are revegetation plants on the land of former sand and stone mines Cipancur village, Kalimanggis district, Kuningan regency. Sampling using the composite sampling method, FMA isolation technique using the wet pour method is then continued with the centrifugation technique and root colonization observation technique using the root staining technique (staining). The temperature of the ex-mining soil ranges from 28C – 29C, the soil pH is 5.5 including acidic, the soil moisture varies between wet, medium (slightly wet) and dry (dry) but during the rainy season the soil will become wet and even flooded. The results showed that there were 5 genera, it are Acaulospora, Glomus, Gigaspora, Sclerocytis and Scutellospora. The dominant genus is Glomus. The highest spore density was found in Sengon stands with 1,157 spores per 50 grams of soil. The highest percentage of AMF infection was at the root of Sengon stands at 69% from the ex-sand mining area.*

Keywords: *FMA; Sand Mining; rhizosphere*

1. INTRODUCTION

Mining is an activity that utilizes natural resources. Mining activities cause damage to the surrounding environment or reduce biodiversity. One of them is the decline in soil productivity and plant growth that grows around mining areas (Sihombing et al., 2015). Prevention and reduction of damage must be immediately carried out in former mining areas by carrying out land rehabilitation. The impact of mining is a decrease in soil quality, water and air pollution, climate change, topography, and hydrogeology. Damaged and critical land will experience a decrease in the physical, chemical, and biological properties of the soil (Sihombing et al., 2015).

The damage to the physical and chemical properties of the soil is caused by the excavation of the top layer of soil which is then removed until the excavation reaches the desired layer of mining material (Herjuna, 2011). Ex-mining sandy soil has a rough and loose texture because it is dominated by sand fractions that are lifted upwards, has low water retention capacity, and low nutrient and organic material content due to the top layer. Vegetation (trees) or even grass covering the land is lost, so that the land becomes difficult to grow plants like in the beginning before mining (Prasetyo et al., 2019)

Kuningan Regency is one of the districts that has many sand mines. There are 13 mining entrepreneurs in Kuningan Regency. The majority are in East Kuningan, such as Cidahu, Kalimanggis and Luragung Districts (Prianto, 2019).

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One of them is a sand excavation mine or group C mine located in Cipancur Village, Kalimanggis District, Kuningan Regency. To reduce the negative impacts that occur and so that the former sand mining land can be reused, revegetation activities are carried out. Revegetation is one way to speed up the rehabilitation process on mining land which has physical and chemical soil properties that do not support plant growth (Anggreiny et al., 2017). On former sand mining land in Cipancur Village, revegetation activities utilize plants including Acacia (*Acacia mangium*) and Sengon (*Paraserianthes falcataria*). Acacia and sengon plants are appropriate plants to plant on crisis land (Anggreiny et. al., 2017).

One factor that greatly influences plant growth is soil. Fertile soil is determined by the existence of a mutually beneficial relationship between roots and fungi which are usually called mycorrhiza (Kurnia et al, 2019). Arbuscular Mycorrhizal Fungi (AMF) are soil microorganisms that have an important role in an ecosystem, including marginal land. Mycorrhizal fungi play an important role in facilitating the absorption of nutrient elements by plants, increasing growth and yield of plant products (Upadhayaya et al., 2010). AMF encourages plant growth on low soil fertility levels, degraded land and AMF hyphae help expand the function of the root system in obtaining nutrients (Garg and Chandel, 2010). Therefore, the role of mycorrhiza in helping revegetation of sand mining land is important.

Objective

The aim of this research was to determine the genus of AMF and the percentage of root infection in the rhizosphere of acacia (*Acacia mangium*) and sengon (*Paraserianthes falcataria*) plants on ex-sand mining land in Cipancur Village, Kalimanggis District, Kuningan Regency.

2. RESEARCH METHOD

This research was carried out on former sand mining land belonging to Cipancur Village, Kalimanggis District, Kuningan Regency and at the Silviculture Laboratory, Faculty of Forestry, Kuningan University. This research was conducted from November 2021 to December 2021.

Tools and materials

The tools used in this research for taking soil and plant root samples were GPS, plastic bags, hoes, and markers. The tools used for observations in the laboratory are graduated sieves with sizes of 1 mm, 425 µm, 106 µm, 45 µm and 0.0308 µm. Centrifuge, test-tube, test-tube rack, computer, digital microscope, digital probe, tube needle, dropper, tweezers, preparation dish, petri dish, spray bottle, measuring cup, back mirror, small bucket, gloves. Soil testing tool to collect pH, temperature, and soil moisture data.

The materials used in this research were soil and root samples from stands of acacia (*Acacia mangium*) and sengon (*Paraserianthes falcataria)* trees, 60% granulated sugar solution, 10% KOH, 2% HCL, Aquades.

Working Method

Sengon and acacia located in a former sand mine in Cipancur Village, Kalimanggis District, Kuningan Regency, are known to be 8 months old. Soil and root samples were taken from the rhizosphere of sengon and acacia by taking 5 soil samples from each tree, consisting of 5 acacia trees and 5 sengon trees. Each soil and root sample was taken at 4 repetition points with a depth of 0 – 20 cm and a hole diameter of 20 cm with a distance of 3/4 from the outermost canopy.

Spore Isolation

Spore isolation was carried out using the wet pour technique (Pacioni, 1991) and continued with the centrifugation technique (Brundrett et al. 1996).

Steps to isolate spores:

1. A 50-gram sample of soil that has been mixed with water is stirred and left for a few minutes so that the heavy particles that make up the soil settle.
2. Pour the soil sample into a graduated sieve with sizes of 1 mm, 425 µm, 106 µm, and 45 µm respectively from top to bottom.
3. Soil samples that were filtered and retained on a 45 µm sieve were transferred into 8 centrifuge tubes by spraying them using a water spray. Insert the centrifuge tube then

Quagga
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spin using centrifuge for 3 minutes at a speed of 2000rpm so that the heavy particles settle.

4. The centrifuged soil suspension was filtered again using a 0.0308 µm sieve, then transferred back into 6 centrifuge tubes and added with a sugar solution, using the modified centrifuge method from (Ansiga et al., 2017) with the addition of sugar. centrifuge to release spores from pelleted soil particles and centrifugation again.
5. Pour the suspension back into a 0.0308 µm sieve and rinse gently with a little water then filter through filter paper so that all spores are retained in the filter paper.
6. Then the filter paper was transferred to a petri dish to be observed under a digital microscope.

Observation of root colonization

Observation of the colonization of Arbuscula Mycorrhizal Fungi on sample plant roots was carried out using the root staining technique. To allow root colonization to be clearly visible, roots were cleared and stained with trypanblue and observed under a microscope.

Procedure:

1. Root processing – take young root samples from five points on the root system. Wash clean then cut into 1 cm long pieces. Weigh 2 g each of Purification and staining without repeated heating and put it in a test tube.
2. Clarity and color without heating
   This method takes longer than heating dyeing. The coloring process is as follows. This method requires more time than heating and dyeing. The coloring process is as follows:
   - Clean the plant roots in 20% KOH solution in 1 – 3 days.
   - Pour in the KOH solution, then rinse the roots with tap water until clean. Then acidify the roots by adding 0.1M HC1 solution.
   - Pour the HC1 solution, then apply the anilie blue dye solution and leave for 1-3 days.
   - Pour the remaining dye solution into a special glass container for collection.
   - Apply the color washing solution to each test tube, then leave it overnight so that the dye absorbed by the roots has a chance to dissolve into the color washing solution.
3. Microscopy and establishment of % colonization
   - Cut the roots 1 cm long, place them in a row on a glass containing 7 pieces. Then cover with a glass of preparation. Make 10 preparations as a test.,
   - Root sections were observed under a stereo microscope of the mycorrhizal root segments of each root containing AMF ornaments marked with mycorrhizal roots.

Data analysis

The data obtained was analyzed descriptively and data was displayed in the form of identification results of arbuscular mycorrhizal fungi starting from spore morphology based on: color and shape. Spore morphology was identified using. Calculation of spore density (Koske, 1987), as well as calculation of the percentage of AMF infection on plant roots (Ansiga et al., 2017) is calculated using the formula:

1. Spore Density = $\frac{\sum \text{spore}}{\text{Soil weight}}$

2. Infected roots (%) =

$$\frac{\Sigma \text{ infected roots}}{\Sigma \text{ the whole observed root}} \text{ x } 100\ \%$$

3. RESULTS AND DISCUSSION

Table 1. Results of analysis of soil physical properties at the former sand mining site in Cipancur Village.

Sampel	Suhu Tanah	Ket*	pH	Ket*	Kelembapan Tanah	Ket*
Akasia 1	28°	Optimum	5,5	Asam	1 (wet)	Basah
Akasia 2	28°	Optimum	5,5	Asam	1 (wet)	Basah
Akasia 3	28°	Optimum	5,5	Asam	1 (wet)	Basah
Akasia 4	28°	Optimum	5,5	Asam	1 (wet)	Basah
Akasia 5	28°	Optimum	5,5	Asam	1 (wet)	Basah
Sengon 1	28°	Optimum	5,5	Asam	1 (wet)	Basah
Sengon 2	28°	Optimum	5,5	Asam	1 (wet)	Basah
Sengon 3	29°	Optimum	5,5	Asam	1 (wet)	Basah
Sengon 4	28°	Optimum	5,5	Asam	1 (wet)	Basah
Sengon 5	28°	Optimum	5,5	Asam	1 (wet)	Basah

Data collection on environmental parameters was taken from a soil depth of 20 cm. Seen from table 1 above, the soil temperature ranges between 28°C – 29°C with the highest temperature found in the Sengon stand reaching 290C. The soil pH in each stand has the same pH, namely 5.5, which is included in the acid category, this pH is caused by a lack of nutrients in the soil. Soil moisture

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consists of 3 levels, namely 1 wet (wet), 2 medium (slightly wet), and 3 dries (dry). Meanwhile, in the table above, the results for each stand are at level 1 wet.

Soil sampling at the research location was carried out during the transition from the rainy season to the dry season with the soil temperatures obtained ranging from 28^0C – 29^0C. During the rainy season, soil moisture will also be high, which will have an impact on soil temperature. If the soil temperature is low, AMF spores cannot germinate. AMF can develop at a temperature of 30°C, according to (Prasetyo et al., 2019) a temperature of 15°C-30°C is apparently good for spore production.

Judging from the results of table 1 above, it can be concluded that the temperature and humidity of the soil in the former sand mine are at normal levels, but the low pH still causes the soil to become less fertile in the area.

Mycorrhiza in Soil Samples

The observation results showed that the mycorrhizal spore genera found at the research location were the *Acaulospora, Glomus, Gigaspora, Sclerocytis* and *Scutellospora genera*. The five of them have different morphological characteristics. There are 2 types of *Acaulospora* variations found with different characteristics, some are yellowish brown, and some are bright yellow, *Glomus* has 13 different shape and color variations, **Gigaspora** has 4 different shape and color variations, *Sclerocytis* and *Scutellospora* has only 1 variation. The *Sclerocytis* genus is not found in the rhizosphere of sengon and is only found in the rhizosphere of acacia plants, it is oval shaped, yellow in color, the spores are clustered, round which functions as a support for the spores in the hyphae.

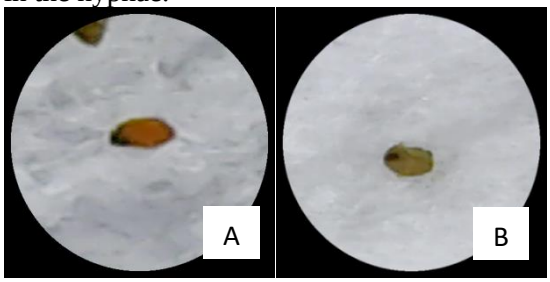

Figure 1 . The genus *Acaulospora* is found in acacia (a) and sengon (b) plants.

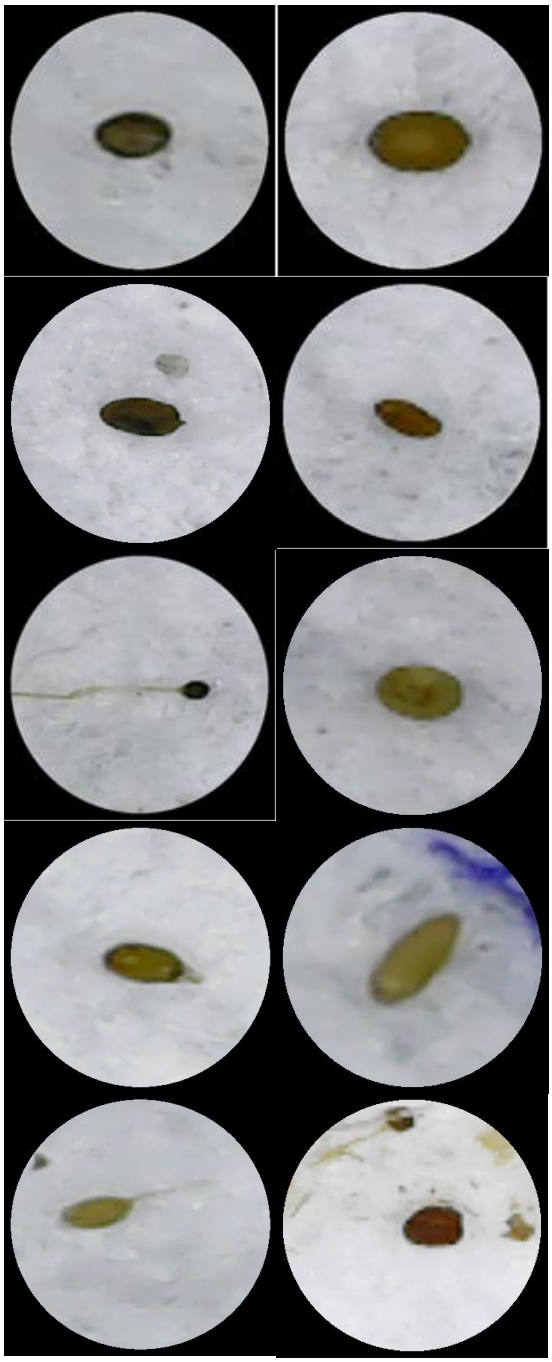

Figure 2. The *Glomus* genus is found in acacia and sengon plants

Quagga
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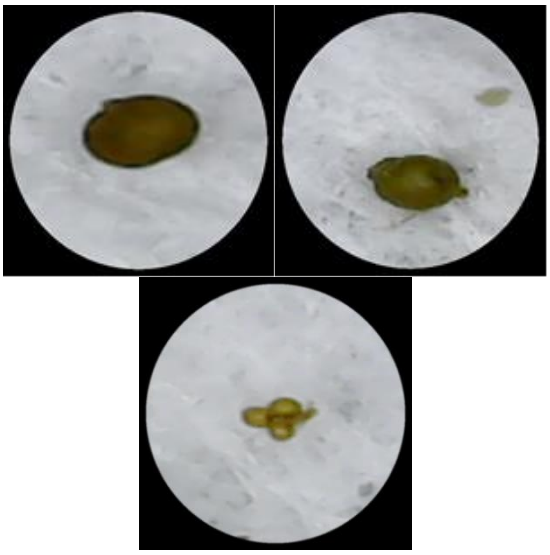

Figure 3. The *Glomus* genus is found in acacia trees

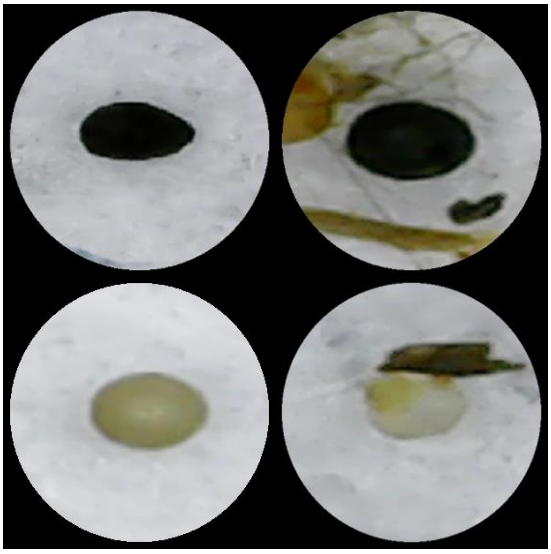

Figure 4. The *Gigasopra* genus is found in acacia and sengon plants

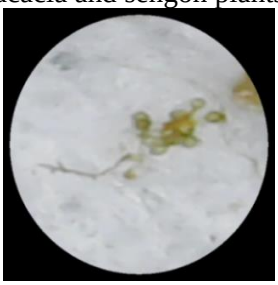

Figure 5 . The *Sclerocytis* genus is found in acacia

Figure 6. The *Sclerocytis* genus is found in acacia and sengon plants

Abundance of AMF

Table 2. AMF genus names and their abundance (per 50 grams of soil) in each sample plant.

NO.	Genus Name	Plant Type: Acacia	Plant Type: Sengon
1.	*Acaulospora* sp.	4	10
2.	*Gigaspora* sp.	106	117
3.	*Glomus* sp.	868	1,024
4.	*Scutellospora* sp.	5	5
5.	*Sclerositis* sp.	1	-
	Amount	984	1,157

From Table 2, based on the results of identifying AMF spores, the most common spores were found in sengon plants with the number of spores reaching 1,157. In acacia plants, 5 genera of AMF were found, while in sengon plants, 4 genera of AMF were found. Of the five genera found, *Glomus* was the genus with the most spores found with 1,892 individuals. Apart from *Glomus*, there are genera found in each sample, namely the genera *Acaulospora*, *Gigaspora* and *Scutellospora*. There is a genus that is only found in one plant, namely the *Sclerocytis* genus which is only found in acacia plants which consists of 1 individual.

Glomus is the most found genus with characteristics of round, slightly rounded, irregular, and oval shapes or with spore walls consisting of more than one layer. The color of the spores of the *Glomus* genus varies from brownish yellow, brownish brown, light brown, to dark blackish brown. This shows that the *Glomus* genus has a high level of distribution and adaptation to extreme environments compared to other genera. According to Wanda et al. (2015), *Glomus* genus spores are the most dominant spore genus found in several ecosystem conditions,

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because this type of ecosystem has a wide host range. In this study, 5 AMF genera were found from acacia plants and 4 AMF genera were found in sengon. This result was higher than in research conducted by Anggreiny et al. (2017), which was carried out in a tin mining area from acacia plants, found 4 genera of AMF and 3 genera of AMF in sengon. More results from research by Khoirunnisa (2015) who found 5 AMF genera associated with sengon in peatlands. Thus, differences in land influence the number of genera found. According to Sundari (2011) the presence of AMF in an area is influenced by environmental factors and soil type.

The soil characteristics at the sampling location of the former sand mining land, Cipancur Village, Kalimanggis District, Kuningan Regency, are clay and the soil pH is in the acidic range. This is in accordance with the opinion of (Lica et al., 2022) who stated that soil samples dominated by the clay fraction are very appropriate for the life of *Glomus* spores. The pH acidity at the research location is 5.5 which is included in the acid category. This shows that the soil acidity value is generally not good for plant growth. The population and diversity of AMF in acid mineral soils in Indonesia is quite high, but is generally dominated by the genera *Glomus*, *Acaulospora, Gigaspora* and *Scutellospora*.

Structure of AMF colonization in the roots

The results of staining using trypan blue AMF structures, both on sample plant roots and microscope observations, revealed AMF structures, namely hyphae, arbuscules and vesicles. The hyphal structures were found to be long like threads, the arbuscular structures were found to be lumpy and dark in color, and the vesicular structures were found to be round and blue in color because these structures absorbed the trypan blue dye solution.

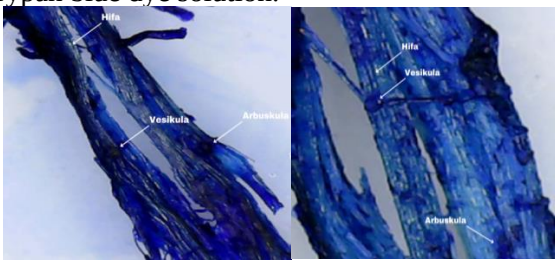

Figure 7. AMF colony structure on the roots of Acacia (a) and Sengon (b) plants.

Based on Figure 7, in the roots of the samples of acasia and sengon plants, AMF structures were found, namely hyphae, arbuscules and vesicles. Thus, both sample plants were infected with AMF, this shows that there is a relationship between AMF and revegetation plants in post-sand mining land.

According to Karyaningsih et al. (2022) AMF is characterized by the presence of vesicles and arbuscules. These two structures are very important in the life of FMA. In the root tissue, mycorrhiza forms arbuscules which function as a place of exchange between the fungus and the roots of the host plant. Arbuscules are branching hyphae that enter the host plant cells. Arbuscula are finely branched hyphae that are formed from repeated dichotomous branching that resembles a tree in the host cell (Suharno et al. 2020). The entry of nutrients into host plant cells will be followed by an increase in cytoplasm volume, formation of new organs, division of the cell nucleus, increased respiration, and enzyme activity. Then the vesicle is a structure that originates from swelling of intraradical hyphae terminally and intercalally.

According to Suharno et al. (2020) the working principle of mycorrhiza is to infect the root system of the host plant, producing intensive hyphae so that plants containing mycorrhiza will be able to increase their nutrient absorption capacity. Other facts show that there is a large contribution of AMF to plant N absorption and assimilation. External hyphae play a role in increasing plant efficiency in absorbing and translocating nutrients and soil water.

Percentage of AMF infections

Staining technique, the average percentage of infection in each sample plant can be seen in Figure.

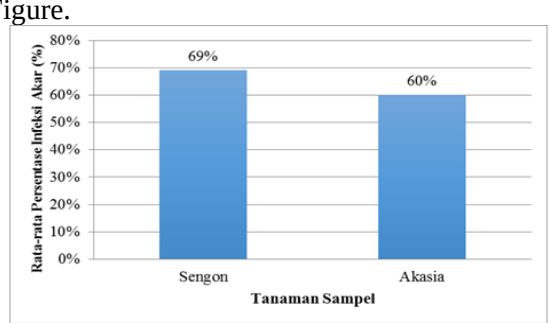

Figure 8. Average Percentage of Mycorrhizal Infections at PT

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Examples of Sengon and Acacia Standing Roots
>75 %: high infection rate
51-74 %: moderate infection level
<50 %: low infection rate (Setiadi and Setiawan, 2011)

Based on Figure 8, root AMF infection has occurred in the sample plants with different percentages. One of the differences in the percentage of root colonization is influenced by the type of host plant. The highest infection was found in sengon plants (*Paraserianthes falcataria*) and the lowest in acacia plants (*Acacia mangium*). Both sample plant roots had root infections that were included in the moderate category.

After stning was carried out on the two types of sample plants, AMF structures were found, namely hyphae, vesicles and arbuscules in the two sample plants, which can be seen in Figure 22. Sampling of acacia and sengon roots was carried out in the rainy season. According to Delvian and Elfianti (2016), in high rainfall conditions the percentage of colonization generally increases, and spore formation decreases, whereas in the dry season the formation of new spores will increase. The results of calculating the percentage of infection in the roots showed that the plant with the highest infection rate was the sengon plant with a percentage of 69% and the lowest was the acacia plant with an infection percentage of 60%. Each type of plant has a different response to AMF according to its characteristics.

According to Smith and Read (2008) the most important structures in the symbiotic system with plant roots are the arbuscules which are in the roots. Arbuscula are structures formed by internal hyphae and connect the fungus to the root cortex cells. This structure plays a role in the movement of nutrients from fungi to plants and vice versa.

Based on the results of research at a former sand mining location in Cipancur Village, Kuningan District, Kuningan Regency, it was found that the highest number of spores was on sengon plants with a number of 1,157 spores per 50 grams of soil with an average percentage of root infection of 69% and the lowest was on aasi plants with a number of 984 spores per 50 grams of soil with an average root infection percentage of 60%. The presence of several spores will increase the chance of root infection, but the number of spores does not necessarily indicate the diversity of the AMF genus at the location depending on the suitability of the association.

4. CONCLUSION

In the rhizosphere of *sengon*, *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora* were found, while in the rhizosphere of *acacia mangium*, *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocytis* and *Scutellospora* were found. The genus *Sclerocytis* is only found in the rhizosphere of acasia. The most dominant genus in ex-sand mining land is Glomus.

The percentage of AMF infection in the roots of acacia and sengon trees in ex-sand mining land is moderate. The highest infection was obtained on sengon trees with an infection percentage of 69% and the lowest was on acacia trees with a percentage of 60%.

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