Mycorrhizal dependency and growth response of *A. chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) Pierre in soil media with low pH and high aluminium

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Abstract. Silica sand post mining has low nutrient soil, acidic soil pH (3.2), high Al content (7.70 cmol/kg) which are the limiting factors of plant growth. Therefore, soil quality might be improved by the addition of soil ameliorant and fertilizer application. MycoSilvi is consortium of Arbuscular Mycorrhizal fungi and Mycorrhizal helper bacteria (MHBs). This study aimed to analyze the dependence of plants against FMA contained in MycoSilvi. There are three variants of MycoSilvi, both single or in combination with soil ameliorant to enhance *Albizia chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) Pierre growth. The research design was a factorial scheme with two factors, MycoSilvi (M) consists of four levels (M0=Without MycoSilvi, M1=MycoSilvi variant 1, M2 = MycoSilvi variant 2 and M3 = MycoSilvi variant 3) and Soil ameliorant (LC) consisting of four levels (L0C0 = Without soil ameliorant, L1C0 = Lime, L0C1 = Compost, L1C1 = Lime and Compost). Data were analysed using analysis of variance (ANOVA). Dry weight of shoot and root, top root ratio, percentage of mycorrhizal colonization, growth response and the relative dependence of mycorrhiza were observed after 12 weeks of planting. The results showed that combination of MycoSilvi and soil ameliorant can made dry weight of a shoot, root and top root ratio better than all treatments. Whereas the best growth response and mycorrhiza dependency was shown in MycoSilvi variant 3 without lime and compost (M3L0C0). The results showed that *A. chinensis* and *P. pinnata* plants had a high degree of dependence on FMA for their life force.

1. Introduction

The barriers to plant growth on post mining soil are the presence of high Al content and low pH which are toxic to plants and low solubility of nutrients so that deficiency occurs. One alternative that can be used to provide nutrients for plants while improving the chemical and biological properties of the soil is by adding biotechnology inputs such as MycoSilvi. MycoSilvi is consortium of Arbuscular *Mycorrhizal* fungi and *Mycorrhizal* helper bacteria (MHBs). FMA is a reciprocal relationship between fungi and host plants that brings positive benefits for both (mutual symbiosis). For host plants, the existence of this association can provide great benefits to growth, both directly and indirectly. Those fungi are widely used as natural biofertilizers [1] due to their contribution to alleviating water stress [2–4], and aluminum stress [5], prevent nutrient loss from soil [6], improve soil chemical properties [7] and
soil aggregation [8], increase phosphate uptake [9] and nitrogen uptake [10]. Mycorrhizal plants contribute to enhanced plant biomass production [11,12] and resistance to root disease [13].

Leguminosaeae tree are very well used in improving soil quality because they can symbiosis with nitrogen fixing bacteria, rhizobium which is able to form root nodules in its root system, deep roots can control erosion, soil building and ground cover [14] growth is relatively fast (fast growing species), as seeds and leaves as well as animal feed [15]. Leguminosaeae tree used in this study were A. chinensis and P. pinnata which were known to grow on various soil variants and at the level of tillers tolerant to shade.

In relation to plant growth, Plenccete et al. (1983) proposed a formula known as the term mycorrhizal dependency (MD) [16]:

$$\text{Mycorrhizal Dependency (\%)} = \frac{\text{dry weight of mycorrhizal plant} - \text{dry weight of mycorrhizal plant}}{\text{dry weight of mycorrhizal plant}} \times 100$$

Mycorrhizal dependence is the level of plant dependence on mycorrhiza to produce maximum growth in the soil fertility rate given [17]. However, the response of plants is not only determined by the characteristics of plants and fungi, but also by the soil conditions in which the experiment was carried out. The effectiveness of mycorrhizas is influenced by soil environmental factors which include abiotic factors (nutrient concentration, pH, water content, temperature, tillage and fertilizer / pesticide use) and biotic factors (microbial interactions, fungi species, host plants, host root variants, and competition). Among mycorrhizal fungi, mycorrhizal growth responses (MGR) can be calculated based on the total dry weight of each plant colonized by FMA and the mean dry weight of plants that are not colonized by FMA at harvest, using the following equation:

The mycorrhizal response (%) of each plant species is expressed and the formula is like this [18]:

$$\text{Mycorrhizal Growth Response (\%)} = \frac{\text{dry weight of mycorrhizal plant} - \text{dry weight of non-mycorrhizal plant}}{\text{dry weight of mycorrhizal plant}} \times 100$$

2. Materials and methods

2.1. 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 than transported to Laboratory, air dried, sieved (2 mm) and autoclaved at 121°C for 1 hour. The chemical properties of soil were analysed following standard method in Soil Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agricultural University. The chemical properties of soil medium are pH H<sub>2</sub>O = 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 = 7.70 cmol/kg. Lime was obtained from the farmer market as dolomite. Compost with the trade name of agro flower was produced by CV. Laksmi Prima Bogor Indonesia. The characteristic of compost are: pH 7.71; C-organic 48.79%; N 0.58%; K 1.00% and P<sub>2</sub>O<sub>5</sub> 2.90% that was determined at Soil Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agricultural University. The sterilized soil were than mixed with lime and compost in accordance with the treatment.

2.2. Seedling and MycoSilvi inoculum

Seed of Albizia chinensis (Osbeck) Merrill and Pongamia pinnata (L.) Pierre were obtained from Center of Research and Development for Forest Seed, Bogor Indonesia. 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. MycoSilvi was produced in zeolite medium with Purarea javanica as host plant for two months in the green house. There were three variant of MycoSilvi produced. MycoSilvi variant 1 containing Glomus mossea (Gerd. & Trappe), MycoSilvi variant 2 containing Glomus mossea (Gerd. & Trappe) and Acaulospora sp. and...
MycoSilvi variant 3 containing *Glomus mosseae* (Gerd. & Trappe), *Acaulospora* sp. and *Gigaspora margarita* (Becker & Hall).

2.3. *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. Plants were weather inoculated with *MycoSilvi* or not as a control at transplanting. Each inoculated plant received 5 g of *MycoSilvi* inoculum (containing environ 50 spores of Arbuscula Mycorrhizal Fungi). Plants were grown for twelve weeks in the green house and watered as needed.

2.4. *Harvesting and parameter measurement*

Roots were sampled before harvest for evaluating their mycorrhizal colonization at twelve weeks old plants. Mycorrhizal colonization was evaluated according to the method of Clapp et al. (1996) [19]. Percentage of mycorrhizal colonization was determined according to the method of O’connor et al. (2001) [20]. Plants were harvested twelve weeks after planting and evaluated for their shoot and root dry weight (g). Shoot and root dry weights were recorded after drying at 70°C to constant weight is attained. Then, evaluating their MD dan MGR was used method of Plencce et al. (1983) and Hetrick et al. (1996) [16,18]

2.5. *Experiment design*

The experiment were done with factorial design with 2 factors, *MycoSilvi* and Soil ameliorant. The *MycoSilvi* (M) has four level (M0 = uninoculated, M1 = *MycoSilvi* variant 1, M2 = *MycoSilvi* variant 2 and M3 = *MycoSilvi* variant 3), and soil ameliorant (LC) have 4 level (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 replicate. All data were analyzed by Analysis of Variance Procedure.

3. **Results and discussion**

3.1. Estimation of mycorrhiza colonization and mycorrhizal dependency

The results of the study (Table 2) showed that FMA contained in *MycoSilvi* had successfully colonized the roots of *A. chinensis* and *P. pinnata*. This is indicated by the presence of FMA structures such as vesicles and internal hyphae found in the roots of both plants. The large colonization in the *A. chinensis* roots was shown in *MycoSilvi* variant 1 with the addition of lime and compost (80%) while in *P. pinnata* seedlings was shown in *MycoSilvi* variant 3 with the addition of lime and compost (48%). This indicates that the combination of *MycoSilvi* and ameliorant soil can improve root colonization which is positively correlated with shoot and root dry weight and top root ratio. The increase in colonization is caused by an improvement in nutrient and water absorption status related to a decrease in Al content and an increase in soil pH, so that plant growth takes place well.

3.2. Growth response

The results of growth response due to *MycoSilvi* inoculation showed that were negatively correlated with the addition of ameliorant soils such as lime and compost. Addition of soil ameliorant in the form of lime and compost to *MycoSilvi* can reduce the growth response of *A. chinensis* and *P. pinnata* seedlings. The best growth response was shown in *MycoSilvi* variant 3 without the addition of lime and compost (M3L0C0). Effect of *MycoSilvi* and soil ameliorant on shoot and root dry biomass, and top root ratio of *A. chinensis* and *P. pinnata* (Table 1), if combined *MycoSilvi* and soil ameliorant will show significant results on shoot weight and root *A. chinensis* different from *P. pinnata* which shows significant results if applied separately. This indicates that the FMA contained in *MycoSilvi* has a positive role in increasing the growth response of the two seedlings.
Figure 1. Effect of MycoSilvi and soil ameliorant on mycorrhizal growth response of *A. chinensis* and *P. pinnata* (12 weeks after planting). 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.

3.3. Relative mycorrhizal dependency

The relative mycorrhizal dependency of *A. chinensis* and *P. pinnata* was determined by expressing the dry weights of mycorrhizal plants as percentage of the dry weight of non-mycorrhizal plants (Figure 2).

Figure 2. Effect of MycoSilvi and soil ameliorant on mycorrhizal dependency of *A. chinensis* and *P. pinnata* (12 weeks after planting). 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).

The relative mycorrhizal dependence on both variants of plants changes with the addition of soil ameliorant in the form of lime and compost. *A. chinensis* and *P. pinnata* showed the highest mycorrhizal dependence on the treatment of MycoSilvi variant 3 without lime and compost (M3L0C0) which was 81.73% and 43.73% followed by MycoSilvi variants 1 and 2 which were positively correlated with growth response.
Table 1. Effect of MycoSilvi and soil ameliorant on shoot dry biomass, root dry biomass and top root ratio of A. chinensis and P. pinnata (12 weeks after planting).

| Treatment  | Shoot dry biomass (gram) | Root dry biomass (gram) | Top root ratio (gram) |
|------------|--------------------------|-------------------------|-----------------------|
|            | A. chinensis             | P. pinnata              | A. chinensis          | P. pinnata          |
| M0L0C0     | 0.11 ± 0.07f             | 1.23 ± 0.14g            | 0.09 ± 0.07f          | 1.10 ± 0.22c        |
| M1L0C0     | 0.35 ± 0.14ef            | 1.97 ± 0.26f            | 0.21 ± 0.07ef         | 1.37 ± 0.16bc       |
| M2L0C0     | 0.51 ± 0.04e             | 2.19 ± 0.26ef           | 0.31 ± 0.03cdef       | 1.48 ± 0.21abc      |
| M3L0C0     | 0.68 ± 0.10de            | 2.57 ± 0.07def          | 0.40 ± 0.08cde        | 1.57 ± 0.03abc      |
| M0L1C0     | 0.38 ± 0.09ef            | 2.02 ± 0.92f            | 0.27 ± 0.09def        | 1.43 ± 0.50bc       |
| M1L1C0     | 0.92 ± 0.24cd            | 2.35 ± 0.37ef           | 0.51 ± 0.13bcd        | 1.50 ± 0.20abc      |
| M2L1C0     | 0.97 ± 0.31cd            | 2.37 ± 0.64ef           | 0.52 ± 0.16bcd        | 1.54 ± 0.50abc      |
| M3L1C0     | 1.67 ± 0.28a             | 2.73 ± 1.05ef           | 0.92 ± 0.19a          | 1.59 ± 0.58abc      |
| M0L0C1     | 0.66 ± 0.02de            | 2.16 ± 0.43ef           | 0.44 ± 0.02cde        | 1.48 ± 0.25abc      |
| M1L0C1     | 0.91 ± 0.52cd            | 2.70 ± 0.18cdef         | 0.57 ± 0.27bc         | 1.62 ± 0.22ab       |
| M2L0C1     | 1.19 ± 0.21bc            | 2.90 ± 0.23bcde         | 0.74 ± 0.20ab         | 1.82 ± 0.24ab       |
| M3L0C1     | 1.48 ± 0.37ab            | 3.21 ± 0.46abcd         | 0.90 ± 0.40a          | 1.78 ± 0.19ab       |
| M0L1C1     | 0.69 ± 0.10de            | 2.50 ± 0.76def          | 0.43 ± 0.14cde        | 1.74 ± 0.69ab       |
| M1L1C1     | 1.51 ± 0.34ab            | 3.43 ± 0.40abc          | 0.85 ± 0.15a          | 1.96 ± 0.28a        |
| M2L1C1     | 1.63 ± 0.62a             | 3.52 ± 0.24ab           | 0.97 ± 0.07a          | 1.99 ± 0.04a        |
| M3L1C1     | 1.75 ± 0.20a             | 3.77 ± 0.43a            | 0.81 ± 0.21a          | 1.94 ± 0.42a        |

Significance

| M    | ** | ** | ** | * | * | * | ** |
|------|----|----|----|---|---|---|----|
| LC   | ** | ** | ** | ** | * | * | ** |
| M x LC | * | ns | * | ns | ns | ns | ns |

Note: 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

Table 2. Effect of MycoSilvi and soil ameliorant on total dry biomass, Mycorrhizal colonization, Mycorrhizal dependency and Mycorrhizal Growth Response of A. chinensis and P. pinnata (12 weeks after planting).

| Treatment  | Total dry biomass (gram) | Colonization (%) | MD (%) | MGR (%) | Total dry biomass (gram) | Colonization (%) | MD (%) | MGR (%) |
|------------|--------------------------|------------------|--------|---------|--------------------------|------------------|--------|---------|
|            | A. chinensis             | P. pinnata       |        |         | A. chinensis             | P. pinnata       |        |         |
| M1L0C0     | 0.56ef                   | 8.00e            | 64.52  | 181.82  | 3.34fg                   | 6.00de           | 30.18  | 43.22   |
| M2L0C0     | 0.82e                    | 8.00e            | 75.74  | 312.12  | 3.67ef                   | 18.00de          | 36.46  | 57.38   |
| M3L0C0     | 1.08de                   | 20.00de          | 81.73  | 447.47  | 4.14def                  | 14.00de          | 43.73  | 77.70   |
| M1L1C0     | 1.43cd                   | 36.00cd          | 54.42  | 119.38  | 3.85def                  | 12.00cd          | 10.49  | 11.72   |
| M2L1C0     | 1.70c                    | 36.00cd          | 61.67  | 160.92  | 3.92def                  | 12.00cd          | 12.00  | 13.64   |
Silvi-
d abiotic pressure addition to this role, FMA can also increase plant resistance to biotic an
phosphates and other nutrients or only given by MycoSilvi. This is associated with the role of FMA in absorption of nutrients, especially phosphates and other nutrients [28], water [29] and photosynthetic carbohydrate translocation [28]. In addition to this role, FMA can also increase plant resistance to biotic and abiotic pressure [30],

|    | M3L1C0 | M1L0C1 | M2L0C1 | M3L0C1 | M1L1C1 | M2L1C1 | M3L1C1 |
|----|--------|--------|--------|--------|--------|--------|--------|
|  2.59a | 78.00a | 74.94  | 299.08 | 4.32bcdef | 30.00abc | 20.19  | 25.30  |
|  1.47cd | 50.00bc | 28.13  | 39.13  | 4.32bcdef | 26.00bcd | 15.71  | 18.64  |
|  1.97bc | 14.00de | 46.40  | 86.58  | 4.74abcde | 28.00abcd | 23.22  | 30.24  |
|  2.38ab | 56.00abc | 55.51  | 124.76 | 5.02abcd | 28.00abcd | 27.59  | 38.10  |
|  2.36ab | 80.00a | 52.55  | 110.73 | 5.40abc | 22.00cde | 21.34  | 27.13  |
|  2.60a | 66.00ab | 56.97  | 132.38 | 5.51ab  | 44.00ab  | 22.88  | 29.67  |
|  2.82a | 60.00abc | 60.30  | 151.88 | 5.71a  | 48.00a  | 25.64  | 34.48  |

Note: 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. Values in column followed by same letter are not significantly different (P ≤ 0.5).

3.3. Discussion
Silica sand post-mining soil has acidic soil pH (3.20) and high aluminum content (7.70 cmol/kg). This property is one of the limiting factors for plant growth. With the presence of MycoSilvi inoculation on A. chinensis and P. pinnata can improve plant growth, provide nutrients back by releasing P bonds by Al metal so that it is available to plants. In this study, we observed that the combination of the treatment of MycoSilvi and ameliorant soil could increase shoot and root dry weight in both variants of plants, especially in MycoSilvi variant 3.

We found that MycoSilvi variant 3, which is combination of three AMF species and MycoSilvi variant 2 which is combination of two AMF species had better effect on shoot and root dry weight of both tropical trees species. These results were in agreement by Kohl and van der Heijden (2016) that AMF variented in their effects on plant nutrient acquisition and growth. This was positively correlated with colonization of mycorrhiza in A. chinensis and P. pinnata. The largest mycorrhizal colonization in A. chinensis was seen in the treatment of MycoSilvi variant 1 with the addition of lime (M1L1C1) which was 80% and not significantly different from MycoSilvi variant 3 with lime addition (M3L1C0) which was 78%. Whereas P. pinnata was shown in the treatment of MycoSilvi variant 3, lime and compost (M3L1C1), which was 48%. The results of the study by de Oliveria (2017) showed that Jatropha curcas L. sob which was inoculated with 3 variants of mycorrhizae namely Rhizophagus intraradices, Gigaspora albida, Claroideoglomus etunicatum, succeeded in colonizing the roots more than only one species.

Relative dependence on mycorrhiza shows the degree of a host in a mycorrhizal condition to produce maximum growth in the range of certain soil fertility. A. chinensis seeds and P. pinnata inoculated with MycoSilvi variant 3 showed the highest degree of dependence on FMA at 81.73% and 43.73% (Table 2). Based on these values, A. chinensis and P. pinnata are variants that have a high dependence on FMA. According to Nwoko and Sanginga (1999), the value of RMD (Relative Mycorrhizal Dependency) > 30% shows high dependence, a value of RMD 10-30% indicates moderate dependence, and does not depend on mycorrhizal if the value of RMD is <10% [21]. This indicates that the growth and viability of A. chinensis and P. pinnata plants is very dependent on their association with FMA. The high value of RMD indicates that FMA contained in MycoSilvi is beneficial for the production of quality seedlings on the nursery and field scale and is strong against heavy metal stress, drought, soil nutrient deficiencies and attacks on root pathogens [22]. The high dependence of plant species on FMA was also reported in several tropical tree species including Acacia nilotica [23], Acacia melanoxylon [24], Acacia mangium [22], Hancornia speciosa [25], Dyera polyphylla and Aquilaria fijaria [26]. The results of the same study were shown by Tawaraya (2003), the largest mycorrhizal dependence was obtained from the tree species group compared to wild grasses and field crops and forage crops [27].

The combination of MycoSilvi and ameliorant soil showed greater weight than those not inoculated or only given by MycoSilvi. This is associated with the role of FMA in absorption of nutrients, especially phosphates and other nutrients [28], water [29] and photosynthetic carbohydrate translocation [28]. In addition to this role, FMA can also increase plant resistance to biotic and abiotic pressure [30],
stimulation of nodulation and supply of growth regulators [31,32]. This study is similar to several previous studies on legume plants, including *Erythrina terrana* [33], *Acacia nilotica* and *Albizia lebbeck* [23], *Biglobosa Parkia*, *Tamarindus indicus* and *Zizyphus mauritiana* [34], *Gliricida sepium* and *Leucaena leucocephala* [35], *Sesbania aegyptiaca* and *S. grandiflora* and *Cassia siamea* [36,37]. Soil ameliorant with limiting reduces the fixation of P by Al plant growth and finally increases nutrient uptake by plant roots.

The top root ratio of *A. chinensis* and *P. pinnata* inoculated by MycoSilvi tended to increase with the addition of soil ameliorant. The top root ratio of *A. chinensis* and *P. pinnata* applied with MycoSilvi variant 3 were 1.73 and 1.63 respectively, and the highest at the time was still within the normal range of the vegetative growth period of the plant. This shows that the results of photosynthesis are more translocated proportionally to the shoots and roots. Top root ratio illustrates the relative abundance of above-ground resources for resources in the root area.

4. Conclusion

Inoculation of MycoSilvi variant 3, lime and compost (M3L1C1) showed a significant effect in triggering shoot and roots dry weight of *A. chinensis* and *P. pinnata* aged 12 weeks after planting. The *A. chinensis* and *P. pinnata* plants have a high level of dependence and growth response to FMA found in MycoSilvi especially MycoSilvi variant 3 (M3L0C0), which has a positive correlation with the percentage of root colonization.

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