



## Effectiveness of several methods of mycorrhizal inoculation and inoculum doses on growth of red meranti (*Shorea leprosula* Miq.) wildlings

Basir Achmad<sup>1\*</sup>, Faisal<sup>2</sup>, Suhartati<sup>3</sup>

<sup>1</sup>Faculty of Forestry, Lambung Mangkurat University, Jl. A. Yani, Km 36, PO Box 19, Banjarbaru 70714, South Kalimantan, Indonesia

<sup>2</sup>Faculty of Mathematics and Natural Sciences, Jl. A. Yani, Km 36, PO Box 19, Banjarbaru 70714, South Kalimantan, Indonesia

<sup>3</sup>Badan Riset dan Inovasi (BRIN), Jl. Pajajiang No. 13 Sudiang Raya Makassar, Indonesia

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### Abstract

Micorrhizae have been proved to increase the growth of trees from Dipterocarpaceae family, but the research about applying several methods and inoculum levels has not been done. The purpose of this study was to determine the effective method of applying mycorrhizae inoculation and optimum levels of inoculum for the growth of red meranti wildlings. Experimental design used was a  $4 \times 3$  Nested Randomized Design, which consisted of two factors: inoculation methods and inoculum doses nested in the inoculation methods. Inoculation methods consisted of capsules, spores, spore suspension, and mycorrhizal soil. Inoculum doses consisted of 1 capsule, 2 capsules, and 3 capsules; 15 mg spores, 20 mg spores, and 25 mg spores; 3 ml of spore suspension, 4 ml of spore suspension, and 5 ml of spore suspension; 120 g soil, 160 g soil, and 200 g soil. Total seedling used was  $12 \times 3 \times 5 = 180$  wildlings. The results showed that the method of inoculation significantly affected the increase in height; and the levels of inoculum significantly increased the diameter of wildlings. The highest height of wildlings was the wildlings provided mycorrhizae spores (3,269 cm). The highest diameter increment was reached by the wildlings treated with mycorrhizal soil 200 g (0.374 cm). Mycorrhizae spores and mycorrhizal soil are available in large quantities in nature so they are feasible to use.

\* **Corresponding Author:** Basir Achmad ✉ [basir.achmad@ulm.ac.id](mailto:basir.achmad@ulm.ac.id)

## Introduction

Indonesia has a large tropical forest which is dominated by the *Dipterocarpaceae* family. This family is one of the most important tropical tree species that produce wood that can generate large foreign exchange for the country in addition to oil and mining goods. *Dipterocarpaceae* family has been widely exploited to meet the demand for wood, both domestically and abroad. The rapid rate of exploitation of natural forests, especially for the *Dipterocarpaceae* family, is feared to deplete the supply of wood in natural forests in a shorter time. To compensate for this, it is necessary to develop *Dipterocarp* species so that these species can still be sustainable. However, the planting of *Dipterocarpaceae* family still has many failures because the appropriate silvicultural system has not yet been fully controlled, both in nurseries and in planting and maintaining it in the field. One of the obstacles faced in the development of *Dipterocarpaceae* family is the procurement of seedlings in large quantities and at the right time. One of the species in question is red meranti (*Shorea leprosula* Miq.) which takes a long time to bear fruit, or does not bear fruit every year even though the meranti is needed to supply meranti tree species that have been exploited (Achmad, 2017).

One alternative to meet the needs of seedlings is to utilize natural seedlings that grow naturally in forest areas. However, sometimes plants in a place cannot grow well, this fact happens unexpectedly and can lead to failure in reforestation and planting efforts on dry land.

This failure is often caused by biotic factors, namely the presence or absence of mycorrhizal-forming fungi in the soil which plays a significant role in plant survival. It is known that *Dipterocarpaceae* family have symbiosis with several mycorrhizal fungi. This symbiosis has a positive effect on the growth rate and plant health. The ability of mycorrhizae to increase the absorption of nutrients and water is an important mechanism for forest plants growing on nutrient-poor soils. In wet tropical forests, mycorrhizae play an

important role as direct nutrient cyclists from organic matter in litter to plant roots. In critical soils, mycorrhizal plants have the ability to absorb nutrients more efficiently (Gunawan, 1993 in Achmad and Faisal 2021).

Mycorrhizal potential has important meaning as a factor that can be considered to be utilized for the benefit of forestry plants, especially when dealing with unproductive (critical) lands. Several researchers have proven that with the use of the method and in certain doses of mycorrhizae can increase the growth of several types of meranti. Achmad (2020) has proven that giving one mycorrhizal tablet to six species of *Dipterocarpaceae*: *Shorea leprosula* Miq., *Shorea ovalis* Korth., *Hopea mangerawan* Miq., *Shorea palembanica*, *Shorea macroptera* Dyer, and *Shorea parvifolia* can increase the growth of these species. Achmad and Faisal (2021) have also proven that providing mycorrhizae in the form of pollen/powder as much as 15 g/seedling can increase the growth of sengon (*Paraseriathes falcataria* Neils). However, the study about the most effective inoculation method has not been tested, including the dose for each of these inoculation methods.

On the basis of the description above, the purposes of this study is to analyze the effectiveness of several inoculation methods and their respective dose levels on the growth of natural meranti wildlings obtained by the extraction system.

## Method

### *Materials and tools*

The materials used in this study were red meranti wildlings, topsoil, black plastic bags (polybags), mycorrhizal capsules, mycorrhizal fungus spores from mycorrhizal fruiting bodies, mycorrhizal soil, and suspending agents (Tween 20). The tools used were soil tillers (soil filter, hoe), plant sprinklers, analytical balance, ruler or meter (to measure wildling height), syringe or pipette (as suspension drops), caliper (a tool to measure diameter), hood plastic, plastic buckets, foam (sponge), plastic jars (storing mold or spores), cuttings scissors, and stationery.

### Procedure

The research procedure started from field preparation which included the preparation of the research site, materials and equipment. Followed by the preparation of growth media, namely topsoil taken around the study site, then the topsoil was cleaned of grass or plant roots by filtering or sifting and sterilized by air-drying for 3 days. The soil was then put in a plastic bag (polybag) according to the number of wildlings. To determine the nutrient content of the soil used, some of the soil was analyzed at the Balai Penelitian Tanaman Pangan Lahan Rawa (Balitra) Banjarbaru. Furthermore, natural wildlings were collected after rain where the soil was still wet and soft so that it will be easier to remove, with the following criteria: they come from the same parent tree, the location of the wildlings was  $\pm 9$  m from the mother tree, has 3-6 leaves, wildlings' height was less than 35 cm, and the appearance of the wildlings was healthy and not bent or the appearance of growth suppressed.

The planting of wildlings on weaning media obtained from the forest was then separated or selected to get viable wildlings. Weaned wildlings were selected with the same number of leaves and height and were growing well, then placed in a simple greenhouse. After 14 days, the 180 wildlings were selected and divided into 4 treatments, where in each treatment there were 3 levels of factors which were repeated 5 times and each replication had 3 wildlings.

The 180 wildlings were each treated as follows: 45 plastic bags (A<sub>1</sub>) filled with soil (topsoil) at each level (B<sub>j</sub>) mycorrhizal capsules (1, 2, and 3 capsules) were given; 45 plastic bags (A<sub>2</sub>) filled with topsoil, at each level (B<sub>j</sub>) was given mycorrhizal spore powder (15, 20, and 25 mg); 45 plastic bags (A<sub>3</sub>) filled with topsoil, at each level (B<sub>j</sub>) was given spore suspension (3, 4, and 5 ml) of 5 g/liter mother liquor; 45 plastic bags (A<sub>4</sub>) filled with topsoil, at each level (B<sub>j</sub>) was given mycorrhizal soil (120, 160, and 200 g).

The preparation of the capsule inoculum was carried out by mixing the spores with sawdust as a "carrier"

and then put it into the gelatin capsule. In this study, mycorrhizal capsules were obtained from the Bogor Forest Soil Biology Laboratory, with spores derived from *Scleroderms* sp. Furthermore, the spore inoculants were taken from ripe fruit bodies and growing around the study site, then the spores were weighed according to the level of the B<sub>j</sub>(i) factor. Spore inoculants are a practical method in the field, considering the potential for large spores such as those in the genera *Scleroderma* and *Pisolithus*. According to Kuswanto (1990), for 1 mg of *Scleroderma* and *Pisolithus* spores there are  $\pm 1.1 \times 10^6$  spores. For spore suspension inoculants, the principle was the same as spore inoculants, except that water was needed in suspension, so it was felt that it was easier to implement (watering or dripping). In this study used 5 g of spores dissolved in 1 liter of water and added 6-8 drops of Tween 20. According to Stiadi *et al.* (1992), in dissolving 2 g of spores in 1 liter of water, it is necessary to add a few drops of Tween 20 solution to help spread the spores evenly. Soil inoculants were taken below the stand in the form of soil and humus. The collection was carried out in the morning followed by weighing the inoculants according to the level of the B<sub>j</sub>(i) factor. This soil inoculant was a classic mass production technique that has been used by several countries for field purposes.

Inoculation was carried out in the morning after the soil inoculants were taken. Each inoculated wildling was in accordance with the treatment A<sub>i</sub> with the factor level B<sub>j</sub>(i). Capsule inoculants were applied by immersing the capsule as deep as  $\pm 3 - 4$  cm with a distance of  $\pm 1 - 2$  cm from the wildling stem. Spore inoculants were carried out by sprinkling the spores evenly around the wildlings as deep as  $\pm 3 - 4$  cm with a distance of  $\pm 1 - 2$  cm from the wildling stems. Spore suspension inoculants were applied by dripping or injecting  $\pm 1 - 2$  cm suspension from wildling with a depth of  $\pm 3 - 4$  cm, and soil inoculants were given by replacing part of the media with soil inoculants. During the study, maintenance was carried out, namely watering twice a day (morning and evening), cleaning of weeds in and around the beds.

### Parameters

Parameters observed were the increase in wildling height measured from the soil surface (marked) to the apical meristem (where the young leaves emerge on the main stem), the increase in the number of leaves, and the increase in diameter, where the three parameters were observed 4 times during the study: beginning of the study, one month running, two months running and the end of the study. The viability of wildlings was determined by counting the number of wildlings that grew divided by the total number of wildlings that were planted expressed in percent (%) and calculated at the end of the study.

### Experimental design and analysis

The experimental design used was a nested experimental design ( $4 \times 3$ ) with 4 factors and 3 levels of nested factors. Each treatment consisted of 3 wildlings and 5 replications. Inoculation methods (Ai) consisted of the inoculation with mycorrhizal capsule (A1), inoculation with mycorrhizal spores (A2), inoculation with spore suspension (A3), and

inoculation with mycorrhizal soil (A4). Inoculant doses or the dose of factor (Bj), consisted of 1, 2 and 3 capsules (Bj(1)), 15, 20 and 25 mg spores (Bj(2)), 3, 4 and 5 ml spore suspension (Bj (3)) from 5 grams/liter of basic solution, and 120, 160 and 200 grams of mycorrhizal soil (Bj(4)). Data analysis was performed by analysis of variance. The significant effects of among treatments were continued with the least significant difference test on the level of 0.05. Data analysis used the SPSS package.

## Results

### Height increment

Based on the tests of between-subjects effect or the F test (Table 1), the inoculation method significantly affected the height increment of red meranti wildlings ( $p\text{-value} = 0.000 < 0.05$ ), while the inoculum levels did not affect the the height increment significantly ( $p\text{-value} = 0.218 > 0.05$ ). The highest height was the wildlings provided mycorrhizae spores (3,269 cm), while the lowest one was the wildlings provided mycorrhiza suspension (2.293 cm).

**Table 1.** Tests of between-subject effects on the height increment of the wildlings.

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculation	7.976	3	2.659	7.300	.000
Dosage (Inoculation)	4.101	8	.513	1.407	.218
Error	17.483	48	.364		
Total	29.560	59			

To test the effects of the inoculation method among the treatments on the height increment of the red meranti wildlings, a pairwise comparison was done based on the least significant difference test at the level of 0.05 as described in Table 2. Based on Tabel 2, the effect of mycorrhiza spores was significantly different from the effect of the mycorrhizae suspension, yet it did not differ significantly from the effect of the mycorrhiza soil, and mycorrhiza capsules.

### Diameter increment

Diameter increment was significantly affected by the levels or the doses of mycorrhizae ( $p\text{-value} 0.012 < 0.05$ ) based on the thest between-subject effects of the diameter increment of red meranti wildlings as

seen in Table 3.

Average diameter increment of red meranti wildlings was 0.284 cm. The highest diameter increment was reached by the wildlings treated with mycorrhizal soil 200 g (0.374 cm).

This diameter increment was significantly different from the diameter increment of the wildlings treated with the mycorrhizal capsule of 1 capsule (0.242 cm), the mycorrhizal spore of 15 mg (2.220 cm), the mycorrhizal soil of 120 g (0.220 cm), the spore suspension of 4 ml (0.214 cm), and the spore suspension of 3 ml (0.200 cm). Yet, it did not differ significantly from the diameter increment of wildlings treated with the mycorrhizal sopres of 25 mg (0.362

cm), the spore suspension of 5 ml (0.356 cm), the mycorrhizal capsule of 3 capsules (0.342 cm), the mycorrhizal spores of 20 g (0.328 cm), the mycorrhizal capsules of 2 capsules (0.276 cm), and the mycorrhizal soil of 160 g (0.276 cm). To test the

effects of the inoculation dosage among the treatments on the diameter increment of the red meranti wildlings, a pairwise comparison was done based on the least significant difference test at the level of 0.05 as described in Table 4.

**Table 2.** Pairwise comparisons of inoculation method effects on the height increment.

Methods	Height increment (cm)	Marks
Mycorrhizal spores	3,269	a
Mycorrhizal soil	3,067	a
Mycorrhizal capsules	2,837	a
Spore suspension	2.293	b

#### *Leaf increment*

Leaf increment of red meranti wildlings has been tested. The tests of between-subject effects on the leaf increment of the wildlings of red meranti was described in Table 5.

Based Table 5, inoculation methods and dosages nested in the inoculation method did not affect significantly the leaf increment of the red meranti wildlings.

### **Discussion**

#### *Height increment*

Height increase is a feature of plant life, where the increase in height is the result of the interaction of the physiological processes of the plant so that the cell development of the plant occurs.

Based on the tests of between-subject effects of the height increment of red meranti wildlings (Table 1), the effectiveness of several ways of mycorrhizal inoculation had a very significant effect on the increase in the height of red meranti wildlings. This is because the inoculum inoculated in different ways is capable of symbiotic relationship with plant roots and has different levels of infectivity, where the highest level of mycorrhizal fungi infectivity was that which was transmitted through spores, followed by the use of mycorrhizal soil media. This is presumably because both methods can transmit mycorrhizae directly to plant roots because both inoculants can easily infect wildlings' roots because the location of the inoculant source is not far from the research location so that the conditions are relatively the same, so the mycorrhizae can develop rapidly.

**Table 3.** Test between-subject effects on the diameter increment of the wildlings.

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculation method	.017	3	.006	.602	.617
Dosage (Inoculation)	.217	8	.027	2.837	.012
Error	.458	48	.010		
Total	.692	59			

Mosse and Hyman (1971) in Soemardi (1986) state that the success of inoculation is highly dependent on the ability of the fungus to live and thrive in a new soil environment, including the ability to compete with existing soil microbes. The competition will be less meaningful if the mycorrhizae have formed first on the plant roots. One of the conditions that must be met by plants to form mycorrhizae in a place is the

availability of suitable mycorrhizal fungi in that place. According to Birch (1986), the poor correlation between the number of spores and mycorrhizal initiation, and the rapid initiation (within days) of vesicular-arbuscular mycorrhizas (VAM) infections that often occur in ecosystems suggest that a pre-existing network rather than soil hyphae is more common is the main source of VAM inoculum. Even

dead root parts infected with soil-dwelling mycorrhizal fungi can also initiate VAM as long as they are in close proximity to new roots (McGee, 1987).

In Table 2 (the pairwise comparisons of inoculation method effects) it can be seen that inoculation through spores had the greatest effect on increasing the height of meranti wildlings, which means that it has the best transmission power because transmission through spores is very easy to occur. This was stated by several researchers such as Koske and Gemma (1990) who have observed that fungal spores produced by rhizome leaf sheath or quiescent fungal structures in old roots can function as inoculum, even after exposure to seawater. Furthermore, Allen (1988) stated that fungal spores from animal waste can introduce VAM to new locations. Trappe and Maser (1976) have also observed that VAM spores can still grow after passing through the digestive tract of rodents. Trappe and

Maser (1976) continued that fauna that may spread spores are small animals, grasshoppers, worms, ants, wasps, and birds. Then Rabitin and Stinner (1988) explained that macroarthropod detritivores such as woodlice (Isopoda) and millipedes (Diplopoda) consume and disperse mycorrhizal inoculum and may internally be consumed by many small animals which may thus act as vectors for the mycorrhizal fungi. Brundrett (2009) stated that mycorrhizal fungal spores have been found in organisms that can serve as vectors, including in the tropics, but the distance and importance of this dispersal mechanism in the ecosystem is not known with certainty. Fakuara and Setiadi (1986) concluded that the height of mycorrhizal inoculated plant seedlings could increase two to three times compared to the uninoculated plant height.

This indicates that the higher mycorrhizal transmission will increase the absorption of nutrients and water, so that plant growth will be better.

**Table 4.** Pairwise comparisons of inoculation method effects on the diameter increment.

Inoculation methods	Dosages	Diameter increment (cm)	Marks
Mycorrhizal soil	200 g	0.374	a
Mycorrhizal spores	25 mg	0.362	ac
Spore suspension	5 ml	0.356	ace
Mycorrhizal capsules	3 capsules	0.342	aceg
Mycorrhizal spores	20 mg	0.328	acegh
Mycorrhizal capsules	2 capsules	0.276	aceghi
Mycorrhizal soil	160 g	0.272	aceghi
Mycorrhizal capsules	1 capsule	0.242	bcceghi
Mycorrhizal spores	15 mg	0.220	dfghi
Mycorrhizal soil	120 g	0.220	ghi
Spore suspension	4 ml	0.214	hi
Spore suspension	3 ml	0.200	i
Average		0.284	

#### *Diameter increment*

According to Suhardi (1985), the increase in diameter is due to lateral meristem activity which includes primary and secondary cambium. Primary cambium is located between the bark and wood which will cause thickened growth, where every year new xylem and phloem will always be formed. Based on the test between-subject effects of the diameter increment of

red meranti wildlings (Table 3), the inoculation method had a very significant effect on the increase in diameter of red meranti wildlings. Furthermore, based on the diameter increment of red meranti wildlings based on the dosages of mycorrhizae (Table 4), all of the inoculation materials that had the highest dose of each type of inoculation material (mycorrhizal soil 200 g, mycorrhizal spores 25 mg,



spore suspension 5 ml) , and mycorrhizal capsules 3 capsules) had a significant effect on increasing the diameter of meranti wildlings. This was presumably because the highest dose of each inoculation material was able to infect the roots of red meranti wildlings, thus affecting the increase in diameter, while the lower dose was considered insufficient inoculant to infect the roots of meranti wildlings. This situation is in accordance with the results of previous studies conducted by Badaruddin (1997) using spore

inoculants and Lutfian (1991) with soil inoculants reporting that increasing the dose of inoculum will provide growth in plants that continues to increase. According to Seomardi (1986), the lack of inoculum will not guarantee the occurrence of mycorrhizae on plant roots. If there was an infection, it would be so slow that it would be inefficient. This may be due to the inoculants given to the roots of many plants have died, so that inoculants that have a large number can have an infectious effect on plant roots.

**Table 5.** Tests of between-subject effects on the leaf increment of the wildlings.

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculation	3.451	3	1.150	2.637	.060
Dosage (Inoculation)	1.284	8	.161	.368	.932
Error	20.944	48	.436		
Total	25.679	59			

This is in line with the observation by Harley and Smith (1983) that many fungi that form ECM associations have large fruit structures (mushrooms) that produce large numbers of wind-borne spores, but the survival and dispersal of these spores may be limited because they are consumed by other microorganisms. Fungal-eating nematodes and springtails have been observed to reduce their association with mycorrhizae in some cases (Warnock *et al.*, 1982; Ingharn, 1988; Rabatin and Stinner, 1988). VAM fungal spores isolated from soil in natural ecosystems often show signs of predation (Berliner and Torrey, 1989 in Brundrett, 2009) which may influence seasonal fluctuations in their abundance. Hyphal grazing by soil organisms has possibilities to significantly reduce the efficacy of mycorrhizal associations in ecosystems by inhibiting the transport of mineral nutrients to the roots (Finlay, 1985; Ingham, 1988; McGonigle and Fitter, 1988b; Rabatin and Stinner, 1988 in Brundrett, 2009), but also allows nutrients contained in hyphae to be recycled (Coleman, 1985; Perry *et al.*, 1987). Furthermore (Brundrett (2009) stated that the supply of mycorrhizal inoculum could be limited in some recently created or disturbed habitats if these fungi were less readily dispersed than their host plants.

### Conclusions and recommendations

The inoculation method significantly affected the height increment of red meranti wildlings. The highest height was the wildlings provided mycorrhizae spores (3,269 cm), while the lowest one was the wildlings provided mycorrhiza suspension (2,293 cm). The effect of mycorrhiza spores was significantly different from the effect of the mycorrhizae suspension, yet it did not differ significantly from the effect of the mycorrhiza soil, and mycorrhiza capsules. Likewise, Diameter increment was significantly affected by the levels or the dosages of mycorrhizae. The highest diameter increment was reached by the wildlings treated with mycorrhizal soil 200 g (0,374 cm). This diameter increment was significantly different from the diameter increment of the wildlings treated with the mycorrhizal capsule of 1 capsule (0,242 cm), the mycorrhizal spore of 15 mg (2,220 cm), the mycorrhizal soil of 120 g (0,220 cm), the spore suspension of 4 ml (0,214 cm), and the spore suspension of 3 ml (0,200 cm). Yet, it did not differ significantly from the diameter increment of wildlings treated with the mycorrhizal spores of 25 mg (0,362 cm), the spore suspension of 5 ml (0,356 cm), the mycorrhizal capsule of 3 capsules (0,342 cm), the mycorrhizal spores of 20 mg (0,328 cm), the mycorrhizal capsules of 2 capsules (0,276 cm), and the mycorrhizal soil of 160 g (0,276 cm).

It is recommended to use an inoculation system and a dosage as follows: spores 25 mg, mycorrhiza soil 200 g, mycorrhiza spore suspension 5 ml or 3 capsules of micorrhizae. It is better to inoculate the wildlings before planting in the field using soil inoculants or micorrhizal spores, because these inoculants are more effective than mycorrhizal capsules. The soil and micorrhizal spore inoculants can be used at any time and are available in large enough quantities. In certain conditions where the natural conditions/conditions at that time did not allow the use of soil or spore inoculants, among others due to the long dry distance which affects the availability and quality of materials (inoculum), capsule inoculants can be used.

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