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Identification Of Mycorrhizal Fungi Arbuscular Indigenous On Various Land Use Patterns

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ABSTRACT. Information on the existence of indigenous Arbuscular Mycorrhizal Fungi (FMA) is needed as an early stage of FMA biofertilizer production to overcome the scarcity of subsidized fertilizers. The purpose of this study was to determine the type of indigenous FMA and determine the location that can be used as a source of FMA inoculum as a biological fertilizer material. Soil and root samples were observed in 4 types of land use, with 10 repeats. The results showed that for every 100 g of soil, the highest number of spores were found in natural forest types as many as 248 spores, in agroforestry land 138 spores, in ex-mining land 95 spores, and at least in sweet potato-intensive agricultural land only 58 spores. Natural forest areas have a high enough spore count so that natural forest locations have the potential to be a source of indigenous FMA inoculum than other types of land use. There are three genera of FMA found, namely Glomus, Gigaspora, and Acaulospora. Glomus is found on all types of land use except monoculture farmland and is the genus that dominates spore populations. The genus Acaulospora is found only in agroforestry land types. Indigenous FMA was found to be able to infect plant roots in natural forests in as much as 6 % and agroforestry land in as much as 16 % so it was included in the medium and low categories. Meanwhile, in the monoculture type of sweet potato and ex-mining land, no root infection was found..

Keywords : *Biological Fertilizer, Glomus, Inoculum, Root Infection*

INTRODUCTION

The scarcity of subsidized fertilizers is shown by the performance of fertilizer subsidies in the ‘right place’ in each province which is in the range of 30–96 % (Zulaiha et al. 2018). This causes problems in agricultural cultivation such as not precisely the amount, type, and time of fertilizer use (Kautsar et al. 2020). The state budget can only meet about 40 % of subsidized fertilizer needs (Mudassir 2022). The efficient use of inorganic fertilizers can be done by mycorrhizal inoculation. Mycorrhizal applications have been widely tried on various types of annuals and annual crops, especially in nutrient-poor planting media or in ex-mining areas in reclamation activities (Gahidaet al. 2020, Febriyantiningrum et al. 2021). FMA was shown to help increase maize growth, and had a significant effect on plant height, leaf area, and relative growth rate (Suharno et al. 2017), having a significant effect on available P uptake (Nuridayati et al. 2019). Another benefit is that FMA can reduce plant dependence on fertilizer because FMA is more active in developing in less fertile or dry soil conditions so the addition of fertilizer in certain doses will reduce spore density and colonization (Asrianti et al. 2016).

FMA has a symbiotic relationship with higher plants, including endangered species in diverse climatic regions and land types, on heavily metal-polluted lands, even associated with a successful invasion of most exotic plants (Kong et al. 2021, Akib et al. 2022, Tuheteru et al. 2022). FMA is seen as one of the most effective and environmentally friendly management to increase the productivity of legume crops (Hu et al. 2022). FMA inoculation can increase plant height, plant dry weight, root bud ratio, seed quality index, and root colonization and decrease the total number of nematodes in soil and roots (Hindersah et al 2022, Tuheteru et al. 2022). Recent mycorrhizal population studies have mostly been conducted on specific plant rhizospheres, both annuals and annuals on horticultural, agroforestry, or mixed forest lands (Danesh et al. 2022, Jing et al. 2022, Suharno et al. 2022, Susila et al. 2022). Recent research has also shown that mycorrhizal abundance is influenced by several things such as soil and plant age factors (Gao et al. 2021), differences in location and environment (Armansyah et al. 2022, Rasmussen et al. 2022), vegetation diversity (Fei et al. 2022, Guy et al. 2022), and light or heavy tillage rates (Egboka et al. 2022). Recent research has shown that indigenous mycorrhizae have a higher ability to infect plants (Hajoeningtijas et al. 2021). In general, the genus Glomus is the most widely found genus, dominating in the roots and rhizosphere of plants. Other commonly found genera are Acaulospora and Gigaspora (Palwi et al. 2021, Purba and Prasetya 2021, Chikoti et al. 2022, Islam et al. 2022, Sanana et al. 2022). Based on this, the purpose of the study is to identify the types of indigenous FMA found and determine locations that have the potential to be developed as sources of mycorrhizal inoculum. This study used a wider and more diverse sample. The samples used are types of land use, namely: conservation areas, monoculture agricultural land, agroforestry land, and ex-mining land. Thus, information on the existence of mycorrhizal fungi will be more complete so that locations that have the potential to be used as a source of inoculum for the propagation of mycorrhizal fungi as biological fertilizers can be determined.

MATERIALS AND METHODS

The research was scheduled to be conducted from July to November 2023 in four locations, namely Bandorasa Village for monoculture land types, Cidahu Village for ex-mining land, Linggarjati Village for agroforestry land, and Sagarahiang Village for conservation land. The tools used are multilevel sieve tests (1 mm, 425 μm, 106 μm, 45 μm, and 25 μm), centrifuges, centrifuge tubes, microscopes, Buchner funnels, Wheatmann filter paper, digital scales, loose needles, pipettes, tweezers, preparation glass, petri dishes, spray bottles, measuring cups, backerglass. The materials used are soil samples, water, sugar solution, KOH, HCl, trypan blue, and glycerin.

The stages of research are as follows:

1) Determine the location point of soil sampling in each type of land use. In this case, the type of vegetation is not a consideration in determining the sample point, but the vegetation data is recorded and documented. The monoculture agricultural land chosen is sweet potato cultivation land which is a commodity that is widely developed in the Kuningan Regency area, even this sweet potato has penetrated exports to Japan and several other countries in the form of paste. The sweet potato land chosen is in Bandorasa Village, Bandorasa District, Kuningan Regency which is located at coordinates 6.896927° S 108.483425° E. Former sand mining land is the most degraded land in Kuningan Regency, especially the East Kuningan region. This condition causes a large area of land that needs to be reclaimed. Soil samples were taken from former sand mining land that has been abandoned for 5 years, namely in Cidahu Village, Cidahu District, Kuningan Regency which is located at coordinates 6.991470° S 108.647010° E. The agroforestry land chosen is community forest land located in Linggarjati village, Cilimus district, Kuningan regency at coordinates 06º88'29" S 108º46'69" E. The selected conservation area is a natural forest area in Gunung Ceremai National Park in English?! located at coordinates 6º57'14.0" S 108º24'34.3" E. Please present the geographic coordinates uniformly with ??º??'??"
2) The next stage is Taking soil samples from the location with predetermined conditions. Soil and root samples were taken at a depth of 0–20 cm (Kafrawi et al. 2022) at each location many as repetitions How many repetitions are there for each location?, so that there were 40 test samples. Soil samples taken at each point were placed separately where as much as 500 g for soil analysis, 100 g for mycorrhizal identification.
3) Measuring environmental factors at each sampling location which included temperature, humidity, sunlight intensity, vegetation type, altitude, and elevation (Syarif et al. 2007, Syib'li et al. 2013). You write that you will measure, and you cite 2 sources! Did you measure according to the methodology presented in the two sources or did you use data from these two sources?
4) Determination of mycorrhizal fungal infection. Spore isolation was carried out by wet filtration method (Pacioni 1992) and decantation followed by sugar centriffusion centrifugation method (Brundrett et al. 1996) to release spores from particle pellets. Spores are observed under a microscope and identified. Spore identification is done by observing the morphological

characteristics of spores and then comparing them with literature. Next, spores are prepared by dripping Melzer's solution (Nusantara et al. 2012) and PVLG to observe the presence or absence of spore color changes. The preparation is stored in the refrigerator for further observation. Observation of mycorrhizal colonization in roots is carried out if there are mycorrhizal-infected roots. The colonization of hyphae in plants is calculated from the percentage of roots containing hyphae or arbuscular.

The data obtained were analyzed descriptively and displayed data in the form of identification results of arbuscular mycorrhizal fungi, starting from spore morphology based on: color and shape. Spore morphology was identified using INVAM 2008. The calculation of spore density as well as the calculation of the percentage of FMA infection of plant roots was were calculated by the formulas (1) and (2):

$$\text{Spore density} = \frac{\sum spora}{Soil\ weight}, \text{number per 100 g of soil} \quad (1)$$

$$\text{Infected roots} = \frac{\sum infected\ roots}{\sum the\ entire\ observed\ root} \cdot 100, \% \quad (2)$$

Categories Infection rates in roots are classified according to the Instate of Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia (Setiadi et al. 1992) as follows:
a) If the infection is 0 % – 5 %, the category is very low;
b) If the infection is >5 % – 25 %, low category;
c) If the infection is >25 % – 50 %, moderate category;
d) If the infection is >50 % – 75 %, high category;
e) If the infection is >75 % – 100 %, the category is very high.

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi can be found in most soil types and generally do not have a specific host, but certain factors that affect the population level and composition of arbuscular mycorrhizal species such as plant characteristics, topographic shape, plant age, temperature, and soil chemical properties such as soil pH, soil moisture, N and P content as well as heavy metal concentration levels (Kafrawi et al. 2022) and the number of spores in the rhizosphere of a plant not only Influenced by one factor only but influenced by the accumulation of several factors, including mycorrhiza itself, host plant varieties and environmental conditions.

The number of indigenous FMAs found in plant rhizospheres in various types of land use shows the highest number of mycorrhizal spores found in the rhizosphere of natural forest stands of Gunung Ceremai National Park in English?! which is a conservation area of 248 spores per 100 g of soil (Table 1). The number of spores found on agroforestry fields is 138 spores per 100 g of soil, in former sand mining fields, 95 spores per 100 g of soil were found and the lowest number of spores was found in sweet potato farmland with the number of spores only 58 spores per 100 g of soil. The number of spores found per 100 g of soil were 138 on agroforestry fields, 95 – in former sand mining fields, and the lowest 58 number – in sweet potato farmland. This means that the presence of mycorrhiza in each ecosystem shows that differences are influenced by many factors, both biotic and environmental factors. One of the key elements that influences the distribution of mycorrhizal spores is that type of host vegetation, thus influencing the presence of mycorrhizal spores through its ability to form fine roots in the soil. The fine roots in plant roots are easily colonized by mycorrhiza, this research refers to research that has been carried out, by taking soil samples at a depth of 15 cm as many observations at depths of 15–20 cm formed many young roots from several plants by showing a high level of spore density as indicated by Masrikail et al. (2019).

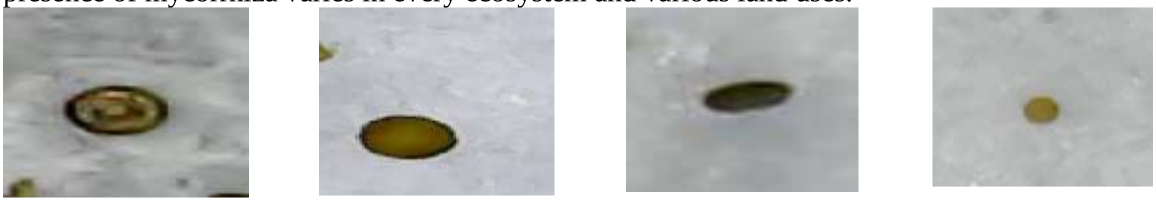
Table 1. Mycorrhizal density in plant rhizosphere in various types of land use.

Types of land use	**Number of spores**
Sweet potato farming	58 spore per 100 g soil
Former sand mine	95 spore per 100 g soil
Agroforestry	138 spore per 100 g soil
Natural forest	248 spore per 100 g soil

Table 1 shows that mycorrhizal spore density shows a clear gradient across different types of land use. Natural forests showed the highest spore counts, reflecting the diversity of undisturbed root structures. Agroforestry, which combines tree species, supports more spores than sand mining and sweet potato farming, where sweet potato farming has the fewest spores, most likely due to intensive land use and monoculture practices.

This study shows the potential presence of mycorrhiza in intensively managed fields such as sweet potato farmland shows a very low spore count of only 58 FMA spores per 100 g of soil, the same as the number of FMA spores found in rice fields which are only found 10 spores per 100 g of soil (Nugroho and Prasetya 2023). Similarly, in disturbed or degraded land such as former sand mining land, the potential for mycorrhizal presence exists but the number is low, with only 95 FMA spores per 100 g of soil. Agroforestry land is land that is rarely cultivated, only at the beginning of planting this land is cultivated then usually this agroforestry land will be left for years. This allows the stable development of mycorrhiza for plant growth. Natural forest areas that are very well maintained and rarely disturb/ process their land have the ability of the largest mycorrhizal association where natural forest ecosystems continue to climax so that there is no disruption of ecosystems in it both above ground and underground. So, the number of FMA indigenous spores found in the rhizosphere of natural forests is quite large reaching 248 spores per 100 g of soil. So, agroforestry land and natural forests in conservation areas have a great opportunity to be used as land to obtain quite a lot of FMA indigenous spores.

"In the field, we observed different types of mycorrhiza by identifying the spores, focusing on their shape, ornamentation, and color to determine their genus." Observation of mycorrhizal types found in the field is carried out by identifying mycorrhizal spores by observing spore morphology, namely shape, ornament, and color to the genus level. This yellowed sentence is methodical! The identification of mycorrhizal spores in the rhizosphere of plants in each type of land use shows that not all mycorrhizal genera are found in the rhizosphere of plants in various types of land. In sweet potato farmland, only the genus Glomus was found, with 4 variations in shape and color found as shown in Figure 1. In the former sand mining area, 3 genera were found, namely Glomus with 2 variations in shape and color, Gigaspora and Aclauspora shown in Figure 2. Similarly, natural forests found 3 genera, namely Glomus, Gigaspora, and Aclauspora as shown in Figure 4, while in Figure 3 in agroforestry land found only the genera Glomus and Gigaspora were found. This shows that the presence of mycorrhiza varies in every ecosystem and various land uses.



a b c d

Figure 1. Types of FMA on sweet potato farmland: Glomus sp. 1 (a), Glomus sp. 2 (b), Glomus sp. 3 (c), Glomus sp. 4 (d).

a b c d

Figure 2. Types of FMA on former sand mining land: *Glomus* sp. 1 (a), *Glomus* sp. 2 (b), *Gigaspora* sp. (c), *Aclauspora* sp. (d).

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a b c d e

Figure 3. Types of FMA on agroforestry: *Gigaspora* sp. 1 (a), *Gigaspora* sp. 2 (b), *Gigaspora* sp. 3 (c), *Glomus* sp. 1 (d), *Glomus* sp. 2 (e).

a b c d e

Figure 4. Types of FMA in the conservation forest area of Gunung Ceremai in English?! National Park: *Glomus* sp. 1 (a), *Glomus* sp. 2 (b), *Glomus* sp. 3 (c), *Gigaspora* sp. (d), *Aclauspora* sp. (e).

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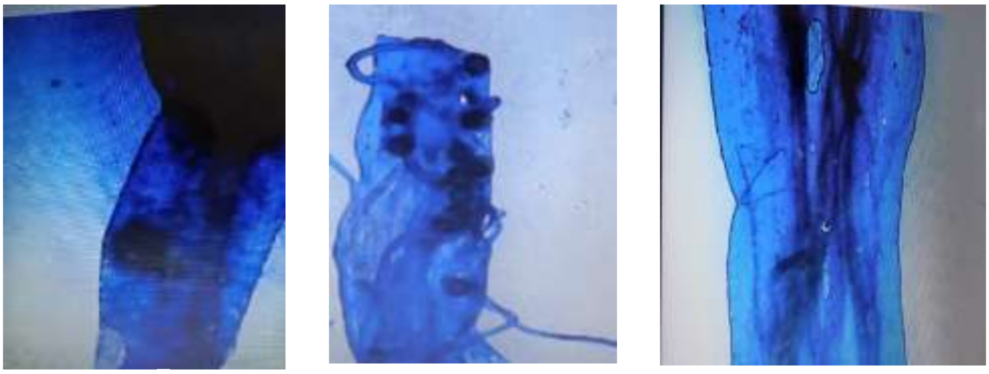
Glomus is found at all observation sites. This shows that *Glomus* lives and adapts to diverse ecosystem conditions, both land with intensive processing patterns, degraded land, and ecosystems without tillage such as natural forest land and agroforestry land. On monoculture land based on forest plants or agroforestry usually found *Glomus* and *Acalauspora* (Parwi et al. 2021, Purba and Prasetya 2021). In monoculture fields based on agricultural tenure with intensive land processing, only glomus is usually found in sweet potatoes and rice fields (Nugroho and Prasetya 2023).

Arbuscular mycorrhizal fungi are widely known for their role in forming mutualistic relationships with approximately 80% of plant species, although their effectiveness can vary between different species. Arbuscular mycorrhizal fungi receive much attention for their ability to form mutualistic symbioses with about 80 % of plant species although their effectiveness is not the same in every plant species (Nuridayati et al. 2019, Susila et al. 2022, Tuheteru et al. 2022). The term Arbuscular Mycorrhizal Fungi is used because this type of mycorrhiza forms arbuscular structures in association with the roots and partially forms vesicles. Mycorrhizal invasion of plant roots in various types of land use is presented in Table 2. In all types of land use mycorrhizal infection of plant roots. This sentence is missing a verb! This suggests that in all types of land use, there are mycorrhizal associations and their constituent plants, although not all forms present in the roots were observed. Naturally, FMA indigenous found in the rhizosphere in the four ecosystems is able to penetrate into plant roots. This penetration suggests that the FMA indigenous found is capable of symbiosis with plant roots. Sweet potato roots and plant roots in natural forests can be seen forming hyphae, spores, and arbuscules in their roots. Arbuscules are tree-like branching structures formed by fungal hyphae within the root cortex of the host plant. This structure functions as a place for nutrient exchange between fungi and plants. In line with the statement by Brundrett et al. (1996) arbuscular is a hyphal structure that branches like a small tree in the host root cortex, where arbuskul has a function as a place of nutrient exchange between fungi and host plants. Meanwhile, vesicles are the ends of hyphae that bubble up in the area of root cortex cells that function as storage organs where they have thick walls. Such as vesiculars seen on plant roots in agroforestry fields which also see hyphae and spores. While plant roots in former sand mining land only show the presence of hyphae and spores. Hyphae are fungal fruit bodies in the form of moving threads that penetrate the roots of plants. The penetration and shape of fungal structures found on plant roots as shown in Figure 5.

Table 2. Root invasion in the rhisozsphere of plants in various types of land use.

Types of land use	Spore	Hyfa	Arbuscular	Vesicular
Sweet potato farming	√	√	√	-
Former sand mine	√	√	-	-
Agroforestry	√	√	-	√
Natural forest	√	√	√	-



a b c

Figure 5. Root invasion form: (a) vesicular, (b) spora, (c) hifa.

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Namely plant type, plant age, mycorrhizal conditions, and soil type. The level of effectiveness of each type of host plant varies when applied with different mycorrhizae, because mycorrhiza itself has specifications in selecting and associating with certain types of plants (Akib et al. 2022, Danesh et al. 2022, Tuheteru et al. 2022). In addition, the type of soil is also an influential factor in the presence of mycorrhiza. This is because each type of soil has a different organic content and acidity so the mycorrhizal effects become diverse. The degree of FMA infection shows mycorrhizal ability to associate with host plants and also shows its ability to support plant growth. The results of root infection observations presented in Table 3 show that the highest degree of root infection occurs in plant roots located in former sand mining land, which is 43 %. Former sand mining land is land that has lost topsoil containing humus and organic matter so it becomes critical land and unsuitable for plant growth. FMA will develop better and play a more important role on nutrient-poor land (Suharno et al. 2017, Asrianti et al 2016). Similarly, the rate of FMA infection in the roots of sweet potato plants is 31 %, this root infection is higher than FMA infection in plant roots in agroforestry land by 16 % and roots in natural forests by 6 % which are categorized as low invasion. This is possible because this sweet potato agricultural land is intensive agricultural land with a large enough chemical fertilizer input as well as chemical pesticide inputs on this land are high enough so that it can be said that this intensive agricultural land is polluted / degraded land so that FMA increases penetration into the roots.

Table 3. Degree of FMA infection of plant roots in various types of land use.

Types of land use	Degree of root invasion, %	Category
Sweet potato farming	31	moderate
Former sand mine	43	moderate
Agroforestry	16	low
Natural forest	6	low

Table 3 shows that more disturbed land, such as sweet potato farms and ex-mining land, promotes higher fungal colonization in response to stress or lack of nutrients in the soil. On the other hand, in more stable environments such as agroforestry and natural forests, the level of root invasion is lower, although the number of mycorrhizal spores is higher in natural forests. This may be due to the high efficiency of the symbiotic relationship that has been established in undisturbed soils, so that extensive root invasion is not necessary.

This difference indicates that land use and vegetation conditions really influence the biological conditions of the soil, as also mentioned by Syarif et al. (2007). However, our results show that in more stable ecosystems, mycorrhizal fungi can thrive without the need for significant root invasion. Therefore, ecosystem stability appears to be a key factor in optimizing the symbiosis between AMF and host plants.

In Table 4 indicated environmental conditions and above-ground vegetation analyzed for FMA ingenus potential. The microclimate formed in this type of land use as a natural forest conservation area of Gunung Ceremai National Park in English?! is at an altitude of 1213–1306 m a.s.l. showing a cool temperature with the highest humidity. This is supported by the types of plants that dominate in the location are mixed plants dominated by tree shapes and undergrowth. The more types of plants that grow on it, the more opportunities for the existence of various types of FMA indigenous that can be associated with various types of mixed trees and also various types of undergrowth.

Table 4. Environmental conditions and above-ground vegetation on various types of land use.

Types of land use	**Temperature, °C**	**Rh, %**	**Altitude, m a.s.l.**	**Elevation, m**	**Plant dominance**
Sweet potato farming	28	79	551–585	529–551	*Ipomoea batatas* (L.) Lam.
Former sand mine	34	54	129–140	47–122	*Cyperus rotundus* L.
Agroforestry	23	77	1017–1040	809–810	Fruit crops
Natural forest	19	91	1213–1306	1171–1299	Mixed

The presence of certain plant species also affects the ability of FMA to associate with soil or environments containing indigeonous FMA (Masrikail et al. 2019, Kafrawi et al. 2022, Nugroho and Prasetya 2023). As in the former sand mining land which is dominated by puzzle grass plants and sweet potato plants. Puzzle grass and sweet potato have enough fibrous roots to show higher FMA infection in the root category with moderate infection (Table 2) than root infection in agroforestry land and natural forest in the low injection category. The fibrous root form has a higher number of fine roots than the taproot form.

CONCLUSION

Our results showed conclusively that the association of FMA with plants growing on it marked by FMA spores in all plant rhizospheres and root infection in various forms of spores, hyphae, arbuscles, and vesicles. Glomus and Gigaspora are the most common genera in all types of observation land use so these two FMA indigenera have great potential to be developed as biofertilizers.

Natural forest areas and agroforestry land have a fairly high number of spores, and very potential as a source of FMA indigenous isolate than sweet potato agricultural land that has a high level of land cultivation or former sand mining land which is disturbed / degraded land. Further research needs to be carried out related to the relationship between vegetation conditions, aboveground environmental conditions and the rhizosphere environment to the presence of FMA indigenous in various land uses.

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