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EXPLORATION OF INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI ON *ARENGA PINNATA* MERR IN POST-MINING LAND

EKSPLORASI FUNGI MIKORIZA ARBUSKULA INDIGENOS PADA Arenga pinnata *MERR DI LAHAN PASCATAMBANG*

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ABSTRACT

Mining activities have a positive impact that can generate income for the state, but the activities also cause negative impacts in the form of soil damage, vegetation and animal losses to disrupt the ecosystems. Therefore, it is necessary to carry out a reclamation with revegetation using local plant species. PT Berau Coal has conducted revegetation using a local sugar palm plant (*Arenga pinnata).* The success of sugar palm plant growth in post-mining reclamation land is influenced by several factors, one of it is the symbiosis of the arbuscula mycorrhizal fungi (AMF) with the sugar palm planted by PT Berau Coal. The purpose of this study was to determine the diversity of indigenous spores in the arenga rhizosphere and root colonization of sugar palm plants. Identification of the AMF diversity was carried out by observing the soil taken from the palm rhizosphere with a depth of 0-20 cm and 20-40 cm. Isolation of spores using the wet pouring technique method with centrifuges and AMF spores were identified using the INVAM methods. The observation of AMF colonization on plant roots was carried out through the root staining technique with the Clapp modification method. The results showed that the AMF spores were found in 3 AMF genera at the observation site, namely genus *Glomus* (15 sp), *Acaulospora* (3 sp), and *Gigaspora* (1 sp). The highest spore abundance is genus *Glomus* sp at a soil depth of 0-20 cm. The AMF structures found colonizing the roots of sugar palm plants are hyphae, vesicles, and spores.

Keywords: indigenous AMF, revegetation, root colonization, spores, sugar palm plant.

ABSTRAK

Kegiatan pertambangan memiliki dampak positif yang dapat menghasilkan pendapatan bagi negara, namun juga menimbulkan dampak negatif berupa rusaknya tanah, hilangnya vegetasi dan satwa hingga mengganggu ekosistem, oleh karena itu perlu dilakukan reklamasi dengan revegetasi menggunakan jenis tanaman lokal. PT Berau Coal telah melakukan revegetasi menggunakan tanaman lokal aren (Arenga pinnata). Keberhasilan pertumbuhan tanaman aren pada lahan reklamasi pascatambang dipengaruhi oleh beberapa faktor, salah satunya adalah adanya simbiosis fungi mikoriza arbuskula (FMA) dengan tanaman aren yang ditanam oleh PT Berau Coal. Tujuan penelitian ini adalah untuk mengetahui keanekaragaman spora indigenous pada rizosfer aren dan kolonisasi akar pada tanaman aren. Identifikasi keanekaragaman FMA dilakukan pengamatan pada tanah yang diambil dari rizosfer aren dengan kedalaman 0-20 cm dan 20-40 cm. Isolasi spora menggunakan metode teknik tuang saring basah dengan sentrifugase dan spora FMA diidentifikasi dengan metode INVAM. Pengamatan kolonisasi FMA pada akar tanaman dilakukan melalui teknik pewarnaan akar (staining) dengan metode modifikasi Clapp. Hasil penelitian menunjukkan bahwa spora FMA yang ditemukan di lokasi pengamatan ada 3 genus FMA yaitu Glomus *(15 sp),* Acaulospora *(3 sp) dan* Gigaspora *(1 sp) dengan kelimpahan spora terbanyak adalah genus* Glomus *sp pada kedalaman tanah 0-20 cm. Struktur FMA yang ditemukan mengkolonisasi akar tanaman aren adalah hifa, vesikula dan spora.*

Kata kunci: FMA indigenos, revegetasi, kolonisasi akar, spora, aren.

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INTRODUCTION

Indonesia is a country that is rich in natural resources, both living and non-biological natural resources**,** including a variety of abundant deposits of mining materials, such as coal, nickel, gold, bauxite, and iron so the mining sector has been the largest contributor to Indonesia's development for more than 30 years (Agus *et al.*, 2014). Mining activities generally use a large area of land which has a destructive effect on land, vegetation, and animals. The negative impacts caused by land damage and loss of vegetation include increased erosion, decreased soil fertility, low or even very low soil pH, low water holding capacity, salinity, coarse texture, inadequate supply of nutrients to plants, erosion, and the presence of acid generating materials formed from the presence of Acid Mining Water, causing disrupting of the environmental ecosystem (Puspanti, 2013; Herlina, Handayani and Iskandar, 2014).

To reduce the negative impact of mining activities, it is necessary to do reclamation. One of it is revegetation. Reclamation is defined as an activity carried out throughout the stages of the mining business to organize, restore, and improve the quality of the environment and ecosystem so that it can function again according to its designation (Menteri Energi dan Sumber Daya Mineral, 2018).

PT Berau Coal (BC) is a mining company, especially coal, located in Berau District, East Kalimantan. PT BC has carried out post-mining reclamation by planting forest types; one of it is sugar palm (*Arenga pinnata*). The sugar palm is a multi-use local plant that is widely developed by the people of East Kalimantan, such as in the areas of Samarinda, Balikpapan, Bontang, Paser, North Penajam Paser, Kutai Kartanegara, East Kutai, West Kutai, Berau, and Mahakam Hulu.

Rehabilitation of damaged post-mining land is a holistic activity with careful planning. So that the problems in the mining areas can be recovered. The use of the alternative treatments and the use of technology can help the plant growth on post-mining critical lands as the use of the local plants that are adaptable and the application of microorganisms such as arbuscular mycorrhizal fungi (AMF) indigenous revegetation plants.

The AMF as soil microorganisms are the key to facilitating the absorption of nutrients by plants (Suharno *et al.*, 2014). 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 and increase the growth as well as yield of plant products. On the other hand, fungi obtain assimilated energy from plants. Although the AMF symbiosis with plants on a fertile land does not have much positive effect, in extreme conditions it can increase most plant growth (Harley & Smith, 1983).

The AMF can be found in most soils and generally does not have a specific host plant. The level and composition of the population vary greatly and are influenced by plant characteristics and environmental factors such as temperature, soil pH, soil moisture, phosphorus, and nitrogen as well as heavy metal concentrations. Thus, each ecosystem is likely to contain AMF of the same or different types. In the revegetation process, indigenous AMF is considered better than using exogenous AMF.

Indigenous AMF is a species found naturally associated with the plant roots without a human intervention in the initial infection process between mycorrhizae and host plants. Indigenous AMF has a high potential to form extensive infections because it is more adaptive and has a higher tolerance for environmental conditions with extreme conditions. AMF exploration and identification is an important step in a reclamation strategy, particularly on post-mining soils. This study aimed to examine the AMF found in the rhizosphere of sugar palm (*Arenga pinnata*) in the post-mining land of PT Berau Coal, East Kalimantan.

METHOD

The research was conducted for 5 months (April – August 2019) at post-mining reclamation site of Binungan I PT Berau Coal, namely the disposal site for IPD (*in pit dump*) D6, IPD C3.1, IPD C3.2, IPD D2, and IPDK K, East Kalimantan. The observation of root and mycorrhizal analysis from soil was carried out at the Silviculture Laboratory, Department of Silviculture, Faculty of Forestry, IPB University, while the identification stage was carried out at the PPSHB Forest Biotechnology Laboratory, IPB University.

The implementation of research activities began with taking soil and root samples, staining and observing roots, isolating and identifying AMF spores. This activity uses the root staining and the wet filter pour methods.

Root Staining and Observation

Observation of AMF root colonization on plant roots was carried out using a modified root staining method (Kurnia, Gusmiaty and Larekeng, 2019). This method is carried out in several stages. The first one begins with the roots that have been separated from the soil and washed thoroughly. In the second stage, the roots were soaked with 10% KOH for ± 2-3 days. Soaking with KOH was carried out to remove all cytoplasmic contents from the root cells so that it would be easier to observe the colonization structure of AMF. In the third stage, the roots were rinsed and soaked again into 2% HCl for ±10 minutes. The fourth stage is the roots that have been cleaned by 2% HCl given liquid with trypan blue and soaked for ± 2-3 days. After that the trypan blue was removed, then the roots were washed and were soaked with 50% glycerin solution for the color reduction process.

Root observations were carried out by cutting the roots around 1 cm long, then the roots were arranged on preparation and covered with a covered glass. The number of roots of each preparation as much as 10 pieces were made per sample with 4 replications. Root infection can be seen through the presence of vesicles, arbuscules, hyphae, and spores that infect roots. The percentage of mycorrhizal infections in a root or root colonization (RC) was calculated by the formula (Birhane *et al.*, 2017):

$$\% \text{ RC} = \frac{\sum \text{colonized field of view}}{\sum \text{overall field of view}} \text{x} 100\% \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Isolation and Characterization of the AMF Spore Types

Isolation of indigenous AMF was carried out by taking soil samples from the sugar palm rhizosphere zone as much as ± 500 g in the composite form at a depth of 0-20 and 20-40 cm. The samples were then wrapped by aluminum foil and put into a plastic and labeled according to the location of the soil collection and the time of the collection. The spore-type characterization of AMF was carried out by extracting and identifying spores from soil samples using a wet-filtered pouring technique with modified centrifugation (Ervayenri, 2020).

This pour-filter technique is conducted first by mixing as much as 20 g soil sample with 200 - 300 ml water. The mixture was stirred until the soil grains were crushed, then filtered in a set of sieves with sizes of 500 mesh, 125 mesh, and 63 mesh, respectively. The water is then sprayed in position from the top of the filter to make it easier for spores to escape. The remaining soil left on the 125 mesh and 63 mesh sieves was transferred to a centrifuge tube.

Spores were isolated by pouring, filtering and centrifugation techniques. The filter results in the form of supernatant added with 60% sucrose by weight/volume and then centrifuged at 3000 RPM for 2-3 minutes. The supernatant solution was poured into a 45 µm filter paper. The precipitate that has been filtered is placed in a petri dish, and then prepared for microscopic observation of density, as well as characterizing the existing AMF spores. Healthy spores are selected and placed on slides that have been treated with Meltzer solution and/or polyvinyl alcohol-lactic acid-glycerol (PVLG).

Spores were determined at the genus and species level. The identification of AMF spores carries out under a microscope at the Mycorrhizal Technology Laboratory, Department of Silviculture, Bogor Agricultural University. The nomenclature of AMF spores follows the pattern published by Schüßler and Walker (2010) and Redecker *et al.* (2013). Identification of morphological types based on scientific literature available online such as International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu and other literature).

Observation

The parameters observed in this study were (1) spore density, (2) relative frequency of spores (RF), (3) relative abundance of AMF genus (RA) (Shi *et al.*, 2007):

$$\text{Spore density} = \frac{\text{number of spores}}{\text{20 grams of soil}} \quad \ldots\ldots\ldots\ldots (2)$$

$$\text{RA } \% = \frac{\text{number of genera}}{\text{total spores}} \text{ x} 100\% \quad \ldots\ldots\ldots\ldots (3)$$

$$\text{RF} = \frac{\text{number sample found}}{\text{total samples}} \text{x} 100\% \quad \ldots\ldots\ldots\ldots (4)$$

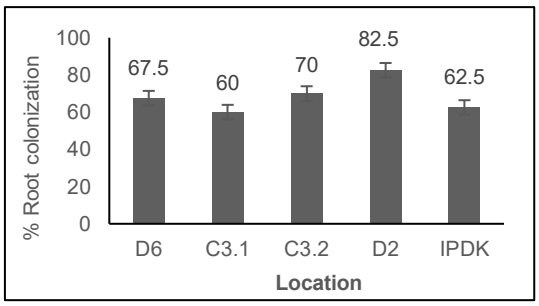
Data analysis

Spore abundance data were analyzed by Analysis of Variance (ANOVA) to see the effect of each sample location. If the test results show a significant effect, then the Tukey test treatment difference test is carried out at the $p<0.05$ level with MINITAB® 16 Software.

RESULTS AND DISCUSSION

AMF Spore Colonization on Palm Roots

AMF root colonization is a structure found in plant roots such as vesicles, external hyphae and arbuscules. The calculation is completed by counting the number of root parts infected by the AMF. Results of observations of percent colonization of roots are presented in Figure 1.



Description:

IPD D6 : pit dump block D6 with dry soil conditions
IPD C3 : pit dump block C plot 1 with flooded soil condition
IPD C3 : pit dump block C plot 2 with flooded soil condition
IPD D2 : pit dump block D2 with very dry soil conditions
IPD K : pit dump block K with wet soil conditions

Figure 1. Percentage of AMF root colonization at the sampling site

Based on the observation of root colonization (Figure 1), it was shown that there was an association between the AMF and the sugar palm roots that formed a hyphae in the root cells. The AMF was able to infect the sugar palm roots at each study site with different plant ages and growing locations. The criteria for the level of root colonization according to Rajapakse and Miller (1992) is that the average percent of AMF root colonization at the study site had a grade 4 percent category and was categorized as high (Hadianur, Syafruddin and Kesumawati, 2016).

If seen from the graph in Figure 1, the highest percent of colonization is found in plot D2 because this location is a place with an elevation of 70% with dry land conditions and palm trees are not shaded. In addition, at sampling locations C3.1 and C3.2, at which the areas close to the water or the puddles, it still shows that the AMF can be symbiotic with the plant roots well by 60 to 70%. This is in accordance with the research conducted by Hermawan, Muin and Wulandari (2015) that in soils with an acidic pH and flooded AMF, it can still develop well. The AMF still shows its existence. Soil conditions with nutrient content, especially low phosphorus elements can cause the increased AMF colonization on the plant roots. Basically the AMF is needed by the plants to absorb the phosphor.

Pulungan (2013) stated that, a high availability of phosphorus in the soil directly reduces the AMF activity so that the presence of the AMF decreases, conversely the low availability of the phosphor in soil increases the formation of AMF in the plants because in soil conditions like this, the plants tend to use the AMF as a source of energy and one way to get the nutrients in the soil. Roots infected by the AMF can absorb nutrients using hyphae from the AMF to absorb all soil and water nutrients. AMF in plant roots can increase the surface area for nutrients and water absorptions. With the increase of root surface area, it can increase the absorption of nutrients and minerals from the soil. Based on the observations, it can be seen that the sugar palm is a plant that is responsive to the AMF. This can be seen from the large percentage of AMF colonization on sugar palm roots which is more than 50.00% which is classified as high to very high because according to Agustin (2011), there are several factors that affect the level of root colonization including the differences in tree genotypes, developmental levels, and proximity to other plants. as well as the environment. Some of the AMF structures found in the roots of sugar palm plants can be seen in Figure 3.

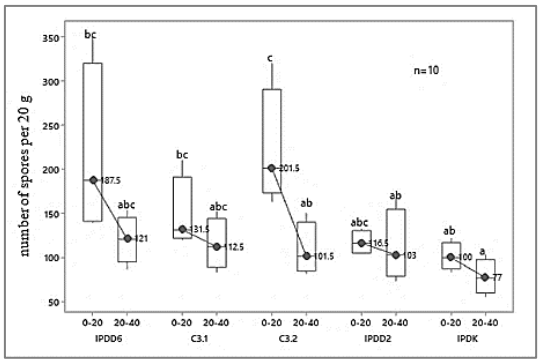
The AMF structures found in stained palm roots were external hyphae (Figure 3A), vesicles (Figure 3B) and spores (3C). The arbuscules were not found in all root samples. The presence of arbuscules in the roots was relatively short, ranging from 1-3 days, but with

the presence of one or more AMF structures, it could be said that the AMF association had occurred with the host plant. The difference in the percentage of AMF root colonization at each research location was thought to be the influence of the chemical properties of the soil.

Abundance and Diversity of Arbuscular Mycorrhizal Fungi (AMF)

Spore abundance describes the number of spores contained in an ecosystem based on soil samples taken. The abundance of spores at two depths at the observation site is presented in Figure 2.

According to Smith and Read (2008), field observations using conventional and molecular detection methods can show that a single root system can have several types of AMF and that plant species in the same place often have the same fungal sequence variance. Mycorrhizal fungi found in plants can help the plant roots take up water and soil nutrients in exchange for carbon (C), which allows plant growth to increase or plants can withstand biotic and abiotic stress conditions. Based on Figure 8, it can be seen that the overall abundance of spores in the five observation areas and the level of soil depth had a significant difference ($p<0.05$). Based on observations, it is known that the IPD C3.1 plot with submerged and a depth of 0-20 cm had the highest number of spores ($p<0.05$) compared to other observation plots and the IPD D6 plot with dry soil condition had the second highest number.



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IPD D6 : in pit dump block D6 with dry soil conditions
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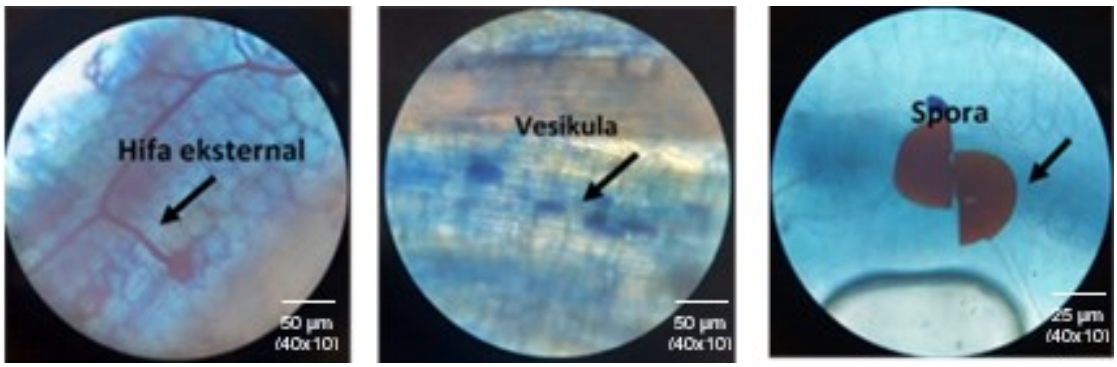
IPD C3 : in pit dump block C plot 2 with flooded soil condition
IPD D2 : in pit dump block D2 with very dry soil conditions
IPD K : in pit dump block K with wet soil conditions

Figure 2. The abundance of spores per 20 g of soil sample at the observation site. Different letters showed signification differences, after one-way Anova ($p<0.05$) and Tukey's test were performed for all comparisons

The results showed that the abundance of spores decreased in density with the depth of the soil layer. The number of spores at a soil depth of 0-20 cm had the highest value and was significantly different ($p<0.05$) compared to a layer of 20-40 cm. According to Lamit *et al.* (2017), stratification of soil depth greatly affects the fungal community four times more at a depth of 10-20 cm than the depth below it (30-40 cm) and the highest spore abundance is generally found in the soil surface layer (Liu *et al.*, 2013).



Source: author's observations

Figure 3. Root infection of sugar palm in the post-mining area of PT Berau Coal East Kalimantan (400x magnification): root structure with external hyphae (A); root structure with vesicles (B); and root structure with spores (C).

The decrease in the number of spores is due to the fact that the deeper the soil, the less oxygen is available and the more water content, in this condition the soil aeration gets worse, especially when it rains, this will cause the soil to lack oxygen, causing less space for spore growth and root colonization. The decrease in the number of spores at the observation site with deeper soil depth was due to soil biophysical factors in the mining area, the presence of the AMF and the reach of the roots of the sugar palm plant.

AMF Spore Diversity

The existence of AMF spores in an ecosystem is influenced by various things, such as the age of the host plant and the condition of the root soil of the host plant. The AMF genus was found in different numbers depending on the biophysical conditions of the land at the observation site and the depth of soil sampling. The abundance of spores per 20 g at the genus level in the five sampling locations namely IPD D6, C3.1, C3.2, IPD K, and IPD D2 is presented in Table 1.

The table shows the dominance of *Glomus* at all observation sites at various soil sampling depths, whereas *Acaulospora* and *Gigaspora* were only found at a soil depth of 0-20 cm except in the IPD D6 plot which was also found at a soil depth of 20-40 cm. The observation sites of IPD D6 and C3.1 were the locations where spores were found the most, due to the abundance of AMF species which were mostly found in 20 g of sugar palm rhizosphere soil samples.

Glomus dominated all observation locations at various depths of soil sampling, whereas *Acaulospora* and *Gigaspora* were only found 0-20 cm except in the IPD D6 plot which was also found at a soil depth of 20-40 cm, The observation sites of IPD D6 and C3.1 were the locations where spores were found the most, due to the abundance of AMF species which were mostly found in 20 g of sugar palm rhizosphere soil samples. The distribution characteristics of the spore genera in the observation area can be seen from the frequency at each observation location (Table 2).

Table 1. Spore abundance per 20 g (mean ± sd) per genus at various observation sites and soil depths

Location	Soil Depth	*Glomus*	*Acaulospora*	*Gigaspora*	Unidentified
IPD D6	0 - 20 cm	32.1±15.8	26.6±14.0	8.3±3.5	0.0±0.0
	20-40 cm	25.5±11.4	7.7±7.4	0.0±0.0	0.0±0.0
C3.1	0-20 cm	27.5±9.3	14.8±6.7	5.5±2.1	0.0±0.0
	20-40 cm	28.8±10.7	0.0±0.0	0.0±0.0	0.0±0.0
C3.2	0-20 cm	36.0±11.3	9.0±1.4	16.7±5.8	19.0±5.7
	20-40 cm	22.4±11.3	0.0±0.0	0.0±0.0	10.7±9.8
IPD K	0-20 cm	20.6±5.8	7.5±3.5	4.5±0.7	6.5±3.5
	20-40 cm	26.4±10.2	0.0±0.0	0.0±0.0	0.0±0.0
IPD D2	0-20 cm	22.6±8.7	0.0±0.0	0.0±0.0	10.5±6.4
	20-40 cm	18.4±9.6	0.0±0.0	0.0±0.0	0.0±0.0

Table 2. Relative frequency (%) of AMF spore genus in the observation area

Location	Soil depth	*Glomus*	*Acaulospora*	*Gigaspora*	Unidentified
IPD D6	0 – 20 cm	100	100	75	0
	20 – 40 cm	100	50	0	0
C3.1	0 – 20 cm	100	100	50	0
	20 – 40 cm	100	0	0	0
C3.2	0 – 20 cm	100	50	50	50
	20 – 40 cm	100	0	0	75
IPD K	0 – 20 cm	100	50	50	50
	20 – 40 cm	100	0	0	0
IPD D2	0 – 20 cm	100	0	0	50
	20 – 40 cm	100	0	0	0

In Table 2, the genus *Glomus* is spread across all observation sites. The presence of AMF spores of the genus *Glomus* in all observation locations indicated that the genus had good adaptation to all conditions in the observation area. Other genera that were also found in almost all the observation sites were the genus *Acaulospora* and *Gigaspora* but their presence was not found at the IPD D2 observation site. According to Hermawan, Muin and Wulandari (2015) *Glomus* has a high level of distribution and adaptation to extreme environments compared to other types, apart from the results of research conducted in other mining areas such as limestone mines (Teixeira-Rios *et al.*, 2013), nickel mines (Teixeira-Rios *et al.*, 2013), nickel mines (Setiadi and Setiawan, 2011; Teixeira-Rios *et al.*, 2013), and gold mines (Suharno *et al.*, 2014) showed that the genus *Glomus* was found in all ex-mining areas.

The types of AMF found in the five observation plots were only 3 genera and some could not be identified due to damaged and incomplete spore conditions, based on the study of Widodo (2011) damaged to natural habitats such as land conversion, chemical and organic pollution, and climate change are the main factors affecting the number of organism, diversity and species dominance, so that according to Säle *et al.* (2015), In agroecosystems, land use types, agricultural systems, tillage systems and fertilization strategies are the main factors that influence the persistence and development of AMF. The local AMF community is regularly challenged by rapid host exchange, crop rotation, and soil management, especially in perennial crops.

Based on field observations, 3 types of spores were found, is *Glomus* sp, *Gigaspora* sp and Acaulospora sp, and one type of spore was not identified. There were found 3 types of *Acaulospora*, 15 species of *Glomus* and 1 species of *Gigaspora* based on differences in color, shape, size and several other characteristics. Several types of AMF found in this study are presented in Figure 4.

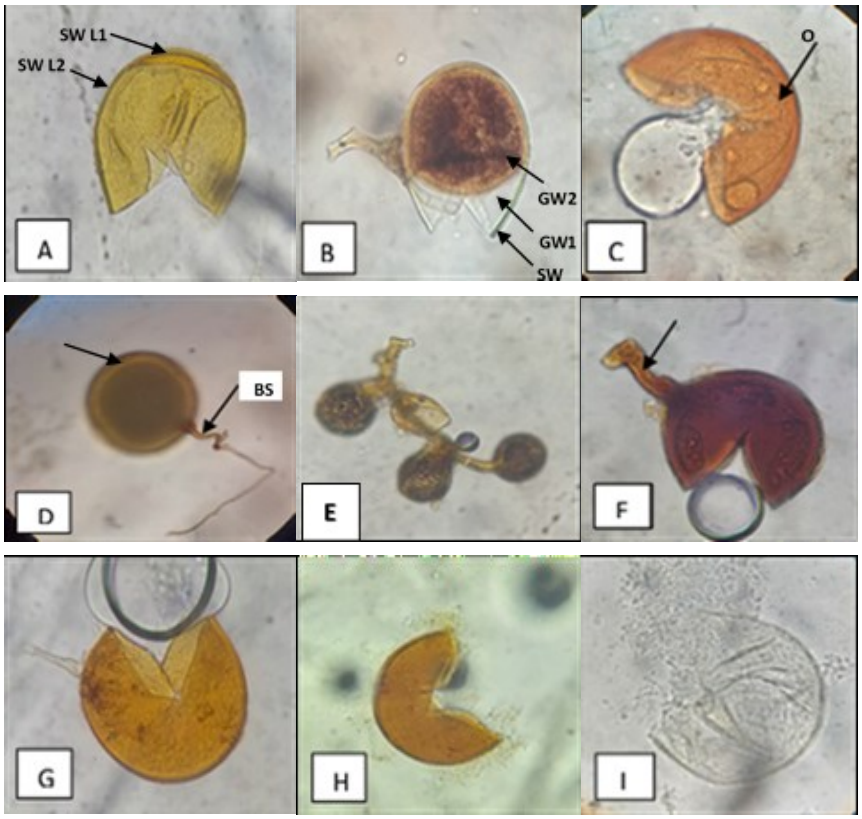


Figure 4. A-C: *Acaulospora* sp: (swL1) thin, hyaline outer layer of spore wall; (swL2) laminated inner layers of spore wall; (gw1) first bilayered hyaline germinal inner wall; (gw2) second hyaline inner wall*;* (O) Spore wall with ornaments. D. *Gigaspora* sp: (BS) bulbous suspensor , the characteristic feature ot *Gigaspora* is that has a bulbous suspensor. E-H: *Glomus* sp: (sh) Subtending hypha- the hypha below or attached to a spore from which the spore arose.

CONCLUSION

The AMF spores were found in 3 AMF genera in 5 observation locations, namely the genus of *Glomus* (15 sp), *Acaulospora* (3 sp) and *Gigaspora* (1 sp) with the highest abundance of spores belongs to the genus of *Glomus* sp at a soil depth of 0-20 cm. This shows that the sugar palm has a symbiosis with the AMF which plays a role in helping the absorption of plant nutrients, increasing growth and yield of plant products even in marginal soil conditions such as ex-mining. So that the sugar palm (*Arenga pinnata*) can be used as a post-coal mining reclamation plant.

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