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The Effect of Arbuscular Mycorrhizal Fungi and Azotobacter on the Growth of *Toona sureni* Merr (Suren) Seedlings on Post-Coal Mining Land

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ABSTRACT. This research aimed to investigate the growth of Toona sureni Merr (suren) seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and azotobacter on land previously used for coal mining. We also investigated the source of AMF and azotobacter isolates, resulting in the best growth of suren seedlings on the post-coal mining land. We used a completely randomized design in a factorial manner with two treatment factors. The first factor was the inoculation of AMF isolates (C), which consisted of 5 levels, namely: without AMF (C0), AMF from the suren rhizosphere (C1), AMF from Gmelina arborea (gmelina) rhizosphere (C2), AMF from Durio zibethinus (durian) rhizosphere (C3), and AMF from Acacia mangium (acacia) rhizosphere (C4). The second factor was the inoculation of azotobacter isolates (A), which consisted of 4 levels, namely: without azotobacter (A0), azotobacter from the suren rhizosphere (A1), azotobacter from the gmelina rhizosphere (A3), and azotobacter from the durian rhizosphere (A4). Each treatment had six replications. Data analysis used ANOVA at a 5% test level. Then, we used the orthogonal contrast test to compare the growth of the inoculated seedlings with AMF and azotobacter with that of the un-inoculated seedlings. Meanwhile, to find out the source of isolates for AMF and azotobacter, which resulted in the best growth of the seedlings, we analyzed them with a two-way Duncan Multiple Range Test (DMRT). The results of the ANOVA showed that the AMF inoculation had a significant difference in height growth, diameter growth, and growth in the number of leaves. In contrast, azotobacter inoculation had no significant difference in the same growth variables. The interaction between AMF inoculation and Azotobacter was not significantly different in height growth but in diameter and leaf number growth. The DMRT analysis showed that AMF from suren rhizosphere isolates resulted in the best growth of the suren seedlings.
Keywords: *arbuscular mycorrhizal fungi, azotobacter, suren, post-coal mining land*

INTRODUCTION

Mining activities, especially coal mining, are among the largest foreign exchange contributor sectors. However, on the other hand, coal mining activities can cause environmental damage. The post-coal mining land can be productive again; its ecosystem resembles a forest as it was initially required for revegetation activities (Woodbury et al., 2019). The silvicultural treatments affect the success and acceleration of revegetation. The selection of adaptive vegetation types to the environment and utilization of soil microorganisms such as arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (NFB) is an alternative way to rehabilitate degraded lands. AMF and NFB derived from local isolates have high effectiveness, so the inoculated vegetation will grow better, and the land quality can be improved (Salim et al., 2020; Nasrudin and Ridwan, 2914). Microorganisms' role in improving the soil's physical condition, especially soil aggregates, has received significant attention. Fungal mycelium, bacterial cells, and metabolic products may affect soil structure. (Costa et al., 2018).

There have been many uses of nitrogen-fixing bacteria (NFB) to help provide nitrogen nutrients for plants. Most NFBs come from symbiotic bacterial groups, such as the Rhizobium group associated with legume plants. Meanwhile, the utilization of NFB from non-symbiotic groups such as Azotobacter species has not been widely carried out, especially for forestry plant species. Like Rhizobium, Azotobacter can assimilate various forms of combined nitrogen, such as nitrate, ammonia, and simple amino compounds. The presence of these compounds can inhibit free nitrogen fixation. Hasanudin (2000) reported that Azotobacter inoculation could reduce the use of artificial fertilizers by around 15 - 30%. Soil reactions, organic matter, and the concentration of certain elements, especially phosphate, may affect azotobacter activity.

Some criteria used in selecting superior isolates are that apart from being effective. Isolates can adapt to the local environment where the inoculation will be carried out, compete with soil microbes, are easily mass-produced, and live in the host plant's root environment. Several preliminary studies proved that a significant growth response in some trees is due to AMF inoculation from local isolates (Smith and Smith, 2012). The importance of using AMF and NFB from local isolates is because each fungus and bacteria require a specific environment to live optimally. Setiadi (2001) explained that AMF exploration in several ecosystems in several regions in Indonesia succeeded in identifying and mapping the dominant and specific types of AMF found in an area.

The post-coal mining land conditions with poor soil physical, chemical, and biological characteristics need proper silvicultural techniques, selection of suitable plant species, fertilization, and organic matter management to overcome it. For this reason, it is necessary to have plant species that can grow in critical land conditions.

Suren (Toona sureni Merr) is one of the plants developed for the reforestation of degraded land. Suren wood is often used for furniture, veneer, and cigar boxes; the bark and roots are used for concoctions for diarrhea medicine; the skin and fruit are used as essential oils (Heyne, 1987; Hua et al., 2017; Latifah et al., 2018).

The aims of this study were: (a) to compare the growth of suren seedlings in post-coal mining land inoculated with AMF and Azotobacter with seedlings without inoculated with AMF and Azotobacter (b) to find out the source of AMF and Azotobacter isolates that performed the best growth in suren seedlings in post- coal mining land.

MATERIAL AND METHODS

The equipment and materials used in this study included analytical scales, 325 mesh sieve, 1 kg polybag, 250 ul micropipette, microscope, 27 cm x 22 cm x 14 cm p; plastic box, 0.5 cm soil sieve, test tube, isolation chamber, 1 kg clear plastic, erlenmeyer, sprayer, autoclave, and veneer caliper. The materials used were suren seedlings, AMF inoculum, Azotobacter inoculum, compost, dolomite lime, mannitol, K_2HPO_4, $MgSO_4$, H_2O, NaCl, K_2SO_4, $CaCO_3$, agar, and distilled water.

The design used was a completely randomized design (CRD) arranged factorially. This study used 2 (two) treatment factors, namely: The first factor was the type of AMF consisting of without AMF (C0), AMF from the suren rhizosphere (C1), AMF from Gmelina arborea (gmelina) rhizosphere (C2), AMF from Durio zibethinus (durian) rhizosphere (C3), and AMF from Acacia mangium (acacia) rhizosphere (C4). The second factor was the type of Azotobacter consisting of without azotobacter (A0), azotobacter from the suren rhizosphere (A1), azotobacter from the gmelina rhizosphere (A3), and azotobacter from the durian rhizosphere (A4). Each treatment was replicated six times; it was 120 sampling plants.

Azotobacter inoculum was prepared using the Ashby method. Ashby media was made by preparing ingredients such as mannitol 20 g, K2HPO4 0.2 g, MgSO47H2O 0.2 g, NaCl 0.2 g, CaCO3 5 g, agar 15 g, and distilled water 1000 ml. Next, we boiled all the mixture and stirred it during heating. After that, the solution was autoclaved and then put in an erlenmeyer tube.

Germination of suren seeds was performed in a 27 cm x 22 cm x 14 cm seed box using sterilized topsoil media. We put the soil into a box 5 cm in height. The seeds that had been soaked and dried were then sown and covered again with soil. The nursery was maintained for three weeks until true leaves grew.

After three weeks, we transplanted the seedlings into polybags. Before planting, we put soil into the polybags and dug a small hole to provide a space for the seedlings to grow. In the planting hole, we put azotobacter inoculum by dripping 10 ml of Azotobacter suspension around the roots. Similarly, we put AMF inoculum in the form of spores (100 spores), which were dripped around the roots using a micropipette. Then, we planted the seedlings in planting holes and covered them with a layer of soil.

After four months in the pots, we transplanted the seedlings in the post-coal mine land. This land with conditions after ± 2 months of exploitation with an altitude of 485 m above sea level. The results of the initial soil analysis showed that the soil had an acidic pH (pH H2O 4.75 and pH HCl 4.22), C-organic (0.95%), N (0.10%), P (10.82 ppm), K (0.12 Cmol+/kg), Ca (3.56 Cmol+/kg), and Mg (2.87 Cmol+/kg).

RESULT AND DISCUSSION

The suren seedlings that lived on the post-coal mine land reached 100%. Based on the ANOVA results, the application of AMF significantly differed in height but not in diameter and the number of leaves. In contrast, the application of Azotobacter is not significantly different in height, diameter, and number of leaves of the suren seedlings.

Table 1. The ANOVA results on the effect of the application of AMF and Azotobacter on the observed variables

	F- value		
Growth Variables	AMF	*Azotobacter*	Interaksi
Height	3.973 *	31.099 ns	1.430 ns
Diameter	2.086 ns	0.245 ns	2.989 *
Number of leaves	1.515 ns	0.973 ns	2.101 *

Table 1 shows that the interaction between AMF and Azotobacter is not significantly different in height growth but in diameter and number of leaves. The interaction between

AMF and Azotobacter in diameter growth shows that each treatment factor, namely AMF (C) and Azotobacter (A), together or acted independently of each other in providing treatment effects on the diameter growth of suren seedlings.

Table 2. Results of contras orthogonal analysis of the comparison between inoculated and un-inoculated seedlings

Growth Variables	F- value	α
Height		
Un-inoculated and AMF inoculated	0.007	0.05*
Un-inoculated and Azotobacter inoculated	0.832	0.05*
Diameter		
Un-inoculated and AMF inoculated	0.144	0.05*
Un-inoculated and Azotobacter inoculated	0.861	0.05*
Number of leaves		
Un-inoculated and AMF inoculated	0.230	0.05*
Un-inoculated and Azotobacter inoculated	0.902	0.05*

Table 2 compares inoculated and un-inoculated seedlings to AMF and Azotobacter application in height, diameter, and leaf number growth significantly contrasts. It means that AMF and Azotobacter effectively improve the seedling's growth.

Table 3. Interaction effect of AMF and Azotobacter on average diameter growth

	A0	A1	A2	A3	Interaction
C0	7.75 *bc* (abc)	7.90 *abc* (abc)	8.30 *ab* (ab)	8.10 *ab* (abc)	+
C1	8.43 *a* (a)	8.31 *a* (ab)	8.40 *a* (a)	7.58 *b* (c)	-
C2	8.2 *ab* (ab)	8.28 *a* (a)	7.85 *abc* (abc)	8.11 *ab* (abc)	-
C3	8.36 *a* (a)	8.23 *ab* (ab)	8.00 *abc* (abc)	8.1 *ab* (abc)	-
C4	7.63 *bc* (b)	7.65 *c* (c)	8.01 *abc* (ab)	8.21 *a* (a)	+
Interaction	-	-	-	+	

Note:

-The numbers followed by the same letter and italicized in the same column are not significantly different at the 5% level.

-The numbers followed by the same letter and in parentheses in the same row are not significantly different at the 5% level.

Table 3 shows that each diameter growth differed in each AMF and Azotobacter treatment. The interaction between AMF from suren isolate (C1) and without Azotobacter isolate (A1) resulted in the highest diameter growth, 8.43 mm. In contrast, the interaction between AMF isolates from suren (C1) and Azotobacter from durian isolates (A3) resulted in the lowest growth. It is 7.58 mm.

The interaction between AMF from acacia isolates (C4) to all Azotobacter isolates is positive. Likewise, there is positive interaction between Azotobacter from durian isolates (A3) to all AMF treatments. However, they are not significantly different in diameter growth statistically.

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Table 4. The interaction effect of AMF and Azotobacter on the average growth in the number of leaves

	A0	A1	A2	A3	Interaction
C0	15.83 *b* (abc)	19.66 *ab* (a)	17.66 *ab* (ab)	16.83 *bc* (abc)	+
C1	18.00 *ab* (ab)	20.33 *a* (a)	19.33 *a* (ab)	16.83 *bc* (b)	-
C2	18.66 *ab* (ab)	16.16 *c* (b)	17.66 *ab* (ab)	17.33 *bc* (b)	-
C3	19.00 *a* (a)	18.16 *abc* (a)	16.66 *ab* (a)	19.16 *ab* (a)	+
C4	18.00 *ab* (a)	18.33 a*bc* (a)	17.50 *ab* (a)	18.16 *abc* (a)	+
Interaction	+	-	-	+	

The interaction between AMF from suren isolate (C1) and Azotobacter from suren isolate (A1) resulted in the highest growth response of the number of leaves. Meanwhile, the interaction without AMF (C0) and Azotobacter (A0) resulted in the lowest seedling growth.

Table 5. DMRT results of mean seedling height with AMF application

No	Treatments	Average
1	C4	54.80 a
2	C1	52.97 ab
3	C3	50.19 bc
4	C2	49.81 bc
5	C0	47.63 c

The ANOVA results show that AMF significantly differs in height growth. Meanwhile, the stem diameter and the number of leaves are not significantly different. The results of the DMRT on plant height growth (Table 5) show that AMF from acacia isolate (C4) gave the highest average growth of 54.80 cm, significantly different from un-inoculated seedlings (C0). AMF from gmelina isolate (C2), and AMF from durian isolate (C3) have the same results.

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The compact structure of the post-coal mine soil causes poor water management and aeration systems, which directly causes disruption of root function and development in nutrient absorption (Ezeokoli et al., 2020). The utilization of mycorrhiza is one of the right solutions to enhance plant growth in post-coal mine land. Mycorrhiza fungi can increase

nutrient absorption, drought resistance, and resistance to root pathogen attacks and toxic elements. In addition, mycorrhiza can produce hormones and growth regulators (Butcher, 2007; Begum et al., 2019; Ezeokoli et al., 2019).

Based on the ANOVA, the AMF is significantly different in height growth but not significantly different in the diameter and number of leaves. AMF caused the best height growth from acacia isolates (C4), with an average height growth of 58.21 cm. The effectiveness of AMF spores in infecting plant roots was quite good, so it can help to absorb nutrients and improve plant growth on post-mining land. Leight et al. (2009) stated that mycorrhizal inoculation could improve plant growth. The results of the initial soil analysis showed that the soil was acidic (soil pH 4.72) and the Al content was 0.40. Therefore, some nutrients became unavailable to plants due to the high Al content. High Al content tends to bind P ions, reducing their availability for plants. The high pH of the soil allows the content of toxic elements in the soil, which can inhibit plant growth. Bucher (2007) stated that in acidic soils, the availability of Al is higher, so it tends to bind P ions and reduce its availability for plants. AMF can increase the absorption of phosphorus (P) nutrients. Mycorrhizal fungi hyphae also secrete phosphatase enzymes that can help provide nutrients, especially P. In addition, it can also help the absorption of N nutrients in a bound form into an available form that plant roots or mycorrhizal fungus hyphae can absorb. The results of the P analysis showed that the P content in plant tissues inoculated with AMF was 0.27%. Compared to the control treatment with a content of 0.23%. AMF can increase plant tissue's P content compared to plants not inoculated with AMF. The results of the N analysis also showed the same thing. The tissue N content of 2.79% was lower than the N in plant tissues not inoculated with AMF, which was 1.71%.

Increased nutrient absorption due to mycorrhiza can increase the solubility and availability of nutrients, and mycorrhizal hyphae will expand the field of nutrient uptake due to the size of the hyphae finer than the root hairs allowing hyphae to infiltrate the smallest soil pores. AMF hyphae can absorb nutrients, especially P, compared to roots not inoculated with AMF. In addition, mycorrhiza can increase plant growth by protecting plants from root pathogens and toxic elements. Mycorrhizal hyphal membranes can function as a protective barrier to pathogens, and mycorrhizas can secrete antibiotics that can kill or inhibit the growth of pathogens. AMF application was not significantly different in the diameter and number of leaves.

Simarmata (2005) stated that suitable host plants strongly influence the effectiveness of AMF. Environmental factors include temperature, soil acidity, humidity, light intensity, and nutrient content. AMF spores will develop at a temperature of 24^{o} C -35^{o} C. Differences in soil pH values will affect spore germination for each AMF genus; the Glomus genus can germinate at pH 4-6. During the study, the environmental conditions in the post-coal mine

land were the average temperature of 24.7^{o} C and humidity of 88.5%, and the average rainfall intensity was 272.426 mm month $^{-1}$.

Based on the ANOVA results, Azotobacter inoculation is not significantly different in suren growth. It happened due to the low pH of the mine soil. Azotobacter is an N-fixing bacterium widely distributed in soils with a pH above 6.0. Soil reaction is a limiting factor in the development and spread of these bacteria (Sutedjo et al., 1996). The Azotobacter isolates used the same type because this study did not identify the Azotobacter types used. Environmental factors also affect the effectiveness of Azotobacter. Azotobacter can live at an optimum temperature of 30^{o} C – 35^{o} C. The average temperature during the study was 24.7^{o} C. However, the role of Azotobacter could not be optimal. Based on plant growth data, we found that height, diameter, and number of leaves variables showed that plants inoculated with Azotobacter grew better than those not inoculated with Azotobacter. It is due to Azotobacter's ability to synthesize biologically active substances such as B vitamins, indole acetic acid (IAA), and gibberellins.

The role of mycorrhiza was more dominating than Azotobacter in impacting the diameter and number of leaves. Mycorrhiza plays a role in the absorption of N and P nutrients (Smith and Smith, 2012). At the same time, Azotobacter can fix N to increase its availability. Azotobacter can secrete the enzyme nitrogenase, which helps increase plant nutrient uptake. While mycorrhiza, with its mycelium, can expand the root contact area and shorten the distance between nutrients in the soil and plants to increase nutrient absorption (Hindersah dan Simarmata, 2004; Aasfar et al., 2021; Aprilya and Mulyawan, 2022)

CONCLUSION

Based on the results and discussion of the research:

1. The suren seedlings grew well on the post-coal mine land with the percentage of live plants 100%.
2. The seedlings inoculated with AMF and Azotobacter showed better growth in height, stem diameter, and the number of leaves than those not inoculated with AMF and Azotobacter.
3. The interaction of AMF from acacia isolate (C4) and Azotobacter from durian isolate (A3) provides a positive interaction in the growth of the suren seedlings on post-coal mine land.

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