Article

Effect of Fungal Inoculation to Resin Content on Gaharu Plants (*Gyrinops Versteegii* (*Gilg.*) *Domke*)

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Received: 13 April 2019; Accepted: 29 May 2019; Available online: 1 June 2019

Abstract

One of the commodities of non-timber forest products (NTFPs) is agarwood sapwood which has high economic value as an export commodity, as well as a good source of income for the community. Agarwood sapwood is an aromatic substance in the form of a lump that is found between wood cells in a variety of distinctive shapes and colors and has a resin content (mastic). Resin is a secondary metabolite compound produced by agarwood plants due to injury or infection by microbes. One of the microbes that infects the agarwood plant is a variety of fungi. This study aims to obtain a type of fungi that is effective in the formation of resin compounds in agarwood plants. This research is a field experiment with randomized block design. The treatments that experimented were mushroom inoculation, namely: J0 (without fungal inoculation), JF (Fusarium solani mushroom inoculation), JR (inoculation of Rhisopus sp), JT (inoculation of Trichoderma sp). Each treatment was repeated 4 times. Parameters observed were: sap color, sap smell, infection width and length, and resin content in sapwood (%). Data were analyzed statistically by variance test (ANOVA). If the treatment has a significant effect, followed by a BNT test at the level of 5%. The results showed that the treatment of fungi inoculation significantly affected the width of the infection and the sap content of sapwood, while the other parameters had no significant effect. The best treatment was obtained in JT (inoculation of mushroom Trichoderma sp.) with the results in the form of sap with: the color of the sap ranged from brown to blackish brown; fragrant aroma, infection length (2.39 cm) and infection width (1.11 cm), and resin content of 7.92%.

Keywords: *type of fungus; resin content; aloes plant*

1. Introduction

Agarwood sap is an aromatic substance in the form of clumps located between wood cells with a variety of shapes and colors and has a content of mastic (resin), derived from trees or parts of agarwood-producing trees that grow naturally and have died as a result of the process infections that occur both naturally and artificially (Syukur, 2015).

Agarwood is a commodity of non-timber forest products (NTFPs) that has high economic value and as one of export commodity, as well as a good source of income for the community (Pasaribu et al., 2013). Since 2000-2002 the export volume of Indonesian agarwood was only reached 30 tons, with an export value of 600,000 US dollars. The high demands of agarwood sap abroad is due to the benefits of gaharu for perfume, cosmetics, medicines and for religious ritual needs (Tarigan, 2004; Akter et al., 2013). However, agarwood production in Indonesia is relatively low and continues to decline with an average production around 45 tons per year.

The forming of agarwood sapwood is influenced by various factors such as: genetic of agarwood trees, inducing microba, environment, and the time length of agarwood sap forming process. Agarwood sap can occur when certain pathogens infect agarwood trees. The response of the tree from the pathogen attack is the forming of secondary generation metabolites or resin compounds that cause a fragrant aroma when burned (Sitepu et al.,

2011). According to research results by Mega and Phabiola (2010), the species of Fusarium sp and Rhizopus sp. causing the forming of agarwood sap on Gyrinops versteegii. Putri, et al. (2016) showed that agarwood-producing plants inoculated with fungi inoculant produced 1.1% higher resin content than non-inoculated plants. Furthermore, the results of research by Budi et al. (2010) showed that there were four Fusarium species that induced Aquilaria spp, which is: F. Solani (Mart) Appel & Wollenw, F. Lateritium Ness, F. Tricinotium (Corda) and F. Moniliformae Sheldon. The mixture of liquid inoculants from Fusarium solani mushroom and Rhizopus sp. succeeded in inoculating agarwood plants (Gyrinops versteegi) of 16 months which is produced agarwood with a resin content of 13.58%. (Mega et al., 2015). The result of Fusarium sp. on the agarwood plants from species Aquilaria beccariana shows that the isolated Fusarium sp. from Gorontalo has the most potential in producing agarwood compared to isolated Fusarium sp. from Parung, Jambi, and the isolated from Banjarmasin (Iskandar and Suhendra, 2012). Likewise, three isolates of Gyrinops versteegii plant fungi belong to Fusarium solani (Nugraheni et al., 2015).

Based on China traditional culture, the quality of agarwood is determined by: resin content, sink in water, fragrant, inoculation-agarwood method, the time length of agarwood forming, where agarwood is formed (Liu et al, 2017). Furthermore, Azah et al (2013) said that the use of color parameters, density, agarwood sap forming and odor was very subjective of agarwood quality classification, and resin content could be used as a guide in the classification of agarwood quality. According to Subasingghe and Hettiarachchi (2013) said that the production of agarwood resin in Gyrinops walla is the same as Aquilaria spp.

Most of the agarwood that cultivated in Bali is from Gyrinops versteegii family. Unfortunately, there are no adequate technology to increase the production of agarwood sapwood in quality an quantity. Mega et al. (2013), said that the resin content of agarwood sap formed resulted from Fusarium solani and Rhizopus sp inoculant on agarwood plants (Gyrinops versteegii) for 5 months is 5.32% and 5.24% respectively. The resin content above is still relative low, so the quality of the sapwood is also relative low. Therefore it is necessary to do research to find another fungal inoculants which are more effective in forming resins in agarwood sapwood so they could improve the quality of the agarwood sapwood.

2. Materials and Methods

The location of the fungal inoculation study was carried out at agarwood farm gardens at the Marga District of Tabanan Regency, Bali Province. The study was conducted from April 2018 to October 2018, start from the preparation, the preparation of fungal inoculants, fungal inoculation in the field, resin analysis in the laboratory, and report. Preparation of inoculants was carried out at the Soil Biology Laboratory at the Faculty of Agriculture, Udayana University, Denpasar, and resin analysis was carried out at the Laboratory of the Land, Agriculture Faculty, Unud, Denpasar.

2.1 Material and Equipment

The materials needed in this study such as: fungal inoculants (Fusarium solani, Rhizopus sp., and Trichoderma sp.), agarwood plants (Gyrinops versteegii) 4-6 years old, alcohol, plasticine and chemicals substances for analysis in the laboratory. The tools used in this research include: inoculation devices (drills, syringes, spatulas), pressure cooker, pan heater, plasticine, electric scales, and other chemical tools for analysis in the laboratory.

2.2 Research Design

The design used in this study was RBD (randomized block design). The treatment that will be tried is:

J0 Non-Inoculation (control)

JF Inoculant Fusarium solani

JR Inoculant Rhizopus sp.

JT Inoculant Trichoderma sp.

Each of above treatment was repeated 4 times, so the total of agarwood plants needed were 16 trees. The parameters observed were: sapwood color, aroma (fragrance level) of sapwood, and sapwood resin content. Quantitative data from observations were analyzed statistically by analysis of variance, if there was a significant result will be followed with the BNT Test (level 5%). The research stages are as follows:

a. The preparation stage is to prepare tools and study materials, which is: a) Preparation of inoculants carried out at the Soil Biology Laboratory of Agriculture Faculty, Udayana University, Denpasar, each type of the isolated fungal are grown with PDAs and inoculant media materials such as wood powder, bran and others for mushroom media in the field; b) selection of agarwood plants which have almost the same diameter (10-15 cm) with ages of 5-8 years, c) preparation of tools for fungal inoculation, such as: wood drill, inoculation needle, spatula, plasticine, alcohol, etc.
b. After the mushroom inoculant is prepared from the agarwood-forming mushroom (JPG), then inoculation is carried out on the agarwood plant (Gyrinops verstegii) in Marga Dauhpuri Village, Marga District, Tabanan district.
c. The plants to be inoculated are plants with age of 5 years old, or plants with a stem diameter around 10-15 cm. The stem of the plant is drilled with a depth of 1/3 (2-4 cm) from the tree diameter, the hole location is more than 20 cm from the ground, in one tree several holes could be created with space of 10 cm in a circular direction at an angle of 30o. Inoculants in bottles are inoculated to the hole and then cover it with plasticine (wax).
d. After 3 months from the time of treatment, the inoculation was observed from the sap formed around the inoculation, by cutting the sap with the tool. The parameter observed was the change in color around the inoculation, the width and length of the color change. Small part of the wood that changing color (sapwood) will be taken as a sample by drying and analyze the aroma and resin content of the sapwood in the laboratory.
e. Color analysis of the sapwood is using the colorimetric method, analysis of sapwood aroma by organoleptic method, and analysis of resin content by extraction method. Furthermore, phytochemical analysis to determine the content of resin compounds was carried out at the Soil Chemistry Laboratory, Faculty of Agriculture, Udayana University, Denpasar. The resin yield level(%) was carried out by extracting sap with Methanol of 150 ml each for 3 hours. The resin yield was calculated by dividing the weight of the resin extracted by the weight of 100 times extracted sap (Pasaribu et al., 2013). The data obtained in the form of quantitative data observations were analyzed statistically by variance analysis (variance), if it occurs significantly followed by the LSD Test (level of 5%).

3. Results

3.1 Infection length, infection width, and resin content

The results of statistical analysis showed that the treatment of fungal inoculation had a significant effect on the width of infection and resin content in agarwood sapwood, but it did not significantly affect the length of infection after three months of fungal inoculation treatment (Tables 1 and Tabel 2). Parameters of sapwood color and sap aroma were not statistically analyzed because the data were qualitative.

Table 1. Significance of treatment for parameters of agarwood plants.

No	Parameters	Significance
1.	Infection length (cm)	Ns
2.	Infection width (mm)	*
3.	Resin content (%)	*

Note: * = significant, ns = non significant

Table 2. Analysis of infection length, infection width, and resin level (%).

Treatments	Infection Length (cm)	Infection Width (cm)	Resin Level (%)
J0	1.74 a	0.65 a	5.25 a
JF	2.96 a	1.00 b	5.31 a
JR	2.88 a	1.06 b	5.24 a
JT	2.39 a	1.11 b	7.92 b

Note: The value followed by the same letter in the same column indicate no significant effect of the treatment.

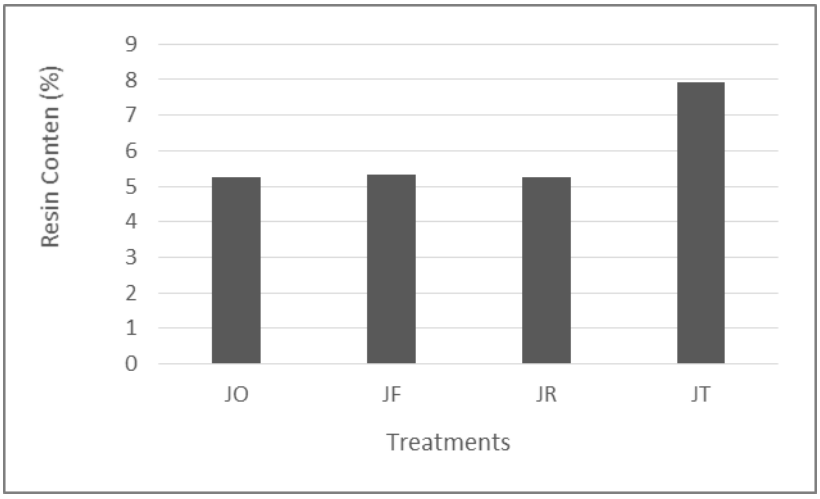

Figure 1. Resin content (%) in Agarwood Sapwood.

3.2 Sapwood Color

The observation of agarwood sapwood color is presented in Table 3 and Figure 2. In the table it is shown that the control treatment (J0) formed a light brown sapwood, while the mushroom inoculation treatment (JF and JR) varied in color from brown to black brown, and in the treatment JT is found mostly blackish brown.

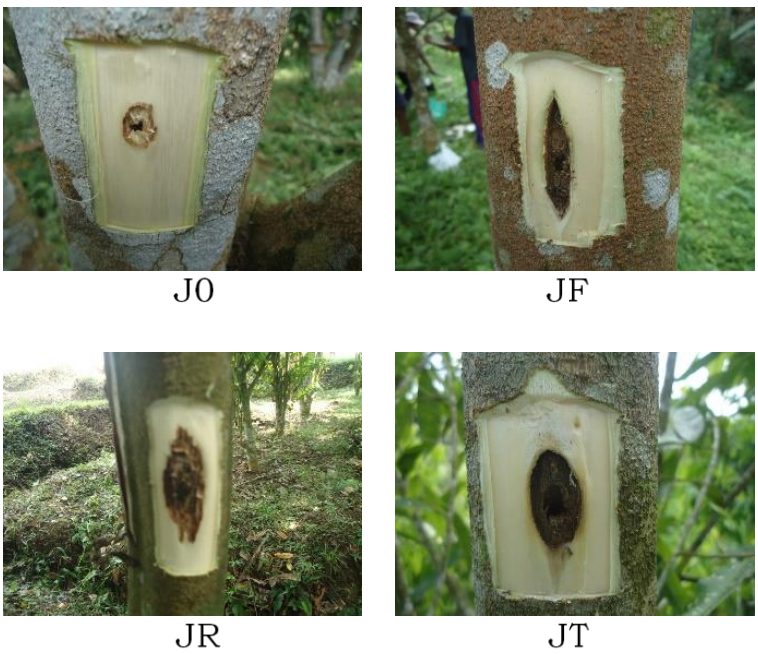

Figure 2. Effect of fungal inoculation treatment on Agarwood Sapwood color.

Table 3. Effect of the treatments to the Sapwood Agarwood Treat¬ments.

Treatments	Repetition			
	I	II	III	IV
J0	Light Brown	Light brown	Light brown	Light brown
JF	Brown	Black brown	Brown	Brown
JR	Brown	Black brown	Black brown	Brown
JT	Black brown	Black brown	Black brown	Brown

3.3 Sapwood Aroma

The fragrant of agarwood sapwood that produced when the sapwood is burned. The results of measuring the aroma of agarwood sap are presented in Table 4. In Table 4, it is shown that the majority of aroma in control treatment (J0) is classified as less fragrant, while in the inoculation treatment of JF and JR mushrooms varies from less fragrant to fragrant, and in the JT treatment all the sapwood of each repetition smells fragrant.

Table 4. Effect of the treatments to the Sapwood Color.

Treatments	Repetition			
	I	II	III	IV
J0	Less fragrant	Less fragrant	Fragrant	Less fragrant
JF	Fragrant	Fragrant	Fragrant	Less fragrant
JR	Fragrant	Less fragrant	Fragrant	Less fragrant
JT	Fragrant	Fragrant	Fragrant	Fragrant

4. Discussion

Based on the results of observations and statistical tests showed that the treatment effect of fungal inoculation had a significant effect on the parameters of infection width, resin content in sapwood, but did not significantly affect the length of infection. The color of the sap varies from light brown to blackish brown, and the aroma of the sap varies from less fragrant to fragrant.

The highest width of infection and resin content in the sap was obtained in the JT treatment (Trichoderma sp. inoculation) around 1.11 cm and 7.92%. The treatment of inoculation of the Trichoderma sp gives the best infection width, resin content, color, and aroma compared to other treatments, moreover to control (without fungal inoculation). It is suspected that the attack of Trichoderma sp. the highest so as to give rise to the response of the agarwood plant (Gyrinops versteegii) which caused the forming of secondary metabolites of resin. This high response is supported by the research of Chhipa and Kaushik (2017) which revealed that Trichoderma virens is the most fungis found at Aquilaria malaccensis stem followed by Lasiodiplodia theobromae, Lasiodiplodia sp. The tree response to the attack of these pathogens is to produce secondary metabolites or resin compounds that cause a fragrant aroma when burned (Sitepu, et al., 2011). This resin is phytoalexin which is a defense compound against pathogenic attacks. The accumulation of resin compounds on the infected stem will form sapwood with several variations in color and aroma.

Based on the data obtained in this study, it turns out that there is a relationship between the color, aroma and content of the resin in the sapwood. The darker or blackish brown agarwood sap makes the aroma gets stronger (fragrant) and the resin content gets higher. This can be seen from the control, JF, and JT treatments with color, aroma and resin content respectively: light brown-brown-blackish brown, less fragrant - fragrant and 5.25 - 5.31- 7.92%.

One criteria in determining the quality of agarwood is the resin content in sapwood (Liu et al., 2017; Azah et al., 2013) is the higher the resin content, the higher the quality of the sapwood. Agarwood sapwood produced in the JT treatment has the best quality compared than other treatments.

5. Conclusions

Fungal inoculation has a significant effect on the width of the infection, and the resin content in the sapwood, whereas it has no significant effect on the length of the infection. The best inoculant for resin formation in gaharu plants (Gyrinops versteegii) is Trichoderma sp. The highest quality of sapwood was obtained in the treatment of Trichoderma sp inoculation with characteristics: blackish brown color, fragrant aroma, and resin content of 7.92%.

References

Akter, S., Islam, M. T., Zulkefeli, M., & Khan, S. I. (2013). Agarwood production-a multidisciplinary field to be explored in Bangladesh. *International Journal of Pharmaceutical and Life Sciences*, **2**(1), 22-32.

Azah, M. A. N., Husni, S. S., Mailina, J., Sahrim, L., Majid, J. A., & Faridz, Z. M. (2013). Classification of agarwood (gaharu) by resin content. *Journal of Tropical Forest Science*, **25**(2), 213-219.

Budi, S. W., Santoso, E., & Wahyudi, A. (2010). Identifikasi jenis-jenis fungi yang potensial terhadap pembentukan Gaharu dari Batang Aquilaria spp. *Jurnal Silvikultur Tropika*, **1**(1), 1-5.

Chhipa, H., & Kaushik, N. (2017). Fungal and bacterial diversity isolated from Aquilaria malaccensis tree and soil, induces agarospirol formation within 3 months after artificial infection. *Frontiers in Microbiology*, **8**, 1286.

Iskandar, D., & Suhendra, A. (2012). Uji inokulasi Fusarium sp. untuk produksi gaharu pada budidaya A. beccariana. *Jurnal Sains dan Teknologi Indonesia*, **14**(3), 182-188.

Liu, Y. Y., Wei, J. H., Gao, Z. H., Zhang, Z., & Lyu, J. C. (2017). A review of quality assessment and grading for agarwood. *Chinese Herbal Medicines*, **9**(1), 22-30.

Mega, I. M., & Phabiola, T. A. (2010). Isolasi Jamur Pembentuk Gubal Gaharu Pada Tanaman Gyrinops Versteegii di Kecamatan Pupuan Kabupaten Tabanan. *Agritrop*, **29**(4), 189-198.

Mega, I. M., Suanda, D. K., Kasniari, D. N., Suena, W., & Parwata, M. A. O. (2012). Formulasi Inokulan Jamur Pembentuk Gubal Gaharu pada Tanaman Ketimunan (Gyrinops versteegii). *Agrotrop: Journal on Agriculture Science*, **2**(2), 139-144.

Mega, I. M., Suanda, D. K., Kasniari, D. N., & Susrama, I. G. K. (2015). Agarwood Producing Fungal Inoculant Formulation in Ketimunan Tree (Gyrinops versteegii DOMKE). *International Journal of Bioscience and Biotechnology*, **3**(1), 22-27.

Nugraheni, Y. M. M. A, Anggadhania, L., & Putranto, R. A. (2015). Identifikasi Tiga Isolat Cendawan Penghasil Gaharu Dari Nusa Tenggara Barat Dengan Menggunakan Primer Its Dan Tef 1-α. *Jurnal Pemuliaan Tanaman Hutan*, **9**(2), 77-90.

Pasaribu, G., Waluyo, T. K., & Pari, G. (2013). Analisis Komponen Kimia Beberapa Kualitas Gaharu dengan Kromatografi Gas Spektrometri Massa. Jurnal Penelitian Hasil Hutan, 31(3), 181-185.

Putri, N., Karlinasari, L., Turjaman, M., Wahyudi, I., & Nandika, D. (2017). Evaluation of incense-resinous wood formation in agarwood (Aquilaria malaccensis Lam.) using sonic tomography. *Agriculture and Natural Resources*, **51**(2), 84-90.

Sangareswari, M., Parthiban, K. T., Kanna, S. U., Karthiba, L., & Saravanakumar, D. (2016). Fungal microbes associated with agarwood formation. *American Journal of Plant Sciences*, **7**(10), 1445-1452.

Sitepu, I. R., Santoso, E., & Turjaman, M. (2011). *Identification of eaglewood (Gaharu) tree species susceptibility*. Bogor, Indonesia: Technical Report No. 1. R & D Centre for Forest Conservation and Rehabilitation Forestry Research and Development Agency (FORDA), Indonesian Ministry of Forestry.

Subasinghe, S. M. C. U. P., & Hettiarachchi, D. S. (2013). Agarwood resin production and resin quality of Gyrinops walla Gaertn. *International Journal of Agricultural Sciences*, **3**(1), 357-262.

Syukur, S. (2015). *Gaharu Dan Cara Penyuntikan Gubal Gaharu Pada Pohon Gaharu*. Muaro Jambi, Indonesia: Agricultural Training Center of Jambi.

Tarigan, K. (2004). *Profil Pengusahaan (Budidaya) Gaharu*. Jakarta, Indonesia: Center for Forestry Extension Services, Indonesian Ministry of Forestry.