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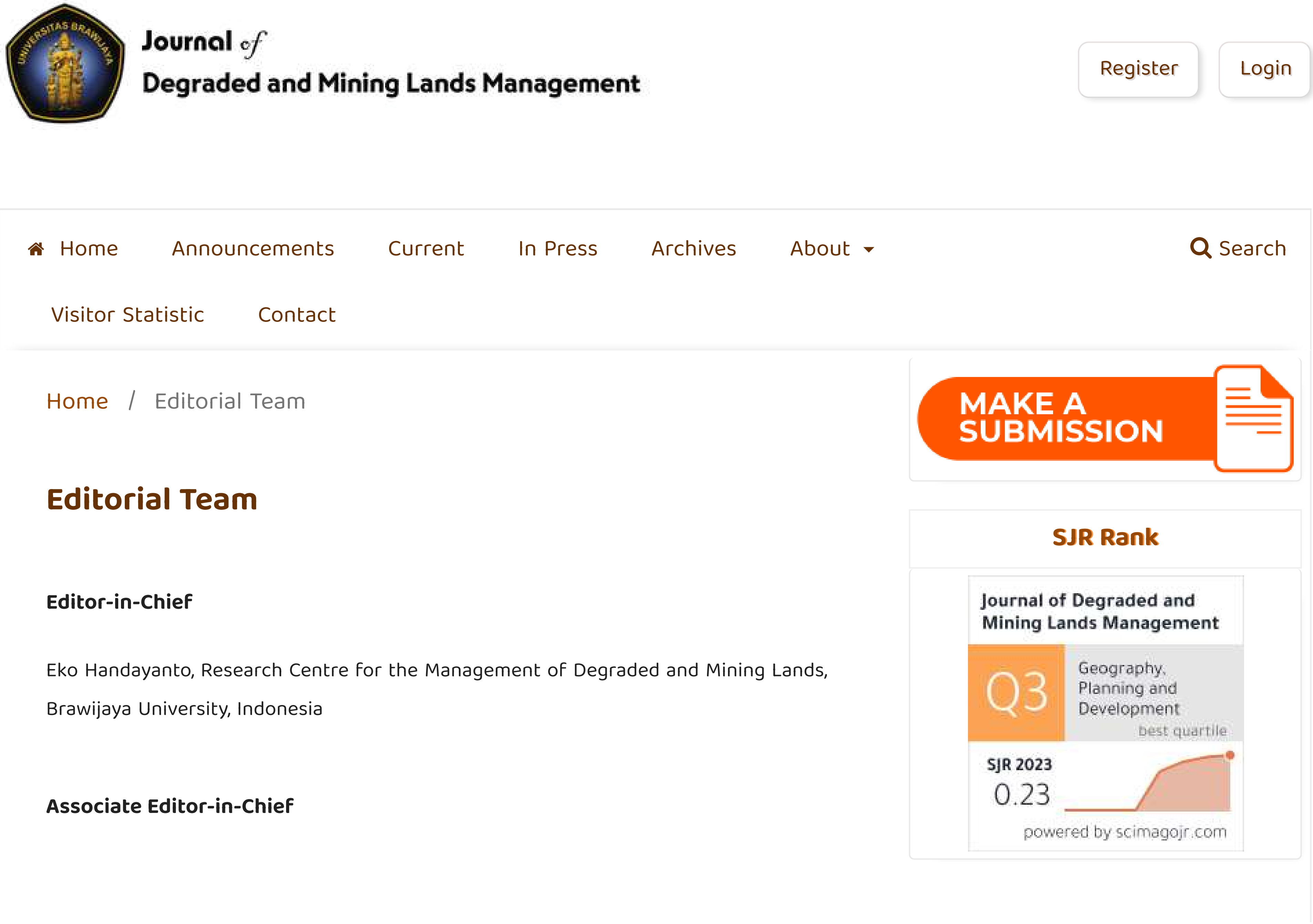

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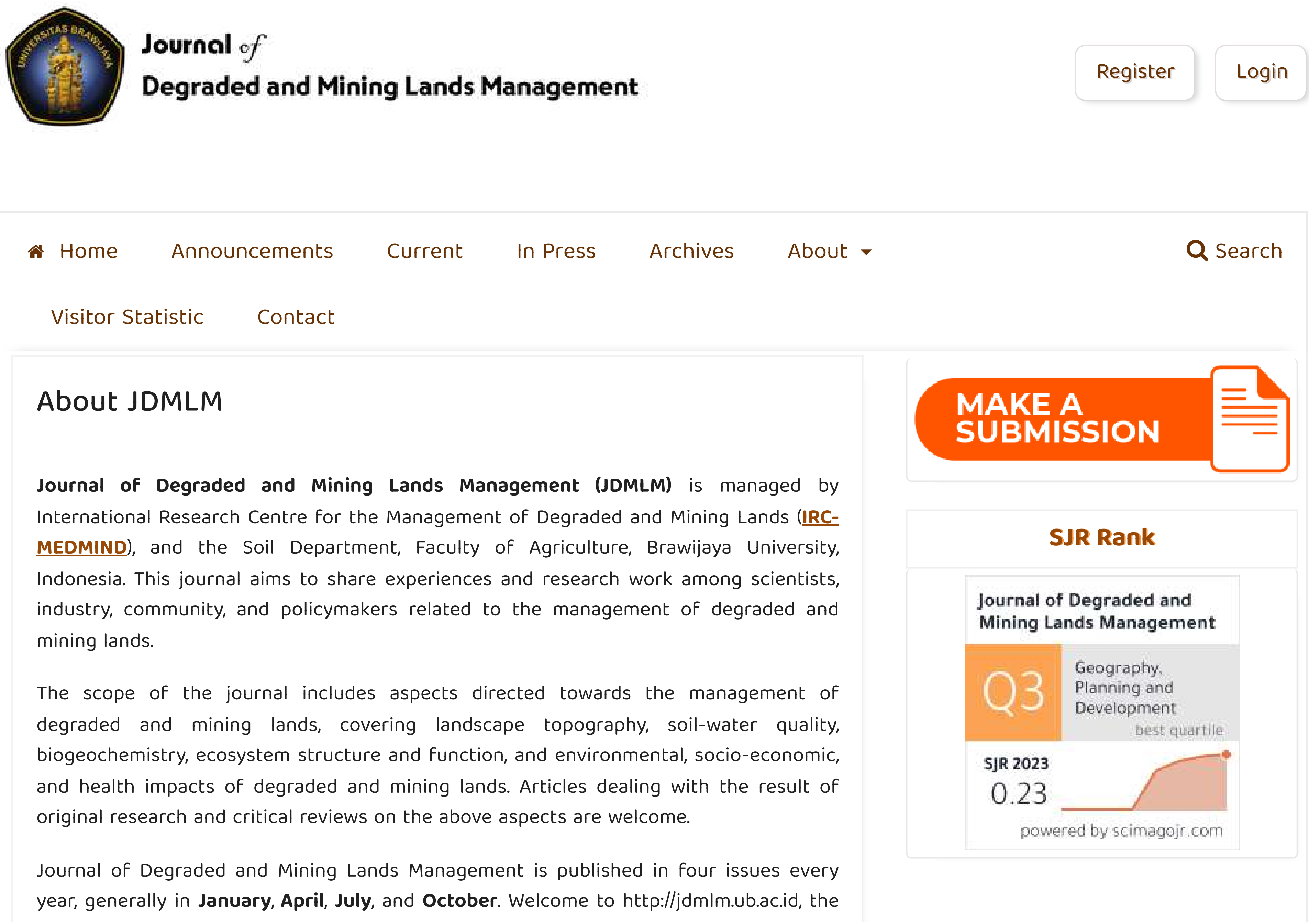

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The scope of the journal includes aspects directed towards the management of degraded and mining lands, covering landscape topography, soil-water quality, biogeochemistry, ecosystem structure and function, and environmental, socio-economic, and health impacts of degraded and mining lands. Articles dealing with the result of original research and critical reviews on the above aspects are welcome.

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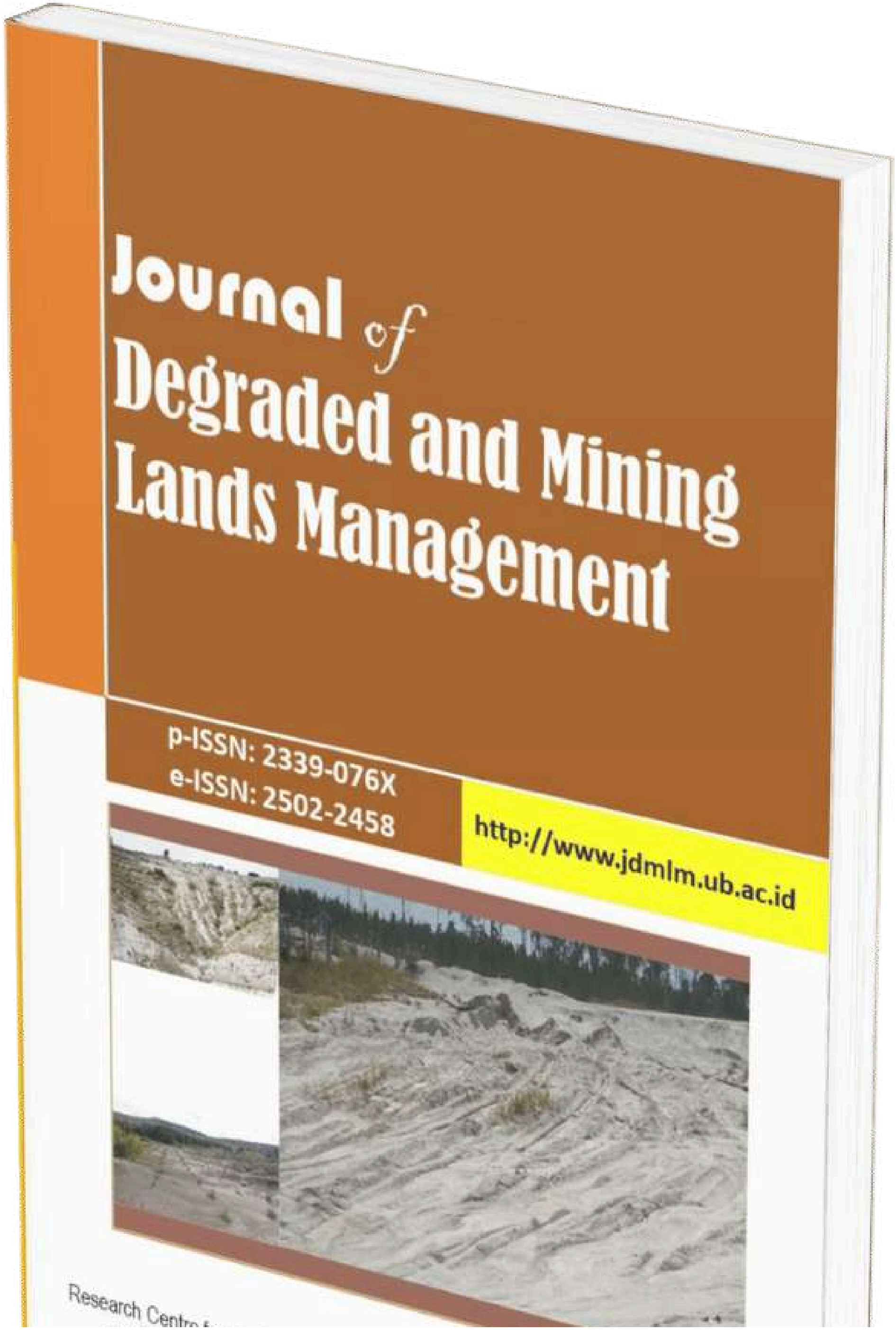

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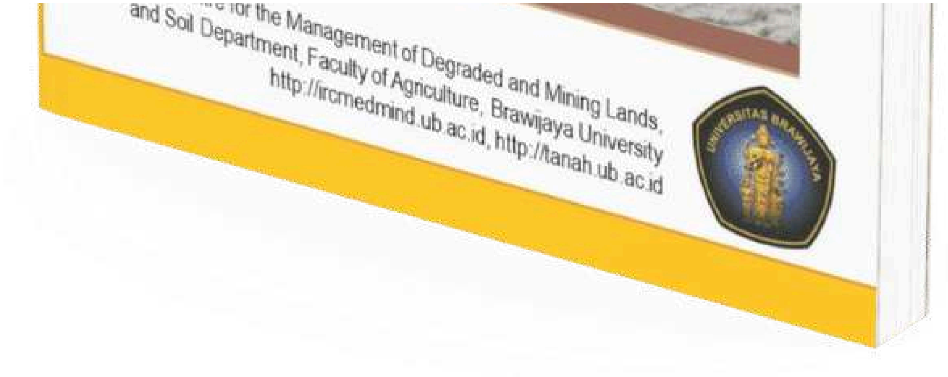

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Research Article

Native arbuscular mycorrhizal fungi promote the growth of *Vitex cofassus* seedlings in post-asphalt mining soil media

Faisal Danu Tuheteru[1*], Husna[1], Wiwin Rahmawati Nurdin[1], Ade Himawan[1], Edy Jamal Tuheteru[2], Albasri[2], Sri Mulyono[3], Asrianti Arif[1]

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Abstract

Post-asphalt mining land is generally damaged and infertile; therefore, restoration efforts are necessary. The use of native Arbuscular Mycorrhizal Fungi (AMF) can improve plant growth and accelerate the success of restoration. This research aimed to elucidate the effect of native AMF inoculation on the growth of *Vitex cofassus* seedlings in greenhouse conditions. In this study, a completely randomized design was implemented with seven treatments, i.e., uninoculated (control), *Racocetra crispi, Glomus intraradices*, *Glomus* sp., *Glomus* sp.-LW10, *Glomus* sp.-SW10 and Mycofer IPB (commercial AMF). The percentage of AMF colonization, plant growth, dry weight as well as P and Ca uptakes were measured after 3 months of planting. The results showed that AMF inoculation significantly increased the percentage of colonization, growth, and dry weight of shoots and total plants of *V. cofassus*. Mycorrhizal Inoculation Effect (MIE) ranged between 92.2% and 94.6%. Native and commercial AMF inoculation increased P and Ca uptakes in the roots and shoots of *V. cofassus*. There is a promising future for native AMF to be developed into a biofertilizer for restoring post-asphalt mining land in Indonesia.

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Introduction

The mining sector contributes significantly to the economic and social aspects of a country (Agboola et al., 2020). Asphalt mining, which uses an open pit mining method, is among the mining sectors that contribute to the national income. On the other hand, mining activities have an impact on damage and loss of natural vegetation, environmental degradation, and human health deterioration, which eventually leave the post-mining land (Worlanyo and Jiangfeng, 2021; Albasri et al., 2023). The physical, chemical, and biological characteristics of the post-mining land do not support plant growth in restoration activities (Pratiwi et al., 2021). These lands need to be restored using low-cost, environmentally friendly approaches. Post-mining land restoration requires appropriate tree species and their symbiosis with beneficial soil microbes (Amir et al., 2023), such as Arbuscular Mycorrhizal Fungi (AMF), which is among important soil microbes that have been utilized in ecosystem restoration (de Moura et al., 2022).

Arbuscular Mycorrhizal Fungi (AMF) belong to the phylum Glomeromycota, which has a symbiotic relationship with 97 families of land plants (Smith and Read, 2008). AMF reportedly are able to improve and accelerate the restoration process and ecosystem services on degraded land, including post-mining land (Asmelash et al., 2016; de Moura et al., 2022) through increasing the uptakes of plant nutrients and water, plant resilience against biotic and abiotic stresses, as well as improving soil quality. Martins et al. (2020) reported that among the use of new biotechnology and phytotechnology in post-mining land restoration, there has been an increase in articles related to arbuscular fungi that provide plant survival, establishment, and growth.

Studies related to active restoration and the use of mycorrhizal biofertilizers to support the success of restoration in both post-asphalt mining soil media and other post-mining lands in the world are still very limited. Previous studies related to the restoration of post-asphalt mining land on Buton Island, Indonesia, include natural regeneration (passive restoration) (Albasri et al., 2023) and AMF biodiversity (Tuheteru et al., 2022). These types of AMF have been collected and reproduced, but studies related to screening native AMF on the growth and survival of local adaptive plants in post-asphalt mining soil media have not been carried out. Wulandari et al. (2024) explained that the effectiveness of AMF varies according to type, so screening is required to find the best AMF for reclamation. Therefore, it is necessary to carry out research to obtain information on the effectiveness of native AMF in post-asphalt mining land on plant growth in post-asphalt mining soil media.

Native arbuscular mycorrhizal fungi (AMF) isolated from degraded areas, such as post-mining sites, exhibit greater tolerance to stress conditions, particularly pollutants, and have good adaptation capability to local habitats due to long-term natural selection (Emam, 2016; Kodre et al., 2017). Previous studies showed that the use of local types of AMF is more effective compared to the exotic ones (Husna et al., 2016; 2019; Tuheteru et al., 2020; Husna et al., 2021a) and is more efficient and cost-effective (de Moura et al., 2022). Development of AMF has the potential for revegetation, reforestation, and environmental damage restoration programs (Asmelash et al., 2016; Husna et al., 2021b; de Moura et al., 2022). Apart from AMF, it is important to have ample knowledge of adaptive plant types in order to support the selection and cultivation of species in post-asphalt mining land restoration programs since selected local species are preferred to be used in post-mining land restoration (Gairola et al., 2023).

One type of local plant that is reported to grow naturally in post-asphalt mining land on Buton Island is *Vitex cofassus* Linn. (Albasri et al., 2023). This species has a limited distribution, namely only spread in Indonesia (Sulawesi, Maluku, Papua) and its surroundings (New Guinea, Bismarcks, and Solomon) (de Kok, 2008). *V. coffassus* is one of Indonesia's leading tree species and has high economic value. *V. coffassus* is also prospective to be used as a plant type for post-mining land revegetation (Pasumbana et al., 2017; Pratiwi et al., 2021; Albasri et al., 2023) and reforestation (Otsamo et al., 1997). This study was carried out to investigate the effects of native AMF inoculation on the growth and nutrient uptake of *V. coffassus* on post-asphalt mining soil media under greenhouse conditions.

Materials and Methods

Preparation of soil media

Post-asphalt mining soil samples were collected from PT's disposal site. Wika Bitumen in Buton District, Southeast Sulawesi, Indonesia, and was kept in a greenhouse for storage. The chemical and physical characteristics of soil media were analyzed at the Soil Laboratory of Soil Research Institute, Bogor, Indonesia. The chemical and physical properties of the soil samples are presented in Table 1.

Table 1. Soil physical and chemical properties from post-asphalt mining land.

Parameter	Unit	Value	Criteria[a]
pH (H_2O)		7.2	Neutral
Organic C (Walkley & Black)	%	7.56	Very high
Total N (Kjeldahl)	%	0.13	Low
C/N ratio		58	Very high
P_2O_5 (HCl 25%)	mg 100 g^{-1}	55	High
K_2O (HCl 25%)	mg 100 g^{-1}	7	Very low
P_2O_5 (Olsen)	ppm	8	Low
K_2O (Morgan)	ppm	49	-
Ca (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	18.58	High
Mg (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	1.47	Moderate
K (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	0.10	Low
Na (NH_4-Acetate 1NpH 7)	cmol kg^{-1}	0.23	Low
CEC (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	23.89	Moderate
Base saturation (NH_4-Acetate 1N. pH 7)	%	85	Very high
Al^{3+} (KCl 1N)	cmol kg^{-1}	0.00	-
H^+ (KCl 1N)	cmol kg^{-1}	0.07	-
Texture (pipet)			
Sand	%	10	Clay
Silt	%	40	
Clay	%	50	
Fe (DTPA)	ppm	7.3	Sufficient
Mn (DTPA)	ppm	9.5	Sufficient
Cu (DTPA)	ppm	1.2	Sufficient
Zn (DTPA)	ppm	0.3	Deficient
Pb (Morgan Wolf)	ppm	0.4	Normal
Cd (Morgan Wolf)	ppm	0.2	Normal
$CaCO_3$ (Titrimetric)	%	2.1	-

[a] Soil Research Institute (2009).

Seed germination

V. coffassus seeds were collected from their parent trees at Wabula Village in Buton District, Southeast Sulawesi. The seeds were soaked in warm water of 50 ºC for 24 hours to gradually cool down and germinated in a plastic sprout maker at the plastic house of the Indonesian Mycorrhizal Association (IMA), Southeast Sulawesi Branch.

Inoculum propagation and inoculation of Arbuscular Mycorrhizal Fungi (AMF)

AMF inoculums used were isolated from the rhizosphere of plants in post-asphalt mining land (Tuheteru et al., 2022). Inoculums of AMF were propagated in zeolite media using *Pueraria javanica* as the host, which was maintained for 3 months. Polyethylene pots (15 x 20 cm) were filled with 1,000 g of sterile soil media (a mixture of soil from post-asphalt mining land, river sand, and husk-charcoals having a proportion of 3:1:1). AMF inoculum was inoculated by placing 10 g of inoculum/plant of each species at 1-3 cm underneath the seedlings. *V. coffassus* seedlings with two new leaves were transplanted into the pots, followed by seedlings maintenance, watering, and observation for 3 months. The seedlings were watered daily to field capacity with tap water. Ten grams of sterilized zeolite was placed into the uninoculated pots as the control treatment. The experiment was carried out in a nursery condition with varied temperatures between 24 and 37 °C, with a relative humidity of 82-95% with a 12-hour photoperiod.

Growth parameter

In this experiment, a completely randomized design was implemented and consisted of 7 treatments of AMF: (A) control (uninoculated), (B) *Racocetra crispa*, (C) *Glomus intraradices*, (D) *Glomus* sp., (E) *Glomus* sp.-LW10, (F) *Glomus* sp.-SW10, and (G) Mycofer IPB (G). Each treatment had three replications. Plant height and stem diameter were measured at a height of 1 cm above the soil medium at 120 days after transplantation. At the end of the study, the number of leaves were counted.

Dry weight determination

Seedlings were harvested after three months of growth, during which the shoots and roots were separated. The samples were then oven-dried at 70 °C for 48 hours to determine their dry weight. Colonization of mycorrhizal fungi was done by using some of the roots. Concentrations of P and Ca were calculated for both the shoots and roots. Formula by Duryea and Brown (1984) was used to calculate the Seed Quality Index (SQI): Seed Quality Index (SQI) = [Shoot dry weight + root dry weight]/[(height/diameter) + (dry weight of shoot/root dry weight)]. Seedlings are of high quality if the value of SQI is ≥ 0.09.

Arbuscular Mycorrhizal Fungi (AMF) colonization and Mycorrhizal Inoculation Effect (MIE)

AMF colonization in *V. coffassus* roots was observed using a trypan blue stain. Formula by Brundrett et al. (1996) was used to count the colony: [Σ number of fields of view colonized/Σ total observed field of view] x 100%. The formula for calculating the Mycorrhizal Inoculation Effect (MIE) was: [total dry weight of mycorrhizal plant - total dry weight of non-mycorrhizal plant/total dry weight of mycorrhizal plant] x 100% (Habte and Manjunath, 1991).

Nutrients

Available P was measured in sodium bicarbonate extracts at pH 8.5 and measured according to the Olsen method at 660 nm by a flow injection automated ion analyzer. Available Ca were extracted with 1 N NH_4-Acetate, pH 7, and determined by atomic absorption. The uptake of P and Ca was calculated by multiplying the nutrient concentrations by the dry weights of the plants. The formula used to calculate nutrient transport factor (TF) was: C aerial/C root, where C aerial is the nutrient concentration in the shoot (stems and leaves) and C root is the nutrient concentration in the root. Additionally, the formula to calculate the increase/decrease of nutrients uptake of AMF-treated seedlings relative to the controls was: [nutrient absorption of AMF plant - nutrient absorption of non-mycorrhizal plant/nutrient absorption of non-mycorrhizal plant] × 100% (Wang et al., 2005).

Statistical analysis

Results of the experiment were first analyzed by comparing means of variance (F test), which were followed by DMRT at a 95% confidence level when the F test results showed a significant effect.

Results and Discussion

Plant growth

Arbuscular Mycorrhizal Fungi (AMF) showed a significant effect on shoot height, stem diameter, and leaf of *V. cofassus* at 12 weeks after transplantation (Table 2, $p<0.01$). The inoculation of *Glomus* sp.-SW10 increased the shoot height of *V. cofassus* and did not differ significantly from other treatments, except *Glomus intraradices* and control treatments. There were no significant differences in leaf number and leaf length among *V. cofassus* inoculated with all AMF treatments. Leaf widths of *V. cofassus* inoculated with *Glomus intraradices* and *Glomus* sp.-LW10 were significantly higher compared to that of *V. cofassus* inoculated with *R. crispa* and control (Table 2, $p<0.01$). The effect of AMF treatments started to show in the 4th week after planting (Figure 1). The performance of the 3-month-old *V. cofassus* seedlings is presented in Figure 2. Inoculation of native AMF on *V. cofassus* seedlings significantly increased plant growth and dry weight. An increase in plant height, stem diameter, and number of leaves of *V. cofassus* plants inoculated with native AMF compared to control was shown by 1,161-1,506%, 743-966%, and 341-581% growth, respectively (Table 2). The growth response of *V. cofassus* to native AMF inoculation shows that improving the *V. cofassus* seedlings' growth in post-asphalt mining soil media can be achieved through native AMF inoculation. The increase in growth and biomass is also reflected in the increased dependence of *V. cofassus* on native AMF (Table 3). Increased plant growth of *V. cofassus* is strongly associated with improvements in plant nutrients and water status, as well as increased plant resistance to biotic and abiotic stresses (Wang, 2017; Begum et al., 2019). Native AMF *G. clarum* improved the growth of *Nauclea orientalis* plant in mixed coal overburden media and soil (1:1 and 3:1) (Wulandari et al., 2024). Before being transplanted onto the degraded land, it is recommended that the plant is inoculated with AMF in the nursery to promote the growth of mycorrhizal tree seedlings (Prematuri et al., 2020).

Table 2. Shoot height, stem diameter, and leaves of *V. cofassus* seedlings after 3 months grown with or without Arbuscular Mycorrhizal Fungi (AMF) under greenhouse conditions.

Treatment	**Height (cm)***		**Stem diameter (mm)**		**Leaf**					
					Number per polybag		**Lenght (cm)**		**Width (cm)**	
Control	2.17±0.46	c	0.30±0.00	c	3.33±0.33	b	2.76±0.45	b	0.84±0.16	c
Racocetra crispa	31.67±2.48	ab	2.70±0.15	ab	16.33±2.03	a	15.20±0.32	a	3.14±0.04	b
Glomus intraradices	27.37±0.71	b	2.53±0.03	b	14.67±1.33	a	14.73±0.75	a	3.68±0.15	a
Glomus sp.	29.73±3.20	ab	2.70±0.31	ab	20.00±3.06	a	16.21±0.67	a	3.39±0.21	ab
Glomus sp.-LW10	33.07±1.35	ab	3.10±0.15	a	17.00±1.00	a	16.37±0.74	a	3.77±0.15	a
Glomus sp.-SW10	34.87±1.62	a	3.00±0.15	ab	15.00±5.13	a	14.79±0.66	a	3.50±0.13	ab
Mycofer IPB	31.57±3.27	ab	3.20±0.00	a	22.67±6.67	a	15.52±0.22	a	3.56±0.13	ab
Pr>F	<0.0001		<0.0001		0.0374		<0.0001		<0.0001	

Notes: Average values followed by different letters in the same column are significantly different at DMRT ($p<0.05$); *Mean±SE.

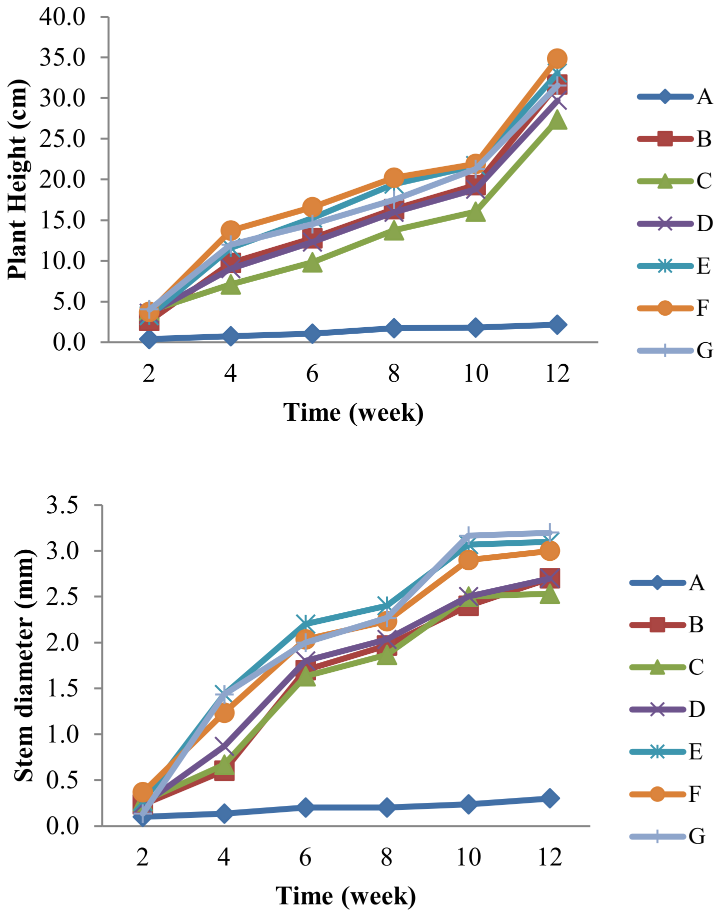

Figure 1. Trend of increasing height and diameter of *V. cofassus* seedlings with or without Arbuscular Mycorrhizal Fungi (AMF). Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB).

Figure 2. Performance of 3-month-old *V. cofassus* seedlings (left) and roots (right).
Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB).

Plant dry weight

AMF colonization significantly increased the roots, shoots, and total dry weight of *V. cofassus* seedlings whose growth was higher than the control (Table 3, p<0.01). The inoculation of *Glomus* sp., *Glomus* sp.-LW10, *Glomus* sp.-SW10, and Mycofer IPB increased roots, shoots, and total dry weight by 471-847, 5,200-6,733, and 1,150-1,720%, respectively. The results of this research show that the application of native AMF is really needed by *V. cofassus* plants. Inoculation of native AMF increases plant growth, leading to time-saving and cost-efficiency increases during the restoration and rehabilitation of post-asphalt mining land. Many publications report that native AMF inoculation can increase plant dry weight in post-mining land media conditions contaminated with heavy metals (Husna et al., 2017; Tuheteru et al., 2017; Wulandari et al., 2024). No significant differences were observed in Shoot Root Ratio (SRR) among *V. cofassus* inoculated with all AMF treatments. All AMF treatments increased the Seedling Quality Index (SQI) of *V. cofassus* by contrast, while *R. crispa* did not increase the SQI of *V. cofassus*. *V. cofassus* seedlings inoculated with all AMF treatments meet SQI standards and are suitable for planting in the field.

Arbuscular Mycorrhizal Fungi (AMF) colonization and Mycorrhizal Inoculation Effect (MIE)

Results of root staining showed that the roots of *V. cofassus* seedlings were colonized by AMF, with Mycofer IPB having the highest average of colonization (96%), followed by *Glomus* sp. (92%) *Glomus* sp.-LW10 (92%) and *Glomus* sp.-SW10. (88%) (Table 2). Significant differences were observed in AMF colonization among *V. cofassus* inoculated with the AMF (Table 3, p<0.01). Common AMF structures observed included internal and external hyphae, coil hyphae, vesicles, and auxiliary cells (Figure 3). Internal hyphae include AMF structures commonly found in the roots of *V. cofassus* plants. The presence of AMF structures in the roots of *V. cofassus* indicates that 3-month-old *V. cofassus* plants are in symbiosis with native AMF, which the native AMF contributes to improving nutrients and water status as well as increasing plant resistance to biotic and abiotic stresses.

Table 3. Dry weight, Shoot Root Ratio (SRR), and Seed Quality Index (SQI) of *V. cofassus* seedlings 3 months grown with or without mycorrhizal fungi under greenhouse conditions.

Treatment	**Dry weight (g)**						**SRR**	**ISQ**	
	Roots		**Shoots**		**Total**				
Control	0.17±0.09	c	0.03±0.00	d	0.20±0.09	d	0.64±0.50	0.007±0.00	b
Racocetra crispa	1.16±0.09	ab	1.60±0.11	bc	2.76±0.09	bc	1.41±0.17	0.34±0.05	ab
Glomus intraradices	0.91±0.38	b	1.59±0.09	c	2.50±0.43	c	3.07±1.74	0.59±0.26	a
Glomus sp.	1.58±0.16	a	1.85±0.24	abc	3.43±0.39	ab	1.17±0.06	0.37±0.06	a
Glomus sp.-LW10	1.33±0.06	ab	2.04±0.13	ab	3.37±0.11	ab	1.54±0.14	0.49±0.07	a
Glomus sp.-SW10	1.61±0.16	a	2.02±0.11	abc	3.64±0.06	a	1.29±0.20	0.41±0.07	a
Mycofer IPB	1.48±0.09	ab	2.05±0.15	a	3.53±0.18	ab	1.39±0.12	0.50±0.04	a
Pr>F	0.0006		<0.0001		<0.0001		0.3825	0.0434	

Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB). Average values followed by different letters in the same column are significantly different at DMRt (p<0.05); *Mean±SE.

The range of MIE values for all AMF treatments was 92,2-94,6% (Table 4). The high MIE value indicates that AMF inoculation is beneficial for the production of quality seeds at the nursery scale. High dependence of plant species on AMF in post-mining soil media conditions that contain heavy metals has also been reported in several plant species, including *Pericopsis mooniana* (Husna et al., 2016; 2019; 2021a), *Pterocarpus indicus* (Husna et al., 2021a), *Nauclea orientalis* (Tuheteru et al., 2020) and *Kalappia celebica* (Husna et al., 2021b). The results of this research showed that native AMF from post-asphalt mining land on Buton Island is compatible with *V. cofassus* and significant in increasing the initial growth and improving the nutrition of *V. cofassus* plants grown on post-asphalt mining soil media. Compatibility of native AMF with *V. cofassus* is possible because of several things, namely 1) native AMF is suitable for media conditions; 2) local AMF is compatible with root exudates produced by *V. cofassus* roots; and 3) genotypically, local AMF has the ability to absorb and conduct water and nutrients to the host plant. Along with improvements in nutrients and water status as well as chelation of heavy metals by AMF, the growth and biomass of mycorrhizal plants can be increased. Several research results showed that AMF can help the growth of plants grown on land contaminated with heavy metals (Bi et al., 2018). This experiment showed that AMF inoculation promotes the growth of seedlings on low-fertility soil media in the nursery.

Table 4. AMF colonization and MIE of *V. cofassus* seedlings 3 months grown with or without Arbuscular Mycorrhizal Fungi (AMF).

Treatment	**AMF Colonization (%)**		**MIE (%)**
Control	2±0.33	d	
Racocetra crispa	77±2.47	c	92.5±3.52
Glomus intraradices	84±7.52	bc	92.2±3.70
Glomus sp.	92±3.12	ab	94.6±2.09
Glomus sp.-LW10	88±4.72	abc	94.1±2.58
Glomus sp.-SW10	92±1.85	ab	94.6±2.38
Mycofer IPB	96±1.87	a	94.2±2.51
Pr>F	<0.0001		<0.0001

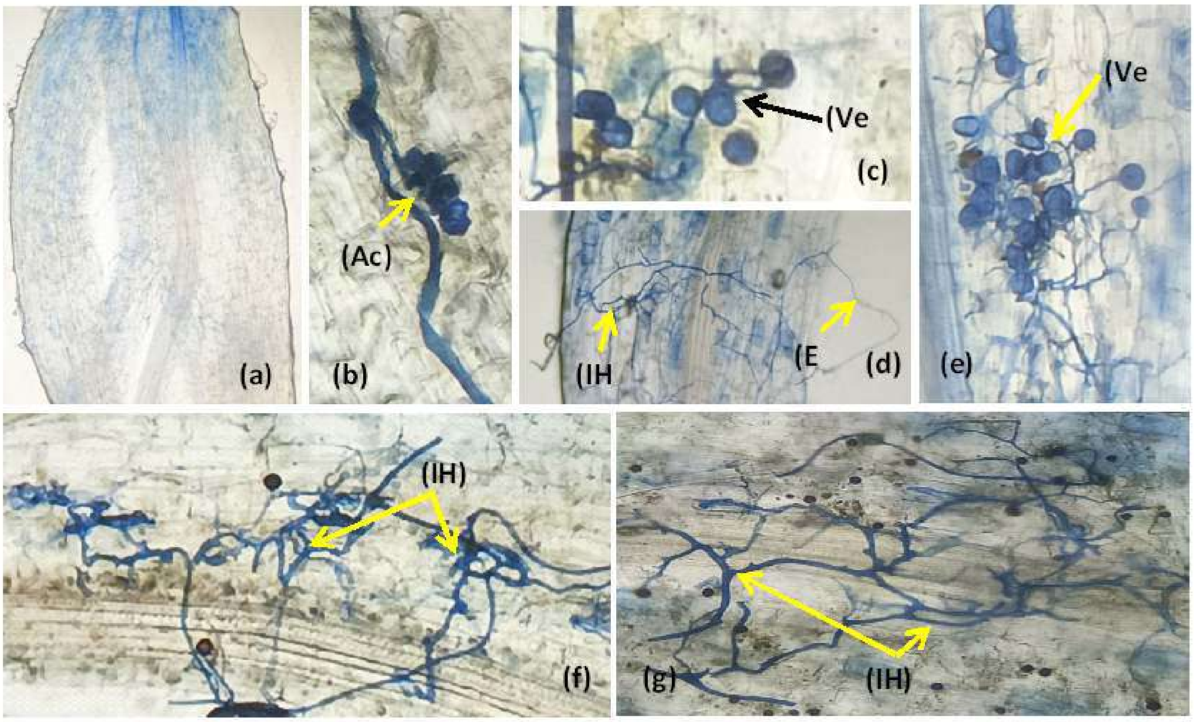

Figure 3. AMF structure in the shoots and roots of *V. cofassus.*
Notes: Control (A), *Racocetra crispa* (B), *Glomus intraradices* (C), *Glomus* sp. (D), *Glomus* sp.-LW10 (E), *Glomus* sp.-LW10 (F), Mycofer IPB (G); IH = Intraradical hyphae; EH = Extraradical hyphae; Ve = Vesicles; and Ac = Auxiliary cells.

Shoot and root nutrient concentration and content

The soil pH was neutral (7.2). The C and C/N ratios were categorized as very high and Ca as high. Total N, P_2O_5 (Olsen), K, and Na were in the low category. Mg level and CEC were in the moderate category. Soil texture was classified as clay (Table 1). Inoculation of native AMF significantly increased P and Ca content and uptakes among AMF treatments, both in roots and shoots of *V. cofassus* (Tables 5 and 6). The control treatment showed significant differences in P and Ca content and uptakes compared to the AMF treatments. Treatments *Glomus* sp. SW10 dan Mycofer IPB significantly increased P uptake in the roots and shoots. Also, both treatments were significantly different from the *G. intaradices* and control treatments in roots (Table 5) and *G. intaradices*, *R. crispa,* and control treatments in shoots (Table 6).

Table 5. Nutrient content and uptake in roots of 3-month-old *V. cofassus* seedlings.

Treatment	**Content (mg kg^{-1})**				**Uptake (mg plant^{-1}x 10^{-3})**			
	P		**Ca**		**P**		**Ca**	
Control	0.26±0.007	b	13.35±2.221	d	0.05±0.024	c	2.51±1.276	b
Racocetra crispa	0.49±0.026	a	73.79±0.557	a	0.58±0.073	ab	85.37±6.929	a
Glomus intraradices	0.52±0.075	a	73.29±18.607	a	0.49±0.166	b	67.10±24.941	a
Glomus sp.	0.51±0.033	a	65.97±6.564	ab	0.81±0.112	ab	104.12±15.946	a
Glomus sp.-LW10	0.52±0.003	a	61.35±1.819	abc	0.69±0.025	ab	81.96±5.570	a
Glomus sp.-SW10	0.55±0.020	a	58.46±6.891	bc	0.89±0.056	a	92.98±8.968	a
Mycofer IPB	0.51±0.027	a	52.77±3.625	c	0.75±0.085	a	77.56±3.092	a

Table 6. Nutrient content and uptake in shoots of 3-month-old *V. cofassus* seedlings.

Treatment	**Content (mg kg^{-1})**				**Uptake (mg plant^{-1} x 10^{-3})**			
	P		**Ca**		**P**		**Ca**	
Control	0.33±0.097	c	25.45±2.427	b	0.01±0.002	c	0.80±0.155	b
Racocetra crispa	0.49±0.054	b	72.87±0.942	a	0.80±0.117	b	116.65±6.548	a
Glomus intraradices	0.68±0.081	ab	74.17±14.851	a	0.85±0.247	b	117.73±35.990	a
Glomus sp.	0.59±0.019	ab	74.39±0.094	a	1.11±0.169	ab	137. 92±.0.169	a
Glomus sp.-LW10	0.61±0.027	ab	74.33±0.042	a	1.25±0.059	a	151.04±9.348	a
Glomus sp.-SW10	0.69±0.074	a	74.29±0.202	a	1.38±0.125	a	150.35±8.279	a
Mycofer IPB	0.60±0.031	ab	69.55±3.901	a	1.22±0.038	a	143.31±15.922	a

Phosphorus (P) and Calcium (Ca) elements had transport factors (TF)>1 (Table 6), except control which showed TF<1. Native and commercial AMF inoculation increased P and Ca uptakes in the roots and shoots of 3-month-old *V. cofassus* under nursery conditions (Table 7).

Table 7. Transport factor of elements and increase/decrease of nutrients uptakes in roots and shoots of 3-month-old *V. cofassus* seedlings.

Treatment	**Transport factor**		**Increase/decrease of nutrient uptake**			
			Roots		**Shoots**	
	P	**Ca**	**P**	**Ca**	**P**	**Ca**
Control	0.22	0.32	-	-	-	-
Racocetra crispa	1.38	1.37	1.184	3.301	7.900	14.481
Glomus intraradices	1.78	1.75	962	2.573	8.400	14.616
Glomus sp.	1.37	1.32	1.704	4.048	11.090	17.048
Glomus sp.-LW10	1.82	1.84	1.429	3.165	12.400	18.780
Glomus sp.-SW10	1.56	1.62	1.871	3.604	13.700	18.694
Mycofer IPB	1.62	1.85	1.576	2.990	12.100	17.814

The results of this study showed that the levels and uptakes of P and Ca in the roots and shoots of *V. cofassus* plants were increased by native AMF. It is well known that AMF can facilitate the acquisition of mineral nutrients (especially P) by plants (Smith and Read, 2008). Seeds of *V. cofassus* plant inoculated with native AMF show a more effective P and Ca allocation. This research agrees with several previous research results on various media conditions of post-mining land, such as high P uptake in *Pericopsis mooniana* plants in serpentine soil media conditions (Husna et al., 2016), *Nauclea orientalis* plants in gold tailings media (Tuheteru et al., 2020), and *Amygdalus pedunculata* in coal mining subfields (Bi et al., 2018). P and Ca are essential nutrients that plants need. Ca plays a role in plants, including being part of cell structure and the formation or division of new cells, and it is a co-factor for many enzymes in plants (Havlin et al., 2017). The most essential function of P is its involvement in the storage and transfer of energy in plants (Havlin et al., 2017). Wang (2017) has summarized the direct and indirect mechanisms of P repair by AMF, including that AMF extraradical hyphae can absorb P directly and translocate it more quickly to AMF structures in the roots, in which AMF secretes phosphatases that hydrolyze organic P sources into available forms. Apart from P, AMF can also absorb other macronutrients (K, Ca, Mg, and S) and micronutrients (Zn, Fe, Cu, and Mn) (Smith and Read, 2008; Husna et al., 2017). In this study, there were differences in the P uptake ability of the two plant parts by each type of AMF. *G. intraradices* had lower P uptake compared to other AMF treatments, even though it was higher than the control. Walder and van der Heijden (2015) stated different AMF species exhibit varying capacities for mycorrhizal phosphate uptake.

Based on this research, it appears that the application of native AMF shows a significant positive effect in increasing plant growth and survival, increasing water and nutrient uptakes, and improving soil structure and quality. Furthermore, native AMF and *V. cofassus* plants can also encourage ecological restoration on degraded land, such as post-asphalt mining land in the world. Thus, providing seedlings with native AMF at the nursery scale is very necessary to support planting on degraded land.

Conclusion

Native AMF from post-asphalt mining land effectively promotes growth and nutrients P and Ca uptakes of *Vitex cofassus* at a 12-week experiment in post-asphalt mining soil media under nursery conditions. Native AMF has the potential to be developed as biofertilizers to support post-asphalt mining land reclamation and forest restoration programs.

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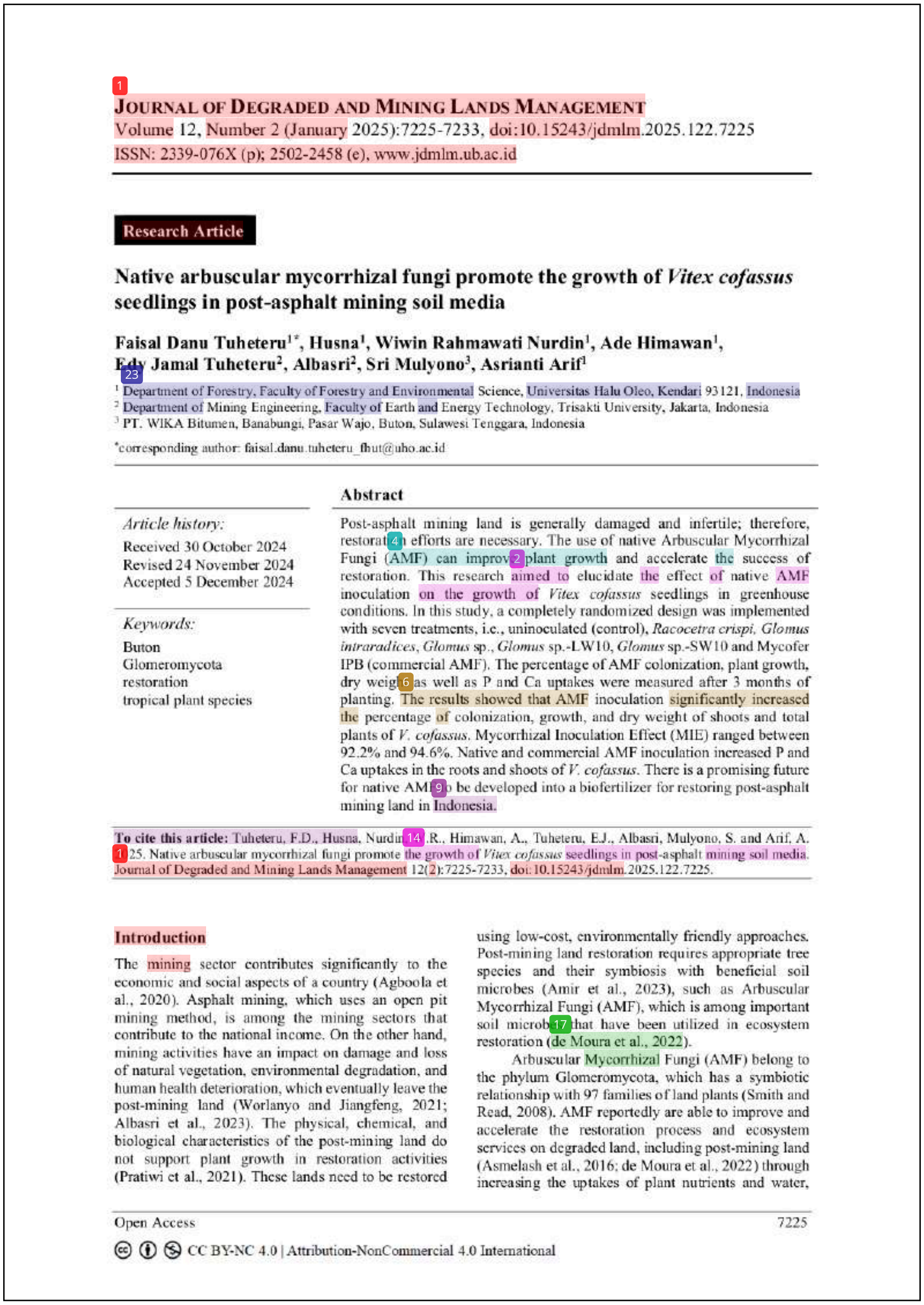

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Research Article

Native arbuscular mycorrhizal fungi promote the growth of *Vitex cofassus* seedlings in post-asphalt mining soil media

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Abstract

Post-asphalt mining land is generally damaged and infertile; therefore, restoration efforts are necessary. The use of native Arbuscular Mycorrhizal Fungi (AMF) can improve plant growth and accelerate the success of restoration. This research aimed to elucidate the effect of native AMF inoculation on the growth of *Vitex cofassus* seedlings in greenhouse conditions. In this study, a completely randomized design was implemented with seven treatments, i.e., uninoculated (control), *Racocetra crispi, Glomus intraradices*, *Glomus* sp., *Glomus* sp.-LW10, *Glomus* sp.-SW10 and Mycofer IPB (commercial AMF). The percentage of AMF colonization, plant growth, dry weight as well as P and Ca uptakes were measured after 3 months of planting. The results showed that AMF inoculation significantly increased the percentage of colonization, growth, and dry weight of shoots and total plants of *V. cofassus*. Mycorrhizal Inoculation Effect (MIE) ranged between 92.2% and 94.6%. Native and commercial AMF inoculation increased P and Ca uptakes in the roots and shoots of *V. cofassus*. There is a promising future for native AMF to be developed into a biofertilizer for restoring post-asphalt mining land in Indonesia.

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Introduction

The mining sector contributes significantly to the economic and social aspects of a country (Agboola et al., 2020). Asphalt mining, which uses an open pit mining method, is among the mining sectors that contribute to the national income. On the other hand, mining activities have an impact on damage and loss of natural vegetation, environmental degradation, and human health deterioration, which eventually leave the post-mining land (Worlanyo and Jiangfeng, 2021; Albasri et al., 2023). The physical, chemical, and biological characteristics of the post-mining land do not support plant growth in restoration activities (Pratiwi et al., 2021). These lands need to be restored using low-cost, environmentally friendly approaches. Post-mining land restoration requires appropriate tree species and their symbiosis with beneficial soil microbes (Amir et al., 2023), such as Arbuscular Mycorrhizal Fungi (AMF), which is among important soil microbes that have been utilized in ecosystem restoration (de Moura et al., 2022).

Arbuscular Mycorrhizal Fungi (AMF) belong to the phylum Glomeromycota, which has a symbiotic relationship with 97 families of land plants (Smith and Read, 2008). AMF reportedly are able to improve and accelerate the restoration process and ecosystem services on degraded land, including post-mining land (Asmelash et al., 2016; de Moura et al., 2022) through increasing the uptakes of plant nutrients and water, plant resilience against biotic and abiotic stresses, as well as improving soil quality. Martins et al. (2020) reported that among the use of new biotechnology and phytotechnology in post-mining land restoration, there has been an increase in articles related to arbuscular fungi that provide plant survival, establishment, and growth.

Studies related to active restoration and the use of mycorrhizal biofertilizers to support the success of restoration in both post-asphalt mining soil media and other post-mining lands in the world are still very limited. Previous studies related to the restoration of post-asphalt mining land on Buton Island, Indonesia, include natural regeneration (passive restoration) (Albasri et al., 2023) and AMF biodiversity (Tuheteru et al., 2022). These types of AMF have been collected and reproduced, but studies related to screening native AMF on the growth and survival of local adaptive plants in post-asphalt mining soil media have not been carried out. Wulandari et al. (2024) explained that the effectiveness of AMF varies according to type, so screening is required to find the best AMF for reclamation. Therefore, it is necessary to carry out research to obtain information on the effectiveness of native AMF in post-asphalt mining land on plant growth in post-asphalt mining soil media.

Native arbuscular mycorrhizal fungi (AMF) isolated from degraded areas, such as post-mining sites, exhibit greater tolerance to stress conditions, particularly pollutants, and have good adaptation capability to local habitats due to long-term natural selection (Emam, 2016; Kodre et al., 2017). Previous studies showed that the use of local types of AMF is more effective compared to the exotic ones (Husna et al., 2016; 2019; Tuheteru et al., 2020; Husna et al., 2021a) and is more efficient and cost-effective (de Moura et al., 2022). Development of AMF has the potential for revegetation, reforestation, and environmental damage restoration programs (Asmelash et al., 2016; Husna et al., 2021b; de Moura et al., 2022). Apart from AMF, it is important to have ample knowledge of adaptive plant types in order to support the selection and cultivation of species in post-asphalt mining land restoration programs since selected local species are preferred to be used in post-mining land restoration (Gairola et al., 2023).

One type of local plant that is reported to grow naturally in post-asphalt mining land on Buton Island is *Vitex cofassus* Linn. (Albasri et al., 2023). This species has a limited distribution, namely only spread in Indonesia (Sulawesi, Maluku, Papua) and its surroundings (New Guinea, Bismarcks, and Solomon) (de Kok, 2008). *V. coffassus* is one of Indonesia's leading tree species and has high economic value. *V. coffassus* is also prospective to be used as a plant type for post-mining land revegetation (Pasumbana et al., 2017; Pratiwi et al., 2021; Albasri et al., 2023) and reforestation (Otsamo et al., 1997). This study was carried out to investigate the effects of native AMF inoculation on the growth and nutrient uptake of *V. coffassus* on post-asphalt mining soil media under greenhouse conditions.

Materials and Methods

Preparation of soil media

Post-asphalt mining soil samples were collected from PT's disposal site. Wika Bitumen in Buton District, Southeast Sulawesi, Indonesia, and was kept in a greenhouse for storage. The chemical and physical characteristics of soil media were analyzed at the Soil Laboratory of Soil Research Institute, Bogor, Indonesia. The chemical and physical properties of the soil samples are presented in Table 1.

Table 1. Soil physical and chemical properties from post-asphalt mining land.

Parameter	Unit	Value	Criteria[a]
pH (H_2O)		7.2	Neutral
Organic C (Walkley & Black)	%	7.56	Very high
Total N (Kjeldahl)	%	0.13	Low
C/N ratio		58	Very high
P_2O_5 (HCl 25%)	mg 100 g^{-1}	55	High
K_2O (HCl 25%)	mg 100 g^{-1}	7	Very low
P_2O_5 (Olsen)	ppm	8	Low
K_2O (Morgan)	ppm	49	-
Ca (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	18.58	High
Mg (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	1.47	Moderate
K (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	0.10	Low
Na (NH_4-Acetate 1NpH 7)	cmol kg^{-1}	0.23	Low
CEC (NH_4-Acetate 1N pH 7)	cmol kg^{-1}	23.89	Moderate
Base saturation (NH_4-Acetate 1N, pH 7)	%	85	Very high
Al^{3+} (KCl 1N)	cmol kg^{-1}	0.00	-
H^+ (KCl 1N)	cmol kg^{-1}	0.07	-
Texture (pipet)			
Sand	%	10	Clay
Silt	%	40	
Clay	%	50	
Fe (DTPA)	ppm	7.3	Sufficient
Mn (DTPA)	ppm	9.5	Sufficient
Cu (DTPA)	ppm	1.2	Sufficient
Zn (DTPA)	ppm	0.3	Deficient
Pb (Morgan Wolf)	ppm	0.4	Normal
Cd (Morgan Wolf)	ppm	0.2	Normal
$CaCO_3$ (Titrimetric)	%	2.1	-

[a] Soil Research Institute (2009).

Seed germination

V. coffassus seeds were collected from their parent trees at Wabula Village in Buton District, Southeast Sulawesi. The seeds were soaked in warm water of 50 °C for 24 hours to gradually cool down and germinated in a plastic sprout maker at the plastic house of the Indonesian Mycorrhizal Association (IMA), Southeast Sulawesi Branch.

Inoculum propagation and inoculation of Arbuscular Mycorrhizal Fungi (AMF)

AMF inoculums used were isolated from the rhizosphere of plants in post-asphalt mining land (Tuheteru et al., 2022). Inoculums of AMF were propagated in zeolite media using *Pueraria javanica* as the host, which was maintained for 3 months. Polyethylene pots (15 x 20 cm) were filled with 1,000 g of sterile soil media (a mixture of soil from post-asphalt mining land, river sand, and husk-charcoals having a proportion of 3:1:1). AMF inoculum was inoculated by placing 10 g of inoculum/plant of each species at 1-3 cm underneath the seedlings. *V. coffassus* seedlings with two new leaves were transplanted into the pots, followed by seedlings maintenance, watering, and observation for 3 months. The seedlings were watered daily to field capacity with tap water. Ten grams of sterilized zeolite was placed into the uninoculated pots as the control treatment. The experiment was carried out in a nursery condition with varied temperatures between 24 and 37 °C, with a relative humidity of 82-95% with a 12-hour photoperiod.

Growth parameter

In this experiment, a completely randomized design was implemented and consisted of 7 treatments of AMF: (A) control (uninoculated), (B) *Racocetra crispa*, (C) *Glomus intraradices*, (D) *Glomus* sp., (E) *Glomus* sp.-LW10, (F) *Glomus* sp.-SW10, and (G) Mycofer IPB (G). Each treatment had three replications. Plant height and stem diameter were measured at a height of 1 cm above the soil medium at 120 days after transplantation. At the end of the study, the number of leaves were counted.

Dry weight determination

Seedlings were harvested after three months of growth, during which the shoots and roots were separated. The samples were then oven-dried at 70 °C for 48 hours to determine their dry weight. Colonization of mycorrhizal fungi was done by using some of the roots. Concentrations of P and Ca were calculated for both the shoots and roots. Formula by Duryea and Brown (1984) was used to calculate the Seed Quality Index (SQI): Seed Quality Index (SQI) = [Shoot dry weight + root dry weight]/[(height/diameter) + (dry weight of shoot/root dry weight)]. Seedlings are of high quality if the value of SQI is ≥0.09.

Arbuscular Mycorrhizal Fungi (AMF) colonization and Mycorrhizal Inoculation Effect (MIE)

AMF colonization in *V. coffassus* roots was observed using a trypan blue stain. Formula by Brundrett et al. (1996) was used to count the colony: [Σ number of fields of view colonized/Σ total observed field of view] x 100%. The formula for calculating the Mycorrhizal Inoculation Effect (MIE) was: [total dry weight of mycorrhizal plant - total dry weight of non-mycorrhizal plant/total dry weight of mycorrhizal plant] x 100% (Habte and Manjunath, 1991).

Nutrients

Available P was measured in sodium bicarbonate extracts at pH 8.5 and measured according to the Olsen method at 660 nm by a flow injection automated ion analyzer. Available Ca were extracted with 1 N NH_4-Acetate, pH 7, and determined by atomic absorption. The uptake of P and Ca was calculated by multiplying the nutrient concentrations by the dry weights of the plants. The formula used to calculate nutrient transport factor (TF) was: C aerial/C root, where C aerial is the nutrient concentration in the shoot (stems and leaves) and C root is the nutrient concentration in the root. Additionally, the formula to calculate the increase/decrease of nutrients uptake of AMF-treated seedlings relative to the controls was: [nutrient absorption of AMF plant - nutrient absorption of non-mycorrhizal plant/nutrient absorption of non-mycorrhizal plant] × 100% (Wang et al., 2005).

Statistical analysis

Results of the experiment were first analyzed by comparing means of variance (F test), which were followed by DMRT at a 95% confidence level when the F test results showed a significant effect.

Results and Discussion

Plant growth

Arbuscular Mycorrhizal Fungi (AMF) showed a significant effect on shoot height, stem diameter, and leaf of *V. cofassus* at 12 weeks after transplantation (Table 2, $p<0.01$). The inoculation of *Glomus* sp.-SW10 increased the shoot height of *V. cofassus* and did not differ significantly from other treatments, except *Glomus intraradices* and control treatments. There were no significant differences in leaf number and leaf length among *V. cofassus* inoculated with all AMF treatments. Leaf widths of *V. cofassus* inoculated with *Glomus intraradices* and *Glomus* sp.-LW10 were significantly higher compared to that of *V. cofassus* inoculated with *R. crispa* and control (Table 2, $p<0.01$). The effect of AMF treatments started to show in the 4th week after planting (Figure 1). The performance of the 3-month-old *V. cofassus* seedlings is presented in Figure 2. Inoculation of native AMF on *V. cofassus* seedlings significantly increased plant growth and dry weight. An increase in plant height, stem diameter, and number of leaves of *V. cofassus* plants inoculated with native AMF compared to control was shown by 1,161-1,506%, 743-966%, and 341-581% growth, respectively (Table 2). The growth response of *V. cofassus* to native AMF inoculation shows that improving the *V. cofassus* seedlings' growth in post-asphalt mining soil media can be achieved through native AMF inoculation. The increase in growth and biomass is also reflected in the increased dependence of *V. cofassus* on native AMF (Table 3). Increased plant growth of *V. cofassus* is strongly associated with improvements in plant nutrients and water status, as well as increased plant resistance to biotic and abiotic stresses (Wang, 2017; Begum et al., 2019). Native AMF *G. clarum* improved the growth of *Nauclea orientalis* plant in mixed coal overburden media and soil (1:1 and 3:1) (Wulandari et al., 2024). Before being transplanted onto the degraded land, it is recommended that the plant is inoculated with AMF in the nursery to promote the growth of mycorrhizal tree seedlings (Prematuri et al., 2020).

Table 2. Shoot height, stem diameter, and leaves of *V. cofassus* seedlings after 3 months grown with or without Arbuscular Mycorrhizal Fungi (AMF) under greenhouse conditions.

Treatment	**Height (cm)***		**Stem diameter (mm)**		**Leaf**					
					Number per polybag		**Lenght (cm)**		**Width (cm)**	
Control	2.17±0.46	c	0.30±0.00	c	3.33±0.33	b	2.76±0.45	b	0.84±0.16	c
Racocetra crispa	31.67±2.48	ab	2.70±0.15	ab	16.33±2.03	a	15.20±0.32	a	3.14±0.04	b
Glomus intraradices	27.37±0.71	b	2.53±0.03	b	14.67±1.33	a	14.73±0.75	a	3.68±0.15	a
Glomus sp.	29.73±3.20	ab	2.70±0.31	ab	20.00±3.06	a	16.21±0.67	a	3.39±0.21	ab
Glomus sp.-LW10	33.07±1.35	ab	3.10±0.15	a	17.00±1.00	a	16.37±0.74	a	3.77±0.15	a
Glomus sp.-SW10	34.87±1.62	a	3.00±0.15	ab	15.00±5.13	a	14.79±0.66	a	3.50±0.13	ab
Mycofer IPB	31.57±3.27	ab	3.20±0.00	a	22.67±6.67	a	15.52±0.22	a	3.56±0.13	ab
Pr>F	<0.0001		<0.0001		0.0374		<0.0001		<0.0001	

Notes: Average values followed by different letters in the same column are significantly different at DMRT ($p<0.05$); *Mean±SE.

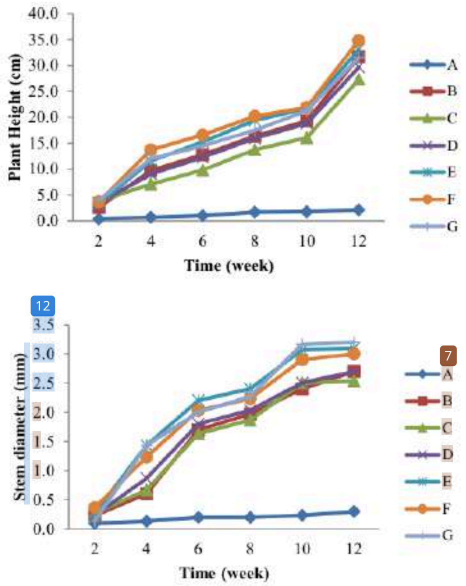

Figure 1. Trend of increasing height and diameter of *V. cofassus* seedlings with or without Arbuscular Mycorrhizal Fungi (AMF). Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB).

Figure 2. Performance of 3-month-old *V. cofassus* seedlings (left) and roots (right).
Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB).

Plant dry weight

AMF colonization significantly increased the roots, shoots, and total dry weight of *V. cofassus* seedlings whose growth was higher than the control (Table 3, p<0.01). The inoculation of *Glomus* sp., *Glomus* sp.-LW10, *Glomus* sp.-SW10, and Mycofer IPB increased roots, shoots, and total dry weight by 471-847, 5,200-6,733, and 1,150-1,720%, respectively. The results of this research show that the application of native AMF is really needed by *V. cofassus* plants. Inoculation of native AMF increases plant growth, leading to time-saving and cost-efficiency increases during the restoration and rehabilitation of post-asphalt mining land. Many publications report that native AMF inoculation can increase plant dry weight in post-mining land media conditions contaminated with heavy metals (Husna et al., 2017; Tuheteru et al., 2017; Wulandari et al., 2024). No significant differences were observed in Shoot Root Ratio (SRR) among *V. cofassus* inoculated with all AMF treatments. All AMF treatments increased the Seedling Quality Index (SQI) of *V. cofassus* by contrast, while *R. crispa* did not increase the SQI of *V. cofassus*. *V. cofassus* seedlings inoculated with all AMF treatments meet SQI standards and are suitable for planting in the field.

Arbuscular Mycorrhizal Fungi (AMF) colonization and Mycorrhizal Inoculation Effect (MIE)

Results of root staining showed that the roots of *V. cofassus* seedlings were colonized by AMF, with Mycofer IPB having the highest average of colonization (96%), followed by *Glomus* sp. (92%) *Glomus* sp.-LW10 (92%) and *Glomus* sp.-SW10. (88%) (Table 2). Significant differences were observed in AMF colonization among *V. cofassus* inoculated with the AMF (Table 3, p<0.01). Common AMF structures observed included internal and external hyphae, coil hyphae, vesicles, and auxiliary cells (Figure 3). Internal hyphae include AMF structures commonly found in the roots of *V. cofassus* plants. The presence of AMF structures in the roots of *V. cofassus* indicates that 3-month-old *V. cofassus* plants are in symbiosis with native AMF, which the native AMF contributes to improving nutrients and water status as well as increasing plant resistance to biotic and abiotic stresses.

Table 3. Dry weight, Shoot Root Ratio (SRR), and Seed Quality Index (SQI) of *V. cofassus* seedlings 3 months grown with or without mycorrhizal fungi under greenhouse conditions.

Treatment	Dry weight (g)						SRR	ISQ	
	Roots		Shoots		Total				
Control	0.17±0.09	c	0.03±0.00	d	0.20±0.09	d	0.64±0.50	0.007±0.00	b
Racocetra crispa	1.16±0.09	ab	1.60±0.11	bc	2.76±0.09	bc	1.41±0.17	0.34±0.05	ab
Glomus intraradices	0.91±0.38	b	1.59±0.09	c	2.50±0.43	c	3.07±1.74	0.59±0.26	a
Glomus sp.	1.58±0.16	a	1.85±0.24	abc	3.43±0.39	ab	1.17±0.06	0.37±0.06	a
Glomus sp.-LW10	1.33±0.06	ab	2.04±0.13	ab	3.37±0.11	ab	1.54±0.14	0.49±0.07	a
Glomus sp.-SW10	1.61±0.16	a	2.02±0.11	abc	3.64±0.06	a	1.29±0.20	0.41±0.07	a
Mycofer IPB	1.48±0.09	ab	2.05±0.15	a	3.53±0.18	ab	1.39±0.12	0.50±0.04	a
Pr>F	0.0006		<0.0001		<0.0001		0.3825	0.0434	

Notes: A (control), B (*Racocetra crispa*), C (*Glomus intraradices*), D (*Glomus* sp.), E (*Glomus* sp.-LW10), F (*Glomus* sp.-SW10) and G (Mycofer IPB). Average values followed by different letters in the same column are significantly different at DMRt (p<0.05); *Mean±SE.

The range of MIE values for all AMF treatments was 92,2-94,6% (Table 4). The high MIE value indicates that AMF inoculation is beneficial for the production of quality seeds at the nursery scale. High dependence of plant species on AMF in post-mining soil media conditions that contain heavy metals has also been reported in several plant species, including *Pericopsis mooniana* (Husna et al., 2016; 2019; 2021a), *Pterocarpus indicus* (Husna et al., 2021a), *Nauclea orientalis* (Tuheteru et al., 2020) and *Kalappia celebica* (Husna et al., 2021b). The results of this research showed that native AMF from post-asphalt mining land on Buton Island is compatible with *V. cofassus* and significant in increasing the initial growth and improving the nutrition of *V. cofassus* plants grown on post-asphalt mining soil media. Compatibility of native AMF with *V. cofassus* is possible because of several things, namely 1) native AMF is suitable for media conditions; 2) local AMF is compatible with root exudates produced by *V. cofassus* roots; and 3) genotypically, local AMF has the ability to absorb and conduct water and nutrients to the host plant. Along with improvements in nutrients and water status as well as chelation of heavy metals by AMF, the growth and biomass of mycorrhizal plants can be increased. Several research results showed that AMF can help the growth of plants grown on land contaminated with heavy metals (Bi et al., 2018). This experiment showed that AMF inoculation promotes the growth of seedlings on low-fertility soil media in the nursery.

Table 4. AMF colonization and MIE of *V. cofassus* seedlings 3 months grown with or without Arbuscular Mycorrhizal Fungi (AMF).

Treatment	AMF Colonization (%)		MIE (%)
Control	2±0.33	d	
Racocetra crispa	77±2.47	c	92.5±3.52
Glomus intraradices	84±7.52	bc	92.2±3.70
Glomus sp.	92±3.12	ab	94.6±2.09
Glomus sp.-LW10	88±4.72	abc	94.1±2.58
Glomus sp.-SW10	92±1.85	ab	94.6±2.38
Mycofer IPB	96±1.87	a	94.2±2.51
Pr>F	<0.0001		<0.0001

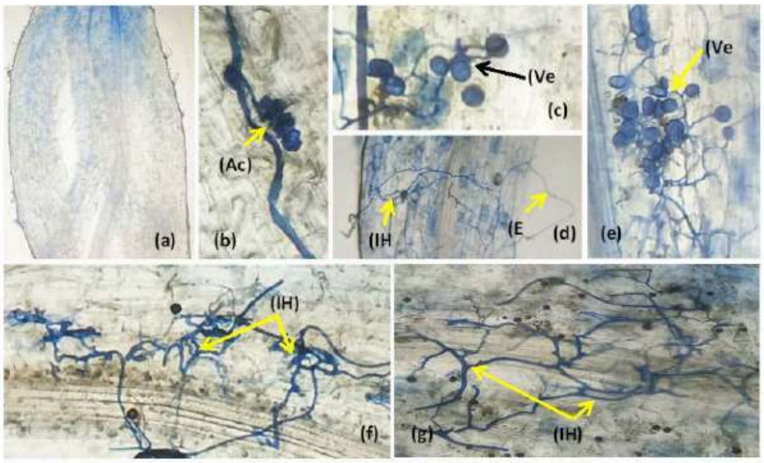

Figure 3. AMF structure in the shoots and roots of *V. cofassus*.
Notes: Control (A), *Racocetra crispa* (B), *Glomus intraradices* (C), *Glomus* sp. (D), *Glomus* sp.-LW10 (E), *Glomus* sp.-LW10 (F), Mycofer IPB (G); IH = Intraradical hyphae; EH = Extraradical hyphae; Ve = Vesicles; and Ac = Auxiliary cells.

Shoot and root nutrient concentration and content

The soil pH was neutral (7.2). The C and C/N ratios were categorized as very high and Ca as high. Total N, P_2O_5 (Olsen), K, and Na were in the low category. Mg level and CEC were in the moderate category. Soil texture was classified as clay (Table 1). Inoculation of native AMF significantly increased P and Ca content and uptakes among AMF treatments, both in roots and shoots of *V. cofassus* (Tables 5 and 6). The control treatment showed significant differences in P and Ca content and uptakes compared to the AMF treatments. Treatments *Glomus* sp. SW10 dan Mycofer IPB significantly increased P uptake in the roots and shoots. Also, both treatments were significantly different from the *G. intaradices* and control treatments in roots (Table 5) and *G. intaradices*, *R. crispa*, and control treatments in shoots (Table 6).

Table 5. Nutrient content and uptake in roots of 3-month-old *V. cofassus* seedlings.

Treatment	Content (mg kg^{-1})				Uptake (mg plant^{-1}x 10^{-3})			
	P		Ca		P		Ca	
Control	0.26±0.007	b	13.35±2.221	d	0.05±0.024	c	2.51±1.276	b
Racocetra crispa	0.49±0.026	a	73.79±0.557	a	0.58±0.073	ab	85.37±6.929	a
Glomus intraradices	0.52±0.075	a	73.29±18.607	a	0.49±0.166	b	67.10±24.941	a
Glomus sp.	0.51±0.033	a	65.97±6.564	ab	0.81±0.112	ab	104.12±15.946	a
Glomus sp.-LW10	0.52±0.003	a	61.35±1.819	abc	0.69±0.025	ab	81.96±5.570	a
Glomus sp.-SW10	0.55±0.020	a	58.46±6.891	bc	0.89±0.056	a	92.98±8.968	a
Mycofer IPB	0.51±0.027	a	52.77±3.625	c	0.75±0.085	a	77.56±3.092	a

Table 6. Nutrient content and uptake in shoots of 3-month-old *V. cofassus* seedlings.

Treatment	Content (mg kg^{-1})				Uptake (mg plant^{-1} x 10^{-3})			
	P		Ca		P		Ca	
Control	0.33±0.097	c	25.45±2.427	b	0.01±0.002	c	0.80±0.155	b
Racocetra crispa	0.49±0.054	b	72.87±0.942	a	0.80±0.117	b	116.65±6.548	a
Glomus intraradices	0.68±0.081	ab	74.17±14.851	a	0.85±0.247	b	117.73±35.990	a
Glomus sp.	0.59±0.019	ab	74.39±0.094	a	1.11±0.169	ab	137. 92±.0.169	a
Glomus sp.-LW10	0.61±0.027	ab	74.33±0.042	a	1.25±0.059	a	151.04±9.348	a
Glomus sp.-SW10	0.69±0.074	a	74.29±0.202	a	1.38±0.125	a	150.35±8.279	a
Mycofer IPB	0.60±0.031	ab	69.55±3.901	a	1.22±0.038	a	143.31±15.922	a

Phosphorus (P) and Calcium (Ca) elements had transport factors (TF)>1 (Table 6), except control which showed TF<1. Native and commercial AMF inoculation increased P and Ca uptakes in the roots and shoots of 3-month-old *V. cofassus* under nursery conditions (Table 7).

Table 7. Transport factor of elements and increase/decrease of nutrients uptakes in roots and shoots of 3-month-old *V. cofassus* seedlings.

Treatment	Transport factor		Increase/decrease of nutrient uptake			
			Roots		Shoots	
	P	Ca	P	Ca	P	Ca
Control	0.22	0.32	-	-	-	-
Racocetra crispa	1.38	1.37	1.184	3.301	7.900	14.481
Glomus intraradices	1.78	1.75	962	2.573	8.400	14.616
Glomus sp.	1.37	1.32	1.704	4.048	11.090	17.048
Glomus sp.-LW10	1.82	1.84	1.429	3.165	12.400	18.780
Glomus sp.-SW10	1.56	1.62	1.871	3.604	13.700	18.694
Mycofer IPB	1.62	1.85	1.576	2.990	12.100	17.814

The results of this study showed that the levels and uptakes of P and Ca in the roots and shoots of *V. cofassus* plants were increased by native AMF. It is well known that AMF can facilitate the acquisition of mineral nutrients (especially P) by plants (Smith and Read, 2008). Seeds of *V. cofassus* plant inoculated with native AMF show a more effective P and Ca allocation. This research agrees with several previous research results on various media conditions of post-mining land, such as high P uptake in *Pericopsis mooniana* plants in serpentine soil media conditions (Husna et al., 2016), *Nauclea orientalis* plants in gold tailings media (Tuheteru et al., 2020), and *Amygdalus pedunculata* in coal mining subfields (Bi et al., 2018).

P and Ca are essential nutrients that plants need. Ca plays a role in plants, including being part of cell structure and the formation or division of new cells, and it is a co-factor for many enzymes in plants (Havlin et al., 2017). The most essential function of P is its involvement in the storage and transfer of energy in plants (Havlin et al., 2017). Wang (2017) has summarized the direct and indirect mechanisms of P repair by AMF, including that AMF extraradical hyphae can absorb P directly and translocate it more quickly to AMF structures in the roots, in which AMF secretes phosphatases that hydrolyze organic P sources into available forms. Apart from P, AMF can also absorb other macronutrients (K, Ca, Mg, and S) and micronutrients (Zn, Fe, Cu, and Mn) (Smith and Read, 2008; Husna et al., 2017). In this study, there were differences in the P uptake ability of the two plant parts by each type of AMF. *G. intraradices* had lower P uptake compared to other AMF treatments, even though it was higher than the control. Walder and van der Heijden (2015) stated different AMF species exhibit varying capacities for mycorrhizal phosphate uptake.

Based on this research, it appears that the application of native AMF shows a significant positive effect in increasing plant growth and survival, increasing water and nutrient uptakes, and improving soil structure and quality. Furthermore, native AMF and *V. cofassus* plants can also encourage ecological restoration on degraded land, such as post-asphalt mining land in the world. Thus, providing seedlings with native AMF at the nursery scale is very necessary to support planting on degraded land.

Conclusion

Native AMF from post-asphalt mining land effectively promotes growth and nutrients P and Ca uptakes of *Vitex cofassus* at a 12-week experiment in post-asphalt mining soil media under nursery conditions. Native AMF has the potential to be developed as biofertilizers to support post-asphalt mining land reclamation and forest restoration programs.

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