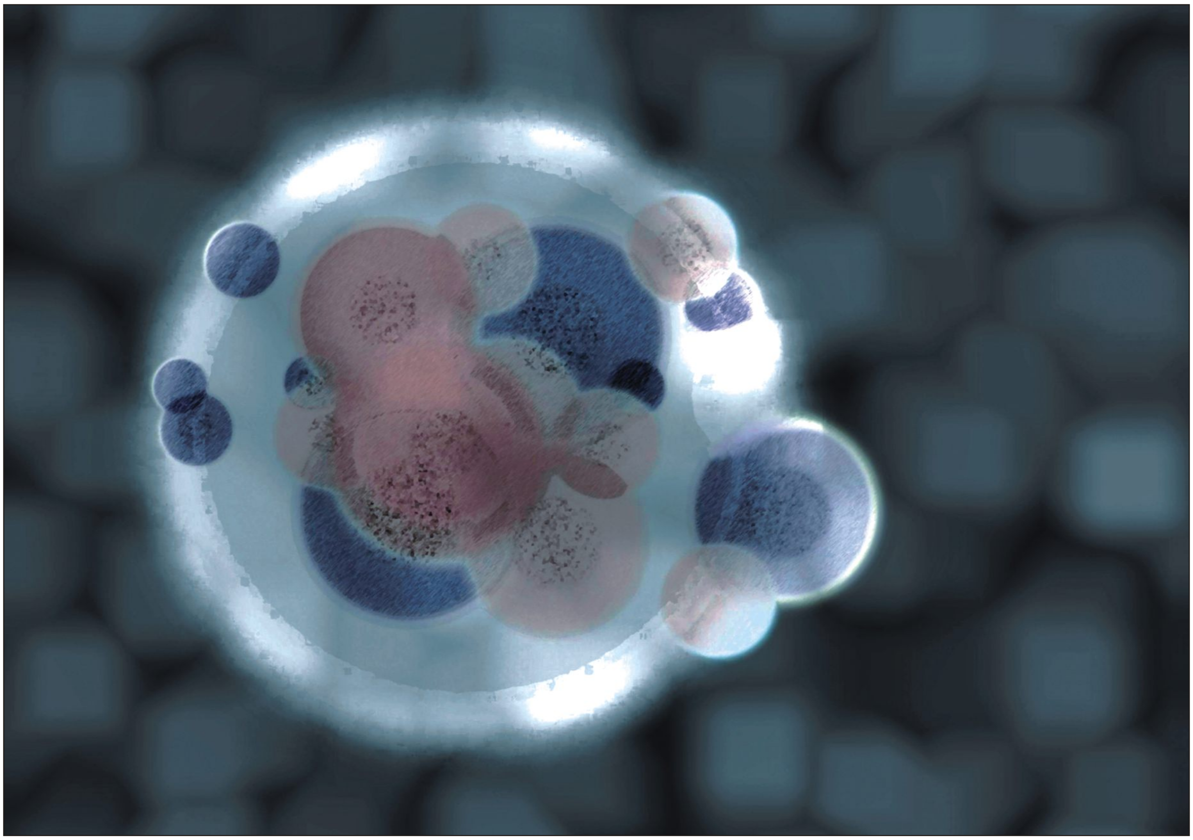

Journal of Biological Sciences

ISSN 1727-3048

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.218.223

Research Article
Exploration of Indigenous Arbuscular Mycorrhizal Fungi on Post Mining Soil as Rehabilitation Strategy

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Abstract

Background and Objective: Activities at mining soil induced extreme complication for environmental, physics, chemical and biology. Arbuscular Mycorrhizal Fungi (AMF) is mutualism symbiosis between fungi and roots of spermatophyta plants. The aim of this study was to assess indigenous AMF in mining contaminated sites Bukit Ngalau PT Semen Padang. The AMF Indigenous were assessed by direct field sampling and trap culture technique. **Materials and Methods:** The symbiosis may affect several other factors that influence the plants ecology, such as water availability, access to other nutrients, grazing resistance and tolerance to soil pathogens and pollutants. **Results:** Identification of indigenous AMF is a necessary step in a phytoremedial strategy in rehabilitation, especially in post mining soil. **Conclusion:** The AMF identified fell within Glomus, Gigaspora and Acaulospora taxa. Glomus was the dominant taxa with the highest spore abundance (38/50 g soil) compared to Gigaspora and Acaulospora.

Key words: Arbuscular mycorrhizal fungi, phytoremedial, rehabilitation, post-mining soil, PT. Semen padang

Citation: Chairul, Zozy Aneloi Noli, Suwirmen, Syamsuardi and Reini, 2019. Exploration of indigenous arbuscular mycorrhizal fungi on post mining soil as rehabilitation strategy. J. Biol. Sci., 19: 218-223.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mining is one of the flagship sectors plays an important role in Indonesian economy[1]. One of the biggest mining activity in west Sumatra is PT Semen Padang which is located in the Indarung Village, Lubuk Kilangan district, Padang. The mining activity negatively impacts the environment. Mining can lead to the destruction of habitats in surrounding areas such as loss of vegetation and destruction of soil horizons[2]. The negative impacts can be prevented and reduced by conducting a rehabilitation, ensuring the area mined is returned close to its original state. Mine rehabilitation have become as an important part of the sustainable development in many countries, especially through revegetation. However, the initiatives undertaken on lands that have been damaged (critical land) often encounter problems. This is because some plant species is difficult to adjust due to drastic changes in habitat. Environmental management is needed to overcome this conditions and minimizing the negative impacts of mining on the environmental also preserving eco diversity[3]. Thus required the use of technology to support the revegetation process as with the application of Arbuscular Mycorrhizal Fungi (AMF).

Arbuscular Mycorrhizal Fungi (AMF) as soil micro-organisms plays an important role in the ecosystem, including marginal land. The AMF provide the plant with water, soil mineral nutrients (mainly phosphorus and nitrogen) and pathogen protection. In exchange, photosynthetic compounds are transferred to the fungus[4]. Mycorrhizal an association between certain fungi with plant roots have an important role in increasing resistance to nutrient deficiency, droughtiness, extreme pH, heavy metals and able to improve the structure and biological properties of the soil[2]. The AMF help plants become established in harsh/degraded ecosystems, such as desert areas and mine spoils[5].

The AMF can be found almost in most soils and generally do not have a specific host plants. The level and composition of the population is very diverse and influenced by the characteristics of the plant and environmental factors such as temperature, soil pH, soil moisture, phosphorus and nitrogen as well as the concentrations of heavy metals. Thus, each ecosystem is likely to contain the AMF of the same type or it could be different. In revegetation process, indigenous AMF is considered better than using AMF exogenous[6]. The AMF indigenous is a type that is found in association with plant roots naturally without human intervention in the process of initial infection between mycorrhizae with host plants. The AMF indigenous has a high potential to form extensive infection because they are more adaptive and have a higher tolerance to environmental with extreme conditions[7]. Identification of indigenous AMF is a necessary step in a phytoremedial strategy in rehabilitation, especially in post-mining soil. This study aims to assess of indigenous AMF in mining contaminated sites Bukit Ngalau, PT Semen Padang.

MATERIALS AND METHODS

Time and study site: The study was conducted from May-July 2018. Soil samples were collected from mining contaminated sites Bukit Ngalau, PT Semen Padang, Indarung, Lubuk Kilangan district, Padang, West Sumatera, Indonesia (Fig. 1).

Isolation of arbuscular mycorrhizal fungi (AMF): Isolation of indigenous AMF was carried out by taking a sample of the soil in the field according to the methods of the ICRAF[8]. The AMF isolate taken from roots of vegetation area with deep of soil about 5-20 cm (rhizosphere area). On each post sample will taken ±500 g of soil (composite). Soil samples were taken put in plastic and labeled in accordance with the type of plant as well as the date of sampling soil (Fig. 2).

Propagation of arbuscular mycorrhizal fungi (AMF)/ trapping: The AMF trapping was done using the "Open pot culture method" according to Brundrett *et al.*[9]. Cultures are grown in a greenhouse at least 1 months. Trapping is necessary step to obtain many healthy spores of colonizing fungi for identification and as inoculum to establish monospecific cultures.

Extraction and identification of arbuscular mycorrhizal fungi (AMF): Extraction and identification of spores from soil samples was carried out by referring to the pour and wet filter method[10] with centrifugation modifications[9]. This pouring and filtering technique procedure, first is mixing the soil sample as much as 50 g with 500 mL of water, then stirring until the granules of the soil are destroyed. Then filtered in a set of filters with a size of 500, 125 and 63 μm sequentially from large to small size of filters set. The top filter is sprayed with water to make it easier for the spores to escape. Then the top filter was removed and a number of residual soil left on the 125 and 63 μm sieve was transferred to the centrifuge tube and centrifugation technique was carried out. Filter results in centrifugal tubes plus 60% glucose using pipettes. The centrifuge tube is closed and centrifuged at 3000 rpm for 5 min. Then the supernatant solution was poured into 45 μm filter paper or Petri dish and observed under a

Fig. 1: Study sites Bukit Ngalau PT Semen Padang, West Sumatera, Indonesian
(Source : www.google.com/maps)

Fig. 2: Isolation of AMF with plot 20×20 m^2 at Bukit Ngalau, PT semen Padang, West Sumatera, Indonesia

binocular microscope for spore density (abundance) calculation. Observation of the characterization of FMA type is based on morphological characteristics of spores. The FMA was identified to the genus level with reference to morphological descriptions of AMF genus.

RESULTS

In this study found 3 genus of the AMF in the rhizosphere of 14 plants species at Bukit Ngalau, PT Semen Padang (Table 1) consists of Glomus, Gigaspora and Acaulospora.

Table 1: Genus of AMF were found associate with vegetation in mining contaminated sites Bukit Ngalau, PT Semen Padang

Plant species	Plant habits	Genus of AMF	Spores abundance/50 g of soil)
Melastoma malabatrichum	Shrub	Gigaspora	28
Commersonia bartramina	Tree	Acaulospora	25
Cassia sp.	Tree	Acaulospora	9
Eupatorium odoratum	Shrub	Glomus	16
Mikania micrantha	Herb	Gigaspora	22
Scleria sumatrensis	Shrub	Glomus	25
Stachytarpheta jamaicensis	Shrub	Glomus	38
Megathyrsus maximus	Shrub	Acaulospora	25
Sp. 9	Herb	Gigaspora	14
Piper aduncum	Tree	Glomus	16
Crotalaria mucronata	Shrub	Glomus	29
Bidens pilosa	Herb	Glomus	13
Cassia mimosoides	Tree	Glomus	26
Pennisetum purpureum	Shrub	Glomus	24

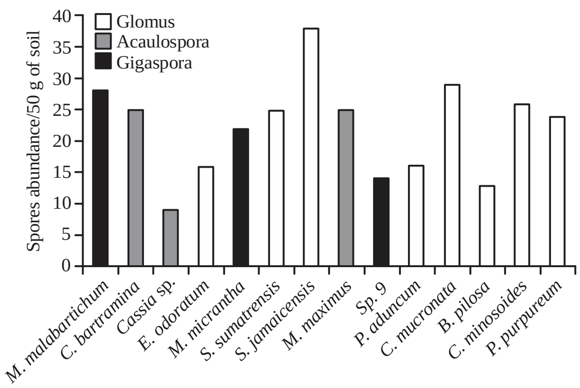

Fig. 3: Genus of spores found associated with plants rhizosphere in Bukit Ngalau PT Semen Padang

These types of AMF are associated with different types of plants. The greatest dominant genus found was from Glomus taxa which is associate with 8 species of vegetation with different habits and has the highest abundance of spore 38/50 g of soil. The Glomus population was found as the highest compare to other mycorrhizal genus Acaulospora and Gigaspora. On the other hand, Acaulospora and Gigaspora genus only found associate with 3 types of vegetation and the lowest value for spores abundance was from Acaulospora (9/50 g of soil) (Fig. 3).

DISCUSSION

This study found the AMF Indigenous from post mining land Bukit Ngalau that can be beneficial for rehabilitation of post mining land especially through revegetation. The result showed that Glomus was the dominant taxa with the highest spore abundance and form the association with most of plants at study site. Glomus is considered as a generalist species at contaminated areas. Glomus is also adapted to the stress caused by different concentrations of heavy metals[11]. Saidi *et al.*[12] mentioned that some of Glomus species produces higher glomalin than the other species. Glomalin serve to protect the hyphae of drought and destruction attack by microbes. According to Husin *et al.*[13], Glomus spores are also found dominant in various rhizosphere in the critical land of Sumatra.

All 14 plants found at Bukit Ngalau, PT Semen Padang showed a strong symbiotic relationship between plants species and AMF but significant differences were observed in the different of host plant species and the spore density varied among plant species. There be selectivity between host plants and AM fungi. The number of AM fungi detected was influenced by host plants under certain conditions[14]. Holste *et al.*[15] also mentioned that the diversity and density of AMF spores would determine by the type of plants on the ground. Both of mycorrhiza and plant diversity can stimulate or retard each other[14]. The exudates that was produced by the roots of the host plant also can affect the germination of spores and the growth of hyphae. The root exudates produce a signal that can be responded by the AMF, if there is a match then the spores will germinate and grow to form hyphae[16-18].

The highest abundance of Glomus spore may also have been influenced by trapping process. Trap cultures is an important factor influencing mycorrhizal development, especially spore formation[19]. Glomaceae isolates generally are the fastest genus to colonize plant roots. Meanwhile, Acaulosporaceae isolates generally colonized roots more slowly, at a rate more similar to spore-regenerating Gigasporaceae. Glomaceae isolates showed more extensive colonization than others isolate[20]. The extensive colonization from Glomus may be one of the reason why Glomus has higher abundance of spore compared to Acaulospora and Gigaspora. On trap culture, there are also some species present in the field soil that were lost after trap culture and the frequency and relative abundance of some species were quite different before and after trap culture[19]. This may due to differences in the ability of AMF to sporulate. According to Wang and Jiang[21], Glomus are thought to require less time to produce spores than others species. Glomus spores germitate faster where it only take 4-6 days of germination[9,22]. The AMF indigenous found in this study is beneficial for the revegetation on post-mining soil. AMF indigenous can guarantee and help plants become established in harsh ecosystems such as post mining soil. In Indonesia, the government recommend to use native plants in land rehabilitation, this study will help the success of using native plants as rehabilitation strategy that associated with AMF.

CONCLUSION

There is three genera of AMF were found in the rhizosphere of vegetation at Bukit Ngalau, PT Semen Padang. The AMF identified fell within Glomus, Gigaspora and Acaulospora. Glomus was the dominant genus with the highest spore abundance (38/50 g soil) compared to both of Gigaspora and Acaulospora.

ACKNOWLEDGMENT

This study was supported by Andalas University who has given financial support for this study with the grant number 4917.01 $and PT Semen Padang for facility and access to study sites.

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