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Application of Arbuscular Mycorrhizal Fungi and Soil Ameliorant on the Growth of *Leucaena leucocephala* in Limestone Post-mining Soil Media

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Abstract

Limestone mining has the potential into environmental damage that involve modify an ecosystem. The attempt that contrived to reduce the disturbances are rehabilitation. This research was conducted to examine the growth response of Leucaena leucocephala inoculated with AMF and soil ameliorant in a limestone post-mining soil. The design used was a split-plot design in a completely randomized design with 3 factors. The first factor was AMF inoculum (Daemonorops draco AMF and MycoSilvi), the second factor was organic fertilizer of compost, and the third factor was inorganic fertilizer. The variables used in this study expressed by height, diameter, biomass, root colonization, and nutrient absorption of the plant. The analysis showed that the combination of MycoSilvi and compost 7.5% gave best result of height, diameter, and biomass, with significantly increased by 962.67%, 899.41% and 1440.67% to control plant. It also gave best result of nutrient uptake N, P, and K, with significantly increased up to 17.64 g plant^{-1}, 2.42 g plant^{-1}, and 18.05 g plant^{-1}. In general, AMF showed a good percentage of root colonization with an average 36.67-86.67%. Further research is needed to determine the response to the growth of seedlings planted in the field.

Keywords: AMF, compost, MycoSilvi, limestone post-mining, Leucaena leucocephala

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Introduction

Indonesia has an adequate wide karst area and it has been recorded 15.4 million ha (Cahyadi, 2010; Rahmadi, 2017). Gunung kapur or limestone mountain in Ciampea, Bogor, West Java, is one of the karst regions in Indonesia with a diversity of ecosystems that play a role in maintaining hydrological functions (Wasis et al., 2019). The increase in the human population causes the utilization of karst areas to also increase, especially on the eastern part of the mountain (Afra et al., 2008).

Mining is an activity accomplish by forest clearing, stripping the soil, dredging, and landfill (Cakyayanti & Setiadi, 2014). The block of limestone from mining results are used by communities to produce lime and raw material for making cement (Aziz, 2010; Prayudyaningsih & Sari, 2016). On other hand, limestone mining can increase income, however, threaten environmental damage, especially underground water sources because mining can immediately damage the forest's hydrological system (Hakim, 2011), and damage to post-mining areas such as topsoil loss, decreased organic matter, low nutrient availability, high pH, low microbial population, and damage to soil structure and texture (Prayudyaningsih et al., 2015; Aprillia et al., 2019).

Karsh ecosystem transformation effect adversity for plants to adapt (Chairul et al., 2019), tree planting activities often fail because post-mining soil conditions are not conducive to supporting its growth. Soil improvement is needed to restore ecosystems (Chen et al., 2011). Various post-mining soil treatments have been accomplished, such as by apply soil ameliorant material or by utilizing microorganisms (Prayudaningsih & Sari 2016). Inoculation of arbuscular mycorrhizal fungi (AMF) and soil ameliorant has the potential to increase soil quality as a result of increased nutrient availability and help plants survive in marginal environmental conditions (Prayudyaningsih & Sari, 2016). AMF is a group of fungi that has a mutual symbiosis (mutual benefit) with plant roots (Smith & Read 2008). The ability of AMF in increasing plant growth can be combined with other microorganisms. MycoSilvi is an AMF inoculum enriched with Mycorrhizal Helper Bacteria (MHBs) which capable to increase the number of AMF spores and mycorrhizal colonization of some legume plants in silica sand post-mining soils (Jayani et al., 2018, Budi et al., 2020).

However, there is no data on the use of MycoSilvi on limestone post-mining soil. Soil ameliorant in the form of compost is useful in improving soil properties that were originally solid to lose, increase the availability of nutrients in the soil, and make microorganisms in the soil becomes developed (Setyorini et al., 2006). Urea fertilizer is useful in accelerating plant growth and photosynthesis (Pramitasari et al., 2016). In *L. Leucocephala* seedlings, AMF plays an

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important role in the early growth of *L. leucocephala* seedlings (Brandon et al., 1997). It helps in increasing plant biomass, P concentration, root colonization, avoiding drought stress (Dixon, 1993; Habte & Antal, 2010). Rani et al., (2019) also stated that a mixture of soil, vermicompost, and AMF can provide optimal *L. Leucocephala* seedling growth in the nursery. Research Puthur et al., (1998) stated that in tissue culture, *L. Leucocephala* plantlets that were inoculated with AMF had better adaptability than without AMF.

L. leucocephala is a pioneer plant from Central America which is currently widely grown and spread in tropical regions such as Indonesia (Wolfe & Van Bloem 2012; Hendrati & Hidayati, 2018). This species is a type of fast-growing legume which plays an important role in restoring soil fertility, prevent erosion, form root nodules, symbiosis with fungi, and to adapt in marginal land (Atawodi et al., 2008; Mansur 2013; Dharmaputri et al., 2016). Therefore, *L. leucocephala* has the potential to improve the limestone post-mining soil. The purpose of this study was (1) examine the role of AMF and soil ameliorant and their interactions in increasing *L. leucocephala* seedlings growth in limestone post-mining soil (2) determine the most optimal treatment to increase the growth of *L. leucocephala* seeds in limestone post-mining soil.

Methods

Time and location of research This research was conducted in April-December 2019. The research location was carried out at the Greenhouse and Mycorrhizal Laboratory, Departement of Silviculture, Faculty of Forestry, IPB University.

Preparation for planting media and the addition of soil ameliorant Soil media (limestone mining soil) was collected from limestone mining in Ciampea, Bogor, West Java. Soil was wrapped in heat-resistant plastic and then sterilized using an autoclave at 121 C for 1 hour (Jayani et al., 2018). The media then weighed 650 g polybag^{-1}. The sterilized soil was then added with soil ameliorant in the form of compost (0%, 2.5%, 5%, 7.5%, and 10%) polybag^{-1} and 0.5 g polybag^{-1} urea fertilizer . The soil was then watered and incubated for 2 weeks (Yulnafatmawita et al., 2005). Incubation serves to perfect the decomposition process and helps microorganisms develop and metabolize to break down organic matter into organic compounds (Siregar et al., 2017).

Sterilization, breaking dormancy, and sowing seeds *L. Leucocephala* seeds were originally from the collection of Silviculture Laboratory, Faculty of Forestry, IPB University. *L. leucocephala* seeds have a germination percentage 68–100% (Suita, 2019). Seed sterilization refers to Nusantara et al., (2012) that was the seeds are sterilized with a solution of NaClO (5.2%) for 5 minutes. The seeds are then cleaned until the odour of the solution disappears. Breaking of seed dormancy refers to Mansur (2013) that was the seeds were soaked in hot water for 3 minutes, the seeds were then soaked in cold water for 24 hours. The seeds were then drained and sown on zeolite media. Sowing was done in the afternoon to avoid excessive evaporation.

Weaning and inoculation of AMF Weaning was done after 10 days of germination. The seeds were then transferred to the planting media that has been prepared previously. *D. draco* indigeneous AMF originally from rhizosphere of *D. draco* in Sarolangun, Jambi and MycoSilvi were taken from the Greenhouse collection of the Faculty of Forestry, IPB University. Seedlings treated with AMF were inoculated with 10 g zeolite polybag^{-1} (±50 AMF spores) of indigenous *D. draco* contains *Glomus* sp. + *Acaulospora* sp. + *Scutelosphora* sp. and *Gigaspora* sp. (Purwanti et al., 2019) and MycoSilvi consist of AMF *Glomus mosseae* + *Acaulospora* sp. and *Gigaspora margarita* (Budi et al., 2020) in the planting hole around the plant roots.

Maintenance and harvesting Seedlings were grown for 14 weeks in the greenhouse conditions (daily light intensity) and watered every day manually as needed to maintain holding capacity planting media. Maintenance was done by watering plants in the morning and evening following the conditions of the growing media. Harvesting was done in the last week of observation by separating plants and planting media.

Observation parameters Measurement of height (cm) and diameter (mm) of seedlings was carried out after weaning. Observations are carried out every two weeks. Plant biomass was measured after the seedlings are harvested and dried in an oven at 80 C for 24 hours (Wasis & Sa'idah, 2019). Seedlings biomass is then weighed. Observation of root colonization refers to the method of Clapp et al. (1996) that was immersed in a 20% KOH solution for 48 hours, then the roots were soaked in 0.1 M HCl solution, then soaked in trypan blue dye solution for 48 hours, then the roots were soaked in a destaining solution for 24 hours.

Nutrient uptake analysis Measurement of nutrient content in seedlings was conducted at the Laboratory of the Department of Soil and Land Resources at the Faculty of Agriculture, IPB University. The nutrient uptake is calculated by multiplying the biomass and percent of plant nutrients (Ardakani et al., 2011).

Experimental design The design used in this experiment is a randomized split plot design in a completely randomized design (CRD) with 3 factors. The first factor was AMF inoculum (M) which consists of 3 levels, namely M0 = without inoculum AMF, M1 = AMF indigenous *D. draco* consist of *Glomus* sp. + *Acaulospora* sp. *Scutelosphora* sp and *Gigaspora* sp. (Purwanti et al., 2019), M2 = MycoSilvi contains *Glomus mosseae* + *Acaulospora* sp. and *Gigaspora margarita* (Budi et al., 2020). The second factor was compost (K) consisting of 5 levels namely K0 = 0%, K1 = 2.5%, K2 = 5%, K3 = 7.5%, and K4 = 10%. The third factor was urea fertilizer consisting of 2 levels, namely N0 = 0 g and N1 = 0.5 g. The total treatments tested were 30 treatments with each treatment consisting of 5 replications.

Data analysis The data that has been obtained is processed using Microsoft Excel software. The effect of the treatment on the observed variables was carried out using analysis of

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variance (ANOVA) at the α level of 5%, followed by Duncan's multiple range test if the data show significantly different effects using SAS 9.1 software. Test the correlation between variables measured using SPSS 18 software.

Results and Discussion

Effect of AMF and soil ameliorant on the colonization of *L. leucocephala* seedlings roots The addition of indigenous *D. draco* AMF and MycoSilvi, in general, showed a percentage of root colonization in *L. leucocephala* seedlings. In general, control seedlings adapted well in post limestone mining soil and has survival rate 100 %, but the indicated growth rates were relatively slower than those treated with AMF and soil ameliorant. Seedlings treated with compost and AMF helped in the formation of plant root nodules, although rhizobium was not researched in this study, the number of seed nodules treated with AMF and soil ameliorant tended to be more than the control plants. Puthur et al., (1998) stated that AMF can increase the number of root nodules of *L. leucocephala* plantlets and produce stronger roots when compared to control plants.

Seedlings treated with AMF (with or without soil ameliorant) showed a high percentage of root colonization with an average of 36.67–86.67%, while seedlings without AMF did not show the percentage of root colonization (control plants and plants with soil ameliorant) (Table 1). This shows that the addition of AMF has a positive effect on the percentage of *L. leucocephala* seedlings root colonization compared without AMF. Mycorrhizal association is indicated by the presence of hyphae, arbuscules, and vesicles that form in plant roots (Puthur et al., 1998). The symbiosis between AMF and plant roots can increase nutrient uptake especially in limestone post-mining soils with a dominant clay texture. (Prayudyaningsih & Sari, 2016). AMF can produce phosphatase enzymes that convert P-bound compounds of Ca into ions and form external hyphae so that it is easier to enter smaller pore spaces and help absorb P nutrients further in the soil (Widiastuti et al., 2003; Manaroinsong & Lolong, 2015; Rosita et al., 2017). It also can maintains higher leaf water potential during periods of peak water stress (Dixon et al., 1993). Mycorrhizae are able to improve physical properties that are by increasing the soil pore which helps in root penetration and respiration in limestone post-mining soil that tends to be dense (Aprillia et al., 2019). Other studies also mention that AMF can increase the percentage of root colonization in *F. moluccana* seeds by 90.00%, *S. saman* by 86.67%, *C. siamea* by 73.33%, *Theobroma cacao* by 73.9%, and *Tectona grandis* by 74.97% (Rahmayanti et al., 2013; Prayudyanignsih & Sari 2016; Jayani et al., 2018).

Effect of AMF and soil ameliorant on height, diameter, and biomass of *L. leucocephala* seedlings The addition of AMF and soil ameliorant plays an important role in repairing soil damage due to mining and can support plant growth (Mansur, 2013). Post-mining soils collected in this study included sandy clay loam with low nutrient composition (0.54% C-organic, 0.06% N, and 7.81 ppm P-available). The results showed that the addition of AMF and soil ameliorant had a significant influence on the growth of *L. leucocephala* seedlings planted in limestone post-mining soils (Table 2). The interaction of compost 7.5% and MycoSilvi capable to provide a more optimal growth response to *L. leucocephala* seedlings when compared to controls. The average height, diameter, and biomass of the control seedlings only reached 7.23 cm, 0.57 mm, and 0.50 g, in contrast to the addition of 7.5% compost and MycoSilvi is able to produce the best average height, diameter, and biomass increase of 76.87 cm, 5.66 mm, and 7.70 g with a percent increase of 962.67%,

Table 1 Mean and DMRT effects of AMF and soil ameliorant on the colonization of *L. leucocephala* seedlings

Treatment	Mean	Criteria	Treatment	Mean	Criteria
K0N0M0	0.00 ± 0.00^{e}	Not colonized	K2N1M1	53.33 ± 32.15^{abcd}	High
K0N1M0	0.00 ± 0.00^{e}	Not colonized	K3N0M1	66.67 ± 25.17^{abcd}	High
K1N0M0	0.00 ± 0.00^{e}	Not colonized	K3N1M1	50.00 ± 43.59^{abcd}	High
K1N1M0	0.00 ± 0.00^{e}	Not colonized	K4N0M1	40.00 ± 26.46^{bcde}	High
K2N0M0	0.00 ± 0.00^{e}	Not colonized	K4N1M1	40.00 ± 17.32^{bcde}	High
K2N1M0	0.00 ± 0.00^{e}	Not colonized	K0N0M2	66.67 ± 15.28^{abcd}	High
K3N0M0	0.00 ± 0.00^{e}	Not colonized	K0N1M2	36.67 ± 37.86^{cde}	High
K3N1M0	0.00 ± 0.00^{e}	Not colonized	K1N0M2	86.67 ± 23.09^{a}	High
K4N0M0	0.00 ± 0.00^{e}	Not colonized	K1N1M2	60.00 ± 0.00^{abcd}	High
K4N1M0	0.00 ± 0.00^{e}	Not colonized	K2N0M2	73.33 ± 25.17^{abc}	High
K0N0M1	60.00 ± 43.59^{abcd}	High	K2N1M2	80.00 ± 10.00^{ab}	High
K0N1M1	53.33 ± 30.55^{abcd}	High	K3N0M2	73.33 ± 11.55^{abc}	High
K1N0M1	70.00 ± 26.46^{abc}	High	K3N1M2	70.00 ± 20.00^{abc}	High
K1N1M1	66.67 ± 11.55^{abcd}	High	K4N0M2	36.67 ± 11.55^{cde}	High
K2N0M1	26.67 ± 15.28^{ed}	Medium	K4N1M2	53.33 ± 11.55^{abcd}	High
P-value			0.735^{ns}		

* = significantly different effect (P -value) <0.05 (α), ns = not significantly different effect (P -value)> 0.05 (α). K = compost, N = urea, M = FMA. The numbers followed by the same letters show no significant effect on the α level of 5%.

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Table 2 Mean and DMRT effects of AMF and soil ameliorant on height, diameter, biomass, and nutrient uptake of *L. leucocephal* seedlings

Treatment	Height (cm)	Diameter (mm)	Biomass (gr)	Nutrient uptake (g plant^{-1})		
				N	P	K
K0N0M0	7.23 ± 0.50^{k}	0.57 ± 0.23^{h}	0.50 ± 0.18^{h}	0.82 ± 0.25^{h}	0.14 ± 0.04^{j}	0.49 ± 0.15^{i}
K0N0M1	19.00 ± 16.47^{ijk}	2.76 ± 0.99^{fg}	0.54 ± 0.08^{h}	1.04 ± 0.09^{h}	0.18 ± 0.02^{j}	0.62 ± 0.13^{i}
K0N0M2	28.23 ± 5.47^{hij}	3.77 ± 0.49^{cdef}	4.10 ± 0.70^{bcdef}	7.15 ± 2.41^{def}	1.04 ± 0.38^{efgh}	5.53 ± 2.52^{fg}
K0N1M0	15.67 ± 3.2^{1jk}	2.41 ± 1.01^{g}	1.10 ± 0.74^{g}	2.34 ± 1.75^{g}	0.28 ± 0.17^{ij}	1.53 ± 1.26^{h}
K0N1M1	28.43 ± 7.59^{hij}	2.26 ± 0.50^{g}	1.15 ± 0.51^{g}	2.19 ± 0.9^{g}	0.39 ± 0.09^{hij}	1.49 ± 0.41^{h}
K0N1M2	49.97 ± 5.95^{efg}	3.68 ± 0.16^{ef}	3.28 ± 0.15^{cdef}	5.96 ± 0.38^{ef}	0.72 ± 0.08^{ghij}	3.24 ± 0.15^{g}
K1N0M0	60.53 ± 7.64^{abcde}	4.51 ± 0.60^{abcde}	3.93 ± 1.4^{bcdef}	8.75 ± 1.30^{abcd}	1.13 ± 0.27^{abcdef}	6.95 ± 3.20^{abcdef}
K1N0M1	59.4 ± 9.32^{abcde}	3.74 ± 0.11^{def}	6.15 ± 0.96^{abc}	16.01 ± 3.31^{ab}	1.88 ± 0.53^{abcd}	12.15 ± 2.34^{abcde}
K1N0M2	72.33 ± 8.81^{abc}	5.03 ± 0.65^{abcd}	5.86 ± 3.85^{abcdef}	13.04 ± 8.07^{abcdef}	1.56 ± 1.01^{bcdef}	9.58 ± 5.98^{bcdef}
K1N1M0	53.43 ± 2.64^{defg}	3.75 ± 0.15^{def}	2.64 ± 0.59^{ef}	5.97 ± 2.23^{f}	0.76 ± 0.36^{ghij}	6.84 ± 2.73^{def}
K1N1M1	33.93 ± 5.87^{hi}	3.63 ± 0.68^{ef}	2.55 ± 1.06^{f}	5.89 ± 2.42^{f}	0.77 ± 0.21^{ghij}	6.53 ± 2.15^{ef}
K1N1M2	59.9 ± 9.69^{abcde}	4.10 ± 0.44^{bcde}	4.04 ± 1.18^{bcdef}	9.17 ± 1.65^{abcdef}	1.30 ± 0.33^{defgh}	8.05 ± 2.12^{bcdef}
K2N0M0	66.53 ± 4.46^{abcde}	4.15 ± 0.25^{bcde}	4.77 ± 0.69^{abcde}	9.42 ± 1.66^{abcdef}	1.17 ± 0.12^{defgh}	10.93 ± 2.19^{abcde}
K2N0M1	41.5 ± 13.48^{fgh}	3.84 ± 0.26^{bcdef}	4.29 ± 0.77^{abcdef}	9.88 ± 1.68^{abcdef}	1.16 ± 0.13^{defgh}	10.67 ± 0.67^{abcde}
K2N0M2	55.7 ± 14.80^{cdefg}	5.07 ± 0.63^{abc}	7.22 ± 1.08^{ab}	16.04 ± 2.26^{ab}	2.31 ± 0.57^{ab}	13.56 ± 1.55^{ab}
K2N1M0	39.83 ± 15.37^{gh}	4.69 ± 0.37^{abcde}	5.74 ± 0.17^{abcd}	13.48 ± 0.43^{abcd}	1.74 ± 0.27^{abcdef}	12.33 ± 0.45^{abcde}
K2N1M1	63.7 ± 4.46^{abcde}	3.95 ± 1.47^{bcdef}	2.59 ± 0.72^{ef}	5.93 ± 1.57^{f}	0.68 ± 0.28^{ghij}	6.81 ± 1.31^{cdef}
K2N1M2	72.4 ± 2.12^{abc}	5.01 ± 0.62^{abcd}	6.06 ± 1.47^{abcd}	14.13 ± 2.35^{abc}	1.88 ± 0.46^{abcd}	12.74 ± 3.28^{abcde}
K3N0M0	59.87 ± 4.98^{abcde}	4.58 ± 0.63^{abcde}	4.64 ± 1.67^{abcdef}	10.85 ± 3.34^{abcdef}	1.35 ± 0.26^{cdef}	10.07 ± 2.68^{abcdef}
K3N0M1	56.87 ± 13.29^{bcdef}	4.25 ± 0.46^{bcde}	5.35 ± 0.60^{abcd}	13.22 ± 3.86^{abcd}	1.72 ± 0.37^{abcdef}	14.52 ± 1.95^{ab}
K3N0M2	76.87 ± 2.44^{a}	5.66 ± 0.35^{a}	7.70 ± 0.27^{a}	17.64 ± 2.04^{a}	2.42 ± 0.30^{a}	18.05 ± 3.88^{a}
K3N1M0	64.83 ± 17.17^{abcde}	3.86 ± 0.44^{bcdef}	5.28 ± 1.28^{abcd}	13.44 ± 2.77^{abcd}	1.61 ± 0.43^{bcdef}	12.68 ± 1.27^{abcd}
K3N1M1	68.73 ± 8.92^{abcd}	4.13 ± 0.90^{bcde}	6.52 ± 0.48^{ab}	13.13 ± 1.11^{abcd}	1.8 ± 0.15^{abcde}	15.09 ± 1.65^{ab}
K3N1M2	73.9 ± 7.02^{ab}	4.10 ± 1.19^{bcde}	6.24 ± 1.10^{abc}	13.06 ± 4.37^{abcd}	1.86 ± 0.27^{abcd}	11.6 ± 2.54^{abcde}
K4N0M0	61.03 ± 0.85^{abcde}	3.97 ± 0.73^{bcdef}	3.99 ± 1.46^{bcdef}	8.89 ± 3.24^{cdef}	1.14 ± 0.5^{defgh}	10.41 ± 5.69^{abcdef}
K4N0M1	69.83 ± 1.59^{abcd}	5.11 ± 0.68^{ab}	6.29 ± 1.10^{abc}	14.08 ± 1.48^{abc}	1.72 ± 0.20^{abcdef}	13.21 ± 1.75^{abc}
K4N0M2	64.07 ± 14.39^{abcde}	4.47 ± 0.85^{abcde}	6.21 ± 0.52^{abc}	11.25 ± 1.22^{abcde}	1.98 ± 0.05^{abc}	12.2 ± 1.21^{abcde}
K4N1M0	61.87 ± 4.67^{abcde}	3.84 ± 0.65^{bcdef}	4.02 ± 1.16^{bcdef}	8.99 ± 2.32^{bcdef}	1.30 ± 0.45^{cdef}	9.24 ± 1.81^{abcdef}
K4N1M1	63.43 ± 13.43^{abcde}	3.8 ± 0.32^{bcdef}	3.26 ± 0.91^{def}	7.39 ± 1.6^{cdef}	0.96 ± 0.35^{fghi}	8.49 ± 3.33^{bcdef}
K4N1M2	69.47 ± 13.71^{abcd}	4.81 ± 0.66^{abcde}	7.39 ± 2.18^{ab}	13.03 ± 1.58^{abcd}	2.42 ± 0.90^{a}	14.01 ± 4.28^{ab}
P-value	0.0008**	0.044*	0.044*	0.029*	0.011*	0.019*

* = significantly different effect (P-value) <0.05 (α), ns = not significantly different effect (P-value)> 0.05 (α). K = compost, N = urea, M = FMA. The numbers followed by the same letters show no significant effect on the α level of 5%.

899.41% and 1,440.67% of the control. This is by research Prayudyaningsih and Sari (2016) that in the limestone post-mining soil that added compost and FMA can increase the height, diameter, and biomass of *T. grandis* seedlings compared without compost and FMA. Compost can improve the physical and chemical properties of the soil (Surya et al., 2017), physical properties such as good porosity making it easier for roots to penetrate the soil (Aminah et al., 2003), chemical properties in the form of the supply of macronutrients used by plants, especially nitrogen which is a compiler of amino acids, proteins and cell protoplasms that function in stimulating vegetative growth of plants (Djamhari, 2010; Putri et al., 2016). AMF is very effective in helping vegetative growth of plants because it helps the roots absorb plant nutrients which are indicated by the increase in biomass produced (Cavagnaro et al., 2003; Ginting et al., 2018b). Some research also shows that seedlings growth will be better if added with compost and AMF both singly and interactively. In silica sand post-mining, the addition of MycoSilvi and ameliorant soil (32.5 g of compost and 7.2 g of lime) increased the growth of *F. moluccana , S. saman*, dan *C. siamea* with a percent increase in height seedlings by 173.19%, 233.71%, 257.56%, diameter of 186.79%, 303.48%, 562.08%, and biomass by 995.93%, 667.86%, 1447.62% (Jayani et al., 2018). In lime post-mining soils, a single AMF produces height, diameter, and biomass of *Alstonia scholaris, Acacia auriculiformis,* and *Mutingia calabura* seedlings better than without AMF, and compost singly produced the best biomass of *P. falcataria* seedlings up to a 23.16% increase from control (Prayudyaningsih, 2014;

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Wasis & Sa'diah 2019). AMF significantly increased the biomass of *L. leucocephala* which was better than without AMF in masand (crushed basalt) (Habte & Antal, 2010). *L. leucocephala* seedlings that were inoculated with AMF (*Gigaspora margarita, Glomus deserticola,* and *G. etunicatum*) also significantly produced better biomass compared to control seedlings in sandy loam media (Dixon et al., 1993). Rani et al. (2019) also stated that a mixture of soil, vermicompost, and AMF (*Acaulospora scrobiculata* + *G. intraradices*) also were able to produce the best biomass for *L. leucocephala* seedlings.

Effect of AMF and soil ameliorant on nutrient uptake of *L. leucocephala* seedlings Nutrient uptake is influenced by the addition of organic matter and AMF inoculation (Biswas et al., 2000; Shao et al., 2018). The results showed that the addition of AMF and soil ameliorant had a significant effect on nutrient uptake of *L. leucocephala* seedlings (Table 2). Interaction of compost 7.5% and MycoSilvi produce the best nutrient uptake of *L. leucocephala* seedlings of 17.64 g plant^{-1} (N), 2.42 g plant^{-1} (P), and 18.05 g plant^{-1} (K), while the nutrient uptake control seedlings produced were only 0.82 g plant^{-1} (N), 0.14 g plant^{-1} (P), and 0.49 g plant^{-1} (K). Base on these conditions, it can be assumed that the addition of 7.5% compost and MycoSilvi can provide optimal nutrient uptake for *L. leucocephala* seedlings in limestone post-mining soils. Bustami et al. (2012) stated that plant growth will reach optimum if growth-supporting factors such as macronutrients N, P, and K are in optimum condition and available to plants. Compost undergoes a decomposition process which then releases N, P, and K, thereby increasing the availability of nutrients for plants (Hasanudin, 2003; Rani et al., 2019). Mycosilvi contains AMF which can absorb N and Mg nutrients as the compiler of chlorophyll. (Jayani et al., 2018). FMA also helps absorb P nutrients which are useful in stimulating root growth especially in young plants and increasing plant resistance when nutrient deficiency and dryness such as in limestone post-mining soil (Manaroinsong & Lolong, 2015; Chairul et al., 2019). The results of the study (He et al., 2017) also mentioned that AMF inoculation had a significant influence on N and P nutrient uptake in limestone soils. Root morphological conditions such as thickness, length, and the number of roots affect optimal nutrient uptake (Biswas et al., 2000). The better the root development, then the better the nutrient uptake that seedlings (Ginting et al., 2018a).

Correlation of root colonization, nutrient uptake, and biomass of *L. leucocephala* seedlings Root colonization has a positive correlation (r) to nutrient uptake of N, P, and K (Figure 1). The correlation magnitude of root colonization and nutrient uptake of N (Figure 1a), P (Figure 1b), and K (Figure 1c) respectively were 0.273 (R^2 = 0.079), 0.314 (R^2 = 0.103), and 0.209 (R^2 = 0.044). These positive correlations indicate that an increase in percent colonization of roots influences on increasing nutrient uptake of N, P, and K seedlings of *L. leucocephala*. MycoSilvi contains FMA which has external hyphae so that it helps in expanding nutrient uptake for plants (Jayani et al., 2018). At roots inoculated with AMF, nutrients available in the soil will be more easily absorbed compared to without AMF inoculation (Ginting et al., 2018b).

The availability of these nutrients is followed by an increase in photosynthetic activity which supports in

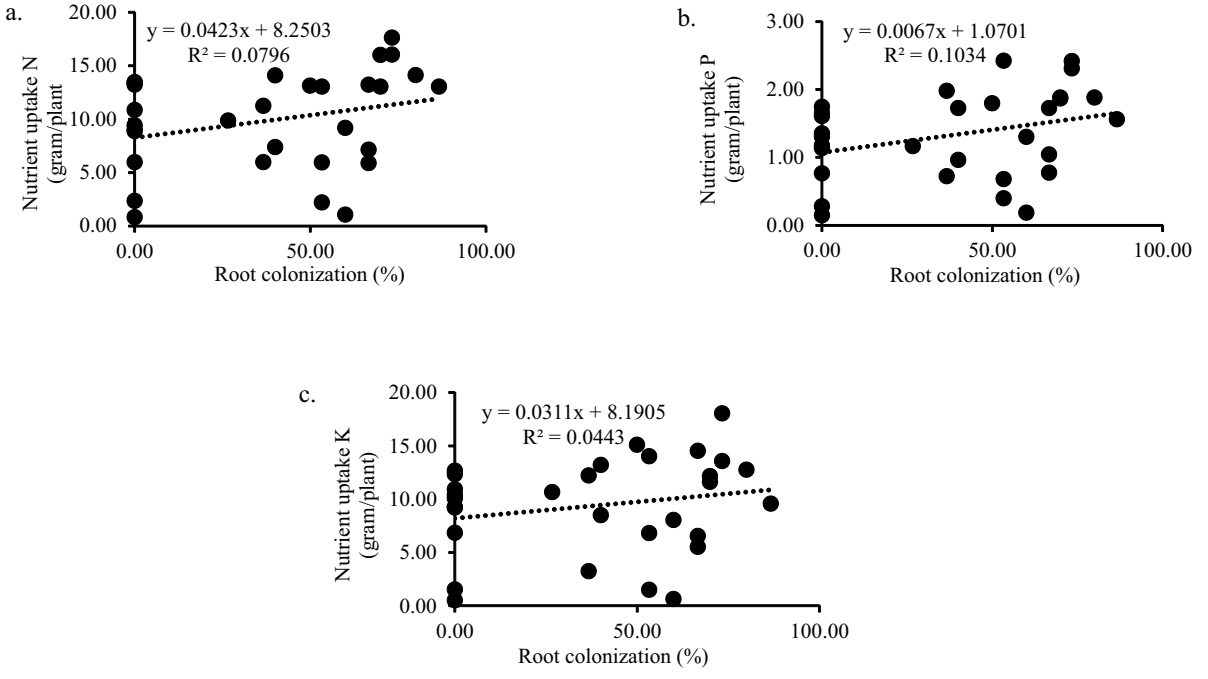

Figure 1 Correlation of root colonization and nutrient uptake of *L. leucocephala* seedlings.

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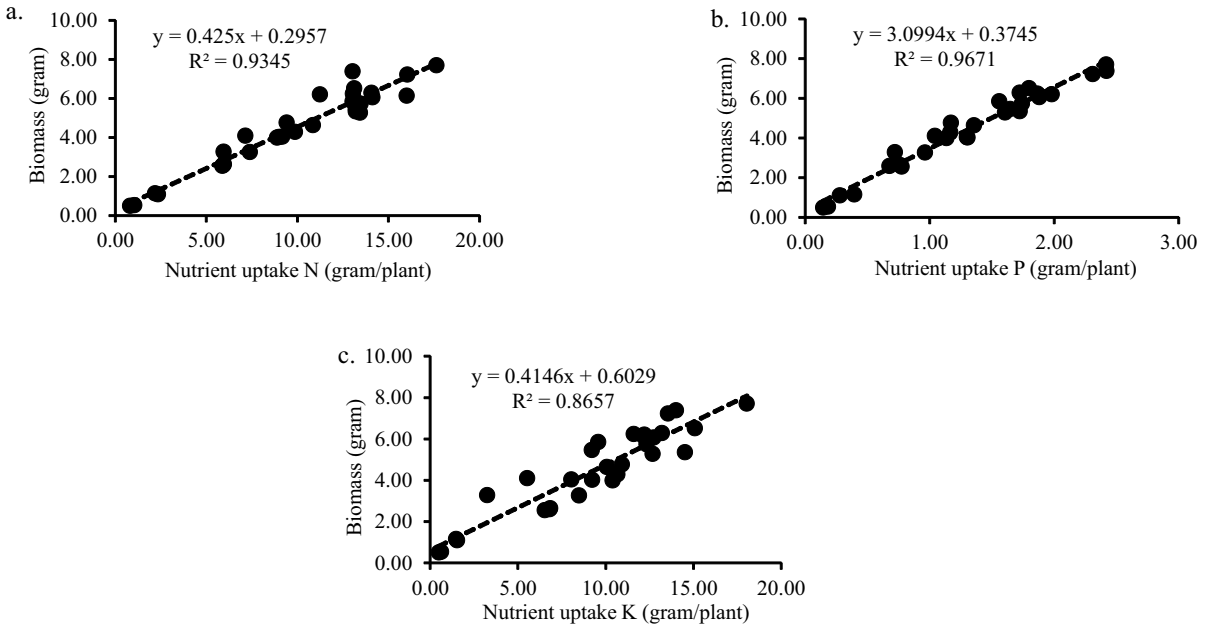

Figure 2 Correlation between nutrient uptake and biomass *L. leucocephala* seedling.

increasing plant biomass (Putri et al., 2016). AMF can significantly help plants absorb nutrients thereby increasing biomass in plants (Bonfante & Genre 2010). The results showed that nutrient uptake had a positive (r) correlation to *L. leucocephala* seedlings biomass (Figure 2). The correlation magnitude of nutrient uptake of N (Figure 2a), P (Figure 2b), and K (Figure 2c) to seedlings biomass respectively were 0.951 (R^2 = 0.934), 0.964 (R^2 = 0.967), and 0.911 (R^2 = 0.865). This shows that there is a very high correlation between plant nutrient uptake and *L.leucocephala* seedlings biomass. The amount of N, P, and K nutrients absorbed by plants has a close relationship with the biomass produced, the higher the nutrient uptake, the higher the biomass produced (Putri et al., 2016; Sumarni et al., 2016).

Conclusion

Arbuscular mycorrhizal fungi and soil ameliorant play an essential aspect in increasing the growth of *L.leucocephala* seedlings in limestone post-mining soils. The interaction of compost dose of 7.5% and MycoSilvi capable to produce the best growth response to height, diameter, biomass, and seedlings nutrient uptake compared to other treatments. There is a positive correlation between root infection and N, P, and K nutrient uptake of 0.273, 0.314, and 0.209. Nutrient uptake of N, P, and K to biomass also has a positive correlation that is equal to 0.951, 0.964, and 0.911.

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