The role of rhizobacteria and ecoenzyme in agarwood growth 

Aquilaria malaccensis Lamk.

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Abstract. Rhizobacteria inoculation and ecoenzyme application can improve seeds' growth. This research aims to study the potency of rhizobacteria and ecoenzyme to improve Aquilaria malaccensis growth. The experimental design used a nested plot design where ecoenzyme as the sub-plot that nested in rhizobacteria inoculation (Serratia marcescens, Stenotrophomonas maltophilia, and the mix of S. marcescens + S. maltophilia) as the main plot. The results showed that rhizobacteria inoculation and ecoenzyme application were no significant effect on improving the plant height, diameter, amount of new leaves, total wet weight, total dry weight, and seed moisture content. The new shoots on S. marcescens inoculation without ecoenzyme increased 112.5% compared to the control. Application of ecoenzyme to the plants inoculated by rhizobacteria promoted root growth and made the leaves got greener. Shoot root ratio and seedling quality index showed that seeds were ready to transplant in the field. The results of this study can be used as a reference in applying biofertilizers to forestry plants, especially the slow-growing species.

1. Introduction

Agarwood is a type of tree that produces sapwood containing secondary metabolites from microbial infection in tree tissue [1]. Agarwood sapwood contains sesquiterpene compounds (terpenoid) which give rise to a fragrant aroma in gaharu sapwood [2]. The compounds produced by agarwood sapwood are widely used in the perfume industry, cosmetics, medicines, and religious ritual purposes such as incense and incense [3]. Agarwood sapwood can be produced from the genera Aquilaria, Aetoxylon, Gyrinops, and Gonystylus [4].

Aquilaria malaccensis Lamk. is one of the producers of sapwood from the Thymelaeaceae family which is spread in South and Southeast Asia, including Indonesia. Agarwood sapwood is one of the high-value non-timber forest product commodities. The selling price of high-quality agarwood sapwood in the market reaches Rp. 400 million per kg. The global market agarwood sapwood demand reaches 4000 tons/year, while the realization of Indonesian agarwood sapwood exports from January to March 2021 is only 328.9 tons [5]. The high price of agarwood sapwood and high market demand are the reasons for increasing agarwood sapwood production. One of the obstacles in meeting the global market is the slow growth of agarwood. Efforts that can be made to increase the growth of agarwood are by using rhizobacteria and ecoenzymes.

Rhizobacteria are non-pathogenic bacteria that aggressively colonize the rhizosphere [6], e.g. Serratia marcescens and Stenotrophomonas maltophilia. The use of rhizobacteria can increase growth,
crop yields, resistance to pests and diseases, can reduce the use of chemical pesticides in controlling plant diseases [7].

Ecoenzymes are organic fertilizers produced from the fermentation of kitchen waste such as fruit peels and vegetable residues. Ecoenzymes are used to increase plant growth by accelerating biochemical processes. The benefits of ecoenzymes include increasing fruit, flower, and harvest yields, as well as repelling nuisance insects. Enzymes produced from fermentation can convert ammonia (NH\textsubscript{4}) into nitrate (NO\textsubscript{3}). Nitrates function as important compounds in protein synthesis in plant growth. This study aims to examine the potential of rhizobacteria and ecoenzymes on agarwood growth.

2. Method

2.1. Materials
The tools in this study were polybags, styrofoam, 60% paranet, ruler, pH meter, digital callipers, digital scales, oven, autoclave, laminar airflow, Petri dishes, Erlenmeyer flasks, beakers, shakers, test tubes, micropipettes, triangular cell spreaders, envelopes, cameras, stationery, and laptops. The materials used in this study were seeds of A. malaccensis, rhizobacteria isolates of S. marcescens and S. maltophilia, alcohol, NaOH, sterile water, culture media in the form of nutrient agar (NA) and nutrient broth (NB) and an ecoenzyme solution. The planting media used were soil, cocopeat, cow manure, and husk charcoal.

2.2. Materials
Research preparation begins with the preparation of new planting media, rhizobacteria inoculum, and ecoenzyme solution. The new planting medium for seeds is a mixture of soil, cocopeat, cow manure and husk charcoal in a ratio of 6:2:2:1 (v/v/v/v), then the mixture is put into polybags measuring 20 x 15 cm. Isolates S. marcescens and S. maltophilia were subculture on NA medium and incubated at room temperature for 24 hours. The isolates were propagated by growing rhizobacteria on NB media and placed in a shaker for 24 hours. The population density used in this study was 10\textsuperscript{10} cfu/mL. The ecoenzyme solution used was made from a mixture of mango peel + papaya, molasses, and water in a ratio of 3:1:10 (w/w/w). Mango and papaya are types of fruit that are easy to obtain and easy to ferment because they have a peel that is easily crushed. The mixture is fermented for 40 days, then filtered to separate the solids and liquids. The concentration of the ecoenzyme solution used was 5% with a pH of 5.6–6.

The Palembang provenance agarwood seeds used came from the nursery in Situ Gede, Dramaga. The criteria for the seeds used were 16–47 cm high, 2.0–6.6 mm in diameter, straight and healthy. Seedlings were transferred into polybags containing new media by including the default media. The seeds that have been transferred to new media are then placed in the Silviculture Nursery, Faculty of Forestry and Environment, IPB. Agarwood seedlings were shaded with 60% paranet and styrofoam pads to minimize treatment contamination (through irrigation water that comes out from under the polybag). Seedlings are left for up to 3 weeks to adapt to the new environment.

Rhizobacteria inoculation was started on seedlings that had undergone an adaptation period (for 3 weeks) and were counted as the 1st week of observation. Subsequent inoculations were carried out at 5 and 9 weeks. The amount of bacterial solution inoculated was 20 mL (equivalent to 10\textsuperscript{10} cfu/mL). Rhizobacteria were inoculated on agarwood seedlings by sprinkling a bacterial solution on the media that had been perforated around the seedlings. Seedlings were not watered on the day of bacterial inoculation.

The application of ecoenzymes was started on seedlings that had been inoculated with rhizobacteria at a distance of 2 weeks and counted as the 3rd week of observation. The next application of ecoenzymes was carried out at the 7th and 11th weeks. The ecoenzyme solution that has been diluted with a concentration of 5% is sprinkled on the planting media that has been perforated around the seeds as much as 20 mL. Seedlings were not watered on the day the ecoenzyme was applied.
Observations and data collection was carried out for 16 weeks with the observed variables namely height, diameter, number of new leaves, number of new shoots, total wet weight (TWW), total dry weight (TDW), root shoot ratio (SRR), seedling moisture content, and seed quality index (SQI). Height, diameter, number of new leaves, and number of new shoots agarwood seedling were observed per week, whereas TWW, TDW, SRR, seedling moisture content, and SQI were measured at the end of the observation. High variable observations are carried out once a week, diameter, number of new leaves, and number of new shoots are carried out every 4 weeks, total wet weight, total dry weight, root shoot ratio, seedling moisture content, and seed quality index were calculated after harvesting. Total dry weight using 80 °C for 72 hours until it reaches a constant weight [8]. The root shoot ratio (SRR) is the comparison of the value of the total dry weight of the shoot with the value of the total dry weight of roots. The seed quality index (SQI) was determined after measuring height, diameter, and seedling biomass. The value of the seed quality index (SQI) is calculated according to the following Dicson formula:

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\text{SQI} = \frac{\text{seed dry weight (g)}}{\text{seed height (cm)} + \frac{\text{shoot dry weight (g)}}{\text{root dry weight (g)}}}
\]

Data were processed using IBM SPSS Statistics 25. The F test is carried out if the data meets the ANOVA test requirements, namely in the form of normality test and homogeneity test. Data that do not meet the requirements of the ANOVA test are only processed using Microsoft Excel by displaying the average value of the treatment, while data that meet the requirements of the ANOVA test are carried out using the F test using IBM SPSS Statistics 25. The results that have a significant effect on the F test are further tested using Duncan Multiple Range Test (DMRT) at an error rate of 5%.

3. Results and Discussion

Rhizobacteria inoculation and ecoenzymes application did not affect agarwood seedlings growth (Table 1). Growth performance is the interaction of plants, rhizobacteria and ecoenzymes, and the environment. Environmental factors in this study were uniform because the research was conducted in the nursery.

| Variable                     | Main plot: rizobakteri | Subplot (nested): ekoenzim |
|------------------------------|------------------------|-----------------------------|
| High                         | 0.960<sup>m</sup>      | 0.737<sup>m</sup>           |
| Diameter                     | 0.886<sup>m</sup>      | 0.221<sup>m</sup>           |
| Number of new leaves         | 0.724<sup>m</sup>      | 0.671<sup>m</sup>           |
| Total wet weight (TWW)       | 0.820<sup>m</sup>      | 0.963<sup>m</sup>           |
| Total dry weight (TDW)       | 0.674<sup>m</sup>      | 0.948<sup>m</sup>           |
| Moisture content             | 0.080<sup>m</sup>      | 0.863<sup>m</sup>           |

The figures in the table are significant values. <sup>m</sup> = treatment has no significant effect on the 95% confidence interval with a significant value (<sup>P-value</sup>) > 0.05.

The response of agarwood seedlings to the treatment given is one of the factors influencing growth. Type <i>A. malaccensis</i> is included in the type of slow-growing. Observations for 16 weeks on agarwood seedlings did not give optimal results on the growth of agarwood seedlings. This species naturally grows slowly, so that the effect of the treatment is visible after a certain period. The same results were found in the study of [9] which showed that foliar fertilizer treatment did not affect agarwood seedling growth after 2 months of treatment. Longer observation time after treatment can increase the growth of agarwood, this is found in the results of the study by [10] agarwood seedlings experienced an increase in growth 5 months after being inoculated with rhizobacteria.
The response of agarwood seedling growth to the treatment was reflected in the value of the standard deviation for each observed variable. A large standard deviation value indicates that agarwood seedlings have various growth responses to the treatment. According to [11], a large standard deviation value indicates a broad plant growth response, so the results are not significant. Agarwood seedlings that inoculated with rhizobacteria and treated with ecoenzymes had a trend of higher height and diameter growth compared to agarwood seedlings inoculated with rhizobacteria but not treated with ecoenzymes. The ecoenzymes application increased the increase in height and diameter in the single rhizobacteria treatment but decreased the growth in height and diameter of the seedlings in the mixed rhizobacteria (treatment \( S.\ marcescens + S.\ maltophilia \)) (Figures 1a and 1b).

**Figure 1.** The growth of agarwood seedlings for 16 weeks: (a) increase in height (b) increase in diameter. B0 = control, B1 = *Serratia marcescens*, B2 = *Stenotrophomonas maltophilia*, B3 = *S. marcescens + S. maltophilia*.

The growth of agarwood seedlings can also be measured by the number of new leaves. Agarwood seedlings that inoculated with rhizobacteria and treated with ecoenzymes had a trend of higher leaf count compared to seedlings without ecoenzymes (Figure 2).

**Figure 2.** Effect of rhizobacteria inoculation and ecoenzymes on the number of leaves of agarwood seedlings for 16 weeks.

Rhizobacteria inoculation and ecoenzyme treatments can affect the number of new shoots of agarwood seedlings (Figure 3). Agarwood seedlings with no ecoenzyme treatment in the bacterial plots of *S. marcescens* showed the highest increase in the number of shoots, which was 112.5% compared to the control. Agarwood seedlings with rhizobacteria inoculated (in plot *S. maltophilia* and mixed bacteria *S. marcescens + S. maltophilia*) without ecoenzyme treatment decreased the number of new shoots. The ecoenzymes application to agarwood seedlings that inoculated with rhizobacteria decreased the number of new shoots in single rhizobacteria inoculation but increased the number of new shoots in mixed bacterial (inoculation *S. marcescens + S. maltophilia*).
Rhizobacteria can increase plant growth. Rhizobacteria produce plant growth regulators (PGR) such as auxins, gibberellins, and cytokinins [12]. In addition, rhizobacteria also play a role in controlling plant pathogens. According to [13] bacterial isolates of *S. marcescens* and *S. maltophilia* isolated from the roots of the pasak bumi plant have the potential to produce enzymes and antibiotics that have the potential to inhibit pathogens.

Inoculation of *S. marcescens* without ecoenzymes application can increase the growth of new shoots. Bacteria *S. marcescens* produce auxin exogenous which is physiologically active in plant growth [14]. Auxin can trigger the growth of apical shoots of seedlings so that the plot of *S. marcescens* without ecoenzyme agarwood seedlings have high new shoot growth.

Another factor that affects the growth of agarwood seedlings is ecoenzymes. The application of ecoenzymes had a positive effect on rhizobacteria and agarwood seedlings. Agarwood seedlings that inoculated with rhizobacteria and ecoenzymes application had better growth compared to the seedlings inoculated with rhizobacteria without the ecoenzymes. Ecoenzymes contain organic matter needed by plants to supply nutrients. According to [15] ecoenzymes can be used as liquid organic fertilizers and disinfectants to increase rice growth.

Figure 3. Increase in the number of shoots agarwood seedlings treated rhizobacteria inoculation and ecoenzymes application for 16 weeks.

Positive responses were shown by agarwood seedlings inoculated with rhizobacteria and ecoenzymes, i.e. the growth of new shoots, seedling leaf colour, and seedling root performance. Agarwood seedlings that inoculated with rhizobacteria and ecoenzymes application had a greener leaf colour response and better root performance. The ecoenzyme used in this study was made from a mixture of mango and papaya peels. According to [16], fruit peels can be used as organic liquid fertilizers because they can supply macronutrients (N, P, K) for plant growth. One of the macro elements that play a role in plant growth is nitrogen. According to [17] nitrogen deficiency can cause stunted plant growth, yellowing leaves, and limited root system.

The application of ecoenzymes affects the leaf colour of agarwood seedlings. Agarwood seedlings treated with rhizobacteria inoculation without ecoenzyme treatment had paler leaf colour (Figure 4a), while agarwood seedlings treated with rhizobacteria and ecoenzyme inoculation had greener leaf colour (Figure 4b).
Figure 4. Leaf colour of agarwood seedlings: (a) without ecoenzymes (b) with ecoenzymes for 16 weeks. B0 = Control, B1 = *Serratia marcescens*, B2 = *Stenotrophomonas maltophilia*, B3 = *S. marcescens* + *S. maltophilia*.

The application of ecoenzymes also affects the root growth of agarwood seedlings. Agarwood seedlings that were not treated with ecoenzyme (Figure 5a) had fewer roots than seedlings that were treated with ecoenzyme (Figure 5b). Agarwood seedlings inoculated with rhizobacteria *S. marcescens* and a mixture of *S. marcescens* + *S. maltophilia* had more roots than controls, while agarwood seedlings inoculated with rhizobacteria *S. maltophilia* relatively the same roots as controls.

Figure 5. Performance of agarwood seedlings roots during 16 weeks: (a) without ecoenzymes (b) with ecoenzymes.

Rhizobacteria inoculation and ecoenzymes application on agarwood seedlings have not been able to increase the biomass (Figure 6a and Figure 6b). This can be seen from the results of the analysis of variance on the TWW and TDW variables which did not significantly affect the treatment of rhizobacteria inoculation and application of ecoenzymes. The results were not significantly different, it could also be seen in the variable of seed moisture content (Figure 6c).
Figure 6. Biomass of agarwood seedlings for 16 weeks of observation: (a) total wet weight (TWW) (b) total dry weight (TDW) (c) seed moisture content.

The addition of new shoots on agarwood seedlings has not been accompanied by an increase in height, diameter, number of new leaves, and seed biomass. The increase in diameter is difficult to measure when the plant phase is still young and in primary growth. The primary growth phase is the phase when the plant shows shoot and root growth as a means of photosynthesis and the absorption of nutrients that are prioritized for long-term plant growth such as diameter and biomass [18]. The seed biomass value is related to the metabolic ability of the seedling, the greater the biomass value in the seedling, the more efficient the seed metabolism process [19].

Agarwood seedlings treated with rhizobacteria and ecoenzymes for 16 weeks had SRR values ranging from 1.51–2.57 (Figure 7a), while the SQI values for agarwood seedlings in all treatments ranged from 0.46–0.60 (Figure 7b). The application of ecoenzymes resulted in a decreasing trend of SRR and SQI values in agarwood seedlings.
SRR and SQI values describe the quality of seedlings based on their adaptability to the environment. The SRR value refers to the classification of [20] which states that the ideal SRR value for seedlings is 1-3. The results showed that the SRR value of agarwood seedlings ranged from 1.51-2.57. Seedlings that have an SRR close to 1 are those that have a balanced ratio of shoots and roots. It is also stated by [21] that balanced seedlings are those that have a shoot-to-root ratio value of 2-3 in seedlings under controlled environmental conditions. The SQI value refers to [22] which states that the SQI value is good, the value is > 0.09. The results showed that the SQI value of agarwood seedlings produced from this study was > 0.09; so that it is expected to have a high survival rate in field conditions. The higher SQI value indicates the seed has high adaptability to environmental conditions. Based on the results of the SRR and SQI values, it can be said that the agarwood seedlings are ready to be transferred under field conditions.

In general, rhizobacteria inoculation and ecoenzyme application were not able to increase the growth of agarwood seedlings during 16 weeks of observation. However, the treatment of rhizobacteria inoculation and the application of ecoenzymes was able to increase the number of new shoots, leaf colour quality, and the number of roots of agarwood seedlings. Agarwood is a slow-growing species, so the observation time of 16 weeks is not sufficient to observe the response of agarwood seedlings due to the effect of the treatment treated.

4. Conclusion

Rhizobacteria inoculation and ecoenzymes application did not affect the height, diameter, number of new leaves, total wet weight, total dry weight and moisture content of agarwood seedlings. Inoculation treatment S. marcescens without ecoenzyme gave a response to increases in new shoots by 112.5% compared to control. The application of ecoenzymes can stimulate root growth and got the leaf colour greener and fresher. The SRR values for agarwood seedlings ranged from 1.51–2.57 and the SQI values ranged from 0.46–0.60; so that agarwood seedlings can adapt to field conditions.

References

[1] Leksonowati A 2016 Interaksi antara biak suspensi sel gaharu (Aquilaria malaccensis Lamk) dan Fusarium sp dalam menghasilkan senyawa seskuiterpena (Bogor: Institut Pertanian Bogor)
[2] Totok K, Waluyo, and Anwar F 2012 Identifikasi komponen kimia empat kelas mutu gaharu (Kacangan A, Teri B, Kamedangan A dan Kamedangan B) Jurnal Penelitian Hasil Hutan 30:291-300
[3] Pratama A 2015 Eksplorasi dan identifikasi morfologi tanaman penghasil gaharu (Aquilaria spp) di Kabupaten Sarolangun Provinsi Jambi (Padang: Universitas Andalas)
[4] Womsiwor D, Dimara P A, and Mofu WY 2018 Klasifikasi kualitas dan nilai komersial gaharu pada klaster pedagang pengumpul di Kabupaten Sorong Jurnal Kehutanan Papuasia 4:19-33
[5] [BPS] Badan Pusat Statistik 2021 Buletin Statistik Perdagangan Luar Negeri 2021 (Jakarta: Badan Pusat Statistik)
[6] Adzhani F I 2015 Aplikasi rizobakteri dan pupuk nitrogen untuk meningkatkan produksi dan mutu fisiologi benih jagung (Zea mays L) (Bogor: Institut Pertanian Bogor)

[7] Ernita M, Zanhanis dan Jamilah 2016 Aplikasi rizobakteri dalam meningkatkan pertumbuhan, hasil, dan ketahanan pada tanaman bawang merah Jurnal Pengabdian Kepada Masyarakat 22 131–4

[8] Liihliang A and Lumingkewas 2020 Efisiensi waktu pemberian pupuk nitrogen terhadap pertumbuhan dan produksi jagung lokal kuning Jurnal Sainsmat 9 144–58

[9] Mentang A H, Rombang J A, Lasut M T, Thomas A 2016 Pengaruh pupuk daun dan naungan terhadap pertumbuhan bibit gaharu Gyrinops versteegii (Gilg) Domke di bawah cekaman air COCOS 7 1-9

[10] Sitepu I R, Aryanto H Y, and Turjaman M 2010 Aplikasi rizobakteri penghasil fitohormon untuk meningkatkan pertumbuhan bibit Aquilaria sp di Persemaian Jurnal Pusat Penelitian dan Pengembangan Hutan dan Konservasi 7 107-16

[11] Rulianti F 2018 Pengaruh pupuk organik cair limbah tebu (Bagasse) terhadap pertumbuhan tanaman cabai (Capsicum frutescens) sebagai penunjang praktikum mata kuliah fisiologi tanaman (Banda Aceh: Universitas Islam Negeri Ar-Raniry)

[12] Simanjutak D R, Halimursyadah, and Syamsuddin 2019 Perlakuan rizobakteri pemacu pertumbuhan tanaman (RPPT) dengan beberapa tingkat kerapatan inokulum rizobakteri terhadap viabilitas dan vigor benih cabai merah kadaluarsa (Capsicum annuum L) Jurnal Ilmiah Mahasiswa Pertanian 4 229-38

[13] Sulistiyani T R, Meliah S, and Damayanti 2020 Bakteri endofit yang diisolasi dari akar Eurycoma longifolia dan potensinya sebagai pengendali jamur patogen tanaman Jurnal Bioteknologi & Biosains Indonesia 7 37-47

[14] Astriani M 2015 Seleksi bakteri penghasil indole-3-acetic acid (IAA) dan pengujian pada bibit kelapa sawit (Elaeis guineensis Jacq) (Bogor: Institut Pertanian Bogor)

[15] Hasanah Y 2020 Eco enzyme and its benefits for organic rice production and disinfectant Journal of Sainstech Transfer 3 1-9

[16] Marjenah, Kustiawan W, Nurhiiftiani I, Sembiring K H M, and Ediyono R P 2017 Pemanfaatan limbah kulit buah-buahan sebagai bahan baku pembuatan pupuk organik cair Jurnal Hutan Tropika 1 120-127

[17] Kaya E 2013 Pengaruh kompos jerami dan pupuk NPK terhadap N tersedia tanah, serapan-N, pertumbuhan, dan hasil padi sawah (Oryza sativa L) Agrologia 2 43-50

[18] Campbell N A, Reece J B, Urry L A, Cain M L, Wasserman S A, Minorsy P V, and Jackson R B 2012 Biologi Jilid 2 Edisi 8 (Jakarta: Erlangga)

[19] Prayudaningsih R 2014 Pertumbuhan semai Alstonia scholaris, Acacia auriculiformis dan Muntingia calabura yang diinokulasi fungi mikoriza arbuskula pada media tanah bekas tambang kapur Jurnal Penelitian Kehutanan Wallace 3 13-32

[20] Duryea M L and Brown N 1984 Seedling physiology and reforestation succes Proceeding og the physiology working group technical session (Boston: DRW Juck Publisher)

[21] Haase D L 2008 Understanding forest seedling quality: measurement and interpretation Tree Planters’ Notes 52 24-30

[22] Rosdiansyah, Muin A, and Iskandar 2016 Pengaruh frekuensi pemberian dan dosis pupuk organik air terhadap pertumbuhan dan indeks mutu bubut gaharu (Aquilaria malaccensis Lamk) di persemaian Jurnal Hutan Lestari 4 185-192