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Biodiversity Arbuscular Mycorrhizal Fungi in the Former Gold Mine Area in North Sumatra

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Abstract. This study aims to determine the diversity of arbuscular mycorrhizal fungi in the area of the former gold mine in North Sumatra. This research was conducted with 5 soil sampling points. The research method used began with calculating the percentage of root colonization, spores density of arbuscular mycorrhizal fungi and types of arbuscular mycorrhizal fungal spores. In addition, physical and chemical conditions of the soil are also measured. The results showed that the rate of root colonization was at moderate to high with an average rate of root colonization at 22.8%. The density of arbuscular mycorrhizal fungi spores in this study averaged 10/50 g of sample soil. the number of spores in this study belongs to the moderate category, however the characteristics of the study location show the uniqueness of its own area that makes the population of mycorrhizal types not too much. Spore types were found in 4 types with each Glomus and Acaulospora. The conclusion obtained that there was a symbiosis between fungi and plants in the former gold mining area in North Sumatra.

1. Introduction

Mining activities are activities that exploit natural resources. Mining activities will cause environmental damage or biodiversity will decrease. One of them is decreasing soil productivity and plant growth. Prevention and reduction of damage must be done immediately for the former mining area by doing land reclamation. Generally mining areas such as gold mines are damaged as a result of mining activities. Chemical, physical and biological properties of the soil will be damaged and become a problem in the existing ecosystem. The damage caused must be followed by land reclamation efforts.

One of the efforts to reclaim the land can be done with the help of microorganisms. One type of microorganisms that play a role in this life is Arbuscular Mycorrhizal Fungi (FMA). This fungus has mutualistic symbiotic properties with plants, is antagonistic to parasites and lives freely naturally in the rhizosphere [1]. FMA is also known to be able to colonize almost all the roots of agricultural crops [2], and is able to improve soil conditions [3].

The improvement of the soil aggregate system is done through an external hyphae network that can improve and stabilize the soil structure. Secretion of polysaccharide compounds, organic acids and phlegm by external hyphal tissue that is able to bind the primary grains into micro aggregates. This capability is very important in stabilizing micro aggregates. Then micro aggregates through the process of "mechanical binding action" by external hyphae will form a steady macro aggregate.

Arbuscular mycorrhizal fungi produce glyomalin glycoprotein compounds which are highly correlated with increased aggregate stability. Factors involved in the formation of structures are organisms, such as fungal threads that can bind one soil particle and other particles. As a result of the extension of external hyphae in mycorrhizal fungi, secretions from polysaccharide compounds, organic acids and mucus that also produced by external hyphae, will be able to bind primary grains / soil micro aggregates into secondary grains / macro aggregates. This organic agent is very important in stabilizing micro aggregates and through the adhesive strength and binding of the acids and hyphae will form a stable macro aggregate.

Formation of a good soil structure is a capital for improving the physical properties of other soils. The physical properties of the soil are improved due to the formation of good soil structure such as soil porosity improvement, soil permeability improvement and improvement of the soil air system [4]. Improvement of soil structure will also directly affect the development of plant roots. In dry land with the better development of plant roots, it will make it easier for plants to get nutrients and water, because indeed on dry land the main limiting factors in increasing productivity are nutrient deficiency and lack of water. Another result of the lack of water availability on dry land is lacking or poor organic material. Poverty of organic matter will worsen the structure of the soil, especially in coarse-textured soil due to the low weathering level.

The potential possessed by the arbuscular mycorrhizal fungi can be used as an alternative to land reclamation. Soil reclamation activities with the help of mycorrhizae can be carried out by exploring specific indigenous arbuscular mycorrhizal fungi in an area. The exploration is to find and determine which species can adapt to an environment. The working principle of this mycorrhizae is to infect the root system of the host plant, producing hyphae intensively so that plants containing mycorrhiza will be able to increase the capacity in nutrient absorption.

The initial step in the utilization and exploration of the potential AMF is to isolate and identify the type of AMF present in a plant in an area. One area of concern is the location of the former community gold mine. The damage caused needs to be a serious concern, repairs are needed and one of them is with the presence of FMA in the roots of plants that live in the former mining area. Overall FMA does not have the same morphological and physiological characteristics, therefore it is very important to know its characteristics.

Previous studies have shown that mycorrhizae can increase plant nutrient uptake which will further improve soil conditions. The results showed that mushrooms had a greater contribution from bacteria, and their contribution was increasing with increasing levels of heavy metals.

Based on the background described above, it is necessary to explore the indigenous arbuscular mycorrhizal fungi at the site of the former gold mine which can later be used as a biological agent for land reclamation. In addition, the spores obtained as a result of FMA exploration can be used as learning media for students taking mycology courses.

2. Materials and Method

In this study samples of plant roots were used from the sampling site. For root staining, 60% glucose solution (spore extraction), polovinyl alcohol solution, lactic acid, glycerin, aquades, iodine, KI (spore staining), 10% KOH, 2% HCl, trypan blue, lacto glycerol (root staining)), red hyponex solution, hypochlorite.

The tools used for soil sampling and plant roots are plastic ropes, hoes, plastic bags and markers and label paper, glass covers, glass objects, spore tweezers, microscopes, petri dishes, measuring flasks, spray bottles. Spore isolation equipment in the form of a 150 mesh size screen, 250 mesh size screen, 300 mesh size screen, 400 mesh size screen, centrifuge tube.

2.1. Sampling of soil and plant roots

Sampling of plant roots that predominantly grow on ex-mining land. The root of the sample is the most delicate root. Root sampling method is done diagonally. Each root sample is done by cutting off the young root tip. Soil samples used were taken from the rhizosphere area of plant roots of 500 g for each type of plant with a maximum depth of 20 cm.

2.2. Observation of FMA Colonization at the Root

Observation of AMF colonization in plant root samples was carried out using root staining techniques. Root colonization is characterized by hyphae, vesicles and arbuscules or one of the three. Every field of view of a microscope showing the sign of colonization is given a symbol (+) and not given a symbol (-). Observation of AMF colonization in the roots of sample plants was done through root staining techniques, because the anatomical characteristics that characterize the presence or absence of AMF colonization cannot be seen directly. The method used in the sample root coloring technique is the Kormanik staining method.

2.3. Spore Extraction and FMA Observation

FMA spore extraction was carried out to separate FMA spores from soil samples so that FMA identification could be done to determine the number and type of FMA spores contained in each sample plot. The technique used in extracting FMA spores is the filter pouring technique and followed by centrifugation techniques [5].

3. Results and Discussion

Soil samples were obtained from the location of the former community gold mine in the Mandailing Natal area of the North Sumatra province of Indonesia. At this location, the community left the gold mining area without making land improvements.

Figure 1. the location of the former gold mine in Mandailing Natal, North Sumatra Province of Indonesia

Analysis of soil and root samples was carried out from 5 plots. Calculation of the percentage of the degree of root colonization is seen through the root field of view in a number of 5 root pieces and divides the field of view into 10 fields of view. Every field of view that contains vesicles or hyphae FMA is given a positive sign and if neither of them is given a negative sign, the results of the observation can be seen in the table below.

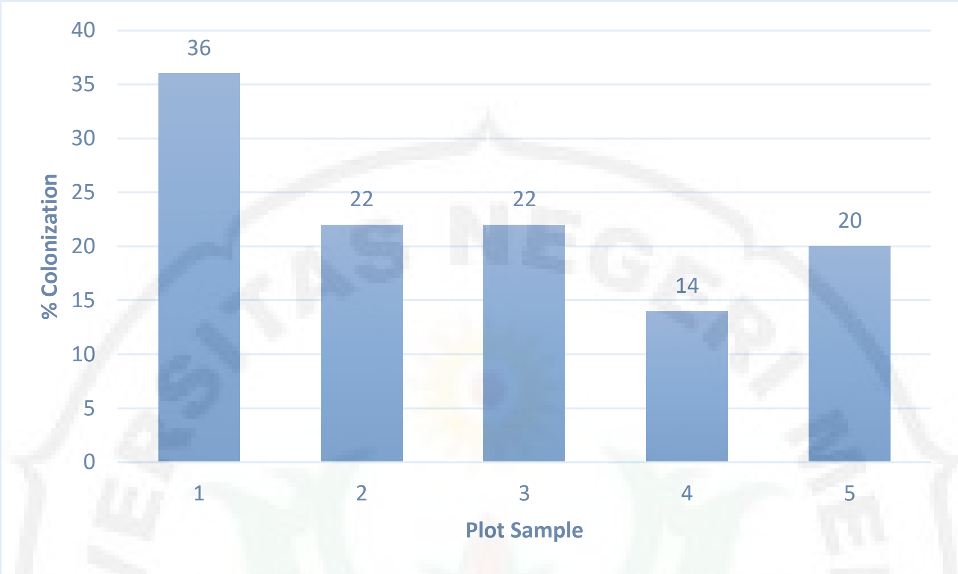

Figure 2. Percentage of colonization FMA in plot sample

The types and characteristics of spores found in soil around plant roots have both color and shape characteristics. Three types of spores were found namely Glomus sp1, Glomus sp2, Glomus sp3 and Glomus sp4. The density of spores can be seen as follows in table 1.

Table 1. Density of spores FMA in plot sample/50 g soil sample

Plots	number of spores/50 g soil
I	7
II	10
III	9
IV	13
V	11

Mycorrhizal spores taken from the field cannot be fully identified because not all mycorrhizae are active at the same time. According to research that has been carried out the density of FMA spores is in line with the level of content available in the soil. Data on the percentage of mycorrhizal colonization in plant roots obtained is directly proportional to the condition of the chemical properties of the soil samples used parameters, namely the condition of soil acid which is rather acidic and the availability of low available so that it supports mycorrhizal colonization in plant roots.

Table 2. physical and chemical properties of the sample soil

Parameter	**Plot**				
	I	II	III	IV	V
pH	5.3	5.6	6	5.7	6
P (ppm)	18.3	17.6	15.7	18.2	15.4
C (%)	3.0	3.1	2.9	2.5	3.0
N (%)	0.9	0.8	0.9	0.9	0.9

Spores are FMA structures that have high resistance to marginal environmental conditions and in certain conditions represent FMA infective propagules in the field ie in conditions after a long period without vegetation or after a long dry season. The number of spore species found in only 4 species with a relatively midle density level. This shows that spores develop in the former mining area.

At low nutrient availability or infertile soil, hyphae can absorb nutrients from the soil that cannot be absorbed by the roots so that the effect of FMA on high nutrient uptake. But in fertile soil conditions with high P content in the soil, plant roots act as nutrient absorbing organs so plants accumulate high amounts of P. This situation makes the FMA still get photosynthate results from plants for life, resulting in a rejection of the response to colonization that affects plant metabolism. This causes a very high P content will be a barrier to plant growth [6].

The presence of AMF infection in plant roots can be characterized by external hyphae, vesicles and arbuscules [7] However, usually the most frequently encountered are hyphae and vesicles. The main function of external hyphae is to absorb nutrients, especially phosphorus from the soil [8]. Hyphae FMA contains the enzyme phosphatase which is able to break covalent bonds of Al3 +, Fe3 +, Ca2 +, and clay with P, so that the element P can be available to plants [9]. The P element available to plants is then absorbed by external hyphae at the root, then channeled into internal hyphae that are exchanged with root cells through arbuscules. In arbuscular, polyphosphate compounds are broken down into organic phosphates which are then released to all host plant cells [10]. At the root then the element is channeled to the xylem to be transported to the leaves and other plant parts.

High available phosphorus content in the soil will inhibit the growth of AMF, because plant roots are able to absorb the nutrients found in the surrounding area without further assistance from AMF [11]. Arbuscular Mycorrhizal Fungi (FMA) which have infected plant roots become malfunctioning in the process of absorption of nutrients that cause AMF not to develop, so AMF can be a parasite for plants because FMA also utilizes photosynthates from plants without the need to assist plants in the process of nutrient absorption.

The density of spores can be seen from only 4 species found with an average amount of 7/50g of sample soil. Suggested that the density of mycelium spores and biomass in the soil is related to the photosynthetic activity of host plants [12]. Low density can be caused by the high Pospor content in the soil so that the work of FMA is not optimal [13-14]. Colonization of AMF is often hampered by the provision of large amounts of available P. Furthermore, the amount of N and P will directly affect root colonization by FMA which will ultimately affect the production of FMA spores.

Of all FMA morphotypes observed, Glomus sp. is the most dominant FMA species found in this study. Glomus sp. it has been reported as the most common and most widely spread AMF species than other species [15], and is the dominant group of AMF especially in less cultivated soils [16]

The distribution of the genus Acaulospora and the genus Glomus in this study cannot be identified more accurately about the distribution and name of the species, because of the total number of spores found, only a few can be identified. This condition is also due to the fact that many dirty spores have not been separated from the soil and spore conditions are damaged (lysis due to sugar solution). Spore identification is also constrained by the limited equipment in the laboratory so that the naming of spores has not been able to reach the naming of species.

4. Conclusion

The conclusion of this study is that there is a symbiosis between fungi and plants in the former gold mining land. This symbiosis is evidenced by the presence of hyphae and vesicles on plant roots and spores on the land of the former gold mine land.

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