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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL AMELIORANTS ON THE MYCORRHIZAL COLONIZATION, CHLOROPHYLL CONTENT, AND PERFORMANCE GROWTH OF TWO TROPICAL TREE SEEDLINGS GROWN IN SOIL MEDIA WITH HIGH ALUMINUM CONTENT

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ABSTRACT

Aluminum is one of heavy metals and its availability is correlated with low soil pH, such as in acidic soil as well as post mining soil and become limiting factors for plant growth. MycoSilvi is a biofertilizer inoculant product containing arbuscular mycorrhizal fungi enriched with Mycorrhizal Helper Bacteria's (MHBs) designed for improving plant growth on post-mining soil media with low pH and high aluminum content. This study was conducted to determine the potential use of three variants of MycoSilvi, both single or in combination with soil ameliorant to enhance *Albizia chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) Pierre growth. This study was conducted by randomized complete design with factorial scheme in a greenhouse for 4 months. The first factor consisted of four different levels based on MycoSilvi (M) inoculation: control (M0); MycoSilvi variant 1 (M1), MycoSilvi variant 2 (M2) and MycoSilvi variant 3 (M3). The second factor consisted of four levels based on the addition of lime and compost to soil medium: control (L0C0), addition of lime (L1C0), addition of compost (L0C1) and addition of lime and compost (L1C1). Data was analyzed using analysis of variance (ANOVA). Total chlorophyll content as indicated by leaf greenness index, height, steam diameter, biomass, root colonization and P accumulation on both plant species were observed after 12 weeks of planting. The results showed that the MycoSilvi inoculation differently colonized the roots and increased P uptake, leaf greenness index and growth of both plants species. The addition of lime and compost increased the mycorrhizal roots colonization, P uptake, leaf greenness index and plant growth of both plants' species. The best result was obtained from the combination treatment of MycoSilvi variant 3, lime and compost.

Key words: Acid soil, aluminum, MycoSilvi, plant growth, post mining soil

INTRODUCTION

Aluminum is a micronutrient needed by plant in small amounts. It plays an important role as cofactors for activating enzyme in the metabolism processes (Zatta *et al.,* 2000). The availability of Aluminium in the soil is greatly affected by soil pH (Dong *et al.,* 1995). In acidic soil the availability of Aluminium in the soil is very large and become toxic for plant growth (Delhaize & Ryan, 1995). In the tropic regions, acidic soil is very abundant, commonly ultisol and oxisol. There are about 500 million hectares (16.2%) of the African continent covered by acidic soil (Bationo *et al.,* 2006) and according to Van Uexkull and Bosshart (1989) about 38% of upland soil in Southeast Asia is covered by ultisol and oxisol. Subagyo *et al.* (2004), stated that in Indonesia, acidic soil covers almost 25% of land surface, which spreads in Kalimantan, Sumatera, Maluku, Papua, Sulawesi, Java and Nusa Tenggara. The acidic soil commonly is not able to support plant growth and development normally because they are not only high in aluminum content but also low nutrient content, high Mn toxicity, low organic matter and biodiversity and very low mineralization and nitrification (Kochian *et al.,* 2004). The high concentration of Aluminum overburdens soil at coal mine, inhibits the plant growth and chlorophyll content of *Pongomia pinata* in the nursery (Agus *et al.,* 2019). The mining activity may alter the soil

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pH as reported by Hudson *et al.* (1999), that affects some environmental impacts such as development of acidic soils and waters, erosion of tailings by wind and water, and physical disturbances to the landscape. Mining activities also increase toxic elements to plants such as Al, Fe, Mn, Ni, Zn, Co and Cd as reported by Wahab and Marikar (2012). Thus, all acidic soil characteristics become a major constrain for plant growth and productivity. Numerous efforts have been made for improving chemical characteristic of acidic soil (Anetor & Akinrinde, 2007). The use of lime in acidic soil and adition of Phosphorous can reduced aluminium toxicity and improve plant growth (Muindi *et al.,* 2015). According to Novak *et al.* (2018), the addition of lime and biochar to acid soils can have a direct effect on metal availability, soil enzyme activity and plant growth. The application of lime and compost in acidic soil is also effective at reducing shoot Mn content of test plant (Chung & Wu, 2008). Lime and compost also can reduce Al toxicity and increase soil pH in acidic soil (Teshome *et al.,* 2017).

Arbuscular Mycorrhizal Fungi (AMF) a symbiotic organism plays an important role in sustainable soil-plant system (Hause & Fester, 2005), and have potential role in the restoration of degraded lands including post mine lands (Asmelash *et al.,* 2016; Wulandari *et al.,* 2016). These fungi have been widely used for improving plant growth and development in degraded lands (Maulana *et al.,* 2017) and acidic soil as they have numerous benefits to the plant, including increased absorption of phosphours nutrition in acidic soil (Smith *et al.,* 2011), better Nitrogen and Potassium uptake (Bucking & Kafle, 2015; Sharma *et al.,* 2017), producing enzyme and plant growth hormones (Bini *et al.,* 2017; Foo *et al.,* 2013), defending root against soil borne diseases (Bakhtiar *et al.,* 2010) and increasing plant growth and productivity (Baum *et al.,* 2015). In the soil-plants systems, AMF interact positively with coal waste and wood charcoal to increase plant growth (Budi & Christina, 2013; Budi & Setyaningsih, 2013), however little data is available on the interactions of lime, compost and AMF in soil-plants systems in acidic soil.

Leguminous trees are known to be very responsive to AMF inoculation (Maulana *et al.,* 2017) and widely used for reforestation in Indonesia. *Albizia chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) Pierre are potential plants for the rehabilitation of degraded land. *A. chinensis* well adapted in poor soil and categorized as a pioneer species in reforestation programme (Manjaribe *et al.,* 2013). The bark contains flavonoids, alkaloids, saponins and proteins, which were used as a medicine (Amudha *et al.,* 2017). *P. pinnata* is a fast growing tree and adapt well in acidic soil and well-drained, lateral roots are numerous and well developed (Bhalerao & Sharma, 2014). This species matures after 4-7 years and produced fruits, which has potential for biodiesel (Karmee & Chadha, 2005). The seeds of *P. pinnata* contain several secondary metabolites having pharmaceutical activity (Yadav *et al.,* 2011).

Our laboratory developed a biological fertilizer named MycoSilvi, an Arbuscular Mycorrhizal Fungi incoculum containing *Glomus mosseae* enriched with Mycorrhizal Helper Bacterias (MHBs). Previous research showed that MycoSilvi improves three species of forest tree grown in marginal soil (Jayani *et al.,* 2018). Severals variants of MycoSilvi inoculum have been produced and tested in *Falcataria molucaana* seedling (Budi *et al.,* 2020). In this research the same MycoSilvi variant was tested in *A. chinensis* and *P. pinata* seedlings were grown in soil medium originally from silica post mining area with low pH and high aluminium content. The aim of this research is to test MycoSilvi variants and soil ameliorant (lime and compost) separately or in combination in improving *A. chinensis* and *P. pinnata* growth in soil medium with low pH and high aluminium content.

MATERIALS AND METHODS

Soil and soil ameliorant

The top soil (0-20 cm depth) was collected from silica post mining area in Cibadak, Sukabumi District West Jawa (06°55'18.1" S and 106°47'10.8" E). The soil was then transported to Laboratory, air dried, sieved (2 mm). The chemical properties of soil were analysed following standard method in Soil Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agriculture University. The chemical properties of soil medium are pH H_2O = 3.20; C-organic (Walkley & Black) = 4.1%; N-Total (Kjeldhal) = 0.19%; C/N = 22.15; P (Bray I) = 13.78; P (HCl 25%) = 278.04 ppm and Al-dd = 690 mg /kg. Lime and compost were widely used as soil ameliorant (Bekele *et al.,* 2018; Haynes & Naidu, 1998). Lime was obtained from the farmers market as dolomite. Compost with the trade name of agro flower was produced by CV. Laksmi Prima Bogor Indonesia. The characteristics of compost are: pH 7.71; C-organic 48.79%; N 0.58%; K 1.00% and P_20_5 2.90% that was determined at Soil Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agriculture University. The soil was sterilized by autoclaving at 121°C for 1 hour. The sterilized soil was than mixed with lime and compost in accordance with the treatment.

Seedling and MycoSilvi inoculum

Seeds of *Albizia chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) Pierre was obtained from Center of Research and Development for Forest Seed, Bogor Indonesia. The nature of both seeds is orthodox. Seeds were soaked in hot water (80°C) for one hour and than soaked in cold water for 24 hours. The seeds than shown in plastict box containing sterilized zeolite and placed in green house for two weeks and watered as needed. The percentage of germination in both species was above 85%. *Glomus moseae* and *Acaulosphora* sp. were isolated from the rhizosphere of *Anthocepalus cadamba*, in Madiun District East Java (Budi & Dewi, 2016). *Gigaspora margarita* was isolated from primary forest, in Batanghari District, Jambi Province, Indonesia (Sahner *et al.,* 2015). MycoSilvi was produced in sterilized zeolite medium with *Puiraria javanica* as host plant for two months (Jayani *et al*., 2018) in the green house, with the daily temperature of 29°C–35°C and relative humidity of 60–90%. There were three variants of MycoSilvi produced. MycoSilvi variant 1, containing *Glomus mossea* Gerd and Trappe; MycoSilvi variant 2, containing *G. mossea* and *Acaulospora* sp. and MycoSilvi variant 3, containing *G. mosseae*, *Acaulospora* sp. and *Gigaspora margarita* Becker and Hall.

MycoSilvi Inoculation

Two weeks old uniform *A. chinensis* and *P. pinnata* seedlings were transplanted into 500 ml polybag containing sterilized mixed silica post mining soil and soil ameliorant. Five grams of fresh MycoSilvi inoculum containing environ 50 spores, mycelium, and mycorrhizal roots and MHBs were placed near the roots seedling at transplanting. *A. chinensis* and *P. pinnata* seedlings without MycoSilvi inoculation was prepared as control. Plants were grown for twelve weeks in the green house and watered as needed.

Harvesting and parameter measurement

Plant height (cm) and steam diameter (mm) were measured at two weeks I interval until twelve weeks old. Plant height was measured using meter rule from plant base to upper tip of the plant. Plant steam diameter was measured by digital caliper at 1 cm above the base of the seedlings stems. At twelve weeks old, SPAD chlorophyll meter was used to measure leaf chlorophyll content. Roots were sampled before harvest for evaluating their mycorrhizal colonization. Mycorrhizal colonization was evaluated according to the method of Clapp *et al.* (1996). Percentage of mycorrhizal colonization was determined according to the method of O'connor *et al.* (2001). Plants were harvested twelve weeks after planting and evaluated for their biomass (g). Biomass weight was recorded after drying at 70°C to constant weight is attained. Biomass P concentration was determined at Soil Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agriculture University. P accumulations was calculated by multiplying biomass P concentration by biomass dry weight.

Experiment design

The experiment was done with factorial design with 2 factors, MycoSilvi and Soil ameliorant. The MycoSilvi (M) has four levels (M0 = uninoculated, M1 = MycoSilvi variant 1, M2 = MycoSilvi variant 2 and M3 = MycoSilvi variant 3), and soil ameliorant (LC) have 4 levels (L0C0 = Lime 0 g and Compost 0 g, L1C0 = Lime 2.078 and Compost 0 g, L0C1 = Lime 0 g and Compost 20 g, L1C1 = Lime 2.078 g and Compost 20 g). The experiment was arranged in a completely randomized design in a polybag culture with 5 replicates. Analysis of Variance Procedure analyzed all of the data.

RESULTS AND DISCUSSION

Arbuscular Mycorrhizal colonization

AM Fungi colonized all seedlings of *A. chinensis* and *P. pinnata.* No AM Fungi colonization was observed in all control plants (Table 1). The single factor of MycoSilvi inoculation significantly affected the arbuscular mycorrhizal colonization of the roots of *A. chinensis* and *P. pinnata*. *A. chinensis* seedling inoculated with MycoSilvi variant 3 had higher AM colonization than that inoculated with MycoSilvi variant 1 and 2, while *P. pinnata* seedling inoculated with MycoSilvi variant 2 had higher AM colonization than that inoculated with MycoSilvi variant 3 and 1 (Table 1), indicating that AM mycorrhizal colonizatioan is affected by plant species as reported by Halder *et al.* (2015). The single factor of soil ameliorant significantly influences the mycorrhizal colonization in both tropical tree seedlings. Lime increased AM mycorrhizal roots colonization in *A. chinensis* seedling inoculated with MycoSilvi variant 1, 2 and 3 increased by 350%, 350% and 875% respectively, while compost increased by 525%, 75% and 180% respectively. Lime also increased AM mycorrhizal roots colonization in *P. pinata* seedling inoculated with MycoSilvi variant 1, and 3 by 100% and 114% respectively, while *P. pinata* inoculated with MycoSilvia variant 2, lime had a negative effect, it decreased the AM mycorrhizal roots colonization by 33% (Table 1). Compost had a positive effect on AM mycorrhizal roots colonization of *P. pinata* which increased by 333%, 55.6% and 100% when inoculated with MycoSilvi variant 1, 2 and 3 respectively.

Table 1. Effect of MycoSilvi and soil ameliorant on mycorrhizal colonization and P absorption of *A. chinensis* and *P. pinnata* (12 weeks after planting)

Treatment	Mycorrhizal colonization (%)		P accumulation (g/seedling)	
	A. chinensis	*P. pinnata*	*A. chinensis*	*P. pinnata*
M0L0C0	0.00 ± 0.00f	0.00 ± 0.00f	0.07 ± 0.05i	0.98 ± 0.14e
M0L1C0	0.00 ± 0.00f	0.00 ± 0.00f	0.25 ± 0.07fghi	1.62 ± 0.67cd
M0L0C1	0.00 ± 0.00f	0.00 ± 0.00f	0.40 ± 0.03efg	1.44 ± 0.32de
M0L1C1	0.00 ± 0.00f	0.00 ± 0.00f	0.42 ± 0.09def	1.57 ± 0.49d
M1L0C0	8.00 ± 3.74e	6.00 ± 2.45de	0.16 ± 0.06hi	1.60 ± 0.29cd
M1L1C0	36.00 ± 9.27cd	12.00 ± 7.35cde	0.30 ± 0.06fgh	1.62 ± 0.29cd
M1L0C1	50.00 ± 14.83bc	26.00 ± 6.78bcd	0.50 ± 0.27de	2.14 ± 0.25bc
M1L1C1	80.00 ± 7.07a	22.00 ± 5.83cde	0.59 ± 0.12cd	2.59 ± 0.33ab
M2L0C0	8.00 ± 2.00e	18.00 ± 4.90cde	0.21 ± 0.02ghi	1.57 ± 0.04d
M2L1C0	36.00 ± 13.64cd	12.00 ± 2.00cde	0.32 ± 0.02efgh	1.64 ± 0.46cd
M2L0C1	14.00 ± 6.78de	28.00 ± 7.35abcd	0.78 ± 0.13b	1.99 ± 0.20cd
M2L1C1	66.00 ± 9.80ab	44.00 ± 9.27ab	1.01 ± 0.25a	2.76 ± 0.14a
M3L0C0	20.00 ± 8.37de	14.00 ± 2.45cde	0.37 ± 0.06efg	1.65 ± 0.03cd
M3L1C0	78.00 ± 9.70a	30.00 ± 14.49abc	0.79 ± 0.14b	1.94 ± 0.73cd
M3L0C1	56.00 ± 8.12abc	28.00 ± 6.63abcd	0.72 ± 0.23bc	2.71 ± 0.39a
M3L1C1	60.00 ± 12.65abc	48.00 ± 9.70a	1.16 ± 0.20a	2.86 ± 0.35a
Significancy				
M	**	**	**	**
LC	**	*	**	**
M x LC	**	ns	**	*

M0, control; M1, MycoSilvi variant 1; M2, MycoSilvi variant 2; M3, MycoSilvi variant 3; L0, without lime; L1, lime 2.078 g; C0, without compost; C1, compost 20 g. Each value is mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.5$). ** = $P \leq 0.01$; * = $0.01 < P \leq 0.05$ and ns = $P>0.05$.

The combination treatment of MycoSilvi and soil ameliorant significantly influences the rate of mycorrhizal colonization on *A. chinensis* seedling species, while for *P. pinata* was not significantly different. *A. chinensis* seedling inoculated with MycoSilvi variant 1 and amended with lime and compost produced the best results with an average mycorrhizal colonization rate of 80%, while the best results of AM Fungi colonization in *P. pinnata* seedling was found in MycoSilvi variant 3 in combination with lime and compost with an average AM Fungi colonization rate of 48%, indicating that there was synergetic effect between lime and compost on the improvement of AM Fungi colonization. The improvement of AM fungi colonization on soil media amended by lime and compost due to the increased of soil pH and decreased Aluminum content (data not presented). This result support previous reports (Jayani *et al*., 2018, Budi *et al*., 2020). The AM Fungi colonization rate in both plants species grown in medium without amended with lime or compost were lower than in other treatment because it contains high aluminum content and low pH. This results in agreement with Agus *et al.* (2019) which stated that the rate of AM Fungi colonization of *P. pinata* was lower in mined-out soil medium than in forest soil medium, due to contamination by heavy metals in mined-out soil which may decreased soil pH. Similar results were also reported by Budi *et al.* (2020) in *Falcataria moluccana* seedling.

Role of AM Fungi on P accumulation

The lone factor of MycoSilvi inoculation and soil ameliorant addition and its interaction significantly influences the P accumulation of *A. chinensis* and *P. pinnata* seedlings (Table 1). *A. chinensis* inoculated with MycoSilvi variant 1, 2 and 3 increased P accumulation by 128.57%, 200% and 428.5% respectively as compared to uninoculated plant, while in *P. pinata* seedling with the same treatment increased their P accumulation by 63.3%, 60.2% and 68.4% respectively as compared to uninoculated plant. Lime exhibited positive effect on P accumulation of *A. chinensis* and *P. pinata* with increased by 257% and 65.3% repectively, while compost contribute to P accumulation by 471.4% and 46.9% on *A. chinensis* and *P. pinnata* seedlings respectively. In general the combination treatments of MycoSilvi, lime and compost had higher P accumulation than others treatments. The combination treatment of MycoSilvi variant 3, lime and compost produced the best P accumulation in *A. chinensis* and *P. pinata* with increased by 1557% and 191.8% repectively as compared to uninoculated plant (Table 1). The increased of P accumulation on several leguminous mycorrhizal plants have been reported by several researchers (Maulana *et al*., 2017; Wulandari *et al*., 2016). It is well known that AM fungi plays an important role on increasing P uptake due to phosphatase enzyme production. According to Sato *et al*. (2015), the extraradical hyphae of AM fungi can release acid phosphatase enzyme in to the hyphosphere. These enzymes play an important role in mineralization fixed P into useful forms, which can be easily absorbed by plants roots (Rhicardson *et al.,* 2009).

Role of AM fungi on biomass and Chlorophyll content

The study also showed that the interaction of MycoSilvi and soil ameliorant significantly increased total plant biomass and chlorophyll content as expressed by leaf greenness index on *A. chinensis* except on biomass of *P. pinnata* is not significantly different (Table 2). Total biomass of *A. chinensis* and *P. pinnata* inoculated by MycoSilvi variant 1, 2 and 3 increased significantly by 180%, 310% and 440% respectively for *A. chinensis* and 43.35%, 57.51% and 77.68% respectively for *P. pinnata* compared to un inoculated plant. While the total chlorophyll content of *A. chinensis* increased by 117.19%, 146.61% and 227.15% respectively and in *P. pinnata* increased by 14.29%, 24.01% and 26.49% respectively compared to un inoculated plant. According to Percival *et al.* (2008), leaf greenness index is a good indicator for N, carotenoid and leaf chlorophyll content. Similar results have been reported by Arumugam *et al*. (2010) on *Vigna unguiculata* and by Sharma *et al.* (2017) on *Vigna mungo* due to increased phosphatase activity in plant inoculated by AMF. Since the chlorophyll plays an essential role for plant photosynthesis, increased chlorophyll content in the leaf cause increase in plant biomass (Table 2).

Correlation between AM Fungi colonization and P acumulation and Chlorophyl content

Positive correlation between AM Fungi colonization rate and biomass P accumulation were observed for *A. chinensis* (R^2 = 0.3181, P< 001), and *P. pinata* (R^2 = 0.3152, P< 001) (Figure 1 & Figure 2). This result exhibited that the increase of P absorption could be related to the degree of AM fungi colonization. This data is in agreement with previous reports (Fini & Ferrini, 2011; Mirkalaei *et al.,* 2013; El-Kinany *et al.,* 2019). According to Furtini-Neto *et al.* (2004), liming reduced the fixation of P by Al and Fe, stimulated roots growth and finally increased nutrient uptake by plant roots. Our study also confirms the previous reports on *Albizia saman* and *Mollatus paniculatus* (Dewi *et al*., 2014), *Paraserianthes falcataria* and *A. saman* (Dewi *et al.,* 2016) and in four legunimous trees;

Table 2. Effect of MycoSilvi and soil ameliorant on biomass and total chlorophyll content expressed by leaf greenness index of *A. chinensis* and *P. pinnata* (12 weeks after planting)

Treatment	Total Biomass (g)		Leaf Greenness Index SPAD	
	A. chinensis	*P. pinnata*	*A. chinensis*	*P. pinnata*
M0L0C0	0.20 ± 0.14f	2.33 ± 0.34g	4.42 ± 1.09g	31.07 ± 1.21h
M0L1C0	0.65 ± 0.18ef	3.45 ± 1.42fg	11.35 ± 1.41f	32.43 ± 1.07gh
M0L0C1	1.06 ± 0.09de	3.64 ± 0.42ef	14.40 ± 1.32e	34.13 ± 1.01fg
M0L1C1	1.12 ± 0.23de	4.25 ± 1.33f	14.68 ± 1.05e	33.97 ± 0.57fg
M1L0C0	0.56 ± 0.20ef	3.34 ± 0.41fg	9.60 ± 0.46f	35.50 ± 1.32ef
M1L1C0	1.43 ± 0.37cd	3.85 ± 0.57def	13.13 ± 0.32e	38.33 ± 1.76cd
M1L0C1	1.47 ± 0.79cd	4.32 ± 0.37bcdef	17.83 ± 1.48cd	37.00 ± 0.46de
M1L1C1	2.36 ± 0.47ab	5.40 ± 0.68abc	19.83 ± 1.15ab	44.90 ± 0.46a
M2L0C0	0.82 ± 0.06e	3.67 ± 0.42ef	10.90 ± 1.03f	38.53 ± 1.56cd
M2L1C0	1.70 ± 0.10c	3.92 ± 1.10def	16.97 ± 0.46d	39.17 ± 1.76c
M2L0C1	1.97 ± 0.33bc	4.74 ± 0.47abcde	18.53 ± 0.90bcd	41.97 ± 1.32b
M2L1C1	2.60 ± 0.63a	5.51 ± 0.28ab	20.70 ± 1.38a	46.00 ± 1.11a
M3L0C0	1.08 ± 0.16de	4.14 ± 0.05def	16.67 ± 0.60d	39.30 ± 0.96c
M3L1C0	2.59 ± 0.46a	4.32 ± 1.63bcdef	17.93 ± 0.40bcd	40.00 ± 0.61c
M3L0C1	2.38 ± 0.75ab	5.02 ± 0.72abcd	19.40 ± 1.13bc	42.63 ± 0.64b
M3L1C1	2.82 ± 0.48a	5.71 ± 0.82a	21.20 ± 1.00a	46.33 ± 0.76a
Significancy				
M	**	**	**	**
LC	**	**	****	
M x LC	*	ns	**	**

M0, control; M1, MycoSilvi variant 1; M2, MycoSilvi variant 2; M3, MycoSilvi variant 3; L0, without lime; L1, lime 2.078 g; C0, without compost; C1, compost 20 g. Each value is mean of five replicates ± SD. Values in column followed by same letter are not significantly different (P ≤ 0.5). ** = P ≤ 0.01; * = 0.01 < P ≤ 0.05 and ns = P > 0.05.

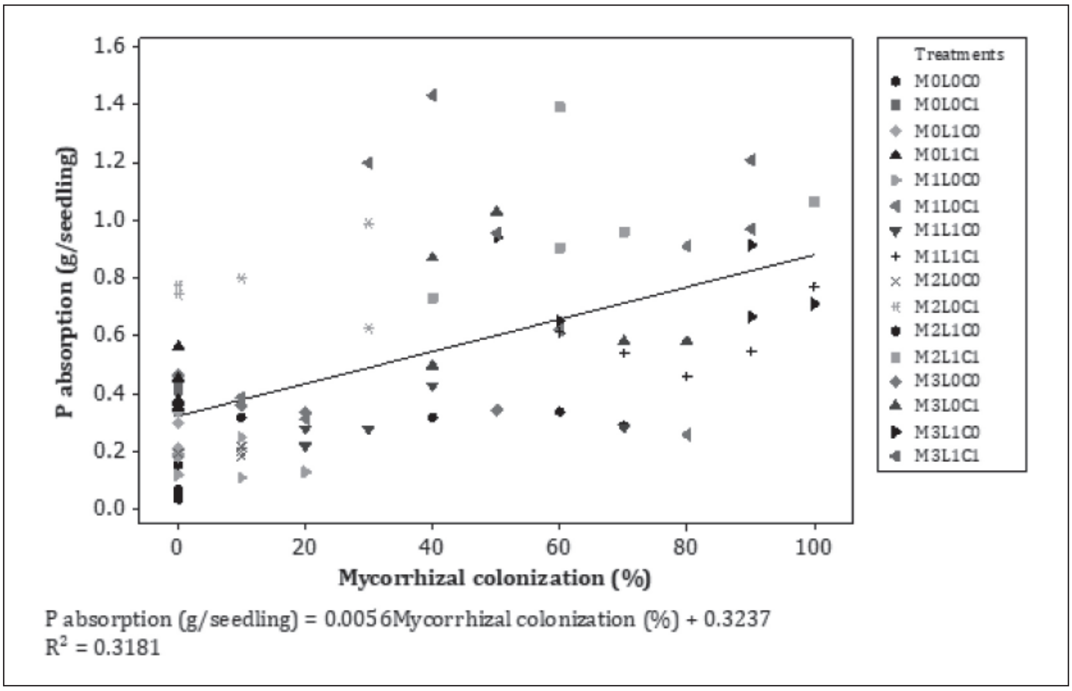

Fig. 1. Correlation between AM Fungi colonization rate and P accumulation of *A. chinensis.*

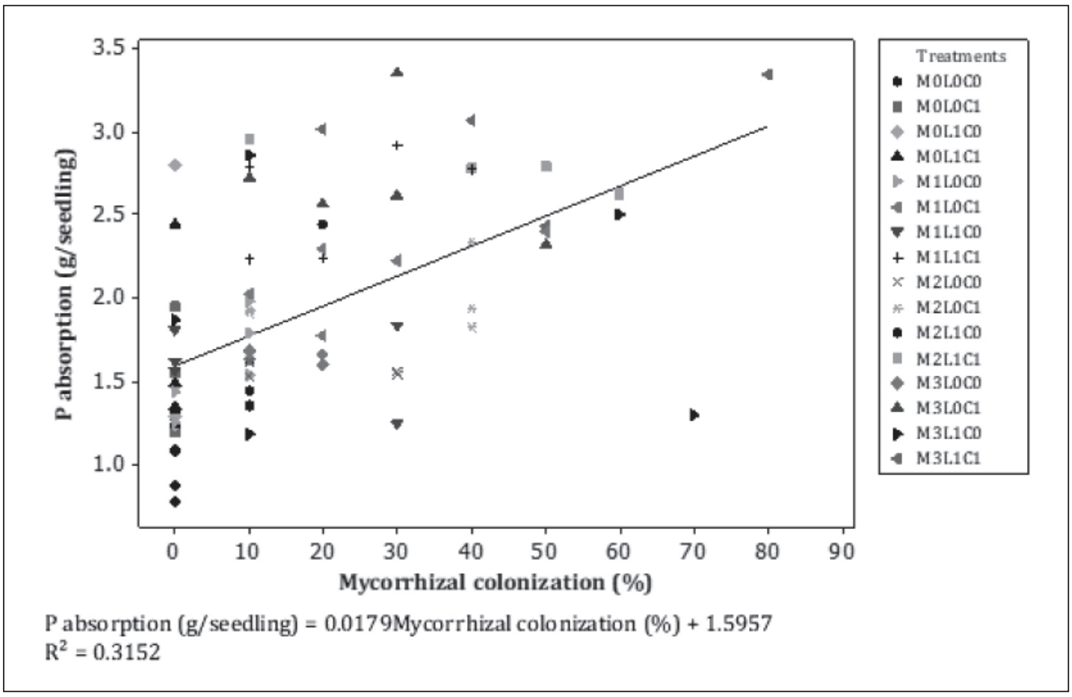

Fig. 2. Correlation between AM Fungi colonization rate and P accumulation of *P. pinata.*

P. falcataria, Calliandra caothyrsus, Cassia siamea and *Sesbania grandiflora* (Maulana *et al*., 2017), which sated the positive correlation between mycorrhizal roots colonization and P accumulation. AM Fungi colonization also positively correlated with chlorophyll content of *A. chinensis* ($R^2 = 0.413$, P< 001), and *P. pinata* ($R^2 = 0.3885$, P< 001) (Figure 3 & Figure 4). According to Zhu *et al.* (2014), the increase in chlorophyll content in mycorrhizal plant is related to the increase in absorption of P and Mg from the soil. This support our data, which demonstrate that P absorption is positively correlated with AM Fungi colonization.

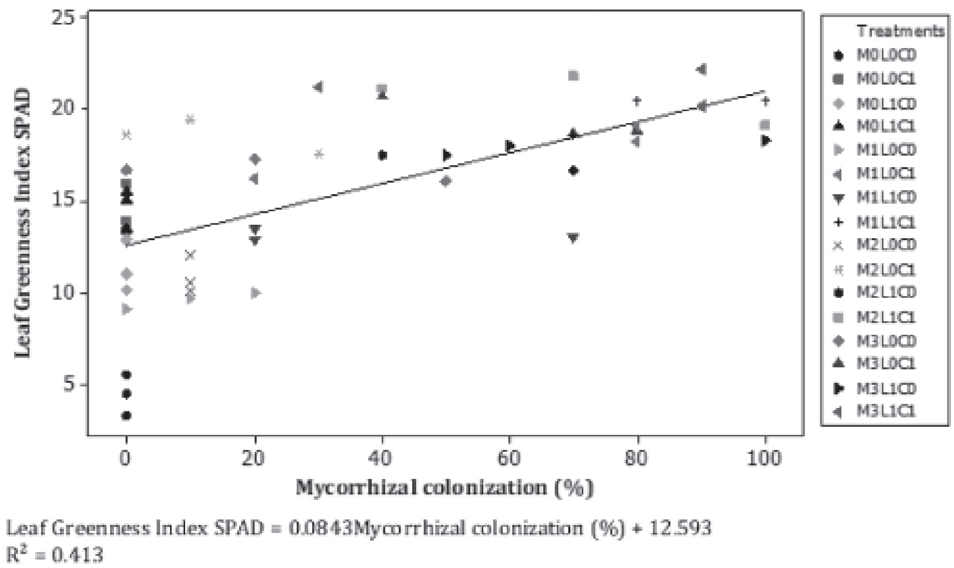

Fig. 3. Correlation between AM Fungi colonization rate and Chlorophyl content of *A. chinensis.*

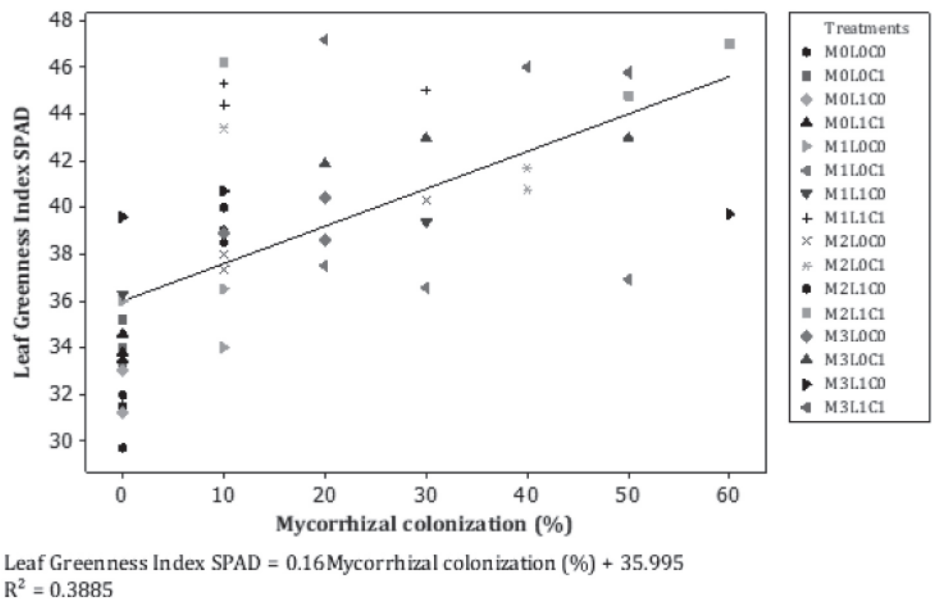

Fig. 4. Correlation between AM Fungi colonization rate and Chlorophyl content of *P. pinata.*

Role of AM Fungi on plant growth

Data for seedling height and steam diameter growth is presented in Table 3. There was a significant interaction between MycoSilvi inoculation treatment and soil ameliorant addition to the soil media ($p< 0.05$) for *A. chinensis* while for *P. pinnata* there was no significant difference. *A. chinensis* inoculated by MycoSilvi variant 1, 2 and 3 significantly increased height growth by 185.7%, 210% and 248.57% respectively and steam diameter growth by 362.96%, 401.85% and 520.37% respectively compared to control plants. Plant height and diameter of *A. chinensis* inoculated by three MycoSilvi variants was not significantly different. The addition of lime to soil media increased significantly height and steam diameter growth of *A. chinensis* by 415.71% and 668.52% respectively compared to the control, and higher than those plant inoculated by MycoSilvi alone. Interactions of lime and MycoSilvi significantly

Table 3. Effect of MycoSilvi and soil ameliorant on height and steam diameter of *A. chinensis* and *P. pinnata* (12 weeks after planting)

Treatment	Height growth (cm)		Steam diameter growth (mm)	
	A. chinensis	*P. pinnata*	*A. chinensis*	*P. pinnata*
M0L0C0	1.40 ± 0.96e	3.36 ± 1.07f	0.11 ± 0.08i	1.02 ± 0.22f
M0L1C0	7.22 ± 1.51c	9.10 ± 2.30de	0.84 ± 0.07defg	1.54 ± 0.48cdef
M0L0C1	10.12 ± 0.28b	9.86 ± 2.12cde	0.77 ± 0.18efgh	1.91 ± 0.61abcd
M0L1C1	10.76 ± 0.38b	10.48 ± 1.71bcd	0.98 ± 0.24bcde	1.65 ± 0.57bcde
M1L0C0	4.00 ± 1.80d	5.56 ± 2.87ef	0.50 ± 0.04h	1.10 ± 0.38f
M1L1C0	10.32 ± 1.51b	13.78 ± 3.71abc	1.13 ± 0.33abcd	1.68 ± 0.33bcde
M1L0C1	10.32 ± 2.38b	11.34 ± 3.88bcd	0.84 ± 0.38defg	1.83 ± 0.32de
M1L1C1	14.68 ± 4.25a	14.00 ± 3.506abc	1.25 ± 0.41ab	1.88 ± 0.25abc
M2L0C0	4.34 ± 0.74d	8.42 ± 3.25de	0.54 ± 0.05gh	1.33 ± 0.23abcd
M2L1C0	10.82 ± 0.28b	14.66 ± 1.52ab	1.17 ± 0.04abc	1.81 ± 0.35ef
M2L0C1	10.72 ± 1.64b	11.48 ± 4.06bcd	0.93 ± 0.29cdef	2.07 ± 0.09abc
M2L1C1	15.92 ± 0.49a	14.86 ± 3.29ab	1.28 ± 0.20ab	1.91 ± 0.45abcd
M3L0C0	4.88 ± 0.50d	8.58 ± 2.45de	0.67 ± 0.11fgh	1.37 ± 0.14def
M3L1C0	15.28 ± 2.44a	16.34 ± 3.16a	1.21 ± 0.24abc	1.90 ± 0.22abcd
M3L0C1	10.90 ± 1.36b	13.92 ± 1.58abc	0.98 ± 0.10bcde	2.11 ± 0.24ab
M3L1C1	16.68 ± 1.68a	17.06 ± 3.08a	1.36 ± 0.14a	2.28 ± 0.44a
Significancy				
M	**	**	**	*
LC	**	**	**	**
M x LC	*	ns	*	ns

M0, control; M1, MycoSilvi variant 1; M2, MycoSilvi variant 2; M3, MycoSilvi variant 3; L0, without lime; L1, lime 2.078 g; C0, without compost; C1, compost 20 g. Each value is mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.5$). ** = $P \leq 0.01$; * = $0.01 < P \leq 0.05$ and ns = $P > 0.05$.

increased height and steam diameter growth of *A. chinensis*. Combination treatment lime and MycoSilvi variant 1, 2 and 3 increased height growth of *A. chinensis* by 637.34%, 672.86% and 991.43% respectively and steam diameter growth by 944.44%, 985% and 1020.37% respectively compared to control plant. These results demonstrated that there was synergetic effect between MycoSilvi and lime for improving plant growth, while MycoSilvi variant 3 gave the best results.

The addition of compost to soil media increased height and steam diameter growth significantly of *A. chinensis* by 622.86% and 614.81% compared to control plant and higher than plant inoculated by MycoSilvi alone or soil media added by lime. There is no significant difference on the interaction of compost and Mycosilvi to the plant height and steam diameter growth, even though the combination of compost and Mycosilvi variant 1, 2, 3 increased plant height and steam diameter. Similar to this result, treatment with compost alone also gave no significant difference, indicating there is no synergetic effect between compost and MycoSilvi. Nevertheless, synergetic effect was obtained when compost, lime and MycoSilvi were combined. The MycoSilvi inoculation significantly influenced the growth of *P. pinnata* seedlings (Table 3). *P. pinnata* inoculated by MycoSilvi variant 1, 2 and 3 increased height by 65.48%, 150.60% and 155.36% respectively and steam diameter growth by 7.84%, 30.39% and 34.31% respectively compared to control plant. *P. pinnata* treated with MycoSilvi and soil ameliorant gave similar resulta as *A. chinensis*. Synergetic effect was observed when compost, lime and MycoSilvi were combined.

Previous study demonstrated that soil ameliorant could improve acid soil properties and plant growth (Teshome *et al.,* 2017). Our results demonstrate that the addition of lime and compost increased soil pH and decreased Al-dd in soil medium (data not presented). Plant growth is influenced by genetic and environmental factor such as sunlight, water and nutrients both macro and micro. In nature, the presence of sunlight and water is very abundant, but the presence of nutrients is often become limiting factor for plant growth, especially in acidic soil and in post mining soil. In this study, post-mining soil used as growing media categorized as very low-fertility soil. The chemical characteristics of these soil are; pH 3.20, C-organic 4.21%, P available 13.78 ppm and total P 278.04 ppm and Al 690 mg/kg. The low pH and high aluminum content in these soil growing media become limiting factors for plant growth. In this study, the growth of *A. chinensis* and *P. pinnata* are very poor in control treatment. Incoulation of MycoSilvi to the plant resulted in increasing plant height and steam diameter of *A. chinensis* and *P. pinnata* (Table 3). MycoSilvi is a biological fertilizer product containing arbuscular mycorrhizal fungi. Those fungi is widely used as natural biofertilizers (Berruti *et al.,* 2016) due to their contribution to alleviate water stress (Sharma *et al.,* 2017), and aluminium stress (Alori & Fawole, 2012), prevent nutrient loss from the soil (Kohl & van der Heijden, 2016) increasing phosphate uptake (Hart & Forsythe, 2012), increasing nitrogen uptake (Bucking & Kafle, 2015), improve soil chemical properties (Pal & Pandey, 2017) and improve soil agregation (Borie *et al.,* 2008). The use of lime in acidic soil to reduced Al toxicity were well documented (Muindi *et al.,* 2015; Teshome *et al.,* 2017). In this study, the application of lime increased height and steam diameter growth significantly in both plants (Table 3), increased soil pH and reduced Aluminium content (data not shown). This result confirms the study by Bambara and Ndakidemi (2009), showing that lime increased the rate of photosynthesis in *Phaseolus vulgaris* L. Our finding suggests that the combination of lime and MycoSilvi synergistically increase height and steam diameter growth of both plant trees species. Our results are contrary to Guo *et al.* (2010), who found that there was no synergetic effect when lime and AMF applied in acid purplish soil. This is due to different chemical soil properties used. In our study the pH H_2O of soil media was 3.20; P (Bray I) = 13.78; P (HCl 25%) = 278.04 ppm and Al-dd = 690 mg/kg while Guo *et al.* (2010) used soil media with low Aluminum content (0.7 mg/kg) and pH 5.45, and after lime treatment, the pH increased to 6.45. Probably by increasing soil pH, the soluble soil P would be quite high which may inhibit mycorrhizal colonization and plant growth. According to Amijee *et al.* (1989), the soluble soil P 140 ppm inhibited mycorrhizal colonization, while in our study the addition of lime increased soluble soil P between 1.56–3.27 ppm (data not presented). In acidic soil, Phosphorus is bound by Aluminum and cannot be absorbed by the roots of the plant. With the addition of MycoSilvi, ion phosphate can be released through an enzymatic phosphatase process which is released by the AMF (Sharma *et al.,* 2017). Furthermore, according to Jung *et al.* (2003), mycorrhizal plant can decrease heavy metals content by organic acids which exudates by plant roots. The application of lime aims to increase soil pH and liberate soil Phosphorous which is bound by aluminum, thus becomes available and can be absorbed by the roots of the plant.

The positive effects of compost amendment on degraded soil are well known. Our study demonstrated that acidic soil amended with compost increased significantly height and steam diameter growth of *A. chinensis* and *P. pinnata* (Table 3). This data is in accord with Mirkalaei *et al.* (2013) and Mrabet *et al.* (2014), describing significant plant growth upon addition of compos to soil media. However, the combination of compost and MycoSilvi had only slight additive effect on height and steam diameter growth of both plant which is in agreement with previous reports (Mrabet *et al.,* 2014). Meanwhile, when three treatments were combined, synergetic effect was determined. *A. chinensis* inoculated by MycoSilvi variant 1, 2 and 3 grown in soil media incorporated by compost and lime significantly increase height growth by 948.57%, 1037.14% and 1091.43% respectively and steam diameter growth by 1057.41%, 1085.19% and 1159.26% respectively as compared to control plant and *P. pinnata* inoculated by MycoSilvi variant 1, 2 and 3 grown in soil media incorporated with compost and lime increase height growth by 316.67%, 342.26% and 407.74% respectively, and steam diameter growth by 84.31%, 87.25% and 123.53% respectively as compared to control. Indicating that the role of AMF and lime is very important. It also important to note that the combination of MycoSilvi variant 3, lime and compost gave the best results for improving height and steam diameter growth of *A. chinensis* and *P. pinnata.* This due to the number of AMF species in MycoSilvi variant 3 higher than in MycoSilvi variant 1 and 2. These results were in agreement with the previous finding that Cucumber seedling inoculated with combination of five and three AMF species had better growth than inoculated by single species (Chen *et al.,* 2017). Similar results also reported by Kohl and van der Heijden (2016) that AMF variantied in their effects on plant nutrient acquisition and growth, and Budi *et al.* (2020), stated that MycoSilvi varaint 3 gave the best result on *F. moluccana* growth. In addition MycoSilvi variant 3 gave the best in the improvement on P accumulation and chlorophyll content of both two tropical tree species which have been discussed previously. The growth performance of *A. chinensis* and *P. pinata* presented in Figure 5 and Figure 6.

CONCLUSION

MycoSilvi inoculation colonized the roots of *A. chinensis* and *P. pinnata* and positively correlated with P accumulation and leaf greenness index of both plant species. MycoSilvi variant 2 which contained two AMF species gave the best mycorrhizal roots colonization (18%) in *A. chinensis* while MycoSilvi variant 3 which contain three AMF species gave the best mycorrhizal roots colonization (20%) in *P. pinnata*. The addition of lime and compost increased mycoorhizal roots colonization in both plant species. The best mycorrhizal roots

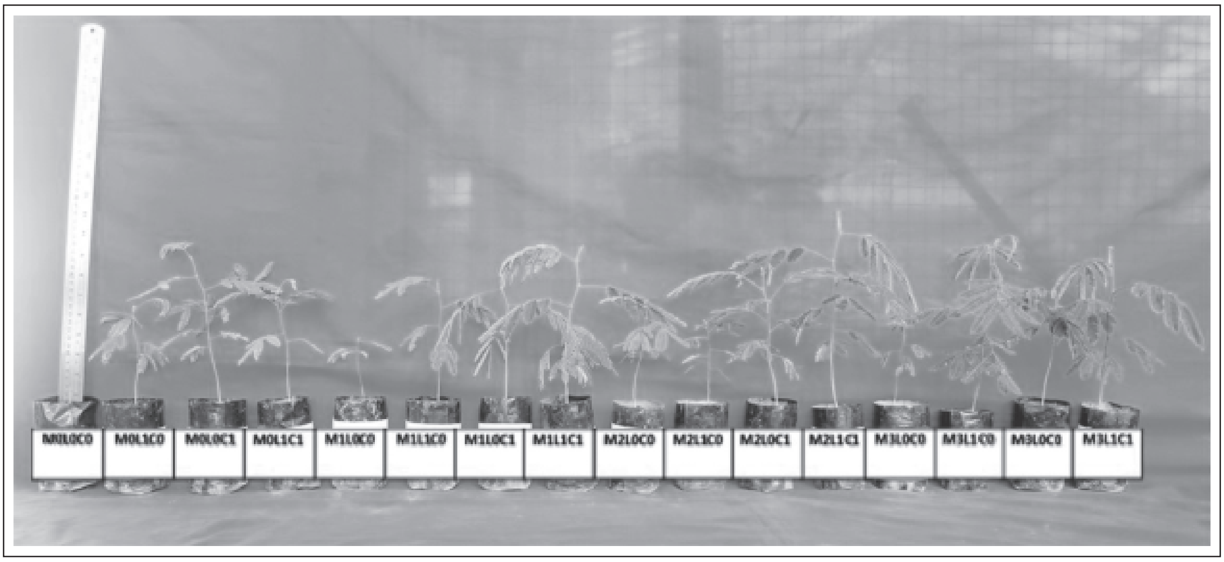

Fig. 5. Growth performance of *A. chinensis* as affected by MycoSilvi and soil ameliorant. M0= Without MycoSilvi; M1= MycoSilvi variant 1; M2=MycoSilvi variant 2; M3=MycoSilvi variant 3; L0= without lime; L1: with lime; C0= without compost; C1 = with compost.

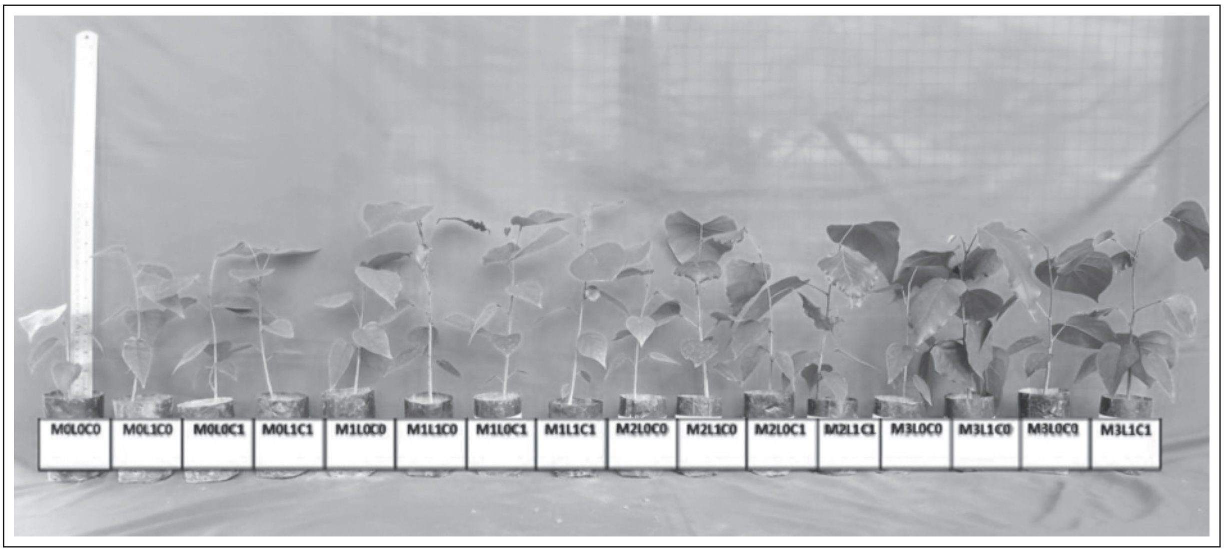

Fig. 6. Growth performance of *P. pinata* as affected by MycoSilvi and soil ameliorant. M0= Without MycoSilvi; M1= MycoSilvi variant 1; M2=MycoSilvi variant 2; M3=MycoSilvi variant 3; L0= without lime; L1: with lime; C0= without compost; C1 = with compost.

colonization (80%) obtained from the combination of lime and compost with MycoSilvi variant 1 in *A. chinensis* and the combination of lime and compost with MycoSilvi variant 3 in *P. pinnata*. The highest P accumulation, leaf greenness index and total biomass growth were obtained from the combination of lime and compost with MycoSilvi variant 3, with the value of 1.6 g/seedling, 21.20, and 2.82 g in *A. chinensis* respectively and 2.86 g/seedling, 46.33 and 5.71 g respectively in *P. pinnata*. The important findings of this research is that post mining soil with low pH and high Aluminium content could be improved for *A. chinensis* and *P. pinnata* plant growth by application of MycoSilvi, lime and compost alone or in combination. The positive effect of MycoSilvi or compost can be maximized by addition of lime. These findings imply the prospective and potential use of MycoSilvi and soil ameliorant for the succesful of post mine land rehabilitation.

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