

Arbuscular mycorrhizal fungi inoculum and soil ameliorants enhance the growth of *Falcataria moluccana* in revegetation of post-silica sand mining land in Sukabumi, Indonesia

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Abstract. *Bekti HS, Budi SW, Wibowo C. 2022. Arbuscular mycorrhizal fungi inoculum and soil ameliorants enhance the growth of Falcataria moluccana in revegetation of post-silica sand mining land in Sukabumi, Indonesia. Biodiversitas 23: 1181-1187.* Open-pit mining of silica sand caused several negative impacts on the environment, especially inhibited plant growth. MycoSilvi is Arbuscular Mycorrhizal Fungi (AMF) inoculum enriched with Mycorrhizal Helper Bacteria (MHBs) which could increase plant growth and nutrient uptake in degraded soils. A field experiment was conducted to study the growth response of *F. moluccana* (Miq.) Barneby & JW Grimes seedlings treated with MycoSilvi inoculation and soil ameliorants (lime and compost) in post-silica sand mining land. The experimental design used in this study was a completely randomized block design with a factorial scheme. The results showed that MycoSilvi inoculation combined with soil ameliorant significantly improved plant growth of *F. moluccana* and assisted plant survival within nine months after transplantation in the field. MycoSilvi inoculation and soil ameliorants also increased the number of bacteria in the soil rhizosphere compared with control treatment. This study indicated that revegetation with MycoSilvi inoculation and soil ameliorants could influence plant establishment and development of microbial soil communities in infertile soils, suggesting that it could be an effective method for further ecological rehabilitation in degraded land areas.

Keywords: *Falcataria moluccana*, MycoSilvi, post-silica sand mining, revegetation, soil ameliorant

INTRODUCTION

Mining activities in Indonesia always increase every year. Based on data from the Indonesian Ministry of Environment and Forestry (2020), Indonesia has a total mining concession area of 810,400 ha in 2019, 346,000 ha of which are forest areas. Open-pit mining of silica sand caused several negative impacts on the environment, i.e., vegetation cover loss, fauna and soil microbes decline, nutrient deficiency, soil acidification, and soil contamination with heavy metals, which are toxic for plant growth (Cristescu 2012; Huot 2015; Gastauer 2018; Diatin 2018; Syauqie 2019).

Revegetation is one of the main ways to improve and restore post-mining land. The vegetation can stabilize the soil by developing extensive root systems (Sheoran et al. 2010) and prevent the spread of heavy metal particles carried by erosion (Arienzo et al. 2004). Therefore, revegetation is an important indicator in determining the success of post-mining land rehabilitation. However, post-mining land is a very unfavorable environment for plants due to many inhibiting factors for plant growth (Sudrajat et al. 2019; Tordoff et al. 2000).

The symbiosis of Arbuscular Mycorrhizal Fungi (AMF) with plants could support plant growth by increasing nutrient uptake, especially P (phosphorus). MycoSilvi is an AMF inoculum enriched with Mycorrhizal Helper Bacteria (MHBs), which we developed, to significantly increase phosphorus uptake, plant height, and stem diameter in post-silica sand mining in greenhouse experiments (Budi et al.

2020; Munawaroh et al. 2020). Therefore, it is necessary to test their effectiveness in the field. In acidic post-mining soils, the addition of soil ameliorants such as lime and compost also play a role in increasing soil pH, soil organic carbon, and soil macro-nutrients (Córdova et al. 2011; Wasis and Andika 2017).

Apart from soil improvement, the selection of plant species is another important part of the revegetation stage in post-mining land. *Falcataria moluccana* (Miq.) Barneby & JW Grimes is generally recognized as an adaptive fast-growing tree species that can be planted intensively for post-mining land rehabilitation (Prematuri et al. 2020; Suita et al. 2018). This plant can fix nitrogen into the soil and has a mutualistic symbiosis with AMF, making it better to be used for revegetation (Ramos et al. 2019).

The objectives of this study were to analyze the growth response of *F. moluccana* seedlings inoculated with various variants of MycoSilvi in post-silica sand mining land amended with soil ameliorants and to analyze the soil bacteria communities in post-silica sand mining land after being treated with soil ameliorants and MycoSilvi inoculation.

MATERIALS AND METHODS

Preparation of planting area

This experiment was carried out in silica sand post-mining land of PT Solusi Bangun Indonesia, which was located in Cibadak, Sukabumi District, West Java,

Indonesia. The first step of revegetation was land clearing, followed by digging 160 planting holes, measuring 30 cm x 30 cm x 30 cm each. In these field trials, dolomite lime and organic compost were put into all planting holes and mixed with the soils as a booster for initial rhizosphere stabilization. The dose of compost was 900 g per planting hole, while the dose of dolomite lime was 200 g per planting hole. Preparation of the planting area was carried out one week before planting time.

Preparation of *F. moluccana* seedlings

Seedling preparation was carried out in a greenhouse at the Department of Silviculture, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia. The number of *segon* seedlings used in this experiment was 160 plants. The seedlings were grown in silica sand post-mining soil medium amended with dolomite lime and compost. MycoSilvi were inoculated to *F. moluccana* seedlings when the seedlings were two weeks old. The dose of MycoSilvi inoculum used contained 50 spores of arbuscular mycorrhizal fungi. There were three variants of MycoSilvi being used in this experiment, namely MycoSilvi variant 1 containing *Glomus mosseae*, MycoSilvi variant 2 containing *Glomus mosseae* and *Acaulospora* sp, and MycoSilvi variant 3 containing *Glomus mosseae* and *Gigaspora margarita*. The seedlings were grown until six months old in the greenhouse and then moved out for the acclimatization process as long as one week before planting time.

Transplanting of *F. moluccana* seedlings

There were two soil characteristics in the planting area, which were determined based on the physical and chemical properties of the soil, namely Marginal Land (ML) and Extreme Marginal Land (EML). The soil characteristics had been analyzed at Soil Laboratory, Faculty of Agriculture, IPB University (Table 1). Each block was planted with 80 *F. moluccana* seedlings. The seedlings were planted into the planting holes by removing the polybags and also planted according to the planting code name that had been arranged for each planting hole.

Table 1. Chemical and physical characteristics of post-silica sand mining soil

Soil characteristics	Marginal land	Extreme marginal land
pH (H ₂ O)	3.56	2.99
C-organic (%)	1.65	1.51
N-total (%)	0.29	0.20
P-available (mg/100g)	4.25	4.29
P-total (mg/100g)	46.18	89.33
Ca (cmol/kg)	0.26	2.50
Mg (cmol/kg)	0.29	1.42
K (cmol/kg)	0.10	0.04
Na (cmol/kg)	0.04	0.06
CEC (cmol/kg)	11.02	16.07
Al (cmol/kg)	5.75	7.94
Fe (mg/100g)	182.98	286.81
Soil texture		
Sand (%)	37.95	22.03
Silt (%)	27.01	30.83
Clay (%)	35.04	47.14

Variables evaluated

The variables evaluated in this experiment were plant height, stem diameter, plant survival rate, mycorrhizal root colonization, and soil bacteria communities. These evaluations were carried out for nine months. Plant heights were determined with a measuring tape, and stem diameters were determined using a digital caliper. The measurements were carried out once a month. The survival rate was determined by counting the total number of plants that survived at the end of the experiment divided by the total number of plants at the beginning of the experiment. The quantification of mycorrhizal roots colonization was performed by the method of Brundrett et al. (1996). Soil samples for determining bacterial communities were collected from plants rhizosphere and were analyzed at ICBB Laboratory, Bogor.

Experimental design and data analysis

The experimental design used was a completely randomized block design with the factorial scheme, which consisted of four types of MycoSilvi treatment (control / not inoculated, inoculated with MycoSilvi variant 1, inoculated with MycoSilvi variant 2, inoculated with MycoSilvi variant 3); two lime doses (0 g and 7.2 g)/650 g growth medium; and two organic compost doses (0 g and 32.5 g)/650 g growth medium. In the Marginal Land Block and the Extreme Marginal Land Block, all treatments were replicated five times. In this study, analysis of variance was carried out using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Plant growth of *F. moluccana*

In recent years, revegetation around mines has attracted the scientific community's attention. Most studies have reported that revegetation is the main way for ecological restoration of mining areas (Chen et al. 2017). However, plant establishment and growth for revegetation are difficult because post-mining soils have some limiting factors, such as low pH, low content of nutrients (N, P, K), and high solubility of toxic metals (e.g., Al, Fe, Mn) that make the availability of P is limited due to fixation by Al and Fe oxides (Carvalho et al. 2013; Zhen et al. 2015; Kochian et al. 2004). According to Węzowicz et al. (2017), AMF inoculation in post-mining soil has a beneficial role in improving plant growth and fitness under metal toxicity.

In this field trial, there were two soil characteristics for transplanting of *F. moluccana*. These soils were acidic, with low nutrient contents and high concentrations of Al and Fe, and the soil properties showed that EML had worse conditions than ML (Table 1). Those poor soil conditions caused stunted growth of control plants without any treatments at EML, and control plants at ML could not survive until the end of the experiment (Table 2). Meanwhile, inoculation of all MycoSilvi variants to *F. moluccana* seedlings increased plant height and stem diameter at ML and EML significantly (Table 2). MycoSilvi inoculation alone without soil ameliorants at ML increased plant height by 3825% and 4813% when

inoculated by M1 and M3, respectively, and increased stem diameter by 1064% 1054%, respectively as compared to control. The same treatment at EML could increase plant height by 2004%, 2080%, and 787% when inoculated by M1, M2, and M3, respectively, and also increased stem diameter by 928%, 946%, and 450%, respectively, as compared to control.

The association of plant roots with AMF is an important ecological strategy to improve plant growth in post-mining soil (Rosita et al. 2017). Previous studies reported that AMF could enhance nutrient acquisition, especially phosphorus (Sari et al. 2002; Wang et al. 2019), nitrogen (Bonfante dan Genre 2010), and also water absorption of host plants by extending hyphae beyond the nutrient depletion zone of roots (Phillips et al. 2013). Available phosphorus is vital to the growth and health of plants and favors the vegetation reconstruction in mining subsidence areas (Bi et al. 2018). In addition, phosphorus causes microbial communities, such as fungal propagules, to thrive and leads to soil fertility (Sudrajat et al. 2019). Furthermore, AMF also plays important roles in increasing plant tolerance to stress conditions such as salinity and drought (Latef and He 2011), toxic metals (Wężowicz et al. 2017), plant root pathogens (Cordier et al. 1998) and helping to improve soil structure (Rillig et al. 2010). In this study, plant heights and stem diameters of all MycoSilvi inoculation treatments were significantly higher than control plants, indicating that inoculating MycoSilvi could increase plants growth in post-silica sand mining land.

According to O'Connor et al. (2001), the percentage of mycorrhizal roots colonization is categorized as high if it has a value >30%. The high AMF infection in plant roots indicates a match between AMF and its host plant (Smith and Read 2008). MycoSilvi inoculation treatments to *F. moluccana* seedlings at ML showed a higher mycorrhizal

roots colonization percentage than at EML (Table 2). These results were supposed to be due to poorer soil properties of EML than ML. Budi et al. (2020) reported that MycoSilvi inoculation of *F. moluccana* seedlings on post-silica sand mining soil medium showed a high percentage of mycorrhizal colonization. In this study, the percentage of mycorrhizal colonization on *F. moluccana* seedlings without MycoSilvi inoculation (M0) indicated that plant roots had been colonized by native soil AMF, but the plants which were colonized by MycoSilvi treatments showed better plant growth. These results indicated that MycoSilvi could be positively associated with *F. moluccana* plants in post-silica sand mining soil; therefore, the plant height and diameter growth could be improved significantly.

The addition of soil ameliorants such as lime and compost for amending soils has positive effects on improving plant growth which is effective for phytostabilization of metal-polluted and acidic soil (Córdova et al. 2011). In this study, the addition of lime alone at ML increased plant height and stem diameter by 4960% and 1124%, respectively, while the same treatment at EML increased plant height and stem diameter by 1328% and 770%, respectively as compared to control plants. M1, M2, and M3 treatments combined with lime at ML increased plant height by 6317%, 5360%, and 7410%, respectively, and increased stem diameter by 1361%, 1536%, and 1754%, respectively, as compared to control plants. The same treatments at EML also increased plant height by 1204%, 1501%, and 2124%, respectively and increased stem diameter by 439%, 717%, and 920%, respectively, compared to control plants. In acidic soil, lime addition has a beneficial role in decreasing the heavy metal mobility such as Al and Fe in soils and their accumulation in the plant as it increases the pH of the soil (Sheoran et al. 2010; Budi et al. 2020).

Table 2. Effect of MycoSilvi and soil ameliorants on plant growth, nine months after transplantation

MycoSilvi	Soil ameliorant		Marginal land			Extreme marginal land		
	Compost	Lime	Height (cm)*	Diameter (mm)*	Mycorrhizal colonization (%)	Height (cm)*	Diameter (mm)*	Mycorrhizal colonization (%)
Without	-	-	0.00c	0.00b	60	2.50b	0.87bc	50
MycoSilvi	-	+	60.73abc	8.57a	43	35.70ab	7.57abc	20
	+	-	36.25bc	7.75a	10	0.00b	0.00c	0
	+	+	0.00c	0.00b	40	0.00b	0.00c	0
MycoSilvi var 1 (M1)	-	-	47.10abc	8.15a	35	52.60a	8.94a	30
	-	+	77.00ab	10.23a	100	32.60ab	4.69abc	25
	+	-	87.43ab	13.70a	50	52.45a	9.75a	20
	+	+	110.53a	12.97a	90	30.75ab	8.63ab	27
MycoSilvi var 2 (M2)	-	-	0.00c	0.00b	35	54.50a	9.25a	20
	-	+	65.53abc	11.45a	80	40.03ab	7.11abc	50
	+	-	44.25abc	8.17a	47	45.35a	7.15abc	20
	+	+	64.63abc	13.22a	85	35.30ab	8.88a	25
MycoSilvi var 3 (M3)	-	-	58.95abc	8.08a	80	22.17ab	4.79abc	30
	-	+	90.13ab	12.98a	60	55.60a	8.87a	27
	+	-	60.93abc	9.16a	65	0.00b	0.00c	40
	+	+	43.38abc	10.65a	35	30.63ab	6.92abc	57

Note: *Values followed by the same letter (s) in the same columns are not significantly different (s) at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

Meanwhile, the addition of organic compost improves soil structure, water holding capacity, cation exchange capacity, soil biological activity and provides macro-and micro-nutrients (Tordoff et al. 2000; Wasis and Andika 2017). The addition of compost alone in this study also could increase plant growth, but the addition of lime alone showed a better result (Table 2). The treatment of compost alone at ML increased plant height and stem diameter by 2921% and 1006%, respectively, as compared to control. However, in the same treatment at EML, no plants could survive. M1, M2, and M3 treatments combined with compost at ML increased plant height by 7185%, 3588%, and 4978%, respectively, and increased stem diameter by 1857%, 1067% and 1208%, respectively, as compared to control. The same treatments at EML except M3 combined with compost that could not survive, M1 and M2 combined with compost could increase plant height by 1998% and 1714%, respectively, and increased stem diameter by 1021% 722%, respectively, as compared to control.

Lime combined with compost without MycoSilvi inoculation showed bad plant growth results because no plants at ML and EML could survive until the end of the field trial (Table 2). However, when MycoSilvi inoculation was combined with lime and compost, the results showed a significant increase in plant growth. M1, M2, and M3 treatments combined with lime and compost at ML increased plant height by 9111%, 5286%, and 3512%, respectively, and increased stem diameter by 1753% 1788%, and 1422%, respectively, as compared to control. The same treatments at EML also increased plant height by 1130%, 1312%, and 1125%, respectively, and increased stem diameter by 892%, 920%, and 695%, respectively, compared to control. In this study, *F. moluccana* plant growth at ML and EML could be improved by the treatments of MycoSilvi and lime alone or the combination of MycoSilvi, lime, and compost. The interaction of MycoSilvi, lime and compost to *F. moluccana* plant growth

in post-mining soil medium has been reported by Jayani et al. (2018) and Budi et al. (2020). Under greenhouse conditions, Jayani et al. (2018) reported that MycoSilvi (*Glomus mosseae*) and soil ameliorant (32.5 g of compost and 7.2 g of lime) was the best treatment to increase plant height and stem diameter. Furthermore, Budi et al. (2020) reported that MycoSilvi containing more than one AMF species combined with lime (7.2 g) alone showed the best plant height and stem diameter results.

The survival rate of *F. moluccana*

Revegetation in post-mining soils has an important role in reversing the degradation process by stabilizing soils through the development of extensive root systems (Sheoran et al. 2010). The survival rate is crucial for the initial establishment of plants transplanted in the field to revegetation activities (Wulandari et al. 2016). However, the removal of upper soil horizons in mining activities leads to inhibition of plants survival and establishment (Dugbley et al. 2015).

Nine months after the transplantation of *F. moluccana* to the field, the results showed that the plants without MycoSilvi inoculation treatment had the lowest survival rate by 30% at ML and 15% at EML. While the plants inoculated with MycoSilvi variant 3 showed the highest result in survival rate by 75% at ML and 50% at EML (Figure 1). In this study, the plants with MycoSilvi inoculation combined with soil ameliorant treatment showed better plant survival rates than the treatment of MycoSilvi inoculation alone or soil ameliorant alone. The inoculation of M1 containing one AMF showed the best result in survival rate when combined with lime and compost, while inoculation of M2 and M3 containing more than one AMF showed the best results when combined with lime only (Figure 2).

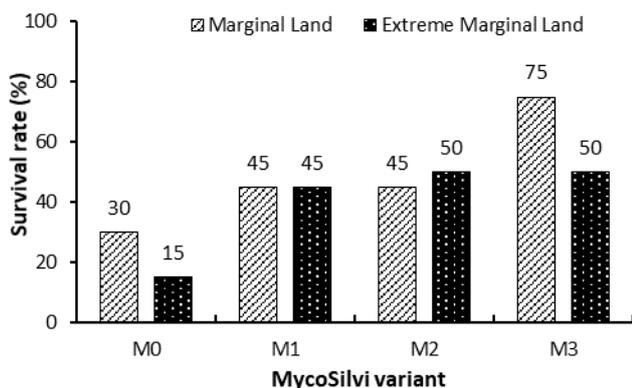


Figure 1. Influence of MycoSilvi treatment (M0: not inoculated, M1: MycoSilvi variant 1, M2: MycoSilvi variant 2, M3: MycoSilvi variant 3) on the survival rate of *F. moluccana*

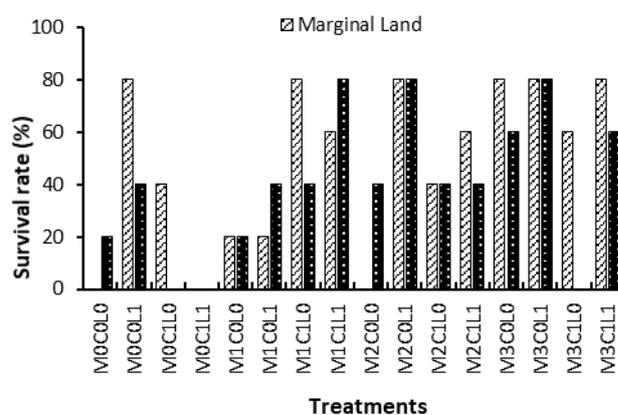


Figure 2. Effect of MycoSilvi (M0: not inoculated, M1: MycoSilvi variant 1, M2: MycoSilvi variant 2, M3: MycoSilvi variant 3), compost (C0: 0 g compost, C1: 32.5 g compost) and lime (L0: 0 g lime, L1: 7.2 g lime) on the survival rate of *F. moluccana*

Table 3. Effect of MycoSilvi and soil ameliorants on soil bacteria communities

MycoSilvi	Soil ameliorant		Soil bacteria (CFU/g)	
	Compost	Lime	Marginal land	Extreme marginal land
Without	-	-	250 x 10 ³	2 x 10 ³
MycoSilvi	-	+	2480 x 10 ³	464 x 10 ³
	+	-	233 x 10 ³	-
	+	+	-	-
MycoSilvi 1	-	-	301 x 10 ³	18 x 10 ³
	-	+	205 x 10 ³	19 x 10 ³
	+	-	1054 x 10 ³	20 x 10 ³
MycoSilvi 2	+	+	1880 x 10 ³	232 x 10 ³
	-	-	-	19 x 10 ³
	-	+	2525 x 10 ³	195 x 10 ³
MycoSilvi 3	+	-	181 x 10 ³	25 x 10 ³
	+	+	1050 x 10 ³	259 x 10 ³
	-	-	2550 x 10 ³	18 x 10 ³
	-	+	2535 x 10 ³	276 x 10 ³
	+	-	176 x 10 ³	-
	+	+	427 x 10 ³	202 x 10 ³

Under field conditions, some studies have been reported that AMF inoculation to plants could increase plant survival and establishment in the post-mining site (Dugbley et al. 2015; Wulandari et al. 2016). Mycorrhizal association with plant roots is indispensable for plant survival and growth (Buta et al. 2019). AMF plays a key role in improving the resilience of plant communities against environmental stresses, including nutrient deficiency, drought, and soil disturbance, and in promoting the growth rate of the plant and hormone activity (Bi et al. 2018; Chandanie et al. 2009). It is well known that AMF hyphae could extend beyond the rhizosphere, thus allowing AMF to acquire resources independently for plant growth and fitness (Węzowicz et al. 2017). In this research, the survival rate of *F. moluccana* could be increased significantly by MycoSilvi inoculation combined with soil ameliorant, indicating that inoculation of MycoSilvi combined with soil ameliorant could enhance plant survival and establishment under metal toxicity and acidic soil in post-silica sand mining land.

Soil bacteria communities

It is well known that soil bacteria play an important role in secreting enzymes as catalysts to decompose organic residues in the soil (Chodak et al. 2015; Lladó et al. 2017). Therefore, soil bacteria and other microorganisms play a crucial role in nutrient cycling, plant nutrition, health, and soil structure and fertility. The effect of different treatments on soil bacteria communities were shown in (Table 3).

In this study, the treatments of MycoSilvi inoculation and soil ameliorant addition showed higher results on soil bacterial quantity than the control treatment. It indicated that MycoSilvi inoculation combined with soil ameliorant effectively increased plant growth and establishment in post-silica sand mining site and, accordingly, also increased bacteria communities at the rhizosphere. Bi et al. (2018) also reported that, at poor soil quality, AMF could

increase the number of soil microbes, especially bacteria. According to (Toljander et al. 2007), exudates from AMF give a substantial quantitative and qualitative impact on bacterial communities in the mycorrhizosphere. In addition, the number of soil bacteria at EML was lower than at ML, indicating that the development of soil bacteria could be inhibited in low pH soil conditions and high heavy metal content. Shuaib et al. (2021) reported that increased metal concentrations would lead to the loss of the bacterial community and the reduction of the microbial community. Previous studies had demonstrated that soil pH was determinant for soil microbial community development, especially bacteria (Rousk et al. 2010; Wu et al. 2018).

Open-pit silica sand mining caused numerous environmental problems, which negatively affected plant growth. In this study, we found that inoculation of MycoSilvi and the addition of lime and compost could enhance plant growth, survival rate, and soil bacteria communities in degraded soil of mining fields. Inoculation of MycoSilvi variant 3, which contains *Glomus mosseae* and *Gigaspora margarita* combined with lime, showed the best result of *F. moluccana* growth. These results indicated that the application of MycoSilvi and soil ameliorants positively impacted initiating revegetation under infertile environments of mining fields and could be an effective method for further ecological rehabilitation in degraded land areas.

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