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The Role of MycoSilvi, Lime, and Compost on The Growth of Balsa (*Ochroma bicolor* Rowlee.) Seedling in Post Silica Sand Mine Media

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Abstract. Problems arising from silica sand mining are decreasing soil fertility, as well as the presence of high heavy metals such as Fe and Al, which inhibits revegetation plant growth. The purpose of this study was to analyze the growth of balsa seedlings (*Ochroma bicolor* Rowlee.) on silica sand post-mining media treated with MycoSilvi, compost, and lime, and to determine the most optimal combination of MycoSilvi, compost, and lime treatment. This study was designed using a completely randomized design (CRD) with a split-plot design with 3 treatment factors, namely the addition of MycoSilvi, compost, and lime. Each treatment consisted of five replications. The results showed that the untreated (control) was not able to support the balsa seedling's growth. The interaction between MycoSilvi, compost, and lime significantly affected the height, diameter, biomass, and percentage of mycorrhizal colonization parameters. The combination of MycoSilvi type 1 treatment, namely *Glomus mosseae* and lime (C0K1M1) resulting in the highest total growth rate, diameter, biomass, and colonization percentage compared to other treatments. The addition of lime and compost to the growth medium can reduce the degree of mycorrhizal dependency of balsa seedling.

Keyword: Compost, Lime, MycoSilvi, Mycorrhizal Dependency, Post-mine

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1 Introduction

Mining activities in Indonesia have increased every year. Before 1970, mining activities were generally still on a relatively small scale [1], but since the mid-1970s larges scale mining activities began. As one of the natural resources exploitation activities, mining activities can have positive or negative impacts [2]. The positive impact is increasing human needs for energy and provides devices for our country. While of the negative impact is soil damage. The soil will damage after mining activities, while an open mining system can reduce the soil fertility and soil structure [3].

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One of the mining activities is silica sand mining. According to the Central Statistics Agency [4], the sand mining materials in 2015 amounted to 59,709 business unit, in 2017 is 59,540 business units. Mining activities of quartz sand in 2015 were 414 business units and decreased in 2017 to 185 business units. Silica and sand mining often commonly resulted from open mining system. Silica sand is a mining product consisting of silica crystals (SiO_2) and contains other compounds that are carried during the mining process. The composition of silica sand is a combination of SiO_2, Fe_2O_3, Al_2O_3, TiO_2, CaO, MgO, and K_2O [5]. This mining material is widely used for cement-making mixtures. Problems arising from silica sand mining are limited nutrient in soils with low pH and organic matter content, high soil density, and the presence of heavy metals such as Al and Fe. These conditions can inhibit plant growth because it causes many plant cells to die [6].

Improving soil conditions and damaged vegetation by mining activities can be conducted through revegetation activities. One plant species that are commonly used for revegetation activities is balsa (*Ochroma bicolor* Rowlee.). Balsa is a fast-growing species belonging to a member of the Malvaceae family. However, for the success of the revegetation effort, it is necessary to improve the growth site. Previous studies have shown that soil improvement can be done through soil enhancers addition using compost [7], or dolomite [8]-[10] which have functions to add nutrients to the soil and improve soil properties and increase soil pH. The use of microbes especially arbuscular mycorrhizal fungi (FMA) and biological fertilizers (MycoSilvi) has also been shown to increase plant growth in infertile soils [7], [11], [12]. MycoSilvi is an AMF inoculum enriched with Mycorrhizal Helper Bacteria (MHBs) [8]. The results of [11] study showed that MHBs were able to increase the colonization of AMF in plants, and enhance spore formation process. Thus, MycoSilvi has an advantage compared to other biological fertilizer inoculums based on FMA and zeolites. This study aims to analyze the response of balsa (O. *bicolor*) seedlings growth inoculated with various types of MycoSilvi, in the post-silica sand soil media by adding organic Argo Flower compost produced by CV. Surya Gemilang Bogor and dolomite lime.

2 Research Procedure

This research was conducted in May 2018 to February 2019, in a greenhouse of the Mycorrhizal Technology Laboratory and Seed Quality Improvement owned by Silviculture Department, Faculty of Forestry, Bogor Agricultural University. The post-silica sand mining was taken PT Holcim Indonesia Tbk, Cibadak, Sukabumi-West Java. Soil was taken at a depth of 0-20 cm from four different points. Then the soil is compiled to produce one soil sample for physical and chemical analysis. Analysis of physical and chemical soil properties consist of soil texture using the pipette method, soil pH, C-organic by the Walkey and Black method, P and K by the 25% HCl extract method, exchanged bases of Ca, Mg, Na, K, base saturation and CEC with NH_4OAc extract method with pH 7, exchanged acidity, Al-dd and H-dd 1M KCl extract, levels of Fe-d

extract of dithionite-citrate extract [13]. At the end of the study, pH analysis of each growth media was conducted. Analysis of chemical and physical soil properties conducted in Soil Science Laboratory, Faculty of Agriculture, IPB University, Bogor.

The MycoSilvi inoculum in this study consisted of 3 types, those were inoculum fungal arbuscular mycorrhizae containing the fungus *Arbuscular mycorrhizae Glomus mosseae*, (type 1), inoculum containing G. *mosseae* and *Acaulopspora* sp. (type 2) and inoculum containing

G. mosseae and Gigaspora margarita (type 3). The inoculum was propagated on zeolite media with host plant *Pureria javanica* and kept in a greenhouse for two months. The three types of FMA obtained from the deposit of Mycorrhizal Technology Laboratory and Seed Quality Improvement, Department of Silviculture, Faculty of Forestry, IPB University. The 10-gram MycoSilvi inoculum was taken as 10 grams and then the number of spores was calculated as a basis for determining the dose of MycoSilvi inoculums.

Balsa seeds are obtained from the Center for Forest Research and Development, Ministry of Environment and Forestry, Bogor. Seeds soaked in hot water with a temperature of 80 ^{o}C for 15 minutes and cold water for 24 hours. The soaked seeds are then sown on a zeolite medium that was previously moistened with water spray. Seeds that have been germinated are watered every morning and evening.

The soil that was used as a planting medium is put in a heat-resistant plastic and then sterilized by putting it in an autoclave for 1 hour with a temperature of 121 ^{o}C and a pressure of 1 atm. Then the planting media is added with compost and dolomite lime according to the treatment. The soil weight on a polybag measuring 15 cm x 20 cm is 650 g/polybag. The dosage of compost used for treatment is 5% of the weight of polybags or 32.5 g/polybag and dolomite lime dose of 7.2 g/polybag [8]. Seedlings that have germinated, have high and homogeneous health are then planted on prepared media. MycoSilvi inoculation is done by placing MycoSilvi around the seedlings roots according to treatment, then filled with soil media.

The observed parameters in this study were plant height, stem diameter, biomass, percentage of mycorrhizal colonization, plant growth response and plant dependence on mycorrhizae. Parameters were measured at 16 weeks after planting (WAP). Plant height measure using a ruler from the base of stem to the tip where the shoots grow. Plant diameter measured using caliper at a height of 1 cm above the base of the seedling stem. Measurement of plant biomass was done by then weighing wet sample (roots and shoot plants) weight before the oven. After being put into the envelope, the roots and shoot plants are dried in an oven at 80 ^{o}C for 72 hours until a constant dry weight is achieved. Observation of FMA colonization based on [14] methods with the coloring stages, 1) roots are washed thoroughly with distilled water; 2) roots soaked in 5% KOH for 48 hours; 3) then roots are washed with water thoroughly using a filter, then soaked in 0.1 M HCL solution, without washing; 4) roots are soaked in aniline blue dye solution for 5

days; 5) roots soaked with staining solution for 24 hours; 6) roots are cut ± 1 cm in size, then roots are arranged parallel to glass object and covered with a slipcover, the number of roots in each glass object (one glass object or treatment for 10 pieces of root). Each treatment consisted of 5 replications, then observed with a compound microscope. Root on glass preparations was observed in every section. The observed infected area is indicated the presence of spores, hyphae, arbuscular or vesicles. The percentage of root colonization was calculated using [15].

$$\%\text{Colonization} = \frac{\sum \text{colonized field of view}}{\sum \text{whole field of view}} \text{ x } 100\% \quad (1)$$

The percentage of FMA colonization is classified based on [16] classification, that is, 0%: not colonized, <10%: low, 10-30%: moderate, and> 30%: high. Growth Response (GR) is calculated [17] formula.

$$GR(\%) = \frac{Dry\ weight\ of\ plants\ with\ mycorrhizae - Dry\ weight\ of\ plants\ without\ mycorrhizae}{Dry\ weight\ of\ plants\ without\ mycorrhizae} \text{x}100 \quad (2)$$

Mycorrhiza Dependency (MD) dependence was measured by [18] formula

$$MD\ (\%) = \frac{Dry\ Weight\ of\ plants\ with\ mycorrhiza - Dry\ weight\ of\ plants\ without\ mycorrhizae}{Dry\ weight\ of\ plants\ with\ mycorrhizae} \text{ x } 100 \quad (3)$$

The research designed using a completely randomized design (CRD) factorial pattern with 3 treatment factors. The first factor is MycoSilvi (M) treatment consisted of four levels (M0, M1, M2, and M3). The second factor is compost (C) addition, which consists of two levels (C0 and C1). The third factor is dolomite lime (K) addition consists of two levels (K0 and K1). Each treatment was repeated five times. The data obtained were processed using Microsoft Excel software and SAS 9.4 software. The effect of treatment on the observed variables was carried out using analysis of variance (ANOVA) at a 95% confidence interval.

3 Result and Discussion

The physical and chemical properties analysis of post-mining soil obtained from PT Holcim Indonesia Tbk. presented in Table 1. Based on Table 1, the main problem of post-silica sand mining soil is the low availability of nutrients with high Al and Fe content that is toxic to plants. This condition will inhibit the plant's growth [6]. The observations showed that plant growth in soils without treatment, either dolomite lime or compost could not support balsa growth. It can be shown in Figure 1, where no balsa seedling can growth in those media. High Al content in growth media can poisoning plant root cells through various mechanisms, namely (1) damage to plasma membrane caused by increased lipid peroxidase triggered by high Fe, (2) mitochondrial dysfunction due to production of Reactive Oxygen Species (ROS) and (3) the destruction of vacuoles which destroys the integrity of the plasma membrane [6].

Table 1 Physical and chemical properties of soil

Nature of soil	Analysis result	Criteria	Source
pH (H_2O)	2.99	Very acid	[13]
pH (KCL)	2.65	Very acid	[13]
C-organic (%)	1.51	Low	[13]
N-total (%)	0.20	Low	[13]
P-total (ppm)	89.33	Very high	[13]
Ca (cmol/kg)	2.50	Low	[19]
Mg (cmol/kg)	1.42	Medium	[19]
K (cmol/kg)	0.04	Very low	[19]
Na (cmol/kg)	0.06	Very low	[19]
KTK (cmol/kg)	16.07	Low	[19]
KB (%)	25.03	Low	[13]
Al (cmol/kg)	7.94	Very high	[13]
Fe (ppm)	286.81	Very high	[13]
Cu (ppm)	2.04	Normal	[13]
Zn (ppm)	3.29	Normal	[13]
Sand (%)	22.03		
Dust (%)	30.83		
Clay (%)	47.14		

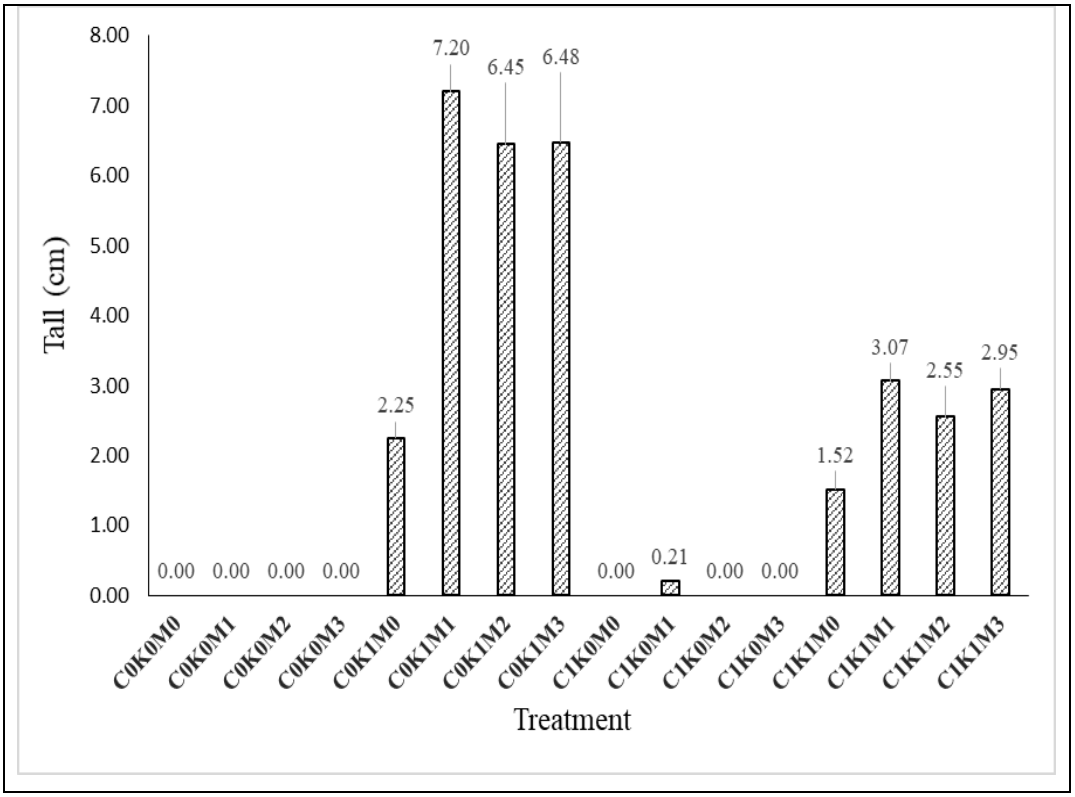

Figure 1 Growth rate of O. bicolor seedling after 16 WAP in all treatments. C0 = No compost; C1 = Compost 32.5 g; K0 = without dolomite lime; K1 dolomite lime 7.2 g; M1 = MycoSilvi type 1 type G. *mosseae*. (3 g); M2 = MycoSilvi type 2 type G. *mosseae* and *Acaulospora* sp; M3 = MycoSilvi type 3 type G. *mosseae* and G. *margarita*.

Dolomite addition on growing media can increase soil pH from very acidic to slightly acidic and normal (Table 2) and it produces the plants can grow well (Figure 1). The results of this study are in accordance with [8]. The pH value affects by CEC soil, on low CEC soils unable to

absorb and provide nutrients, especially P if dominated by acid cations, Al, H (low base saturation) can reduce soil fertility [19]. It was due to nutrients are present in a complex amount of colloidal sorption so that they are easily leached by water [19]. Height is a product of the primary growth that occurs due to apical meristem cell division on the tip and plant shoots that will form new tissues and organs in the vegetative phase. It indicates that height is strongly influenced by nutrient content that plays a role during the division of plant shoot cells [20]. Calcification will also produce hydroxyl (OH) ions which can bind acidic cations in the form of Al and H so that they become inactive [19]. The interesting about this study is dolomite addition combined with MycoSilvi both types 1, 2, and 3 can significantly increase growth in height, 182.4%, 152.9%, and 154.1% when compared with plants that were only given dolomite on the growing medium (Figure 1). This is because liming can increase soil pH (Table 2) so that it can increase the availability of nutrients needed for plant growth [16].

Table 2 Results of analysis of media pH at the end of the research

Treatment	pH*	Category*
C0K1M3	7.06	Normal
C1K1M3	6.95	Normal
C1K1M2	6.76	Normal
C1K1M1	6.70	Normal
C1K1M0	6.41	Rather acidic
C0K1M2	6.15	Rather acidic
C0K1M1	6.03	Rather acidic
C0K1M0	5.57	Very acid
C1K0M3	3.63	Very acid
C1K0M2	3.60	Very acid
C1K0M0	3.57	Very acid
C1K0M1	3.51	Very acid
C0K0M1	3.49	Very acid
C0K0M0	3.49	Very acid
C0K0M2	3.45	Very acid
C0K0M3	3.44	acid

*source [13]

Increased root growth causes the development of FMA properly and mycorrhizal colonization occurs as presented in Table 3. FMA with external hyphae can produce enzymatic acid phosphatase [21] so that it can help absorb P elements that were not initially available to become more available to plants.

Table 3 Percentage of mycorrhizal colonization of O. bicolor 16 MST seedlings in various treatments

Treatment	Average Mycorrhizal Colonization (%)	Criteria*

C0K1M1	94±5	High
C0K1M2	88±18	High
C1K1M1	86±17	High
C0K1M3	86±17	High
C1K1M3	76±11	High
C1K1M2	74±15	High
C1K0M1	20±9	Medium
C0K0M1	0	Not colonized
C0K0M2	0	Not colonized
C1K0M2	0	Not colonized
C0K0M3	0	Not colonized
C1K0M3	0	Not colonized

C0 = No compost; C1 = Compost 32.5 g; K0 = without dolomite lime; K1 = dolomite lime 7.2 g; M1 = MycoSilvi type 1 type G. *mosseae*. (3 g); M2 = MycoSilvi type 2 type G. *mosseae* and *Acaulospora* sp.; M3 = MycoSilvi type 3 type G. *mosseae* and G. *margarita*. * Source: [16]

In this research, the addition of compost to planting media has not been able to improve the characteristics of ex-mining soils, so that plant growth is disrupted (Figure 1). These results are not in line with several studies conducted by [7]-[8], [11]-[12] who reported that the addition of compost and other organic material with C/N <25 in acid soil media could increase plant growth. The difference in results of the study is assumed causing by the different compost types. In this study, the value of the C/N ratio in compost was very high at 83.43. According to [22] organic material that has a high C/N ratio indicates that the composting process is getting longer because it contains many compounds that have high molecular weight so that nutrients cannot be absorbed by plant roots. The use of organic material with a C/N ratio value > 25 in growing media causes the availability of soil N, P and K nutrients to decrease because it is absorbed and used by decomposer microbes for the decomposition of organic matter [22]. This is also evidenced from the results of this research, that the planting media were given a combination of compost and dolomite and inoculated with MycoSilvi resulting in a smaller growth height when compared to the combination of dolomite lime and MycoSilvi alone (Figure 1).

Measurement results for diameter and biomass parameters also show a linear response with heigh growth (Figures 2 and 3). Diameter growth is secondary growth that occurs due to lateral meristem tissue activity as in cambium cells. The activity of these cells inwardly forms xylem tissue and outwards forms phloem tissues are very influential in plant growth [20].

The addition of all MycoSilvi types and 7.2 g of dolomite to growing media resulted in better growth in height and diameter compared to other treatment combinations. Based on Figures 1 and 2, the average total growth rate of height and balsa diameter shows that the treatment without compost, 7.2 g dolomite lime, and MycoSilvi type 1 (C0K1M1) is the best treatment for increasing height and diameter growth (positive growth response) compared to control treatments (C0K0M0) and other treatments. This is indicated by a high growth rate of 7.20 cm and a diameter of 2.77 mm, within 16 weeks of observation.

According to research conducted by [23], mycorrhizae of G. margarita and G. etunicatum can increase growth in height, diameter and biomass of four legume plant species, namely *Albizia lebbeck*, *Enterolobium contorilisiliquum, Leucaena leucephala*, and *Sesbania virgate*. The growth is increased by the application of dolomite that can increase soil pH to increase the nutrients needed by plants to increase growth [24].

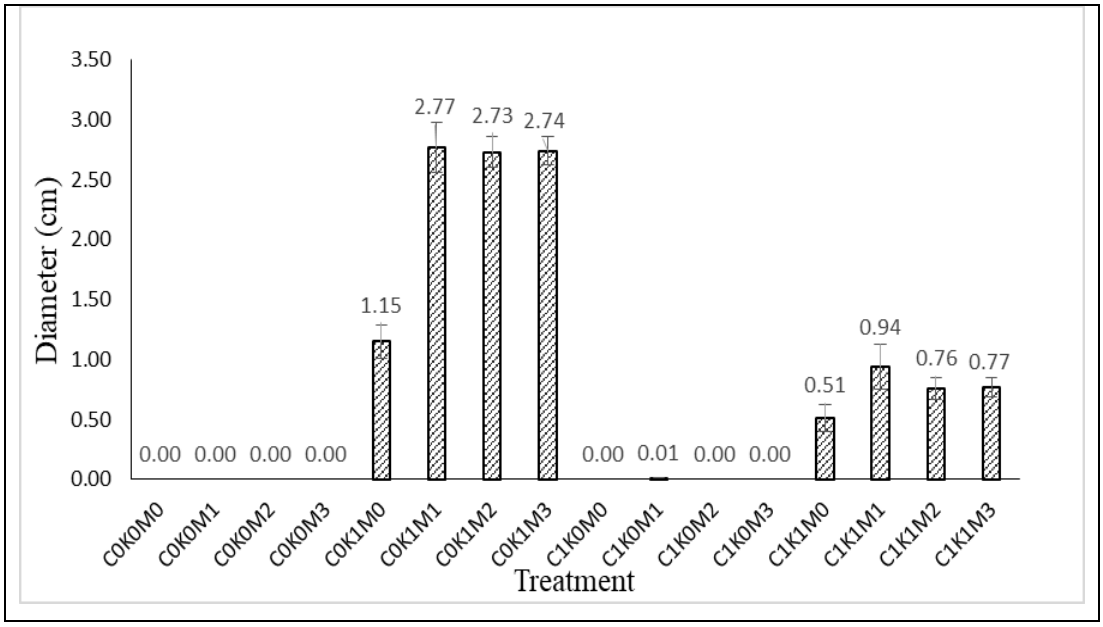

Figure 2 Total growth rate of O. bicolor diameter for 16 WAP in all treatments. C0 = No compost; C1 = Compost 32.5 g; K0 = without dolomite lime; K1 dolomite lime 7.2 g; M1 = MycoSilvi type 1 type G. *mosseae* (3 g); M2 = MycoSilvi type 2 type G. *mosseae* and *Acaulospora* sp .; M3 = MycoSilvi type 3 type G. *mosseae* and G. *margarita.*

Plant biomass is the total dry weight of all parts of living plants derived from the results of photosynthesis, nutrient uptake, and water treated through the biosynthetic process [20]. The content of plant biomass is strongly influenced by the number of nutrients absorbed from growing media for supporting plant growth. The addition of all types of MycoSilvi and 7.2 g dolomite has a positive relationship with the height and diameter growth rate and biomass content of plants. The addition of all types of MycoSilvi and dolomite lime 7.2 g treatment produced better biomass values than the other treatments (Figure 3). The treatment of compost 0, dolomite 7.2 g and MycoSilvi type 1, (C0K1M1) had the highest biomass content, amounting to 1.53 g. That is because the addition of 7.2 g of dolomite lime can increase soil pH (Table 2). Increased soil pH can increase the availability of nutrients C-organic, N-total, and P-available that previously could not be absorbed by plants. The availability of nutrients for plants affects the metabolic processes and production of plants [20].

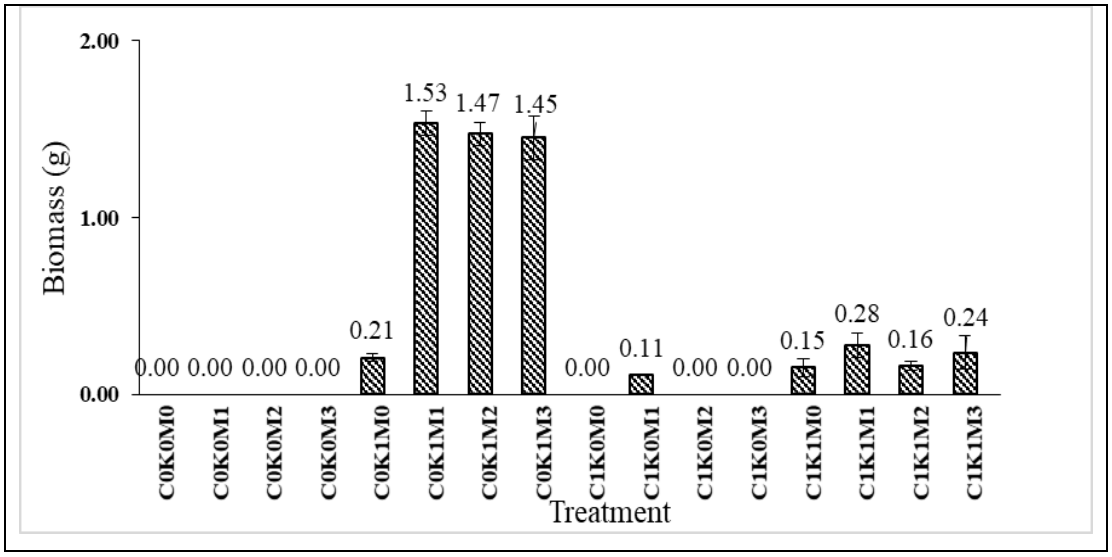

Figure 3 Effects of the interaction of MycoSilvi, compost and dolomite lime treatment on O. *bicolor* seedling biomass aged 16 WAP. C0 = No compost; C1 = Compost 32.5 g; K0 = without dolomite lime; K1 dolomite lime 7.2 g; M1 = MycoSilvi type 1 type G. *mosseae*. (3 g); M2 = MycoSilvi type 2 type G. *mosseae* and *Acaulospora* sp .; M3 = MycoSilvi types 3 type G. *mosseae* and G. *margarita*.

The treatment of MycoSilvi and dolomite lime both single and interaction gave a very significant effect on the percentage of root colonization (Table 3). Treatment without compost, 7.2 g lime and MycoSilvi type 1 (C0K1M1) produced the highest percentage of mycorrhizal colonization compared with other treatments (94%). All treatments with 7.2 g dolomite resulted in a high percentage of mycorrhizal colonization which was more than 30% [16]. This indicates that the addition of dolomite supports mycorrhizae infecting of plant roots. However, the addition of compost into the planting medium inhibited the colonization of AMF (Table 3). The development of AMF is influenced by environmental conditions such as soil temperature, soil acidity, organic matter and other environmental stressors [25]. Soil acidity influence enzyme activity in germination, development and the role of mycorrhizae on plant growth [26]. The presence of mycorrhiza can be known from the presence of hyphae (external and internal), vesicles, and intraradical spores [27].

The addition of AMF is will be effective if able to produce a positive effect on host plants (increase in plant dry weight) or the environmental conditions in which they grow [9]. In this study, the addition of AMF in the form of a MycoSilvi inoculum produced a positive effect on balsa plants as can be seen from the growth response (Figure 4). The combination of dolomite and all types of MycoSilvi provides a high growth response of 636% for type 1 MycoSilvi, 607% for type 2 MycoSilvi and 597% for type 3 MycoSilvi. These data indicate synergy between MycoSilvi and given dolomite. However, on composted treatment, the growth response decreases dramatically to successively for MycoSilvi types 1, 2 and 3 at 84%, 5%, and 58%. This shows the resistance of compost that still has a high C/N, due to the mineral competition between plants and microbial decomposers [22].

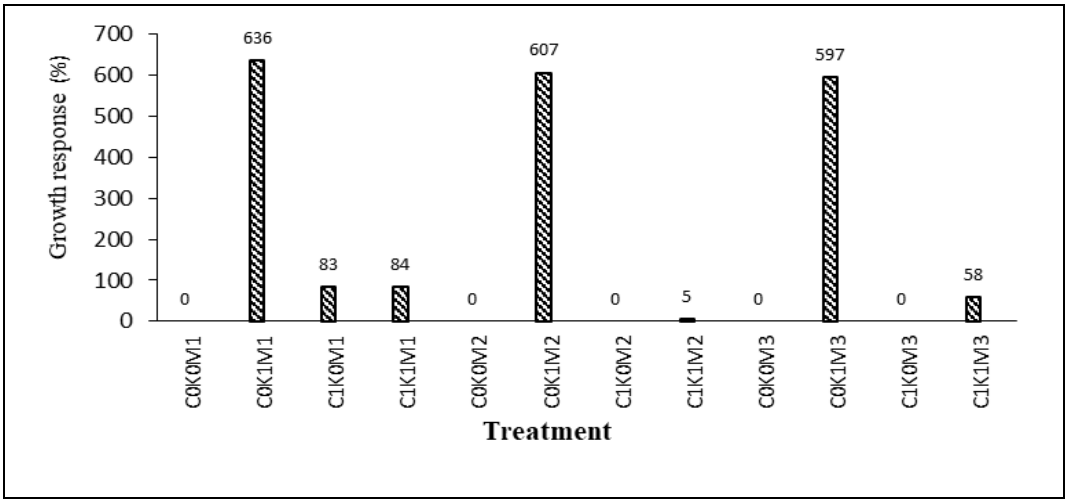

Figure 4 Response of O. *bicolor* plant growth to MycoSilvi 16 MST inoculation. C0 = No compost; C1 = Compost 32.5 g; K0 = without dolomite lime; K1 dolomite lime 7.2 g; M1 = MycoSilvi type 1 type G. *mosseae* (3 g); M2 = MycoSilvi type 2 type G. *mosseae* and *Acaulospora* sp .; M3 = MycoSilvi type 3 type G. *mosseae* and G. *margarita*.

The results of the analysis of plant dependence on mycorrhizae showed that the combination of non-compost treatment, dolomite 7.2 g and MycoSilvi type 1 (C0K1M1), type 2 (C0K1M2), and type 3 (C0K1M3) had a high degree of dependence on mycorrhizae those were 41%, 85.85%, and 85.66%. These results are classified into a very high category (>75%) [28]. Dependence on mycorrhizae shows the degree of a host-adapted to the condition of mycorrhizae so that can produce maximum growth in certain soil fertility conditions. High dependence value indicates that AMF inoculation is beneficial for plant production and resistant to drought conditions, nutrient-poor and root pathogen attack [29].

Growth response and total growth rate of height, diameter and biomass, and mycorrhizal colonization of 16 WAP balsa plants with the addition of 7.2 g dolomite and FMA inoculation in the form of MycoSilvi type 1 inoculum (Table 2, Figure 1, 2, 3, and 4), then it can be concluded that this treatment can be applied to support balsa growth seedling in infertile growing media with high Al and Fe content.

4 Conclusion

The addition of MycoSilvi and dolomite to the post-silica sand soil media produced a positive response to balsa growth. MycoSilvi type 1 and dolomite treatments have a significant effect and produce the best treatment to increase growth in height, diameter, biomass and percentage of mycorrhizal colonization. Balsa has a high value of mycorrhizal dependence on infertile soil conditions, but if the aluminum content is very high and the pH is very low, the plant cannot survive. Further research needs to be carried out to determine the effectiveness of MycoSilvi and soil enhancers in post-mining land reclamation and revegetation activities on a field scale.

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