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Effects of mycorrhizae and rhizobium on the growth of sengon (*Paraserianthes falcataria* Neils) seedlings planted on peat media

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Abstract

The objectives of the research were to analyze the effects of mycorrhizae and rhizobium on sengon seedling growth through the height, and number of leave increments, diameter growth, and dry weight of roots. Factors applied were mycorrhizae and rhizobium. Mycorrhiza factor consisted of without mycorrhizae, 15 gram/polybag, and 30 gram/polybag. Rhizobium factor consisted of without rhizobium, 15 milligram/polybag, and 30 milligram/polybag. The method used was a factorial experiment in a completely randomized design. The number of treatment combinations was 9 combinations. Replications were three times, and the number of wildlings of each treatment was four wildlings. The total number of wildlings observed was 108 seedlings. The results showed that the mycorrhiza factor provided significant effects on the height increments, and dry weight of roots of seedlings; while the rhizobium factor provided significant effects on the number of leave increments, and dry weight of roots of seedlings. There was no interaction effect between the mycorrhizae and rhizobium. The sengon seedlings that had the highest height increment (9.51 cm) were the seedlings provided 15 gram/polybag of mycorrhizae, while that had the highest dry weight of roots (2.20 gram) were the ones provided mycorrhizae of 15 gram/polybag. The sengon seedlings that had the highest number of leaf increments (11.66 leaves) were the seedlings provided 15 milligram/polybag of rhizobium, while that had the highest dry weight of roots (2.05 gram) were the seedlings provided 30 milligram/polybag of rhizobium.

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Introduction

Forestry development activities in Indonesia are inseparable from the current global issue (ecolabeling) which contains one of the conditions for tropical timber trade, namely that it must be harvested from a clear crop. Therefore, the development of industrial forest plantations as a source of wood raw material for various industries is a top priority, considering that the potential for natural forests that previously relied on has now decreased, both in terms of area and volume.

Constraints faced in industrial plantations are the type of soil used is generally less fertile, which is grasslands and critical. Therefore, selecting the types of plants that can utilize the nutrients that come from symbiotic services is very important, considering that fertilization is not possible on a large scale in industrial forest plantations in case of nutrient deficiency. The elements N and P can be overcome by the services of fungi and bacteria.

It is known that several types of forestry plants can associate with to form mycorrhizae, which is a symbiotic mutualism between fungi and plant roots. Fungi get a place to live and carbohydrates from the roots, while plants can increase the uptake of phosphorus (P), especially in nutrient-poor soils (Gunawan, 1993). In addition to mycorrhizae, forestry plants, especially from the Leguminosae family, can make a symbiosis with *Rhizobium radicicola* bacteria, to utilize N_2 in the air in the metabolic process, which cannot be absorbed if there is no active role of bacteria. Soeseno (1974) states that the role of nodules formed as a result of symbiosis can supply approximately 75% of nitrogen requirements for plants.

Sengon (*Paraserianthes falcataria* Neils) is a type that is very likely to be selected because it is a type of Leguminoceae which is known to be able to symbiosis with *Rhizobium radicicola* bacteria to utilize nitrogen (N2) in the air and is also known to be associated with mycorrhizae (Suhardi *et al.*, 1990). As a growth medium that contains a lot of organic, lightweight, practicality in transportation and distribution, peat can be a good alternative that can replace topsoil which is commonly used as a weaning medium. Moreover, the peat in the wetlands is the research center of the Lambung Mangkurat University, so the use of peat as a growth medium is in accordance with the vision and mission of the Lambung Mangkurat University in South Kalimantan province.

The purpose of this study was to analyze the role of mycorrhizae, rhizobium, and their interactions on the growth of sengon seedlings on peat media. The benefits of the research are expected to provide information about the dual effects of mycorrhizae and rhizobium in utilizing nutrient sources from symbiotic services so that some of the nutrient constraints on peat media can be overcome by utilizing mycorrhizal and rhizobium.

Materials and methods

Materials used were sengon seedlings aged 2 weeks, peat soil as growth media of the seedlings, black polybags with the size of 9 cm × 15 cm as containers of the growth media, mycorrhizae with the trademark of Biofer 2000-N, *Rhizobium japonicum* with the trademark of Superizogen, and formalin (2%) for growth media sterilization. Equipment used was germination beds for germinating the sengon seeds, scales for weighting mycorrhizae and rhizobium pollen/powder, a hand sprayer for chemical application, a measurer for measuring the height of seedlings, a caliper for measuring the diameter of seedlings, and a water sprayer for watering the seedlings.

The research procedure was done by preparing a nursery where the research was done. In addition, the peat used as growth media of seedlings was processed first before they were used as growth media. The process was to dry and cut it into pieces. After that, it was mixed with formalin (2%) for fumigation. Also, it was mixed with dolomite to increase the pH of the peat. Besides, the seedlings were from the sengon seeds. The treatment for the seeds was firstly selected and they were germinated in the germination boxes.

Germination media used was sand that had been sterilized by solarization system that is by drying the sand under the sunlight for 3 hours on the temperature about 50 °C to protect the media from the fungi or diseases. Before germinating the seeds, they were soaked in the water for 24 hours to hasten the germination. One week after germination, the seedlings were transplanted to polybags containing peat media/substrate. One week after transplanting, the mycorrhiza and rhizobium were applied to the seedlings as an amount based on research treatments. During observation, tending was done by watering the seedlings and clearing the weeds around the seedlings.

The first factor (A) observed was the mycorrhiza factor consisting of without mycorrhizae (A0), mycorrhizae as the amount of 15 gram/polybag (A1), and 30 gram/polybag of mycorrhizae (A2). The second factor (B) was rhizobium factor consisting of without rhizobium (B0), 15 milligram/polybag of rhizobium (B1), and 30-milligram rhizobium/polybag. Each unit sample consisted of four seedlings, and they were replicated three times so that it was needed seedlings as an amount of 108 seedlings (9 treatment combinations × 3 replications × 4 seedlings). Variables measured were the height, and the number of leave increments which were measured at the beginning and at the end of research. In addition, diameter, and dry weight of roots of the sengon seedlings were measured only at the end of the research. The effects of the treatments on the variables measured were analyzed using a factorial model in a completely randomized design. The Statistical Package for Social Sciences (SPSS) software was used to conduct the analysis.

Results

Height increments

Based on the tests of between-subjects effect or the F test, the mycorrhiza factor significantly affected the height increment of the sengon seedlings (Sig. 0.001 < 0.05), while the rhizobium factor (Sig. 0.471 > 0.05), and its interaction with the mycorrhiza factor (Sig. 0.061 > 0.05) did not affect the height increment of the sengon seedlings. The tests of between-subjects effect of the height increment of the sengon seedlings were presented in Table 1.

Table 1. The tests of between-subjects effects of the height increment of seedlings.

Tests of Between-Subjects Effects					
Dependent Variable: Height Increments					
Source	Sum of Squares	Degree of Freedom	Mean Square	F	Sig.
Mycorrhizae	22.540	2	11.270	11.435	.001
Rhizobium	1.547	2	.773	.785	.471
Mycorrhizae* Rhizobium	10.805	4	2.701	2.741	.061
Error	17.741	18	.986		
Total	52.633	26			

The average height increment of sengon seedlings treated with mycorrhizae of 15 g/polybag (A1) was 9.51 cm; the sengon seedlings treated with mycorrhizae of 30 g/polybag (A2) was 8.43 cm; the sengon seedlings without mycorrhizae treatment (A0) was 7.28 cm. To analyze which treatments providing different height increments from other treatments, the analysis was continued to the multiple comparisons based on the post hoc tests of height increment of sengon seedlings using the least significance test at the 0.05 level as described in Table 2.

Table 2. The multiple comparisons of height increment of sengon seedlings.

Treatments (mycorrhizae)	Height increments (cm)	Marks
A1 (15 g/polybag)	9.51	a
A2 (30 g/polybag)	8.43	a
A0 (0 g/polybag)	7.28	b

Note: The treatments with a different mark were different significantly at the 0.05 level.

Diameter growth

Based on the tests of the between-subjects effect or the F test, the mycorrhizae (Sig. 0.076 < 0.05), rhizobium (Sig. 0.076 < 0.05), and the interaction of both mycorrhizae and rhizobium (Sig. 0.752 < 0.05) did not significantly affect the diameter growth of sengon. The tests of the between-subjects effect of the diameter increment of the sengon seedlings were presented in Table 3.

Table 3. The tests of between-subjects effects for the diameter growth of seedlings.

Tests of Between-Subjects Effects					
Dependent Variable: Diameter Growth					
Source	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Mycorrhizae	.004	2	.002	2.989	.076
Rhizobium	.004	2	.002	2.989	.076
Mycorrhizae * Rhizobium	.001	4	.000	.478	.752
Error	.012	18	.001		
Total	.021	26			

Based on Table 3, there were no effects of the treatments on diameter growth of the sengon seedlings; the analysis was not continued to the multiple comparisons based on the post hoc tests of diameter growth of sengon seedlings using the least significance test at the 0.05 level.

The number of leaf increments

Based on the tests of between-subjects effect or the F test, the mycorrhiza factor did not affect the number of leaf increments of the sengon seedlings (Sig. 0.204 < 0.05). Likewise, the interaction effects between mycorrhizae and rhizobium did not affect the number of leaf increments of sengon seedlings (Sig. 0.078 > 0.05). The only factor affected the number of leaf increment was the rhizobium factor (Sig. 0.013 < 0.05). The tests of the between-subjects effect of the number of leaf increments of the sengon seedlings were presented in Table 4.

Table 4. The tests of between-subjects effects of the number of leaf increment of sengon seedlings.

Tests of Between-Subjects Effects					
Dependent Variable: The number of leaf increments					
Source	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Mikoriza	1.479	2	.740	1.740	.204
Rhizobium	4.773	2	2.386	5.613	.013
Mikoriza * Rhizobium	4.273	4	1.068	2.513	.078
Error	7.652	18	.425		
Total	18.177	26			

The number of leaf increment of sengon seedlings was 11.66 leaves for the seedlings with rhizobium of 15 milligram/polybag (B1), 10.83 leaves for the seedlings with 15 milligram/polybag of rhizobium (B0), and 10.72 leaves for the seedlings with 30 milligrams of rhizobium (B2). To analyze which treatments providing different number of leave increments, the analysis was continued to the multiple comparisons based on the post hoc tests of the number of leaf increments of sengon seedlings using the least significance test at the 0.05 level as described in Table 5.

Table 5. The multiple comparisons of the number of leaf increments of sengon seedlings based on the rhizobium treatments.

Treatments (rhizobium)	Number of leaf increments (leaves)	Marks
B1 (15 mg/polybag)	11.66	a
B0 (0 mg/polybag)	10.83	b
B2 (30 mg/polybag)	10.72	b

Note: The treatments with a different mark were different significantly at the 0.05 level.

The dry weight of roots

Based on the tests of between-subjects effect or the F test, the mycorrhiza factor affected the dry weight of roots of the sengon seedlings (Sig. 0.000 < 0.05). Likewise, the rhizobium factor affected the dry weight of roots of sengon seedlings (Sig. 0.017 < 0.05). However, the interaction between the mycorrhiza and rhizobium factors did not affect the dry weight of roots (Sig. 0.573 > 0.05). The tests of the between-subjects effect of the dry weight of roots of the sengon seedlings were presented in Table 6.

Table 6. The tests of between-subjects effects of the dry weight of roots of the sengon seedlings.

Tests of Between-Subjects Effects					
Dependent Variable: Dry Weight of Roots					
Source	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Mycorrhizae	1.419	2	.709	16.956	.000
Rhizobium	.430	2	.215	5.143	.017
Mycorrhizae * Rhizobium	.125	4	.031	.747	.573
Error	.753	18	.042		
Total	2.727	26			

The average dry weight of roots of the sengon seedlings treated with mycorrhizae of 15 g/polybag (A1) was 2.20 gram; the sengon seedlings treated with mycorrhizae of 30 g/polybag (A2) was 1.96 gram, and the sengon seedlings without mycorrhizae treatment (A0) was 1.64 gram. To analyze which treatments providing different height increments from other treatments, the analysis was continued to the multiple comparisons based on the post hoc tests of the dry weight of roots of the sengon seedlings using the least significance test at the 0.05 level as described in Table 7.

Table 7. The multiple comparisons of the dry weight of roots of sengon seedlings based on the mycorrhiza treatments.

Treatments (mycorrhizae)	Dry weight of roots (gram)	Marks
A1 (15 g/polybag)	2.20	a
A2 (30 g/polybag)	1.96	b
A0 (0 g/polybag)	1.64	c

Note: The treatments with a different mark were different significantly at the 0.05 level.

The average dry weight of roots of the sengon seedlings treated with rhizobium of 30 mg/polybag (B2) was 2.05 gram; the sengon seedlings treated with rhizobium of 15 mg/polybag (A2) was 2.00 gram, and the sengon seedlings without rhizobium treatments (B0) was 1.76 gram. To analyze which treatments providing different dry weight of roots from other treatments, the analysis was continued to the multiple comparisons based on the post hoc tests of the dry weight of roots of the sengon seedlings using the least significance test at the 0.05 level as described in Table 8.

Table 8. The multiple comparisons of the dry weight of roots of sengon seedlings based on the rhizobium treatments.

Treatments (rhizobium)	Dry weight of roots (gram)	Marks
B2 (30 mg/polybag)	2.05	a
B1 (15 mg/polybag)	2.00	a
B0 (0 mg/polybag)	1.76	b

Note: The treatments with a different mark were different significantly at the 0.05 level.

Discussion

Mycorrhizae

Plant growth is the increase of plant dimension as a result of the interaction between the plants and the environment through a physiological process. Based on the F-test, the mycorrhizae inoculation significantly affected the height increment of the sengon seedlings. Likewise, the dry weight of roots was affected significantly by the mycorrhizae. It was assumed that the mycorrhizae were able to do a symbiosis with the roots of sengon effectively so that they supported the plant in increasing nutrient absorption especially phosphorus (P). In the symbiosis between the mycorrhizae and the host plants, mostly the increase of growth because of integration of factors such as the enhancement of nutrient and water absorption, mineral solution, the potential of roots against the pathogen, the growth hormone production, and the movement of carbohydrate from one plant to another one (Hacskaylo, 1972 in Baker *et al*.,1992). Furthermore, the enhancement of growth and the vigor of the host plants were caused by the effectiveness of the root surface for nutrient absorption (Hatch, 1977 cited by Rahmadi 1985). Those all factors that might directly cause the quality of nutrient absorption to become higher and nutrition supply for the sengon seedling growth particularly the height growth became also higher.

Based on the multiple comparisons of height increments of sengon seedlings (Table 2), A1 treatment (15 gram of mycorrhizae/polybag) yielded the highest height increment of sengon seedlings that was 9.51 cm compared to A2 (30 gram/polybag) that was 8.43 cm and A0 (without mycorrhizae) that was 7.28 cm during observation (3 months). The treatment of 15 gram of mycorrhizae/polybag yielded the highest height increment that was significantly different from the height increment of the seedlings without mycorrhizae, although was not different significantly from the height increment of the seedling with mycorrhizae of 30 gr/polybag. It was assumed that the treatment was able to enhance a symbiosis between the mycorrhizae and the roots of the sengon seedlings. The symbiosis resulted in the infection of seedling roots and then resulted in seedling root development. This was proved by the dry weight of seedling roots of sengon (Table 9) showing that the treatment of 15 gram/polybag of mycorrhizae provided the highest increment of the dry weight of roots. This result was in accord with the dosage of mycorrhizae (Biofer 2000-N) recommended by the *Intidaya Agrolestari* Limited Company-the producer of the Biofer 2000-N, i.e.15 gram or one spoon of the product was the optimal dosage for plant growth. The treatment of 30 gram/polybag of mycorrhizae was considered to be too much for the seedlings aged 2 weeks. In the form of mycorrhiza tablet, Achmad (2020) found that one tablet for one seedling was successfully stimulated the growth of the wildlings of the six tree species from the Dipterocarpaceae family, either on the height or on the diameter increment of the wildlings.

The treatments did not affect the diameter growth. This was likely determined by characteristics of small seedlings that are the growth of seedlings is preceded by the height growth and then followed by the diameter growth. According to Kramer and Kozlowski (1960), long-lived species have a longer diameter growth period than short-lived species. Furthermore, it is said that diameter growth requires a longer period than height growth. Some species show growth in diameter always preceded by growth in height; even some species have not shown growth in diameter, even though the leaves have developed. So it is assumed that there is no effect of the treatments applied in the present study because the growth diameter was relatively small.

Rhizobium

In general, rhizobium bacteria are heterotrophs, which mean that the rhizobium's energy source comes from the oxidation of organic compounds such as sucrose and glucose. To increase these organic compounds, rhizobium bacteria need host plants. The rhizobium bacteria obtain food in the form of minerals, sugar/carbohydrates, and water from the host plant, while the host plant receives a reward in the form of nitrogen which is fixated by the rhizobium from the atmosphere.

According to Satria (2020), the composition of the atmosphere is dominated by nitrogen (78.08%), followed by oxygen (20.95%), and argon (0.93%). Furthermore, Nasikah (2007) stated that nitrogen in the air can be a form of urea $CO(NH_2)_2$, N_2, and N, but none of which can be used directly by plants. As a result, farmers have to add nitrogen sources to the soil in the form of inorganic fertilizers containing nitrogen such as urea, ZA, and NPK fertilizers. These inorganic compounds can cause environmental pollution and cost money.

Some of the advantages of utilizing rhizobium bacteria as nitrogen fixers from the atmosphere as biological fertilizers are that they do not pose a danger to the environment. In addition, the cost is relatively cheap with very simple technology (Surtiningsih *et al.*, 2009). Naturally, nitrogen is present in the soil, but this element is quickly lost, either through volatilization, nitrification, denitrification, or drifting with water, and erosion (Ashari, 2006).

Furthermore, Dewi (2007) stated that nitrogen is an element needed by plants to form important compounds in plant cells, including proteins, DNA, and RNA. And according to Nasikah (2007), nitrogen for plants functions as a constituent of protoplasm, chlorophyll molecules, nucleic acids, and amino acids which are constituents of proteins. Likewise, Surtiningsih *et al.* (2009) stated that nitrogen fixation by Rhizobium bacteria can increase the formation of chlorophyll and enzymes which in turn will increase photosynthesis which in turn could increase vegetative and generative growth.

Several researchers have proven that inoculation of rhizobium bacteria can increase plant growth and production. Rizqiani *et al.* (2007) found that the application of liquid fertilizer with a dose of 0.0625 ml of rhizobium bacteria for every 25 cm2 can increase the growth and production of *Phaseolus vulgaris* plants. Surtiningsih *et al.* (2009) also found that rhizobium bacteria were able to significantly increase the growth and production of soybean plants when compared to plants that were not given rhizobium bacteria.

In line with the explanations and findings above, in the present study, it was also found that the treatment of rhizobium as an amount of 15 mg/polybag could significantly increase the number of leaves (11.66 leaves) when compared to the treatment without rhizobium (10.83 leaves) and with the provision of rhizobium 30 mg/polybag (10.72 leaves). The present study also found that with the provision of rhizobium 30 mg/polybag, the dry weight of the seeds could reach 2.05 g, which was significantly different from the dry weight of seedlings not given rhizobium (1.76 g), but not significantly different from the seedlings treated with rhizobium 15 mg/polybag with a dry weight of 2.00 gr. Based on the results of the present study, it was found that a dose of rhizobium bacteria 15 mg/polybag was the best treatment in terms of increasing the number of leaves and overall growth by measuring dry weight. A dose of 30 mg/polybag was considered too high for small seedlings.

Conclusion and Recommendations

The mycorrhizae factor had a very significant effect on the independent variables of the increase in height and dry weight of seedlings. The treatment of 15 g mycorrhizae/polybag had a significant effect on the increase in seedling height (9.51 cm), which was significantly different from that without mycorrhizae treatment (7.28 cm), but not significantly different from the results of the mycorrhizae treatment of 30 g/polybag (8.43 cm). Also, the mycorrhizae treatment of 15 g/polybag gave a very significant effect on the dry weight of the seedlings (2.20 g), which was significantly different from the results of the treatment without mycorrhizae treatment (1.64 g), but not significantly different from the results of the treatment of 30 mg/polybag (1.96 g). The rhizobium factor had a significant effect on the independent variables of increasing leaf number and seedling dry weight. Giving rhizobium 15 mg/polybag resulted in an increase in the number of leaves as the amount of

11.66 leaves, which was significantly different from the results of the treatment without rhizobium (10.83 leaves) and the results of rhizobium treatment 30 mg/polybag (10.72 leaves). Rhizobium with the treatment of 30 mg/polybag gave significantly dry weight results (2.05 g) different from treatment without rhizobium (1.76 g), but not significantly different from the results of treatment with rhizobium 15 g/polybag with a dry weight of 2.00 g. The mycorrhizae and rhizobium factors affected several variables of sengon growth, but on these two factors, there was no interaction effect on the growth of sengon. Based on the results of the present study, it is recommended to use a mycorrhizae dose of 15 g/polybag and or 15 mg rhizobium/polybag to support the growth of sengon seedlings.

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