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RESEARCH ARTICLE **Open Access**

Identification and Diversity of Mycorrhizae in Several Rhizosphere Vegetation Plants in Former Mining Land for Gambir Planting

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Abstract
Post-mining land represents an ecosystem that has undergone severe degradation as a result of mineral resource exploitation. The mining process causes the loss of the topsoil layer, which is rich in organic matter, microorganisms, and essential nutrients, leading to nutrient-poor, compacted, and acidic soils that are often contaminated with heavy metals. The cultivation of gambier has been proposed as a strategy to optimize the use of post-mining land. One initial approach to address this problem is the application of arbuscular mycorrhizal fungi (AMF). Soil conditions and the type of host vegetation strongly influence the diversity of AMF in post-mining areas. Therefore, identifying the presence of AMF in post-mining soils is necessary. Soil samples were collected from PT Atoz Nusantara Mining, located in Kenagarian Tambang, IV Jurai Subdistrict, Pesisir Selatan Regency. This exploratory study involved soil analysis and the identification of arbuscular mycorrhizal fungi. The plant species sampled included alang-alang (*Imperata cylindrica*), resam nail (*Dicranopteris linearis*), caramunting (*Melastoma malabathricum*), telang (*Clitoria ternatea*), sedge grass (*Cyperus rotundus*), and sembung (*Blumea balsamifera*). The results indicated that the occurrence of mycorrhizae in the rhizosphere varied across post-mining sites, with identified species including *Acaulospora* sp., *Glomus* sp. 1, *Glomus* sp. 2, *Sclerocystis* sp., and *Gigaspora* sp. These mycorrhizae demonstrate potential for restoring soil fertility in post-mining areas.

Keywords: Gambir, Mycorrhiza, Post-Mining Land, Soil, Vegetation

1. Introduction

Post-mining areas are ecosystems that have undergone severe degradation as a result of mineral resource exploitation. The mining process leads to the loss of topsoil, which is rich in organic matter, microorganisms, and essential nutrients. As a result, the soils become nutrient-poor, compacted, acidic, and often contaminated with heavy metals. These conditions make post-mining areas highly challenging for plant growth without ecological rehabilitation efforts (Mulyani *et al.*, 2011).

One important approach to improving the quality of post-mining soil is the use of soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF). Mycorrhizae are mutualistic symbiotic associations between soil fungi and plant roots that play a vital role in enhancing water and nutrient uptake, increasing tolerance to heavy metal stress, and improving soil structure (Smith & Read, 2008). The presence of mycorrhizae is crucial in degraded ecosystems because they can enhance the success of revegetation by supporting the adaptation of pioneer plants (Caravaca et al., 2002). Soil conditions and the types of host vegetation present have a strong influence on mycorrhizal diversity in post-mining areas. Pioneer vegetation, such as legumes and grasses, often exhibits a high degree of mycorrhizal association due to its adaptive capacity in nutrient-poor environments. (Asmelash *et al.*, 2016) . The identification of the types and diversity of mycorrhizae in the rhizosphere of various vegetation in ex-mining land will provide important information for ecologically based rehabilitation strategies, especially in selecting appropriate plants and utilizing local mycorrhizal inoculum as a biofertilizer (Gai et al., 2006).

One effort to optimize former mining land is planting gambier plants. Gambier plants have great potential for development on former mining land. Research by Lopita et al. (2024) found that gambier plants can grow well on ex-coal mining land by providing mycorrhizae to the soil. Ex-mining land has a high heavy metal content, so gambier

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plants grown on such land have the potential to have higher levels of secondary metabolites, especially catechins.

The first step is to identify the mycorrhizae present in the post-mining area. Research on mycorrhizae in post-mining sites remains limited in Indonesia. However, a thorough understanding of mycorrhizal diversity and distribution can support sustainable land reclamation and revegetation efforts after mining. Therefore, a study titled "Identification of Mycorrhizal Diversity in Several Rhizospheres of Plant Vegetation in Post-Mining Areas for Gambier Cultivation was conducted, aiming to identify the mycorrhizae present in these post-mining areas.

2. Material and Methods

2.1. Time and place

The research was conducted from February 2024 to June 2024, involving the sampling of ex-coal mining soil in the former mining area of PT. Atoz Nusantara Mining Kenagarian Tambang, IV Jurai District, Pesisir Selatan Regency, coordinates 1° 19′ 8.4″ S, 100° 34′ 33.6″E 1.319 , 100.576 . Observations of root colonization were conducted at the Plant Physiology Laboratory, Faculty of Agriculture, Andalas University, Padang.

2.2. Materials and tools

The materials used in this experiment were ex-coal mining soil, plant samples (Alang-alang, Paku resam, Karamunting, Telang, Rumput teki), water, distilled water, 10% KOH, 2% HCl, and lacto-lys*erol trypan blue*.

The equipment used in this experiment is a soil drill, hoe, shovel, plastic bucket, clear plastic bag measuring 30cm x 45cm, label paper, stationery (pen, book, marker), scissors, spatula spoon, digital scale, spore filter (45 πm, 106 πm, and 300 πm), glass slide, *cover glass*, beaker, *hotplate*, *centrifuge*, film bottle, scissors, petridish, dropper pipette, tweezers, microscope and documentation tools.

2.3. Research procedures

The initial step in this research is the process of taking soil samples, which aims to analyze the soil's properties. The chemical properties of the soil to be analyzed are pH, N-Total, P-Available, P-Total, K-Total, and C-Organic. Soil samples were taken from the former coal mining area located at PT Atoz Nusantara Mining, specifically in Kenagarian Tambang, IV Jurai District, Pesisir Selatan Regency. The soil sampling point used a stratified random sampling method. Three soil sampling points had been carried out. After that, plant samples were also taken, including Alang-alang, Paku resam, Karamunting, Telang, Rumput teki, and Sembung, along with the soil around the roots of the plants. Plant samples were taken by pulling them out to the roots, then placed in plastic bags.

The soil sample, weighing 50 g, was placed into a beaker and then mixed with 500 mL of water. The mixed soil and water solution was filtered through 300 µm, 106 µm, and 45 µm sieves using the wet sieving method (*Brundrett et al.*, 1996). The supernatant solution from each sieve size was transferred into a 50 mL beaker, and each sample extract was diluted to approximately 5 mL. Then, the 5 mL spore extract was mixed with 5 mL of 60% glucose and then centrifuged at 2500 rpm for 10 minutes. After that, the spores were filtered through a 45 µm sieve. The number of spores was then observed and counted using a binocular microscope with a 100x magnification. The following is a flowchart of the research procedure:

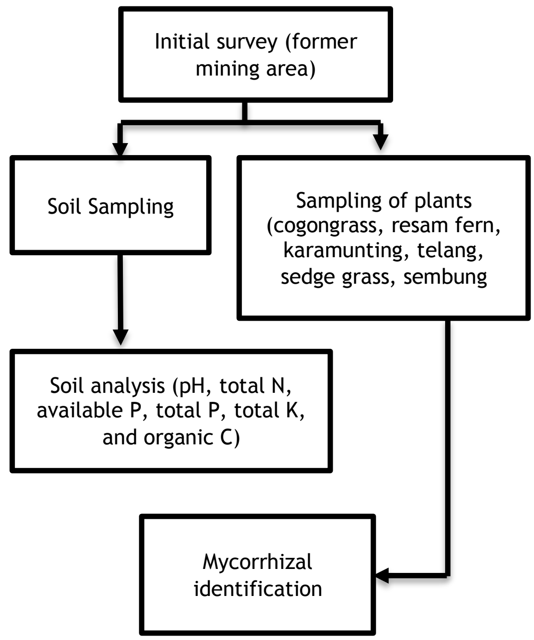


Figure 1. Research flow diagram

3. Results and Discussion

3.1. Soil analysis results

The soil used in this study was ex-coal mine soil, which underwent analysis before the survey. This analysis aimed to determine the initial soil condition, particularly its fertility. Several important parameters measured included pH, nutrient content (nitrogen (N), phosphorus (P), and potassium (K)), and organic matter content (organic carbon). The results of the initial soil analysis are presented in Table 1.

The results of the analysis in Table 1 indicate that the pH of the ex-coal mining soil is 4.42, which falls within the criteria for very acidic soil. Under these acidic soil conditions, microbial activity, including N mineralization from organic materials and nitrification, can be inhibited. (Halvin *et al*, 2005). In addition, soil conditions that are too acidic can hinder plant growth because they can bind important nutrients such as phosphorus, making them unavailable to plants.

The results showed that the soil's N, P, and K nutrient content was low. Low total N content indicates low nitrogen availability in the soil. Nitrogen is an essential nutrient for plant vegetative growth. Low available phosphorus (P) and total phosphorus (P) content will affect plant growth. Phosphorus deficiency in plants will cause stunted growth. The total K content was also low, despite its important role in photosynthesis and nutrient transport in plants. Plants require a balanced intake of nutrients for growth. N deficiency disrupts P and K absorption during the plant's growth phase. P nutrients are needed in large quantities after N nutrients, because P nutrients play a role in plant growth from the vegetative phase to the generative phase.

Table 1. Results of analysis of ex-coal mining soil.

Analysis	Method	Results	Criteria
pH H20 (1:5)	Electromete	4.42	Very sour
N-Total (%)	Kjedhal	0.12	Low
P-Available (ppm P2O5)	Bray 1	6.12	Low
P-Total (mg P2O5/100g)	HCL Extract 25%	17.21	Low
K-Total (mg K2O/100g)	HCL Extract 25%	11.09	Low
C-Organic (%)	Weekly and Black	2.64	Low

Another important parameter for assessing soil quality is organic carbon content. The results of the organic carbon analysis on ex-coal mine soil fall within the moderate criteria. The organic carbon content is crucial for enhancing soil structure, increasing water-holding capacity, and providing a food source for soil microorganisms.

3.2. Identification of AMF in vegetation on ex-coal mining land

Vegetation growing around the soil sampling site on ex-coal mining land is present. This vegetation is illustrated in Figure 2.

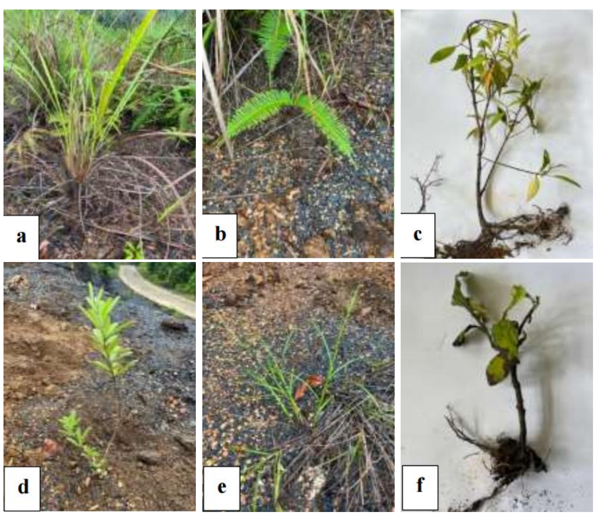


Figure 2. Vegetation on former coal mining land. (a) Cogongrass, (b) Fern, (c) Karamunting, (d) Telang, (e) Nutgrass, (f) Sembung

Observations of local vegetation in former coal mining areas revealed a type of vegetation known as alang-alang (*Imperata cylindrica*). The characteristics of this type of alang-alang (*Imperata cylindrica*) have fibrous roots and many have dense root hairs, the surface of the stem is segmented as a place for the leaves to sit, the leaves of the alang-alang grow upright in the form of lines (*lanceolate*) which narrow towards the base, the edges of the leaves are very rough with wide middle leaf veins.

The characteristics of the type of resam fern (Dicranopteris linearis) that was found include fibrous roots, an inconspicuous stem that grows parallel to the ground, and a growth pattern resembling roots, known as rhizomes. The stem may be spreading or slightly upright. When it appears above ground, the stem is very short; some of the stems are round and flat. The leaves are dark green and have many leaves that are arranged tightly opposite each other.

The characteristics of the Karamunting species (*Rhodomyrtus tomentosa*) found in this study have the attributes of branched taproots, woody stems (*lignosus*) that are round and brown, oval leaves (*ellipticus*), opposite leaf seats, hairy leaf surfaces, and when touched, feel rough, rounded leaf bases, flat leaf edges, and pointed leaf tips, and have purplish pink flowers. In addition, butterfly pea vegetation (Clitoria laurifolia) was also found to have the characteristics of taproots and a white color. The stems are round and dark green, with compound leaves and an oval shape. In one leaf stalk, there are three leaves. The flowers are purplish white.

The characteristics of the nutsedge (*Cyperus rotundus* L.) species found include fibrous roots, numerous branches, and fine hairs. The stems are triangular and green. The leaves are ribbon-shaped and shiny green, located at the base of the stem. In addition, the vegetation of the sembung plant (Blumea balsamifera) was also found to have the characteristics of a taproot and a white color. The stems are dark green, upright, and round. The single leaves are oval in shape, with pinnate leaf veins. The upper leaf surface is slightly rough, the underside is densely and smoothly hairy, and the leaf edges are serrated.

AMF identification was performed on each vegetation rhizosphere found. The purpose of AMF identification was to determine the type of AMF present in the plant's rhizosphere. The results of AMF identification in vegetation from former coal mining areas are presented in Table 3.

Table 3. Mycorrhizal analysis of ex-mining vegetation and its criteria

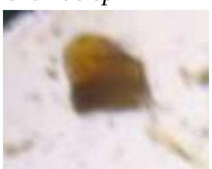
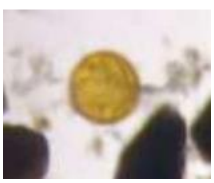
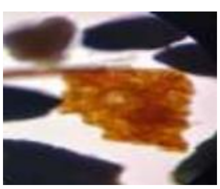
Types of mycorrhiza	**Criteria**
 *Acaulaspora sp*	• Oval-shaped, yellowish brown in color • Has thin cell walls • Found on the 106 πm sieve • Found in the rhizosphere of the fern (*Dicranopteris linearis*)
 *Glomus sp* 1	• Oval shaped • Has thick cell walls • Blackish brown in color • Found on the 300 πm sieve • Found in the rhizosphere of *Imperata* cylindrica
 *Glomus sp* 2	• Oval shaped • Yellowish brown in color • Hyphae attached to the outer wall • Found on the 45 πm sieve • Found in the rhizosphere of butterfly pea (*Clitoria* • *laurifolia*)
 *Gigaspora sp*	• Round shaped • Black in color • Found on the 300πm sieve • Found in the rhizosphere of nutsedge • (*Cyperus rotundus* L)
 *Gigaspora sp*	• Round shaped • Has 2 layers of cell walls • Yellowish brown in color • The surface of the spores is relatively rough. • Hyphae attached to the outer wall • Found on the 45 πm sieve • Found in the rhizosphere of *Blumea* • *balsamifera*)
 *Sclerocytis sp*	• Spores are arranged in a non-compact sporocarp. • Irregular sporocarp shape • Yellowish brown to brown in color • Found on the 106 πm sieve • Found in the rhizosphere of karamunting (*Rhodomyrtus tomentosa*)

Table 3 shows that *Acaulospora sp. AMF spores are present* in the rhizosphere of the resam fern, which are characterized by oval-shaped spores and a yellow-brown color. According to INVAM (2024), *Acaulospora sp. AMF* are round and yellow to brown in color. Alayya & Prasetya (2022) *Acaulospora sp.* generally has an oval shape and is yellow-brownish cream in color.

The rhizosphere of cogongrass contains AMF of *the Glomus sp* 1 type, which has the characteristics of oval spores, thick cell walls, and a blackish-brown color. INVAM (2024) states that the *Glomus sp.* 1 type of spores has the characteristics of a globular, sub-globular, and oval shape, and this type of spore has a brownish to black color and has a nucleus. Bimantara *et al.* (2024) found the genus *Glomus sp* 1 in the rhizosphere of cogongrass with round spore characteristics, reddish-brown in color, and clearly visible spore walls. The results of research by Armansyah *et al.* (2019) stated that there was *Glomus sp* 1 in the rhizosphere of yam plants grown in three types of crop rotation, with the characteristics of oval-shaped spores, passing a 300 μm sieve, light brownish yellow in color, having bulbs, a slightly smooth surface, and a single-layer thick spore wall.

Glomus sp 2 type AMF was found in the rhizosphere of telang plant with the characteristics of oval-shaped spores, brownish-yellow in color, and hyphae attached to the outer wall. Armansyah *et al* (2019) stated that there was *Glomus sp* 2 in the rhizosphere of yam plants grown in three types of crop rotation, with the characteristics of oval-shaped spores, passing through a 300 µm sieve, brownish-yellow in color, a slightly smooth surface, and a two-layered spore wall. Bimantara *et al.* (2024) found that the genus Glomus sp. 2 has the characteristics of brownish-yellow spores, a round shape, a clearly visible spore wall, a smooth spore surface, and no ornamentation, measuring 125.39 µm x 125.39 µm.

In the rhizosphere of nutsedge, there are AMF spores of *the Gigaspora sp type* with characteristics of a round shape and black color. Bimantara *et al.* (2024) found AMF type *Gigaspora sp. 2 in nutsedge, characterized by brown spores with a round shape, clearly visible spore walls, a bulbous suspensor, and spores measuring 146.48 µm* × 146.48 µm. Research by Puspitasari et al. (2012) also discovered the AMF type Gigaspora sp., which is characterized by round spores and a relatively rough spore wall. The spores found have black spore walls, but no attached hyphae are present.

The AMF type *Gigaspora sp* was also found in the rhizosphere of the sembung plant. The characteristics of the AMF spores of *the Gigaspora sp type* found in this study were spherical, had 2 layers of cell walls, were brownish yellow in color, the spore surface was relatively rough, and the hyphae were attached to the outer wall. INVAM (2024) stated that the *Gigaspora sp type* had a spherical shape, had several layers of cell walls, and the hyphae were attached to the outer wall. Karmadibrata (2016) found the AMF type Gigaspora sp. in the rhizosphere of plants on Enggano Island, characterized by yellow spores, spherical in shape, with a smooth spore surface, measuring 200 µm. The "bulbous suspensor" was pale yellow, measuring 50 x 36 µm. (Moh *et al. (*2016) also found Gigaspora sp in the rhizosphere of sugar palm with the characteristics of a round shape, brownish-yellow color, thick spore walls, and rough ornaments.

In the rhizosphere of Karamunting, *Sclerocystis sp. AMF was found—the Sclerocystis sp*. The AMF found in this study exhibits characteristics such as spores arranged in non-compact sporocarps, irregular sporocarp shape, and a yellow-brown to brown color. Tuheteru *et al.* (2019) found *Sclerocystis sp* with characteristics of spores that cluster to form compact, brown sporocarps, and smooth spore surfaces. Kramadibrata (2016) also found *Sclerocystis rubiformis* with characteristics of spores arranged in non-compact sporocarps, yellow-brown to brown in color, irregular sporocarp shape, sporocarp measuring 150-200 µm, smooth spore surface, measuring 30-40 µm. Short hyphae are light yellow to dark yellow.

4. Conclusion

Based on the research conducted, it can be concluded that in former mining land containing cogongrass, resam fern, karamunting, telang, sedge grass, and sembung, mycorrhizae of the following types were found: Acaulospora sp., Glomus sp. 1, Glomus sp. 2, Sclerocystis sp., and Gigaspora sp.

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