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Isolation and Identification of Arbuscular Mycorrhiza (AM) in the Plant Rhizosphere in the Greenbelt Area of PT. Semen Indonesia Tbk Tuban

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Abstract

The greenbelt area serves as a buffer zone within an industrial region, acting as a barrier between mining sites and other zones like residential or agricultural areas. The Greenbelt comprises diverse plant vegetation, absorbing CO2, providing air conditioning, and curbing sedimentation and erosion. Arbuscular mycorrhiza exhibits potential for thriving in this area through symbiotic relationships with local plants. Thus, investigating arbuscular mycorrhiza in the Greenbelt is crucial. It is primarily because certain types of arbuscular mycorrhiza could serve as adaptable biological fertilizers, especially in the environmentally distinct Greenbelt and post-limestone mining regions. This study aimed to identify arbuscular mycorrhizae in the Greenbelt of PT Semen Indonesia Tbk Tuban. Using purposive sampling, we selected three stations: eucalyptus (*Melaleuca cajuputi* Powell), *trembesi* (*Samanea saman* (Jacq.) Merr.), and sapodilla (*Manilkara zapota* (L) P.Royen) rhizospheres. Executed in November 2021, the study encompassed sampling, sample isolation, and species identification phases. Results unveiled seven arbuscular mycorrhiza types across five genera: *Glomus, Acaulospora, Gigaspora, Racocetra,* and *Archaeospor*a. Notably, *Glomus* was the most prevalent genus. This research augments the knowledge pool concerning AMF diversity in PT Semen Indonesia's Greenbelt. It elucidates their potential as biological fertilizers, plant growth catalysts, mine reclamation tools, and defenders against drought stress in mining-affected regions.

Keywords: arbuscular mycorrhiza; greenbelt, *rhizosphere*

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Introduction

Tuban is one of the regencies in East Java, adjacent to the North Coast, and positioned within the northern basin of East Java (1). Owing to its geographical positioning, Tuban Regency possesses a significant reservoir of mineral resources, among which limestone stands out prominently. Limestone constitutes a vital raw material in cement manufacturing, contributing to a substantial 80% of the cement composition in industrial production (2). The cement industry has extensively harnessed lime resources within the Tuban area (3). However, large-scale mining activities have inevitably engendered adverse environmental impacts, resulting in ecosystem deterioration, reduced soil productivity, and compromised environmental quality, leading to critical land conditions and land degradation (4).

The impacts of mining activities on land degradation manifest in a reduction in the variety of flora, fauna, and soil microorganisms. Additionally, the presence of former mining pits disrupts the drainage system and poses obstacles to subsequent land use (5). Soil structure deterioration further contributes to decreased soil stability, alterations in soil pore distribution, impairment of pore channels crucial for water absorption, and heightened vulnerability to erosion (6). Moreover, mining zones and cement industries are suspected culprits behind environmental pollution, notably air pollution, which significantly affects public health (7,8). As a response (7,8), it becomes imperative to establish designated boundary lands separating mining areas from residential zones to mitigate these issues and safeguard the well-being of local inhabitants.

One of the initiatives aimed at demarcating mining zones from residential regions involves establishing greenbelt areas or zones (9,10)(9,10). A greenbelt area constitutes a protective buffer zone meticulously designed to demarcate mining areas from residential ones. This greenbelt zone averts potential landslides along the mining zone's cliffs, enhances the mining area's drainage infrastructure, and contributes to temperature reduction within the mining vicinity (11).

The greenbelt area of PT. Semen Indonesia's Tuban Plant encompasses a space where diverse vegetation flourishes (12). The capacity of this varied plant growth can be attributed to multiple factors, including the ability of plant roots to engage in symbiosis with soil microorganisms like arbuscular mycorrhiza. Such a symbiotic relationship between arbuscular mycorrhizal fungi and plant roots transpires within the vegetation in the Greenbelt area (13). Notably, arbuscular mycorrhiza is recognized for its potential to form symbiotic bonds with nearly 80% of plant species and can thrive across various ecosystems, even in nutrient-deprived mining sites (14). This symbiosis embodies mutualism, with mycorrhiza arbuscular infiltrating plant root cortices to obtain photosynthetically derived carbon from the plants. Mycorrhiza arbuscular also resides within the rhizosphere, encircling the plant's roots (15). As a catalyst, mycorrhiza at the root interface enhances the soil's absorption of phosphorus and nitrogen elements. Furthermore, mycorrhiza offers shielding effects against drought-induced stress and pathogenic assaults on plant roots, demonstrates tolerance to soil metal content, and aids in root access to groundwater. In a broader context, mycorrhiza is extensively employed as a biological fertilizer agent, fostering heightened plant growth (16,17).

This study aims to identify and describe the types of arbuscular mycorrhiza (AM) discovered within the greenbelt area of PT Semen Indonesia Tbk Tuban.

Materials and methods

This research was conducted within the greenbelt area of PT Semen Indonesia Tbk Tuban in November 2021. Mycorrhizal identification occurred at the integrated Biological Laboratory of PGRI Ronggolawe Tuban University. The exploration of arbuscular mycorrhiza employed a purposive sampling technique at three designated stations, selected diagonally, encompassing the rhizospheres of eucalyptus plants (*Melaleuca cajuputi*), trembesi plants (*Samanea saman*), and sapodilla plants (*Manilkara zapota*). Soil samples from the rhizosphere were collected at a depth of 50 cm, amounting to 1000 g each. The research methodology encompassed wet strain preparation followed by centrifugation. The resulting data were subjected to descriptive analysis.

Mycorrhizal isolation was conducted by thoroughly mixing 50 grams of soil samples with 1000 ml of distilled water. Subsequently, a stratified screening process was undertaken using sieves with accuracies of 60 µm, 120 µm, and 230 µm, each repeated twice. The filtrate obtained was then centrifuged at 5000 rpm for 5 minutes. From the resulting centrifuged mixture, 1 ml was extracted and examined under a binocular microscope at a 40x10 magnification level. The observations garnered through the microscopic analysis were aligned with the guidelines provided by INVAM (The International of Vesicular Arbuscular Mycorrhizal) for identification.

Results

The outcomes of the isolation and identification of arbuscular mycorrhiza within the greenbelt area of PT Semen Indonesia Tbk Tuban, specifically in the rhizospheres of eucalyptus plants (*Melaleuca cajuputi*), trembesi plants (*Samanea saman*), and sapodilla plants (*Manilkara zapota*), observed at a magnification of 40x10, are illustrated in Figure 1.

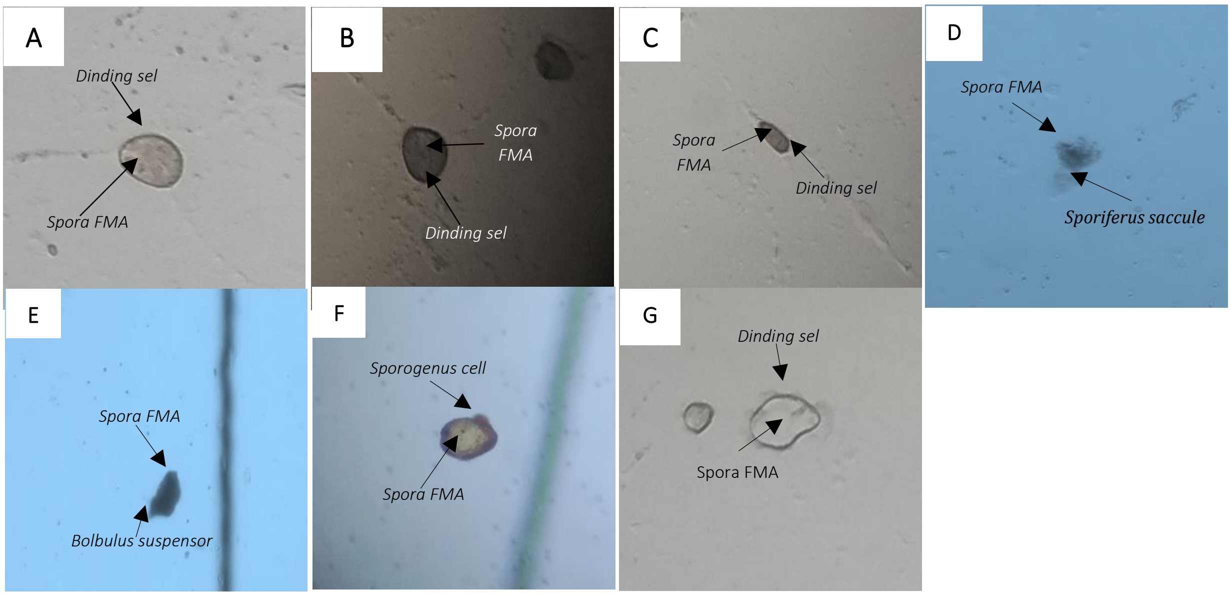

Figure 1. Arbuscular mycorrhizal fungus, A. *Glomus* 1; B. *Glomus* 2; C. *Glomus* 3; D. *Acaulospora*; E. *Gigaspora;* F. *Racocetra;* G. *Archaeospores*

The morphological characteristics of Arbuscular Mycorrhiza (AM), encompassing factors such as spore shape, spore color, and cell wall properties to discern the arbuscular mycorrhizal genus, along with the density of arbuscular mycorrhiza per 1000 grams of soil and the spatial distribution, are detailed in Table 1. The process of arbuscular mycorrhiza identification adheres to the guidelines set forth by INVAM (The International of Vesicular Arbuscular Mycorrhizal).

Table 1. The density of mycorrhizal presence, identified locations, and distinctive characteristics.

No	Genus	Morphological Features			Special Features	Location	Amount^{-1000g}
		Shape	Color	Cell wall			
A	*Glomus* 1	Oval	Faded brown	Clear		*Manilkara zapota*	100
B	*Glomus* 2	Oval	Solid brown	Clear		*Manilkara zapota*	80
C	*Glomus* 3	Elliptical	Solid brown	Clear		*Manilkara zapota*	20
D	*Acaulospores*	Globulus	Solid brown	Not visible	Has *sporiferus sacule*	*Samanea saman*	20
E	*Gigaspores*	Irregular	Solid brown	Not visible	Has a *suspensor bolbolus*	*Melaleuca cajuputi*	20
F	*Racocetra*	Oval	Brownish-yellow	Clear	*Has Sporogenous cells*	*Melaleuca cajuputi*	20
G	*Archaeospores*	Elliptical	Clear white	Clear		*Manilkara zapota*	40

"Mycorrhiza" is derived from "Miko" (fungus) and "rhiza" (root or root area). It signifies a mutually beneficial symbiotic relationship between fungi and plant roots (18). The study's findings, which involved examining three samples from the rhizospheres of eucalyptus, trembesi, and sapodilla plants, revealed the existence of seven mycorrhiza types across five distinct genera. External factors influencing the exploration of arbuscular mycorrhiza included the measurement of soil acidity or pH in the Greenbelt area, which registered at 7. Additionally, the temperature in the Greenbelt region was recorded as 39°C, with soil humidity ranging between 30% and 32%. Figure 1 depicts the genus *Glomus*'s presence in the sapodilla plant's rhizosphere (*Manilkara zapota*). Within the greenbelt area, the identified *Glomus* genus comprises three variations discerned by their morphological attributes. *Glomus* 1 possesses an oval shape and a light brown hue. *Glomus* 2, also oval, exhibits a dark brown color, while *Glomus* 3, characterized by its oblong form, features a solid brown color. Notably, all three variants have transparent cell walls. According to INVAM, *Glomus* is typified by its round to oval shape, appearing as faded brown, brown, or deep brown, with a visible cell wall and branched hyphae (19).

Glomus 1 emerges as the predominant species compared to other genera, constituting 100 individuals per 1000 grams of soil. Amongst the various types of FMA, *Glomus* showcases remarkable resilience to diverse environmental conditions. It thrives in infecting plant roots, even enduring extended periods of aridness. Concurrently, analogous research conducted on the slopes of Bali concurred that the prevalent mycorrhiza type was *Glomeraceae*, precisely the *Glomus* variety (14). Significantly, *Glomus* fulfills a pivotal role as a vital source of phosphorus elements indispensable for plant growth. The capacity of plant roots to absorb nutrients stands to augment in tandem with the number of mycorrhizal infections within the root rhizosphere zone (20).

The notable resilience exhibited by the *Glomus* genus can be harnessed to facilitate the reclamation process of the mining area at PT Semen Indonesia Tbk Tuban. Typically, mining regions are characterized by nutrient-deficient soils (21). *Glomus* can be harnessed as a biological fertilizer agent within the greenbelt area, contributing to enhanced plant growth and developmental processes in challenging terrains. Furthermore, it safeguards against pathogenic attacks on plants (22).

Another genus discovered within the Greenbelt area is *Acaulospora*. Currently, the *Acaulospora* genus comprises 37 known species. *Acaulospora* is distinguished by its germination wall structure and spores devoid of mycelium. In field conditions, the wall structures of *Acaulospora* are prone to damage (23). As per INVAM guidelines, *Acaulospora* displays globule-shaped and oval attributes, presenting colors ranging from dense brown to faded brown, and features a soporiferous saccule attached to the end of its spores. The cell wall is visible, and spores measure 60-380 µm. The *Acaulospora* isolated from the rhizosphere of the trembesi plant (*Samanea saman*) exhibit globule-shaped characteristics, a dense brown color, and are retained by a 230 µm sieve, possessing soporiferous saccule at their ends.

In contrast to *Glomus*, this genus demonstrates a lower tolerance for the typically arid conditions of greenbelt areas (24). It is encountered in numerous wetlands during both rainy and dry seasons. *Acaulospora* also plays a pivotal role in absorbing phosphorus elements within the soil. In Thailand, *Acaulospora* has been employed as a biological agent to enhance the growth of rice plants (25).

Gigaspora represents a mycorrhizal genus found within the *Glomeromicotyna* division of the *Gigasporaceae* family (26). This classification is founded upon analyses of spore development, germination, and cladistics (27). Morphologically, the *Gigaspora* genus displays a densely brown globose shape accompanied by a discernible cell wall. *Gigaspora* spores typically range in size from 100 to 440 µm, showcasing ornaments in shades ranging from yellow to brownish-yellow/deep brown, which exist individually (28). Most spores exhibit a thin composition comprising two spore layers. In *Gigaspora* spores, a bulbous suspensor can be found at the spore's tip (observed under the microscope). These spores feature a single layer of cell walls. The representation of *Gigaspora* is depicted in Figure 1E, which results from isolation efforts within the greenbelt area of PT Semen Indonesia, specifically focusing on a stand of eucalyptus plants (*Melaleuca cajuputi*). Spore identification is executed by aligning morphological characteristics with INVAM's identification guidelines.

Another genus identified within the Greenbelt area is *Racocetra*. These spores exhibit a distinct oval shape and brownish-yellow color, passing through a sieve with a 125 µm mesh size. They possess sporogenous cells at the spores' tips and exhibit a dense brown hue. As elucidated by INVAM, the *Racocetra* genus presents morphological attributes ranging from oval to globose or subglobose. Their color varies from yellow to brownish-yellow, and they fall within the size range of 240-350 µm. These spores consist of two layers of spore walls and two layers of flexible inner germinal walls. The auxiliary cells are characterized by thin walls and a smooth, projecting surface originating from hyphae within the soil near the root surface. *Racocetra* was identified within the greenbelt area, specifically in the rhizosphere of eucalyptus stands (*Melaleuca cajuputi*), numbering 20 occurrences. Research indicates that *Racocetra* can thrive in contaminated terrains, such as former tin and gold mines (29).

The genus *Archaeospora*, discovered within the greenbelt area specifically in the rhizosphere of the sapodilla plant roots (*Manilkara zapota*), displays distinctive characteristics, including a globule-shaped form, a precise white coloration, and visible cell walls. *Archaeospora* is categorized within the order *Glomales*. Its classification as the *Archaeospora* genus was established through molecular, morphological, and biochemical investigations. Notably, this genus is characterized by its single-spore structure (23). Under INVAM, the morphological traits of the *Archaeospora* genus encompass a shiny white appearance (hyaline). Most of these species exhibit a globular shape, while some adopt a subglobose to elliptical morphology. The spore size falls within the range of 50-80 μm. In *Archaeospora*, the spore wall comprises three layers of hyaline material (L1, L2, and L3).

The presence of mycorrhiza in the greenbelt area holds the potential for aiding the post-limestone mining land revegetation process at PT Semen Indonesia Tbk Tuban due to its adaptability to marginal land conditions and minimal water availability. Mycorrhiza is vital in facilitating the phytoremediation process within the reclaimed mining regions, enhancing soil fertility, and preserving soil structure (30). This symbiotic fungal relationship assists plant roots in extending their reach for water and nutrients within the soil, facilitated by lateral hyphae, particularly for absorbing essential phosphorus elements.

Phosphorus is indispensable for synthesizing ATP (Adenosine Triphosphate) compounds in plants, stimulating growth. ATP, a crucial plant compound, is critical in metabolic processes and energy-related reactions (31). Research indicates that phosphorus availability tends to be limited in post-mining regions, underscoring the significance of soil microorganisms like mycorrhiza in aiding plants to access soil nutrients and facilitating plant growth within these areas (32).

Conclusion

A total of 7 distinct mycorrhizal types stemming from 5 different genera were discovered within the Greenbelt region of PT Semen Indonesia's Tuban Factory, specifically within stands of teak, sapodilla, and eucalyptus plants. Mycorrhiza represents a symbiotic relationship between microfungi and plant roots. The microorganisms found within the Greenbelt area offer valuable advantages as biological fertilizers, effectively supporting plant growth. Furthermore, mycorrhiza plays a pivotal role in the bioremediation of post-mining sites, affording protection against drought stress in mining-affected regions, aiding in plant nutrient acquisition, and furnishing protection against pathogens.

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Conflict of interest

All authors declare that they have no conflicts of interest about this matter.

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