



The Effectiveness Analysis of Arbuscular Mycorrhizal Fungi (AMF) and Ameliorant Treatments on the Growth of Red Jabon Seedling on Soil Medium Post-Lime Mining

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ABSTRACT

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The use of AMF and CLW combination in reclamation and revegetation has never been done, so it is expected to provide added value in environmental improvement and accelerate the growth of red Jabon (*Anthocephalus macropyllus*) plants. This study aimed to analyze the effectiveness of AMF inoculants and soil ameliorants on the growth of Jabon red seedlings planted on post-mining soil media of limestone PT. Holcim Indonesia Tbk. The research method used a factorial randomized group design consisting of 3 treatment factors, namely factor 1 mycorrhizae (M₀, M₁, and M₂), factor 2 phosphate (P₀ and P₁), and factor 3 leaching waste cement residue (CLW) from car castings (L₀ and L₁). So there were 12 treatment combinations, each treatment was repeated ten times, and each replicate was 12 plant units, so the total number was 1440 plants. The results showed that the interaction of the three treatment factors of AMF, phosphate, and CLW significantly increased the diameter increase of *A. Macrophyllus* seedlings planted on limestone post-mining soil media. The interaction of AMF treatment with phosphate significantly increased the growth of seedling height and the percentage of AMF colonization. The interaction of phosphate treatment with CLW significantly increased the increase in seedling height and seedling diameter. The single AMF treatment was effective in increasing the average increase in seedling height (18.20 cm), average increase in seedling diameter (2.67 mm), root fresh weight (6.46 g), shoot fresh weight (29.13 g), root dry weight (1, 77 g), shoot dry weight (5.14 g), total dry weight (6.91 g) as well as the percentage value of *A. macrophyllus* colonization on *A. Macrophyllus* plants reached 32.63% -41.00%. This study concludes that treatment with the addition of AMF has a significant effect on the seedling growth of *A. macrophyllus*. Seeing the extent of critical land, marginal land and damaged mining land in Indonesia, the innovation of using biological fertilizers in the form of AMF is one of the most appropriate and promising solutions in the future.

1. INTRODUCTION

Red Jabon (*A. macrophyllus*) is a tree whose distribution is from Sulawesi, Maluku and Papua. The high habitus of the red jabon tree can reach 40 m with a round and upright trunk reaching 70%-80% with a diameter of more than 50 cm. Red Jabon is a pioneer plant that is light tolerant, can live in the lowlands to an altitude of 50-1000 m above sea level. The use of red jabon wood can be used as raw material for plywood, furniture, plywood, home accessories and others [1].

Mining materials are natural resources which in their exploitation or utilization require a complex process from exploration, exploitation to extraction. In addition to increasing state income, improving the community's economy, creating job opportunities and encouraging the development of science and technology, mining activities can cause problems related to environmental damage. In general, post-mining soil conditions are classified as poor. The soil becomes nutrient-poor, topsoil is lost, dense, microbial activity is low, the pH changes, does not have a profile, is polluted by heavy metals, the surface temperature rises, and is easily eroded due to loss

of vegetation, as well as damage to soil structure, texture, and aggregate [2]. Especially for limestone post-mining land the content of organic C, N, P and K is very low [3], therefore it needs to be rehabilitated.

Revegetation of post-mining land is a must. This activity takes a long time so the types of plants planted need to be considered carefully. Another alternative to help plant growth on post-mining land is the addition of soil ameliorant material and creating a suppressive soil (rich in soil microbes such as Arbuscular Mycorrhizal Fungi (AMF). AMF is a form of mutualistic symbiotic relationship between fungi (mykes) and roots (rhiza) of higher plants [4]. In general, AMF can be found in higher plants that grow in various types of habitats and climates. The distribution varies according to climate, environment and land use type. The use of AMF CLW provides water, nitrate, phosphate, potassium and other nutrients, and stabilize soil aggregates [5, 6]. According to Golledge et al. [7], the presence of AMF can accelerate natural succession in habitats that are subject to extreme disturbances.

In the post-mining area of PT. Holcim Indonesia has found 7 types of AMF, namely *Glomus sp-1*, *Glomus sp-2*,

Gigaspora sp., *Acaulospora scrobiculata*, *A. tuberculata*, *A. foveata* and *Sclerocystis sinuosa*, which are naturally symbiotic with the rhizosphere of lower vegetation [3]. Trapping using zeolite media and the host plant *Prureria javanica* found 2 types of AMF (*Glomus* sp-1 and *Glomus* sp-2), which can be used as sources of inoculants. AMF inoculants developed from native propagules are highly recommended because they are more efficient, effective and adaptive to local conditions and do not have a negative impact on the environment [8]. Selection of the right type of AMF in producing inoculants is very important to ensure the successful application of AMF to the plant itself.

Jones Jr [9] stated that AMF can assist in providing auxin, which plays a role in cell differentiation, especially differentiation of carrier bundles, secondary thickening, and activates the cambium to form new cells. Auxin hormone affects the development of the cell wall so that the protoplast has the opportunity to absorb water from the cells below it so that long and large vacuole cells are obtained. The presence of this auxin hormone ultimately plays a role in increasing the average increase in stem diameter. Transport of water, nutrients, and photosynthetic increases due to the activity of the cambium, which forms a phloem towards the outside and xylem towards the inside.

On the other hand, PT. Holcim Indonesia Tbk produces cement leaching waste (CLW) which has the potential as a soil ameliorant. CLW content in the form of calcium carbonate, calcium hydroxide, dolomite, magnesium carbonate, magnesium oxide, and calcium sulfate [10] can increase nutrient availability, improve soil structure and increase infiltration [11]. The use of CLW in reclamation and revegetation is expected to provide added value in improving the environment, as well as accelerating the growth of planted plants. Considering that similar research is still very limited, it is necessary to conduct research on the effectiveness of using AMF inoculants and soil ameliorants on the growth of red Jabon (*A. macrophyllus*) seedlings. The red Jabon was chosen considering that this species is considered a pioneer, fast growing, and multipurpose with good wood quality. The purpose of this study was to analyze the effectiveness of AMF inoculants and soil ameliorant materials on the growth of Jabon red seedlings planted on post-mining soil media of limestone PT. Holcim Indonesia Tbk.

2. MATERIAL AND METHODS

2.1 Study area

The research was carried out at the PT. Holcim Indonesia Tbk Cibinong, Bogor. AMF analysis was carried out at the Laboratory of Forest Microbiology, Center for Forest Research and Development and Nature Conservation, Gunung Batu Bogor and the Laboratory for Botany, Research Center for Biology-BRIN, Cibinong Science Centre, Jalan Raya Jakarta-Bogor km 45, Cibinong. This research was conducted in September 2015~June 2016.

2.2 Experimental design

The design used is the factorial randomized group design. This study consisted of 3 (three) treatment factors, namely the first treatment of AMF (control (M₀), AMF originating from clay areas (M₁) and AMF derived from limestone (M₂),

phosphate treatment (control (P₀) and P₁, and the three treatments washing wastes of the remaining cement from castings (CLW) (control (L₀), and L₁). So a total of 12 treatment combinations, each treatment was repeated 10 (ten) times and each replicated 12 plant units, with a total of 1440 plants.

2.3 Research procedures

1). Germination media prepare: The germination media used fine sand and compost (1:1 v/v), the media was sieved using a micro-sized sieve, then sterilized using an autoclave at 121 °C, pressure of 1 atm for 30 minutes.

2). Germination: The germination media was put into a sowing tub measuring 20 x 25 cm, the media used was sand and compost (1:1 v/v), then the media was doused with water until it dripped. After that the seeds are mixed with fine sand with a ratio of 2:1 v/v so that they are evenly distributed during sowing. Then the seeds are covered with transparent plastic.

3). Growing media: The media used is the soil obtained around the quarries of PT. Holcim Indonesia Tbk Cibinong, at a depth of 0-20 cm. The soil is put into sacks, sifted and sterilized using a roasting system, then the growing media is left to cool. Then put the soil into a polybag measuring 10 cm x 15 cm.

4). Ameliorant prepare: The ameliorant material in the form of CLW is obtained from the washing place for the remaining cement from the cast-out mortar, the CLW is cleaned and air-dried. CLW material was weighed as much as 10 g/seed according to each treatment.

5). AMF trapping: The trapping technique used follows the method of Brundrett et al. (1996) using an open culture pot.

6). AMF inoculation: Mixed AMF inoculants (*Glomus* sp-1 and *Glomus* sp-2) were obtained from culture pots with an average spore density of 19 per 10 g (M1) and 11 per 10 g (M2). The mycorrhizal inoculation technique was carried out by adding 10 g of inoculant to each polybag placed near the roots of the *A. macrophyllus* seedlings and adding 10 g of natural phosphate fertilizer and CLW respectively.

7). Planting: Then the planting hole is closed and the position of the plant must be upright. Plants were planted for 6 months after inoculation. Maintenance is done by watering the plants in the morning or evening according to the conditions of the growing media. Cleaning of weeds and pests if necessary. Plant height and stem diameter were calculated every 2 weeks.

2.4 Data collection

2.4.1 Seedling height

The height was measured from the base of the stem to the highest growing point of the seedling and the measurements were made every two weeks for six months.

2.4.2 Seedling diameter

The seedling diameter data was obtained by measuring the diameter of the seedling at a distance of 1 cm from the root neck using a caliper. Seedling diameter data was measured every two weeks for six months.

2.4.3 Fresh weight of shoots and fresh weight of roots

The measurement of the fresh weight of the plant was carried out at harvest, by separating the shoot and roots of the plant, by cutting between the base of the stem and the root, then weighing the shoot and roots of the plant using an analytical balance.

2.4.4 Total dry weight of plants

The shoots and roots were separated and dried in an oven for 48 hours at 70°C [12]. After drying, the shoots and roots of the plants were weighed using an analytical balance.

2.4.5 Percentage of AMF colonization

It can be calculated using the slide method to find the percentage of AMF colonization, where 10 pieces of colored roots are taken at random and then arranged on a glass object. The percentage of colonization is calculated based on the formula:

$$\% \text{ Colonization} = \left(\frac{\text{number of infected roots cuttings}}{\text{the total number of root pieces observed}} \right) \times 100\% \quad (1)$$

2.5 Analysis data

Analysis of variance (ANOVA) was used using SPSS software version 10.01 to determine the effect of treatment and combination of treatments on the measured variables. Further tests were carried out at a 5% significance level using the Duncan's New Multiple Range test method to distinguish the average effect between treatments or between treatment combinations [13].

Linear formula:

$$Y_{ijkl} = \mu + K_i + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \epsilon_{ijkl}$$

$$i=1, 2, \dots, r \quad j=1, 2, \dots, a \quad k=1, 2, \dots, b \quad l=1, 2, \dots, c.$$

With Y_{ijkl} = the observed value of the 1st group which obtains the j th level from factor A, the k th level from factor B, and the 1st level from factor C.

μ = population mean;

K_i = Additive effect of group i ;

α_j = Additive effect of the j th level of factor A;

β_k = Additive effect of the k -level factor B;

γ_l = Additive effect of the 1st level of factor C;

$(\alpha\beta)_{jk}$ = Effect of the interaction of the j th level of factor A and the k th level of factor B;

$(\alpha\gamma)_{jl}$ = The influence of the interaction of the j th level of factor A and the l th level of factor C;

$(\beta\gamma)_{kl}$ = The interaction effect of the j th level of factor B and the l th level of factor C;

$(\alpha\beta\gamma)_{jkl}$ = Influencer of the interaction of the j th level of factor A, the k th level of factor B, the l th level of factor C;

ϵ_{ijkl} = Random effect of the i th group that gets the j th level of factor A, the k th level of factor B and the 1st level of factor

C;

$$\epsilon_{ijkl} \sim N(0, \sigma^2).$$

3. RESULTS AND DISCUSSION

3.1 Result of the ANOVA test

Based on the results of the ANOVA test at the 5% significant level, it showed that the AMF treatment and soil ameliorant material had a very significant difference in effect on several variables measured in the test plants (Table 1). AMF and ameliorant materials and their interactions on the test plant (*A. macrophyllus*) on indicated significant effect on the several growth parameters measured. The interaction of the three treatment factors of AMF, phosphate and CLW has significantly increased the diameter growth of *A. macrophyllus* seedlings grown on post-mining soil media. The interaction of AMF treatment with phosphate has significantly increased the growth of seedling height, the percentage of AMF colonization. The interaction of phosphate treatment with CLW has significantly increased the growth of seedling height and seedling diameter. While the local AMF single treatment was effective in increasing the growth of seedling height, seedling diameter, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, total dry weight. The effect of single factor phosphate and single factor CLW from car castings on the growth of *A. macrophyllus* plants showed no significant effect on all measured growth parameters.

Table 1. The results of the ANOVA test of the effects of AMF and soil ameliorant materials on several observed variables in the test plant (*A. macrophyllus*)

Variable	F-count						
	M	P	L	M*P	M*L	P*L	M*P*L
Height Increase	22.35**	2.54tn	0.61tn	5.32**	1.56tn	5.12*	0.51tn
Diameter Increase	13.74**	2.12tn	1.51tn	2.90tn	2.71tn	7.05**	3.83*
FW.Root	7.57**	0.02tn	0.91tn	0.16tn	0.17tn	0.57tn	1.28tn
FW.Shoots	32.01**	1.88tn	2.25tn	1.25tn	1.36tn	1.56tn	0.01tn
DW.Root	7.30**	0.02tn	0.19tn	0.70tn	0.09tn	0.6tn	2.02tn
DW.Shoots	28.93**	0.83tn	2.58tn	0.48tn	1.11tn	0.72tn	0.10tn
DW.Total	24.00**	0.34tn	1.75tn	0.26tn	0.76tn	0.07tn	0.67tn
AMF Colonization	59.08**	1.49tn	1.81tn	3.97*	0.15tn	0.08tn	1.43tn

Note: tn=Not significantly different ($P > 0.05$) M=Arbuscular Mycorrhizal Fungi (AMF); * =Significantly different ($P < 0.05$) P=Phosphate; ** =Very significant difference ($P < 0.01$) L=Washing waste of the remaining cement from castings.

3.2 Seedling diameter increase

The interaction of AMF, phosphate and CLW treatment that gave the highest increase in seedling diameter was the $M_2P_1L_0$ treatment with an addition percentage of 82.35% when compared to the control. There is a synergism between the AMF factor (M_2) and phosphate to increase the seedling diameter, meaning that the diameter of the seedling is getting bigger with the interaction of these two factors. The average increase in diameter of the highest *A. macrophyllus* seedlings was 2.79 mm. The average value of the growth in diameter of the seedlings was lowest in the $M_0P_0L_0$ treatment, which was 1.53 mm (Figure 1). Duncan's test on AMF inoculation on *A. macrophyllus* test plants showed that the M_2 type of AMF treatment had a significant effect on the M_0 treatment but was no significantly different from the M_1 treatment. In addition, the average increase in diameter of *A. macrophyllus* seedlings was highest in the M_2 treatment with 2.67 mm (Figure 2).

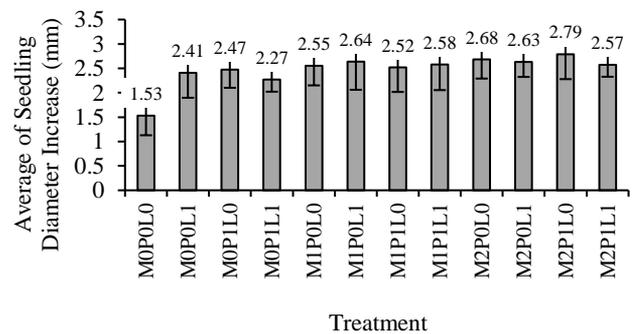


Figure 1. The interaction effect of AMF, phosphate and CLW treatment on the average increase in diameter of *A. macrophyllus* seedlings aged 6 months

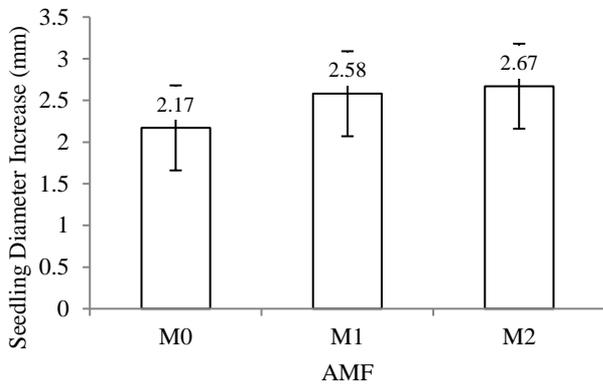


Figure 2. Effect of AMF inoculation treatment on the average increase in diameter of *A. macrophyllus* seedlings aged 6 months

The diameter of the seedlings showed that the interaction of AMF, phosphate, and CLW treatment, and the interaction of phosphate treatment with CLW were indicated significant effect on the increase in diameter of *A. macrophyllus* seedlings at the 5% significant level of ANOVA test. In addition, single factor AMF inoculation showed significant effect on the growth of *A. macrophyllus* seedlings, while single factor phosphate and CLW treatments showed significant effect.

The results of Duncan's test of the interaction of AMF, phosphate, and CLW treatment which gave the highest increase in seedling diameter was the $M_2P_1L_0$ treatment with an additional percentage of 82.35% when compared to the control ($M_0P_0L_0$). The interaction of phosphate and CLW treatment that gave the highest increase in seedling diameter was the P_1L_1 treatment, with a percentage of 53.77% when compared to the control. In comparison, the single factor AMF treatment that gave the highest increase in seedling diameter was the M_2 treatment, with a percentage of 23.04% when compared to the control (M_0). The highest mean seedling diameter of *A. macrophyllus* was 2.79 mm (Figure 1).

The increase in plant diameter is strongly influenced by photosynthesis and the availability of H_2O [14]. AMF can help increase the availability of H_2O with the help of external hyphae that can enter into soil cavities whose diameter is smaller than the diameter of the roots [15]. The increase in plant diameter is a secondary growth strongly influenced by nitrogen [16]; with the presence of AMF, the N content increases and ultimately plays a role in the increase in diameter. N uptake was more significant in mycorrhizal plants than in non-AMF plants. This condition is an evident from the tissue analysis results conducted on *A. macrophyllus* plants.

AMF is one of the microbes capable of producing tryptophan. Tryptophan as a raw material for auxin [16]. Besides the auxin hormone, FMA can also produce other hormones in the form of gibberilin. Gibberilin is a hormone that can work synergistically with auxin in the cambium growth process in plants [17]. According to the document [18], many microbes in the rhizosphere can produce complex organic mixtures such as gibberellins. The cooperation of auxin and gibberellin hormone finally resulted in better stem diameter. The results of other studies have shown that AMF can increase plant growth on disturbed land [19]. For example, AMF inoculation on *Albizia saman* and *Paraserianthes falcataria* seedlings planted on post-coal mining for seven months showed that AMF could increase stem diameter, P and N

content in shoots, shoot dry weight, and high rate survival when compared to control [19].

3.3 The interaction effect of AMF treatment with phosphate

The interaction effect of AMF treatment with phosphate on the test plant *A. macrophyllus*, based on Duncan's test results showed that it had a significant effect on several growth parameters measured, namely the increase in seedling height, percentage of AMF colonization, N-total nutrient uptake and P-total nutrient uptake. The interaction of AMF treatment with phosphate which gave the highest increase in seedling height was the M_2P_1 treatment which was 18.22 cm with an addition percentage of 66.84% when compared to the control (Figure 3). The results showed that there was a synergism between the AMF factor (M_2) and phosphate on the increase in seedling height, meaning that the seedlings would grow more optimally if the two factors were given simultaneously.

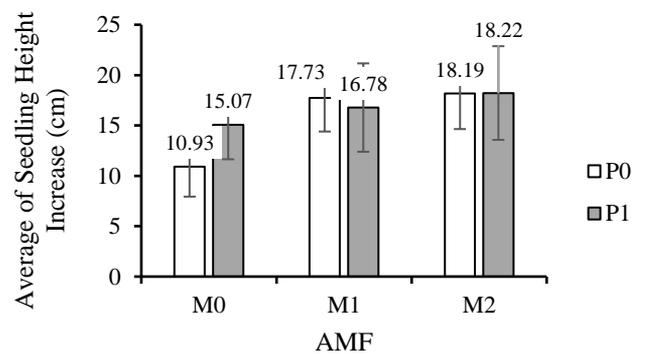


Figure 3. The interaction effect of AMF treatment with phosphate on the average height increase of *A. macrophyllus* seedlings aged 6 months

The interaction of AMF treatment with phosphate which gave the highest percentage of colonization was the M_1P_0 and M_2P_1 treatments, respectively, the percentages of additional colonization were 608.59% and 418.52% when compared to the control (Figure 4). The high percentage of AMF colonization indicated that AMF types M_1 and M_2 were compatible with *A. macrophyllus* plants so that AMF was able to colonize plant roots well.

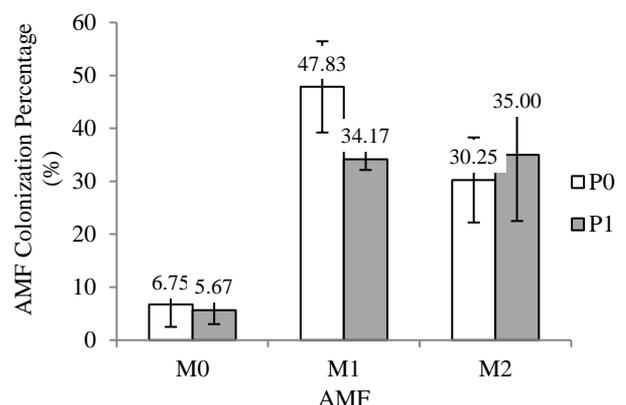


Figure 4. The interaction effect of AMF inoculation treatment with phosphate on the average percentage of AMF colonization of *A. macrophyllus* aged 6 months

3.4 The Interaction effect of phosphate treatment with the provision of cement leaching waste (CLW) from castings

The interaction effect of phosphate treatment with CLW on the test plant *A. macrophyllus*, based on Duncan's test results showed a significant effect on several growth parameters measured, namely the increase in seedling height and increase in seedling diameter. The interaction effect of phosphate treatment with CLW on the test plant *A. macrophyllus* for the parameter of growth in seedling height statistically showed a significantly different effect. The interaction of phosphate treatment with CLW showed a significant difference when compared to the control. The mean value of the highest increase in seedling height was the P₁L₀ treatment, which was 17.72 cm. The average value of the growth in seedling height was lowest in the P₀L₀ treatment, which was 15.11 cm (Figure 5).

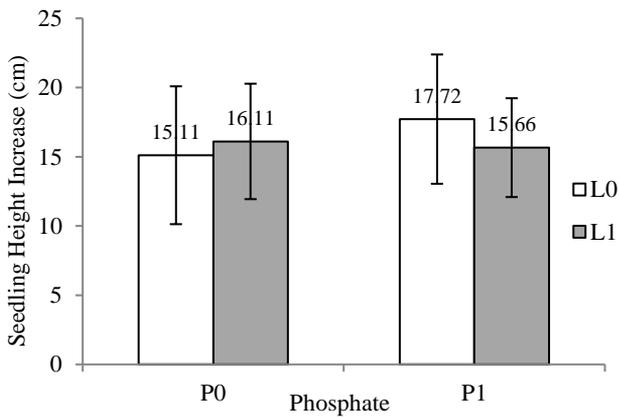


Figure 5. The interaction effect of phosphate inoculation treatment with CLW on the average height increase of *A. macrophyllus* seedlings aged 6 months

The interaction effect of phosphate treatment with CLW on the test plant *A. macrophyllus* for the parameter of increasing seedling diameter statistically showed a significantly different effect. Based on the results of Duncan's test, the interaction of phosphate treatment with CLW showed a significant difference compared to the control. The mean value of the highest seedling diameter increase was found in the P₁L₁ treatment, which was 3.46 mm. The average value of the growth in diameter of the seedlings was the lowest in the P₀L₀ treatment, which was 2.25 mm (Figure 6).

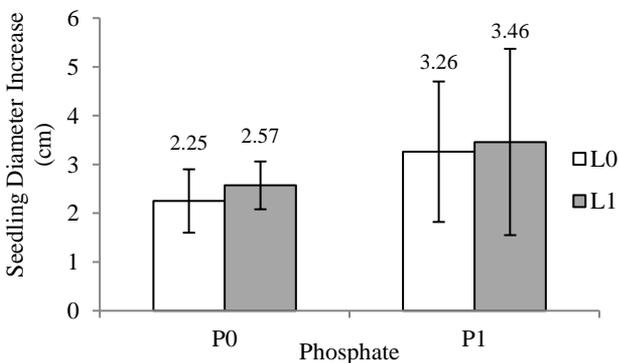


Figure 6. The interaction effect of phosphate inoculation treatment with CLW on the average diameter increase of *A. macrophyllus* seedlings aged 6 months

3.5 Effect of AMF single factor inoculation to seedling height

The ANOVA test results of AMF inoculation on the test plant (*A. macrophyllus*) showed a significant difference result. It is followed by the results of Duncan's test on the effect of single factor AMF treatment on several observation variables of the plant test *A. macrophyllus* showed that the M₂ AMF treatment had a significantly different effect compared to the M₀ treatment (control), but the M₂ AMF treatment showed no significant difference with the M₁ AMF treatment on the average of several variables was measured observations. The M₂ type of AMF treatment gave a significant difference to the M₀ treatment and no significant to the M₁ treatment. The highest mean height increase of *A. macrophyllus* seedlings was found in the M₂ treatment, which was 18.20 cm (Figure 7). Inoculation of AMF single factor can effectively increase the growth of red jaboran (*A. macrophyllus*) seedlings, which are planted in post-mining limestone soil media. There was an interaction between AMF treatment and soil ameliorant to increase several growth parameters of red jaboran (*A. macrophyllus*) seedlings grown on post-mining limestone soil media.

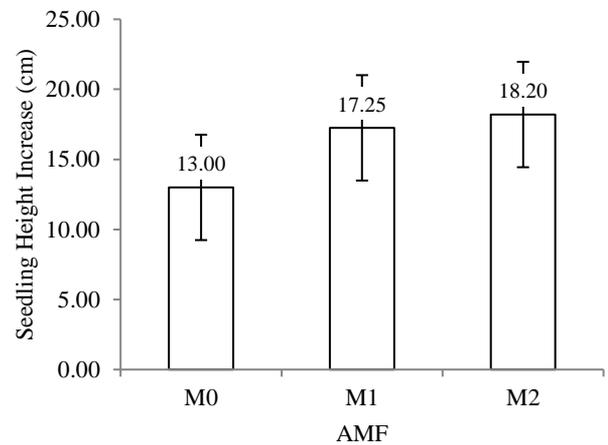


Figure 7. Effect of AMF inoculation treatment on the average height increase of *A. macrophyllus* seedlings aged 6 months

3.6 Root and shoot fresh weight

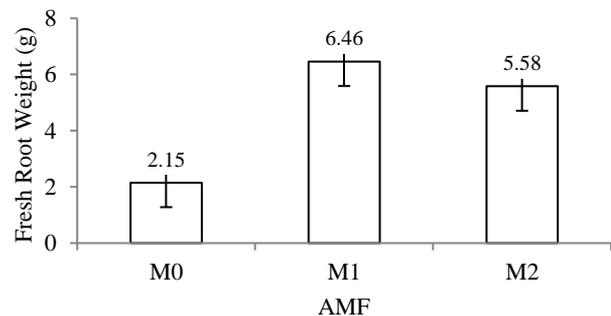


Figure 8. Effect of AMF inoculation treatment on mean fresh weight of roots of *A. macrophyllus* aged 6 months

Fresh root weight is the weight of plant roots when the plant is harvested, separated from the shoots. Based on the results of Duncan's test, the effect of AMF inoculation on the test plant

A. macrophyllum showed that the M₂ type of AMF treatment had a significant effect on the M₀ treatment and no significantly different from the M₁ treatment. The highest mean fresh weight of seedling roots of *A. Macrophyllum* was found in the M₁ treatment, which was 6.46 g (Figure 8).

The fresh weight of the shoot is the fresh weight when the test plant is harvested by separating the base of the stem from the root of the plant. Based on the results of Duncan's test, the effect of AMF inoculation on the test plant *A. Macrophyllum* that the M₂ type of AMF treatment had a significant effect on the M₀ treatment and no significantly different on the M₁ treatment. In addition, the highest mean fresh weight of *A. Macrophyllum* seedlings was found in the M₂ treatment, which was 29.13 g (Figure 9). Based on the ANOVA test at the 5% significance level, the parameters of fresh weight of roots and fresh weight of shoots showed that the interaction of AMF, phosphate, and CLW treatments showed no significant effect. Meanwhile, single factor inoculation of *A. macrophyllum* had a significant effect on fresh root weight and shoot fresh weight of *A. macrophyllum* seedlings, and single factor inoculation of phosphate and CLW had no significant effect on fresh root weight and shoot fresh weight of *A. macrophyllum*. The results of Duncan's test with AMF single treatment that gave the highest fresh weight of seedling roots was treatment M₁ with a percentage increase of 200.46% when compared to control (M₀), and the highest fresh weight value of seedling shoots was treatment M₂ with a percentage increase of 309.12% when compared to control (M₀). As a result, the highest mean fresh root weight of *A. macrophyllum* was 6.46 g, and the highest average shoot fresh weight was 6.46 g.

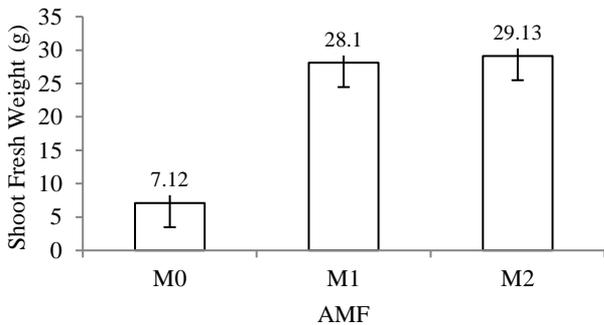


Figure 9. Effect of AMF inoculation treatment on the average fresh weight of shoots of *A. macrophyllum* seedlings aged 6 months

The fresh weight of a plant can characterize the amount of H₂O in plant tissue. Plants with a high fresh weight usually correlate with the H₂O content in them. Water (H₂O) for plants is a vital requirement in the growth process, especially in photosynthesis. The study results show that plants that received AMF treatment had a higher mean value of fresh root weight and shoot fresh weight when compared to treatment without AMF (control). AMF can also increase plant resistance to drought. Another study showed that AMF species *Glomus* sp. and *Gigaspora* sp. influenced the shoot-root ratio of *S. saman* seedlings [20].

3.7 Root and shoot dry weight

Root and shoot dry weight are the weight of plant when the plant is oven-baked at 70°C for 48 hours. Based on the results of Duncan's test, the effect of AMF inoculation on the test

plant *A. macrophyllum* that the M₂ type of AMF treatment had a significant effect on the M₀ treatment and no significantly different on the M₁ treatment. The highest mean root dry weight of *A. macrophyllum* was found in the M₁ treatment, which was 1.77 g (Figure 10).

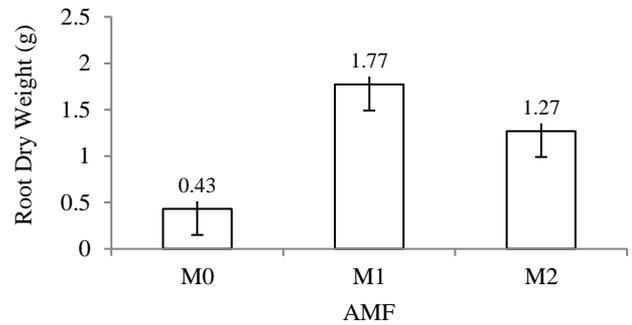


Figure 10. Effect of AMF inoculation treatment on the average dry weight of seedling roots of *A. macrophyllum* aged 6 months

Based on the results of Duncan's test, the effect of AMF inoculation on the test plant *A. macrophyllum* that the M₁ type of AMF treatment had a significant effect on the M₀ treatment and no significantly different on the M₂ treatment. In addition, the highest dry weight of *A. macrophyllum* seedlings was found in the M₁ treatment, which was 5.14 g (Figure 11).

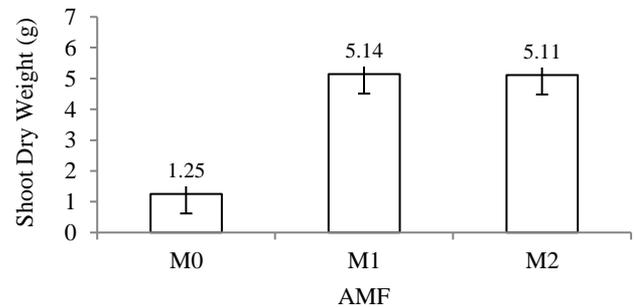


Figure 11. Effect of AMF inoculation treatment on the average dry weight of shoots of *A. macrophyllum* seedlings aged 6 months

The total dry weight includes all plant material derived from photosynthesis and nutrient uptake [11]. In addition, the dry weight is also an integration of almost all events experienced by plants, so this parameter is perhaps the most representative growth indicator.

The ANOVA test at the 5% significance level for the dry weight parameters of plants showed that the interaction of AMF, phosphate, and CLW treatments had no significant effect. Meanwhile, single factor AMF inoculation significantly affected the dry weight of *A. macrophyllum* plants. Meanwhile, single factor inoculation of phosphate and CLW had not significantly affect the dry weight of *A. macrophyllum* plants. Based on Duncan's test results, the single treatment of AMF, which gave the highest plant dry weight, was treatment M₁ with a percentage increase of 311.31% compared to the control (M₀). As a result, the highest mean dry weight of *A. macrophyllum* plants was 6.91 g.

Based on the analysis results, it shows that AMF inoculation

was able to produce a total dry weight that was greater than the control. This condition can happen because, according to Ortaş [21], AMF inoculation can increase magnesium uptake. Magnesium is the central chlorophyll molecule required for photosynthesis and is an activator of enzymes in photosynthesis and respiration [22, 23]. Thus, when magnesium levels increase, the essential processes that are affected will also increase so that plants can grow better than plants without AMF. In addition, the rate of photosynthesis produced will increase so that it plays a role in increasing plant dry weight. Another study also showed that local AMF was significant in triggering the initial growth and biomass of *A. saponaria* plants aged seven months after planting in a greenhouse [24]. Furthermore, another study said that using 5 g AMF and P fertilizer at a dose of 0.6 g on neem and suren seedlings aged five months gave the best results in increasing plant dry weight and could increase root colonization [14].

Besides being caused by an increase in photosynthate, a large dry weight was also caused by an increase in nutrient uptake. The presence of external AMF hyphae that extensively enter the soil volume affects the absorption of nutrients and water. In addition, external hyphae of AMF scattered in the soil can function as root hairs [25], thereby increasing the absorption surface of the roots. Curl and Truelove 2012 stated that increased absorption capacity affected plant growth.

The presence of AMF can increase the dry weight of *A. macrophyllus* test plants directly through the transformation of carbon sources from AMF hyphae, thereby changing the pH in the rhizosphere. Changes in pH, in turn, will change the quantity and quality of other microbes that play a role in plant growth [26] and are indirectly mediated by host plant growth, root exudation, and changes in soil structure [26]. Curl and Truelove [27] stated that microorganisms on the root surface and root hairs could affect the availability and absorption of ions such as zinc, calcium, rubidium, and other ions. The process is strongly influenced by pH. Each type of AMF has functional differences and capacities in mobilizing nutrients in the soil.

Meanwhile, the total dry weight of the plant was obtained from the combination of the dry weight of the shoot and the dry weight of the root of the test plant. Based on the results of Duncan's test, the effect of AMF inoculation on the test plant *A. macrophyllus* that the M₁ type of AMF treatment had a very significant effect on the M₀ treatment and not significantly different on the M₂ treatment. In addition, the highest mean dry weight of *A. macrophyllus* seedlings was found in the M₁ treatment, which was 6.91 g (Figure 12).

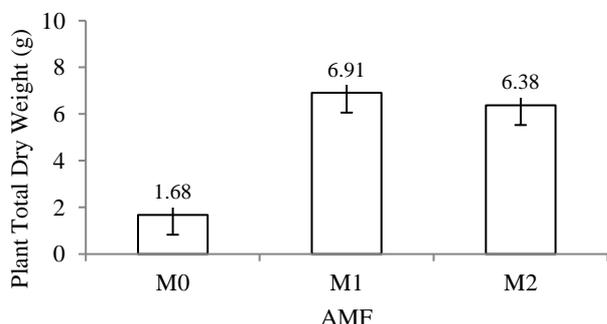


Figure 12. Effect of AMF inoculation treatment on the average total dry weight of *A. macrophyllus* seedlings aged 6 months

3.8 AMF colonization percentage

AMF inoculation significantly affected the parameters of the percentage colonization on the roots of the test plants. The roots of the test plants were declared infected by Arbuscular Mycorrhizal Fungi (AMF); if the roots found the presence of vesicles, arbuscules, and hyphae or one of them, then the percentage was calculated. Based on the results of Duncan's test, the effect of AMF inoculation on the percentage of AMF colonization on the test plant *A. macrophyllus*, M₁, and M₂ treatments showed a very significant effect on M₀ treatment. The mean value of the highest AMF colonization percentage was seen in the M₁ treatment, which was 41.00% (Figure 13).

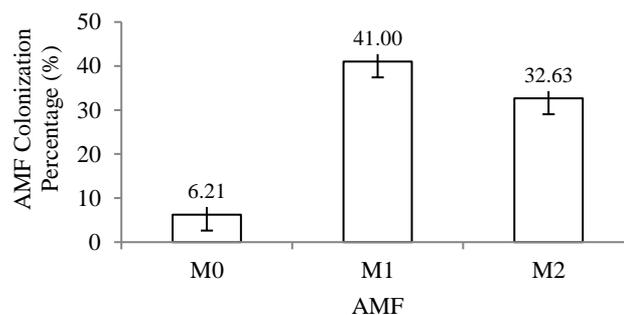


Figure 13. Effect of AMF inoculation treatment on the average percentage of mycorrhizal colonization of *A. macrophyllus* seedlings aged 6 months

Based on the ANOVA test at the 5% significance level, the percentage of AMF colonization parameters showed that the interaction between AMF and phosphate treatments had a significant effect, while the interactions between phosphate and CLW treatments showed no significant effect. AMF single factor inoculation significantly affected the percentage of AMF colonization in *A. macrophyllus*, while single factor phosphate and CLW treatments had no significant effect.

The results of Duncan's test of the interaction of AMF treatment with phosphate, which gave the highest percentage of AMF colonization, was the M₁P₀ treatment with an additional percentage of 608.59% when compared to the control (M₀P₀). While the single treatment of AMF gave the highest percentage of AMF colonization was treatment M₁ with a percentage of 560.23% compared to the control (M₀). The highest mean of AMF colonization in *A. macrophyllus* was 47.83%.

Based on the Pearson correlation analysis results at the 1% level for various parameters of *A. macrophyllus* growth observations, there is a positive and significant relationship/correlation between one observation variable and another. The percentage of AMF colonization had a significant positive correlation with the growth parameters of seedling height ($r=0.005$), seedling diameter ($r=0.014$), fresh root weight ($r=0.000$), shoot fresh weight ($r=0.000$), root dry weight ($r=0.001$), shoot dry weight ($r=0.000$), total dry weight ($r=0.000$), nutrient uptake N ($r=0.000$), and nutrient uptake P ($r=0.000$) (Table 1). Furthermore, the xylem proportion parameter negatively correlates significantly with the phloem, cambium, and pith proportions.

From the results of the study, it is known that AMF can be associated with *A. macrophyllus* plants. The association begins when the AMF propagules respond to roots in the soil. Penetration occurs when the AMF hyphae form an

appressorium attached to the root epidermal cells. The hyphae then spread in the intercellular space and enter the cell [10]. According to the study of Suharno and Sancayaningsih [28], the factors influencing AMF infection are host sensitivity to infection, climatic factors (light), and soil water content. Hyphae that penetrated the roots came from propagules (AMF infected roots, pieces of hyphae, vesicles, spores) germinating. According to Johansson et al. [26] and Molina et al. [29], the compatibility phenomenon between the host plant and AMF was found at the ultra-structural, biochemical, and genetic levels. Therefore, the compatibility between the host plant and AMF will result in better host plant growth.

Prayudyarningsih [30] stated that AMF hyphae that infect plant roots can increase the absorption capacity of phosphate, nitrogen, sulfur, zinc, and other essential elements. According to Puspitasari et al. [31], the number of spores is not only influenced by one factor but by the accumulation of several factors, including; The AMF itself, host vegetation, and soil chemical conditions such as pH, organic C, and P are available. Soil characteristics are one factor affecting the population of AMF in the soil [32].

Permanasari et al. [33] explained that the number of spores is one-factor affecting AMF colonies on plant roots. The higher the number of AMF spores, the higher the level of AMF colonies on plant roots in the soil. This is because AMF colonization in plant roots will induce root hypertrophy, resulting in faster root hair growth stimulation. Plant roots with a high percentage of root infection will secrete more rhizokalin hormone so that the surface area and volume of the root become larger [34], which can help nutrient absorption and increase plant growth.

Besides being influenced by the number of spores, AMF colonization is also influenced by the host plant's roots [35]. Spores are reproductive organs of AMF, formed from extraradical hyphae that have the form of stumps or colonies (sporocarps) [36]. Meanwhile, the arbuscular structures in the root tissue can only survive for a short period, so their presence is difficult to find. Arbuscular is unstable and can only survive for two weeks after colonization [37].

Based on the analysis results, the soil media used in this study was classified as poor in nutrients with a deficient level of fertility. The presence of AMF treatment and the application of natural phosphate and CLW to the test plants of *A. macrophyllum* could produce better growth when compared to the control (Figure 14). The high percentage colonization in *A. macrophyllum* has a prominent role in helping the absorption of essential nutrients such as N and P.

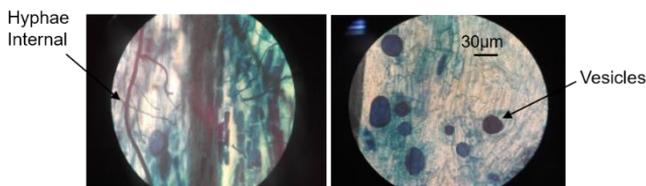


Figure 14. The structure of AMF colonization on the roots of *A. macrophyllum* seedlings aged 6 months after planting in the nursery (internal hyphae, vesicles)

García-Garrido and Ocampo [38] described that the interaction of AMF and host plants involves the recognition of specific molecular signals in order to create a symbiotic relationship of mutualism. The correlation can be seen from the host plant obtaining nutrients and water from AMF and

vice versa, with AMF obtaining carbon from photosynthesis from the host [4]. According to study of Aye [10], the benefits of AMF in ecosystems are significant, namely playing a role in the nutrient cycle and converting nutrients, so they are not lost from the ecosystem due to leaching, improving soil structure, and distributing carbohydrates from plant roots to other soil organisms.

AMF can liberate P that is not available to plants to be available by releasing phosphatase enzymes and organic acids, especially oxalic, which can help liberate phosphate. This role is significant considering that most of the soil in Indonesia has many problems, especially on post-mining land that is poor in nutrients. Phosphorus is an essential part of many phosphate sugars that play a role in nucleotides, such as RNA and DNA, and part of phospholipids in membranes. Phosphorus plays an essential role in energy metabolism due to its presence in ATP, ADP, AMP, and pyrophosphate (Ppi) [11]. In addition, carbon is needed by AMF as an energy source, and nitrogen is needed to form proteins. AMF also play a role in the stability of soil aggregates. Organic matter produced by microorganisms CLW bind soil particles together, while AMF hyphae provide mechanical support.

Therefore, stable soil aggregates can create good soil porosity. The stability of soil aggregates is essential in plant growth because the movement of air, water, and energy transfer are interrelated with soil porosity [39]. Therefore, plants can grow better if the movement of air, water, and energy transfer in the soil runs smoothly. AMF can increase plant resistance to outside soil pathogens. AMF can also help plant growth on soils contaminated with heavy metals [40], such as on post-mining lands. Thus, AMF, besides being beneficial for bio-protection, also functions as an essential bio-remediator for soils contaminated with heavy metals [40]. AMF colonization in plant roots can be observed from several AMF structures, such as internal hyphae, arbuscules, and vesicles (Figure 14). According to Yang et al. [4], internal hyphae function as a means of translocation of nutrients, external functions to absorb nutrients and water, vesicles serve as food reserves, especially lipids, while the arbuscular is a fundamental infection structure in AMF symbiosis because the arbuscular function in the process of nutrient transfer between the two symbionts (fungi with plant roots).

The percentage of AMF colonization in the roots of host plants plays a vital role in a nutrient transfer that is absorbed by external hyphae from the rhizosphere. Although external hyphae have a high ability to absorb nutrients and water, if they fail in the transfer process into root cells, nutrients and water will only be stored in the internal hyphae. Thus, the greater the percentage of AMF colonization, the surface of the contact area of AMF and root cells was more significant so that plants successfully absorbed nutrients and water. AMF is very much needed by *A. macrophyllum*, as evidenced by the high RMD value in the study. The external hyphae of AMF can explore and absorb water, and nutrients plants need [4, 41]. The effectiveness of local AMF in increasing the growth of *A. macrophyllum* seedlings in post-mining soil media was possible because of several things:

1. local AMF was compatible with existing conditions,
2. Local AMF was compatible with root exudates produced by *A. macrophyllum* roots and genotypically.

In addition, local AMFs can absorb water and nutrients from their host plants [26].

3.9 Seedling height growth

Based on the ANOVA test at the 5% significance level, the parameters for seedling height growth showed that the interaction between AMF and phosphate treatment and the interaction of phosphate treatment with CLW were significantly affected the growth of *A. macrophyllum* seedlings. Single-factor AMF inoculation was significantly affected the growth of *A. macrophyllum* seedlings, while single-factor phosphate and CLW treatments had no significant effect.

The results of Duncan's test of the interaction of AMF treatment with phosphate, which gave the highest growth in seedling height, was the M_2P_1 treatment with an additional percentage of 66.84% compared to the control (M_0P_0). The interaction of phosphate and CLW treatment that gave the highest growth in seedling height was the P_1L_0 treatment, with a percentage of 17.27% when compared to the control. While the single factor AMF treatment gave the highest increase in seedling height, the M_2 treatment with a percentage of 40.0% compared to the control (M_0) with the highest mean growth of *A. macrophyllum* seedlings at 18.20 cm (Figure 15).



Figure 15. Growth performance of *A. macrophyllum* seedlings on the interaction of AMF treatment, phosphate and the provision of cement leaching waste (CLW) left over from car castings for 6 months of observation

The increase in plant height is influenced by the activity of several hormones, such as gibberellins and cytokinins [15]. According to Asrul [42], AMF in plant roots can produce many growth-regulating hormones, such as gibberellins and cytokinins, so the presence of AMF in plant roots can play a role in the process of stem elongation. [18] also reported that many microbes in the rhizosphere could produce complex organic mixtures such as gibberellins that can change plant morphology and physiology. Thus, the presence of AMF can increase the average increase in plant height better than the control. The results showed that the test plants inoculated with

AMF with phosphate had better height growth than other treatments.

This condition is due to the role of AMF in plants in terms of helping to increase P uptake [25, 43] due to the presence of external hyphae that have a long reach and spread widely into tiny soil pores (Read and head 1980) so that P initially unavailable becomes available to plants. Besides P, the presence of AMF can also help increase calcium absorption [21], which plays a role in cell division and elongation [23]. Upward or tall growth is primary growth due to meristem activity at the tip of the stem [44]. The activity of the meristem, which always divides, is strongly influenced by phosphorus. Phosphorus is an essential part of many phosphate sugars that play a role in nucleotides, such as RNA and DNA, and part of phospholipids in membranes. Phosphorus also plays an essential role in energy metabolism because of its presence in ATP, ADP, AMP, and pyrophosphate (Ppi) [11]. Thus, if the P level increases, the meristem activity at the tip of the stem also increases so that the stem increases in height. AMF can increase the plant growth of many species found in tropical forests, and the survival/growth value of plants colonized by AMF is higher than without colonization [45].

The results of other studies also showed that inoculation of AMF inoculum on *Albizia saponaria* plants had a better effect on increasing plant height, stem diameter, number of leaves, shoot dry weight, root dry weight, total dry weight, root shoot ratio, number of nodules, and percentage of root colonization. And the relative dependence of mycorrhizae [46]. Other studies also showed that AMF species of *Glomus* sp and *Gigaspora* sp influenced the increasing growth height and diameter of *Falcataria moluccana* seedlings [20]. Another study stated that AMF inoculation at a dose of 20 g/plant on Ambon Jati seedlings could provide good growth [47]. Arbuscular Mycorrhizal Fungi inoculation resulted in better plant vegetative growth than plants without AMF [48]. According to Hadianur et al. [49], the application of AMF to plants can increase the uptake of N, P, and K nutrients so that AMF can increase plant growth. Trisilawati et al. [50] stated that good plant growth in mycorrhizal plants was caused by mycorrhizal growth that could expand the volume of root distribution in the soil so that nutrients were more available to plants.

From the results of the study, it can be seen that the single treatment of AMF was able to have a very significant effect on *A. macrophyllum* seedlings for the parameters of height gain, diameter increase, fresh root weight, shoot fresh weight, root dry weight, average shoot dry weight, total dry weight and mean value. AMF colonization percentage. The interaction of two AMF and phosphate treatment factors was able to provide a very significant and significant increase in seedling height to the percentage of AMF colonization. The interaction of the two phosphate treatment factors and CLW gave a significant increase in seedling diameter to the increase in seedling height. The interaction of the three treatment factors significantly affects the increase in diameter. Meanwhile, every single factor of phosphate and CLW did not significantly affect all growth parameters.

In line with the research results conducted by Vivas et al. [41]; Smith and Read [5] said that the presence of significant AMF external hyphae could explore and absorb water and nutrients plants need. The effectiveness of AMF in increasing the growth of *A. macrophyllum* seedlings on post-mining soil media is possible due to several reasons, namely: 1) AMF matches the existing conditions, 2) AMF matches the root

exudate produced by *A. Macrophyllus* roots and 3) genotypically, local AMF can absorb water and nutrients to its host plants, thereby increasing the effectiveness of plant growth [51].

4. CONCLUSIONS

Arbuscular Mycorrhizal Fungi (AMF) and soil ameliorants were effective in increasing the growth of *A. macrophyllus* seedlings grown on PT. Holcim Indonesia Tbk. A single treatment of AMF was able to provide very significant growth in *A. macrophyllus* seedlings for the parameters of height gain, diameter increase, fresh root weight, shoot fresh weight, root dry weight, average shoot dry weight, total dry weight and the average percentage of AMF colonization. The interaction of the two M₂P₁ treatment factors was able to provide the highest increase in seedling height, namely 18.22 cm, with an additional percentage of 66.84% when compared to the control. The interaction of the two treatment factors (M₁P₀ and M₂P₁) was able to provide the highest colonization percentage values, namely 608.59% and 418.52%, respectively, when compared to the control. The interaction of the three treatment factors, M₂P₁L₀, was able to provide the highest increase in seedling diameter with an additional percentage of 82.35% when compared to the control.

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AUTHOR CONTRIBUTIONS

All authors as the main contributors of this reasearch article. Ceng Asmarahman (C.A) and Hendra Prasetya (H.P) contribute to drafting and reviewing the original article as main contributor. Dian Iswandaru (D.I) and Indra Gumay Febryano (I.G.F) contribute to reviewing the draft article.

REFERENCES

- [1] Bramasto, Y., Sudrajat, D.J., Rustam, E.Y. (2015). Keragaman morfologi tanaman jabon merah (*anthocephalus macrophyllus*) dan jabon putih (*anthocephalus cadamba*) berdasarkan dimensi buah, benih dan daun. In Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia, 1(6): 1278-1283. <https://doi.org/10.13057/psnmbi/m010603>
- [2] Ardhiyansyah, N., Sumarsono, S., Purbajanti, E.D. (2014). Pertumbuhan beberapa jenis leguminosa pakan yang ditanam pada media tanah bekas penambangan batubara dengan perbaikan bahan organik. Jurnal Litbang Provinsi Jawa Tengah, 12(1): 43-54. <https://doi.org/10.36762/jurnaljateng.v12i1.334>
- [3] Amarahman, C., Budi, S.W., Wahyudi, I., Santoso, E. (2018). Identifikasi mikroba potensial fungi mikoriza arbuskula (FMA) pada lahan pascatambang PT. Holcim Indonesia Tbk. Cibinong, Bogor, Jawa Barat. Jurnal Pengelolaan Sumberdaya Alam dan Lingkungan, 8(3): 279-285. <https://doi.org/10.29244/jpsl.8.3.279-285>
- [4] Yang, A.N., Lu, L., Zhang, N. (2011). The diversity of arbuscular mycorrhizal fungi in the subtropical forest of Huangshan (Yellow Mountain), East-Central China. World Journal of Microbiology and Biotechnology, 27: 2351-2358. <https://doi.org/10.1007/s11274-011-0702-x>
- [5] Smith, S.E., Read, D.J. (1997). Mycorrhizal symbiosis. Second edition. San Diego (US): Academic Press. <https://doi.org/10.1016/b978-0-12-652840-4.x5000-1>
- [6] Van Bruggen, A.H.C., Semenov, A.M. (2000). In search of biological indicators for soil health and disease supression. Applied Soil Ecology, 15(1): 13-24. [https://doi.org/10.1016/s0929-1393\(00\)00068-8](https://doi.org/10.1016/s0929-1393(00)00068-8)
- [7] Golledge, R.G., Jacobson, R.D., Kitchin, R., Blades, M. (2000). Cognitive maps, spatial abilities, and human wayfinding. Geographical Review of Japan, Series B., 73(2): 93-104. <https://doi.org/10.4157/grj1984b.73.93>
- [8] Maiti, D., Toppo, N.N., Variar, M. (2011). Integration of crop rotation and arbuscular mycorrhiza (AM) inoculum application for enhancing AM activity to improve phosphorus nutrition and yield of upland rice (*Oryza sativa* L.). Mycorrhiza, 21: 659-667. <https://doi.org/10.1007/s00572-011-0376-0>
- [9] Jones Jr, J.B. (2022). Agronomic handbook: management of crops, Soils and their fertility. 1st edition. CRC Press, 450 (ISBN 9780429120534). <https://doi.org/10.1201/9781420041507>
- [10] Aye, N.S., Butterly, C.R., Sale, P.W.G., Tang, C.X. (2017). Residue addition and liming history interactively enhance mineralization of native organic carbon in acid soils. Biology and Fertility of Soils, 53: 61-75. <https://doi.org/10.1007/s00374-016-1156-y>
- [11] Brundrett, M., Bougher, N., Dell, B., Grove, T., Majalaczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agriculture Research. <https://doi.org/10.13140/2.1.4880.5444>
- [12] Salisbury, F.B., Ross, C.W. (1995). Fisiologi Tumbuhan. Jilid I. Bandung (ID): ITB. 343 pp.
- [13] Gomez, A.A., Gomez, K.A. (1995). Prosedur statistik untuk penelitian pertanian. Universitas Indonesia Press, Jakarta.
- [14] Kozlowski, T.T. (1969). Tree physiology and forest pests. Journal of Forestry, 67(2): 118-123. <https://doi.org/10.1093/jof/67.2.118>
- [15] Kurniaty, R., Damayanti, R.U. (2011). Penggunaan mikoriza dan pupuk p dalam pertumbuhan bibit mimba dan suren umur 5 bulan. Jurnal Penelitian Hutan Tanaman, 8(4): 207-214. <https://doi.org/10.20886/jpht.2011.8.4.207-214>
- [16] Goldsworthy, P.R., Fisher, N.M. (1992). Fisiologi tanaman budidaya tropik. Universitas Gadjah Mada Press. Yogyakarta.
- [17] Gardner, F.P., Pearce, R.B., Mitchell, R.L. (1991). Fisiologi Tanaman Budidaya. Jakarta (ID): UI Press.
- [18] Klein, D.A. (2000). The Rhizosphere. In: Lederberg J. (ed) Encyclopedia of Microbiology. Second Edition. Vol. IV. USA: Academic Press. 177pp.
- [19] Wulandari, D., Saridi, Cheng, W.G., Tawaraya, K. (2016). Arbuscular mycorrhizal fungal inoculation improves Albizia saman and Paraserianthes falcataria growth in post-opentcast coal mine field in East Kalimantan, Indonesia. Forest Ecology and Management, 376: 67-73. <https://doi.org/10.1016/j.foreco.2016.06.008>

- [20] Budi, S.W., Kemala, I.F., Turjaman, M. (2013). Pemanfaatan fungi mikoriza arbuskula (FMA) dan arang tempurung kelapa untuk meningkatkan pertumbuhan semai *Falcataria moluccana* (Miq) Barneby & JW Grimes dan *Samanea saman* (Jacq) Merr. *Jurnal Silviculture Tropika*, 4(1): 11-18.
- [21] Ortaş, İ. (2019). Effect of mycorrhizal inoculation on citrus seedling growth and nutrient uptake. *ISHS Acta Horticulturæ* 1253: 77-84. <https://doi.org/10.17660/actahortic.2019.1253.11>
- [22] Bonner, J. (1952). Principles of plant physiology. *AIBS Bulletin*, 2(2): 14-15. <https://doi.org/10.1093/aibsbulletin/2.2.14-j>
- [23] Gardner, F.P., Pearce, R.B., Mitchell, R.L. (1991). *Fisiologi Tanaman Budidaya*. Jakarta (ID): UI Press.
- [24] Tuheteru, F.D., Husna, H. (2011). Pertumbuhan dan biomassa *Albizia saponaria* yang diinokulasi Fungi Arbuskula Mikoriza Lokal Sulawesi Tenggara. *Jurnal Silviculture Tropika*, 2(3): 143-148. <https://doi.org/10.29244/j-siltrop.2.3.%25p>
- [25] Barber, S.A. (1995). Soil nutrient bioavailability: a mechanistic approach. Canada (CA): John Wiley and Sons, Inc.
- [26] Johanson, J.F., Paul, L.R., Finlay, R.D. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology*, 48(1): 1-13. <https://doi.org/10.1016/j.femsec.2003.11.012>
- [27] Curl, E.A., Truelove, B. (2012). *The Rhizosphere* (Vol.15). Springer Science & Business Media.
- [28] Suharno, S., Sancayaningsih, R.P. (2013). Fungi mikoriza arbuskula: potensi teknologi mikorizoremediasi logam berat dalam rehabilitasi lahan tambang. *Asian Journal of Tropical Biotechnology*, 10(1): 23-34. <https://doi.org/10.13057/biotek/c100104>
- [29] Molina, R., Massicotte, H., Trappe, J.M. (1992). Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implication. In: Allen, M.F. (Ed). *Mycorrhizal Functioning: An Integrative Plant-Fungal Process*. New York (US): Chapman & Hall, Inc. 357-432.
- [30] Prayudyaningsih, R. (2014). Pertumbuhan semai *alstonia scholaris*, *acacia auriculiformis* dan *muntingia calabura* yang diinokulasi fungi mikoriza arbuskula pada media tanah bekas tambang kapur. *Jurnal Penelitian Kehutanan Wallacea*, 3(1), 13-23. <https://doi.org/10.18330/jwallacea.2014.vol3iss1pp13-23>
- [31] Puspitasari, D., Purwani, K.I., Muhibbudin, A. (2012). Eksplorasi vesicular arbuscular mycorrhiza (VAM) indigenous pada lahan jagung di Desa Torjun, Sampang Madura. *Jurnal Sains dan Seni ITS*, 1(1): E19-22. <https://doi.org/10.12962/j23373520.v1i1.1119>
- [32] De Beenhouwer, M., Van Geel, M., Ceulemans, T., Muleta, D., Lievens, B., Honnay, O. (2015). Changing soil characteristics alter the arbuscular mycorrhizal fungi communities of *Arabica coffee* (*coffea arabica*) in Ethiopia across a management intensity gradient. *Soil Biology and Biochemistry*, 91: 133-139. <https://doi.org/10.1016/j.soilbio.2015.08.037>
- [33] Permanasari, I., Dewi, K., Irfan, M., Arminudin, A.T. (2016). Peningkatan efisiensi pupuk fosfat melalui aplikasi mikoriza pada kedelai. *Jurnal Agroteknologi*, 6(2): 23-30. <https://doi.org/10.24014/ja.v6i2.2237>
- [34] Suhardjadinata, S., Kurniati, F., Lulu, D.H.N. (2020). Pengaruh inokulasi cendawan mikoriza arbuskular dan pupuk npk terhadap pertumbuhan dan hasil tomat (*Lycopersicon esculentum* mill.). *Media Pertanian*, 5(1). <https://doi.org/10.37058/mp.v5i1.2131>
- [35] Yurisman, B., Burhanuddin, Wahdina. (2015). Asosiasi fungi mikoriza arbuskula (FMA) pada tanaman bintaro (*cerbera manghas* linn.) di tanah aluvial. *Jurnal Hutan Lestari*, 3(4). <http://dx.doi.org/10.26418/jhl.v3i4.13077>
- [36] Purwati, B., Budi, S.W., Wasis, B. (2019). Status fungi mikoriza arbuskula (FMA) pada rizosfer jernang (*Daemonorops draco* Blume) di Jambi. *Media Konservasi*, 24(3): 261-268. <https://doi.org/10.29244/medkon.24.3.261-268>
- [37] Dharmaputri, N.W.P., Wijaya, I.N., Adiartayasa, W.A.Y.A. (2016). Identifikasi mikoriza vesikular arbuskular pada rhizosfer tanaman lamtoro (*Leucaena leucocephala*) dan kaliandra (*Calliandra calothyrsus*) Serta perbanyakannya dengan media zeolit. *E-Jurnal Agroekoteknologi Tropika*, 5(2): 171-180.
- [38] García-Garrido, J.M., Ocampo, J.A. (2002). Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. *Journal of Experimental Botany*, 53(373): 1377-1386. <https://doi.org/10.1093/jexbot/53.373.1377>
- [39] Rao, N.S.S. (1994). *Mikroorganisme tanah dan pertumbuhan tanah*. Edisi Kedua. Jakarta: Universitas Indonesia Press.
- [40] Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S. (1993). Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Canadian Journal of Botany*, 71(3): 512-518. <https://doi.org/10.1139/b93-056>
- [41] Vivas, A., Biró, B., Németh, T., Barea, J.M., Azcón, R. (2006). Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. *Soil Biology and Biochemistry*, 38(9): 2694-2704. <https://doi.org/10.1016/j.soilbio.2006.04.020>
- [42] Asrul, A. (2019). Pupuk hayati (Biofertilizer) alternatif substitusi penggunaan pupuk kimiawi. *PARTNER*, 24(1): 888-895. <https://doi.org/10.35726/jp.v24i1.340>
- [43] Agrios, G.N. (2005). *Plant Pathology*. Third edition. San diego, California (US): Academic Press, Inc.
- [44] Darmawan, J., Baharsjah, J.S. (1983). *Dasar-dasar Fisiologi Tanaman*. Semarang (ID): PT. Suryandaru Utama.
- [45] Tawarayana, K., Turjaman, M. (2016). Mycorrhizal fungi in Peatland. *Tropical Peatland Ecosystems*, 237-244. https://doi.org/10.1007/978-4-431-55681-7_15
- [46] Tuheteru, F.D., Husna, H., Alimuddin, L.D. (2011). Respon pertumbuhan dan tingkat ketergantungan *Albizia saponaria* (Lour.) Miq terhadap fungi arbuskula mikoriza lokal Sulawesi tenggara. *Biota*, 16(2): 252-261. <https://doi.org/10.24002/biota.v16i2.107>
- [47] Karepesina, S., Umarella, U., Pattiasina, A. (2010). Pengaruh Inokulasi Fungi Mikoriza Arbuskula dan Bahan Organik Terhadap Pertumbuhan Semai Jati Ambon (*Tectona grandis* Linn f). *Jurnal Agrohut*, 1(1): 16-24. <https://doi.org/10.51135/agh.v1i1.26>
- [48] Kumalawati, Z., Baba, B., Misbahyudi, M. (2015). Analisis efektifitas dua jenis cendawan mikoriza arbuskula terhadap pertumbuhan bibit tebu (*saccharum officinarum* L.). *Agrokompleks*, 14(1): 65-68. <https://doi.org/10.51978/japp.v14i1.194>

- [49] Hadianur, H., Syarifuddin, S., Kesumawati, E. (2017). Pengaruh jenis fungi mikoriza arbuskular terhadap pertumbuhan dan hasil tanaman cabai merah besar (*capsicum annum l.*). *Jurnal Agrotek Lestari*, 3(1): 30-38. <https://doi.org/10.35308/jal.v3i1.293>
- [50] Trisilawati, O., Towaha, J., Daras, U. (2012). Pengaruh mikoriza dan pupuk NPK terhadap pertumbuhan dan produksi jambu mete muda. *Jurnal Tanaman Industri dan Penyegar*, 3(1): 91-98.
- [51] Johnson, N.C., Graham, J.H., Smith, F.A. (1997). Fuctioning of mycorrhizal associations along the mutualism-parasitism continuum. *The New Phytologist*, 135(4): 575-585. <https://doi.org/10.1046/j.1469-8137.1997.00729.x>