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The Effect of Fusarium Fungal Inoculation, Hole Position, and Induction Technique on Forming Agarwood in *Gyrinops versteegii* Tree

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Abstract

Gyrinops versteegii, belonging to the Themelaeaceae family, is one of the species producing high-grade agarwood. The induction technique can accelerate the agarwood product. This study inducted the G. versteegii tree with fungal species, a variation of hole position, and an induction technique. The research was done at a domesticated G. versteegii plantation in Sragen and Karanganyar District, Central Java Province. The research material was 18 agarwood trees aged 14 years. Initial inoculation was carried out at a depth of 50 cm from the ground, inoculation holes were made vertically every 50 cm. The distance between inoculation holes was 10 cm horizontally. The depth of the inoculation hole was 1/3 of the tree diameter. The agarwood quality resulted from the inoculated G. versteegii tree characterized by the wood aroma, the discoloration area, wood aroma, and terpenoid content, is significantly affected by the wood position in the stem, the fungal species, and the inoculation technique. The upper stem results in better agarwood than the bottom stem. Using the inoculant of the Fusarium oxysporum can achieve better agarwood than the inoculant of F. solani and mixed F. solani × F. oxysporum. Furthermore, the infusion technique gains a better agarwood result than others.

Keywords: *domesticated Gyrinops versteegii, wood aroma, discoloration, terpenoids*

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Introduction

Agarwood is one of the non-timber forest products with economic value (Karlinasari et al., 2016) and belongs to the Thymelaeaceae family. *Gyrinops versteegii* tree is one of the agarwood-producing trees known to the general public as producing high-quality agarwood (Susilo, 2014). More importantly, agarwood is mainly gained from nature and secondarily harvested from artificial technology engineering (Turjaman, 2011; Mohamed, 2016). Forming agarwood begins from injury at the stem, which allows the pathogen to penetrate the plant tissue (Jayaraman & Mohamed, 2015). A *G. versteegii* tree infected by pathogens will respond to form a phytoalexin compound as a defense mechanism against pests and diseases. It is stated that when the pathogen (microbe) penetrates the plant tissue, it is considered a foreign matter; the plant responds by releasing phytoalexin (Mega et al., 2012). In response to a pathogen attack, the plant will produce a secondary metabolite, producing an aromatic compound when burned (Wahyuni & Prihantini, 2020). A phytoalexin compound can be in the form of an oleoresin compound with a brown to blackish-brown color, and it is known as an aromatic resin that has a pleasant aroma when burned (Mohamed, 2016). The natural process of agarwood formation takes a long time, and its agarwood product is unpredictable. The percentage of agarwood formation is naturally lower, according to Susilo (2014); from twenty agarwood-producing trees grown in their natural habitat, of those are just found one tree which can produce the agarwood for only a few grams.

Even though the agarwood produced from nature has been very small and steadily downward, the demand for agarwood and its product derivatives has quickly risen. Furthermore, engineering technology is urgently needed to increase agarwood productivity. The method accelerating the agarwood formation is generally classified into three forms, i.e., physical-mechanical, chemical, and biological. Local communities and tribes have used a traditional way; the physical-mechanical method is limited to physical stimulation in the form of injury. The chemical method, an expensive method, is carried out by using certain chemical compounds (Chhipa & Kaushik, 2020). Moreover, the biological method, or bio-induction method, the latest method for accelerating the agarwood, is held using pathogens such as *Fusarium* sp. fungi, which attack the agarwood-producing trees (Susilo, 2014; Yang et al., 2014; Tan et al., 2019).

The interaction between the pathogen and the *G. versteegii* tree is crucial in agarwood formation through the bio-induction method. The public community's most usual method, all three methods, can be performed quickly, but the produced agarwood product is relatively small and of a lower grade. The chemical method can promptly have the right quantity of agarwood; however, the agarwood is not healthy enough. Then, the biological method is an environmental friendship agarwood product, even though it needs to take time. The fungus infection on the agarwood-producing tree through injury on the stem either accidentally or intentionally responds to the host tree performing the defense

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mechanism due to attacking the fungi. Many fungi such as *Tarula* sp.*, Aspergillus* sp.*, Botrydiplodia* sp.*, Penicillium* sp.*, Acremonium, Cladosporium* sp.*, Deuteromycota,* and *Fusarium* sp. can invade the agarwood-producing trees, which form a phytoalexin compound with a fragrant aroma (Azren et al., 2019). The fungi of *Fusarium* sp. can accelerate the agarwood formation of the agarwood tree (Mega et al., 2012; Siburian et al., 2013; Karlinasari et al., 2015).

Besides the species of fungi and a pathogen, the inoculation techniques also influence the agarwood formation of the *G. versteegii* tree. The solid and liquid inoculum are used to inoculate the *G. versteegii* (Mega et al., 2012). Inoculation using liquid inoculum is preferable to solid inoculum (Karlinasari et al., 2015). The position of the inoculation hole also determines the process of agarwood formation because it is related to the distance to photosynthate transport. The research objectives were to determine the species of fungi, the inoculation technique, and the hole position at the stem, resulting in the agarwood of the *G. versteegii* tree at several agroforestry practices.

Methods

Research site The research was carried out at an agarwood-agroforestry area located at Sragen and Karanganyar District, Central Java Province, which lay geographically at altitude: S7°28'35.9" latitude; E111°3'5.3" longitude. There were three research; first, in the Sragen, the *G. versteegii* trees were planted with the mixed garden's agroforestry practice. The *G. versteegii* tree was planted in 2004. The biophysical condition of the site is as follows: The second and the third locations were in Karanganyar. The former *G. versteegii* was planted with a home garden system, and the latter with a taungya system. Geographically, the first location lay at S7°37'3.3" latitude and E111°0'53.8" longitude; the second location lay at S7°36'56.2" latitude E111°0'38.2" longitude (Figure 1). The research was held from April 2017 until October 2018.

Making media and multiplying inocula Potato dextrose agar (PDA) was used as the medium for the *Fusarium oxysporum* and *F. solani.* Fungi identification and insolation, and broth was used as a medium for multiplying hype of *F. oxysporum* and *F. solani*. Both media were made according to Leslie and Summerell's protocol (2006). PDA and broth media were filled in the erlenmeyer flask with 1,000 mL, and then the top of the erlenmeyer flask was wrapped in aluminum foil. Both media subsequently were sterilized using the autoclave with a temperature of 121° C for 15 minutes at a pressure of 15 psi. The PDA medium pouring from the erlenmeyer flask into the petri dish of 100 × 10 mm was done in the laminar airflow. The hype of *F. oxysporum* and *F. solani* was PDA medium cultures for three weeks when the hype achieved maximal growth. Then, those mycelia were cut with 1 × 1 cm size, transplanted into a 250-mL erlenmeyer flask containing 100 mL of potato dextrose broth, and incubated at 28 °C at 100 rpm for three weeks. After two weeks of observing spore growth, if the spore population is optimal enough to grow (> 100 spores mm^{-1}) (Sumarna, 2009) or has reached 1.06 × 106 CFU mL^{-1} (Iskandar & Suhendra, 2013), the inoculum media was ready to inoculate in the production process of agarwood.

Implementation of tree induction The inoculation material was a *G. versteegii* tree that was 14 years old with a tree diameter greater than or equal to 10 cm. Each location had nine trees, so the total number of trees inoculated was 18. Other ingredients are *F. oxysporum* liquid inoculum, *F. solani* inoculum, *F. oxysporum* × *F. solani*, 70% alcohol, markers, gasoline fuels, oil, and waxes. The equipment used in conducting induction was an electric drill, 0.5 PK Yamaha Generator, aluminum ladder, nails, cutters, pliers, plastic hose, plastic bottles, spets, and K3 equipment.

G. versteegii tree inoculation was started from the lowest stem (height 50 cm from the ground). The drill was 10 mm, with a depth of 1/3 of the tree's diameter. Drilling was carried out horizontally. Before drilling, the drill holes were

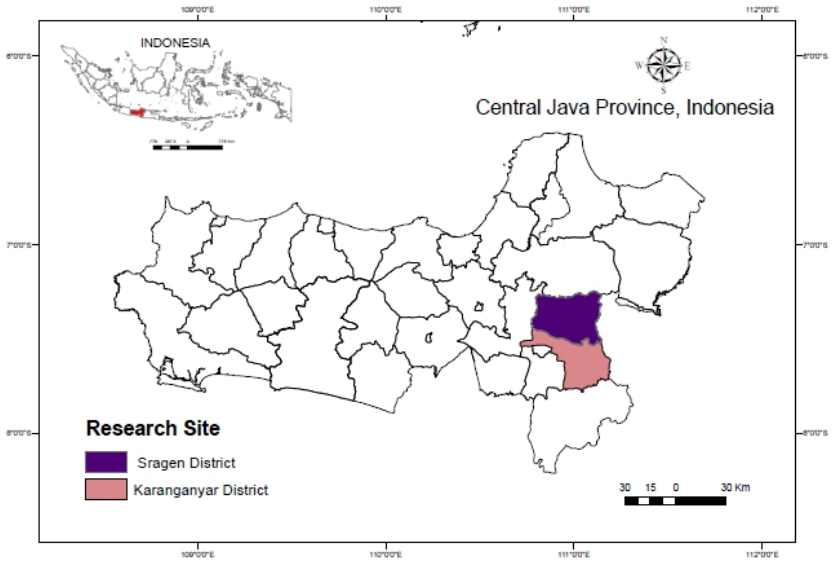

Figure 1 Map of research site.

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sterilized by spraying 70% alcohol, and then the inoculum was inserted into the hole using a syringe (for injection technique). Each hole was given a two-cc inoculum. While the inoculation technique uses the infusion technique, the inoculum was inserted into a plastic bottle with a rubber tube at the end. Each bottle was given 10 mL, and then the rubber tube was inserted into the inoculation hole and closed using wax.

Parameters The parameters used in this research were the wood color, discoloration area ratio, wood aroma, and terpenoid content. Field data was collected 1, 3, and 6 months after induction (6 MAI). The wood color parameter was carried out by slicing and peeling the bark around the inoculation hole (Akhsan et al., 2015) along the 4 cm horizontal direction and 8 cm vertical direction, then to determine the color value used in the color application program meters. The color values stated in the red, green, and blue values were known as the RGB method. Each hole color is determined at 1, 3, and 6 MAI. The measurement boundary in both the horizontal and vertical directions was determined by a different color border with the wood (white). Measurements were made using a ruler for 1, 3, and 6 MAI.

Olfactory test The wood aroma of *G. versteegii* inducted was determined using the olfactory test. The wood sample was taken from infected parts and cut into small pieces. This sample was then burned in a closed room, and each respondent was asked to determine the scent score. The level of aroma was categorized into five scores, namely, score 1 (odorless/only wood odor), score 2 (fragile fragrance), score 3 (medium fragrance), score 4 (rather a strong scent), and score 5 (fragrance scent strong). Respondents tested the scent level of 10 people by filling out the questionnaire provided (Faizal et al., 2017).

GC-MS analysis The sesquiterpene test was carried out using GCMS, which was carried out by the Organic Chemistry Laboratory of the Mathematics and Natural Sciences Faculty of Gadjah Mada University. Samples taken from infected wood are marked with a brownish color. The wood sample was blended until smooth and then extracted using a 10 ml Hexa methanol solution, then incubated using a 100 rpm shaker for 24 hours. Then, it was filtered using filter paper. This extract was then "centrifuged" at 1,000 rpm for 10 minutes and then concentrated by evaporating at a lower pressure. Then, 1 ml was taken to be injected into the GCMS tool.

The GC-MS analysis of agarwood extract used a gas chromatograph interfaced with a mass spectrometer, the GCMS-QP2010S type equipped with silica capillary columns (30 m × 0.25 mm × 0.25 μm). Helium gas was used as a carrier gas at a constant flow rate of 1 mL min^{-1} and an injection volume of 2 μL. Programmable temperatures from 70 °C with an increase of 15 °C min^{-1} to 300 °C. At 70 °C, it is held for 5 minutes; when it reaches 300 °C, it is held for 19 minutes. Each component's relative number (%) is calculated by comparing the average peak area with the total area. The results of the chromatogram are integrated and aligned according to the group. Identifying chemical components is based on comparing the calculation of retention time and authentic mass spectral data with the available library.

Research design A split-plot design was used in this study with three treatments, namely the fungal species, hole position, and inoculation technique. The main plot was the fungal species with three levels, namely *F. oxysporum* (Mo), *F. solani* (Ms), and a mixture of *F. oxysporum* × *F. solani* (Mos). The subplot was the hole position in the stem, which had three levels, namely the top (Pa), middle (Pt), and bottom (Pb) of the stem. The sub-sub plot was the inoculation technique, which had three levels, namely infusion technique (Ti), injection technique (Tj), and control (Tk). The treatment was repeated three times at a time as blocks. The number of samples in this study was 81 (3 replications × 3 main plots × 3 subplots × 3 sub-sub plots). Initial inoculation was carried out at a distance of 50 cm from the soil surface (Pb); subsequent inoculation was carried out at a distance of 100 cm from the ground (Pt) and 150 m from the ground (Pa) (Liu et al., 2013). The layout of the treatments is shown in Figure 2.

Statistical data analysis Analysis of variance was used to know the difference between the treatments. Computation of data was used in the SPSS software program version 22.

Results

The wood color Induction of *G. versteegii* agarwood trees using *F. oxysporum, F. solani*, and *F. oxysporum* × *F. solani* causes changes in the wood color around the induction hole from light-brownish white to brown until blackish brown. Table 1 and Figure 3 show that the RGB value in the multi-layer garden, taungya system, and home garden decreases gradually concomitant with the time increase of 0, 1, 3, and 6 months after induction. Initially, the RGB value in the multi-layer garden is 587, and then six months after induction, the RGB value is 390.

The infection of the fungus of *F. oxysporum* causes the smallest RGB value of 233.93, then the RGB values yielded by mixed *F. oxysporum* × *F. solani*, and *F. solani*, i.e., 304.93 and 307.89, respectively (Figure 4). The smaller the RGB value, the darker the wood color of *G. versteegii*.

Figure 5 shows that the trend of the RGB value of *G. versteegii* inducted by *F. oxysporum, F. solani*, and mixed *F. oxysporum* × *F. solani* in the multi-layer garden, taungya system, and home garden, has the same pattern. In the initial measurement of 0 months, the RGB value is relatively more

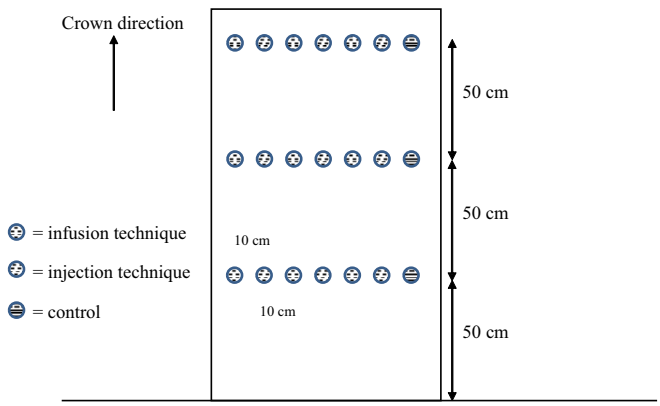

Figure 2 The layout the hole position in the stem of *Gyrinops versteegii*.

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Table 1 An RGB value at 0, 1, 3, and 6 MAI (the month after induction) in three agroforestry practices

Time	Multi-layer garden				Taungya				Home garden			
(MAI)	R	G	B	RGB	R	G	B	RGB	R	G	B	RGB
0	193	202	201	596	185	180	173	538	185	199	200	584
1	149	131	115	395	145	129	111	385	145	114	90	349
3	149	130	114	393	128	113	92	333	128	101	80	309
6	148	129	113	390	127	92	66	285	127	95	82	304

Noted: R = red; G = green; B = blue; MAI = month after induction

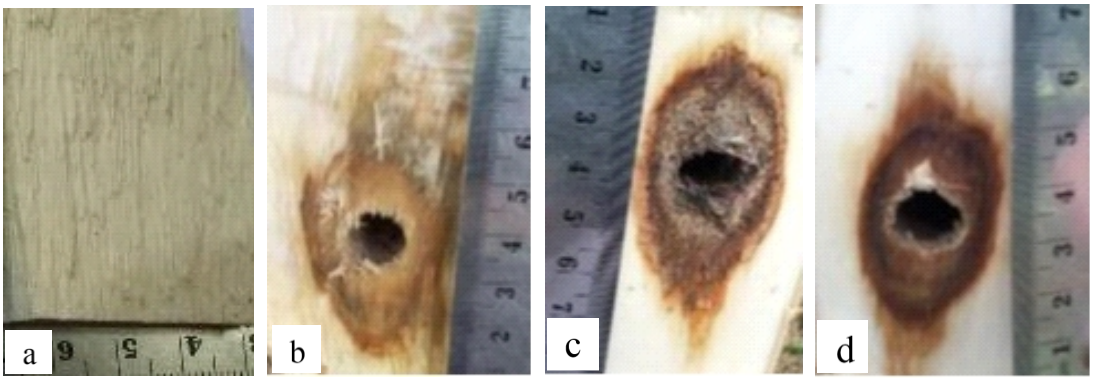

Figure 3 A wood color in the period of (a) 0; (b) 1; (c) 3; and (d) 6 MAI (a month after inoculation).

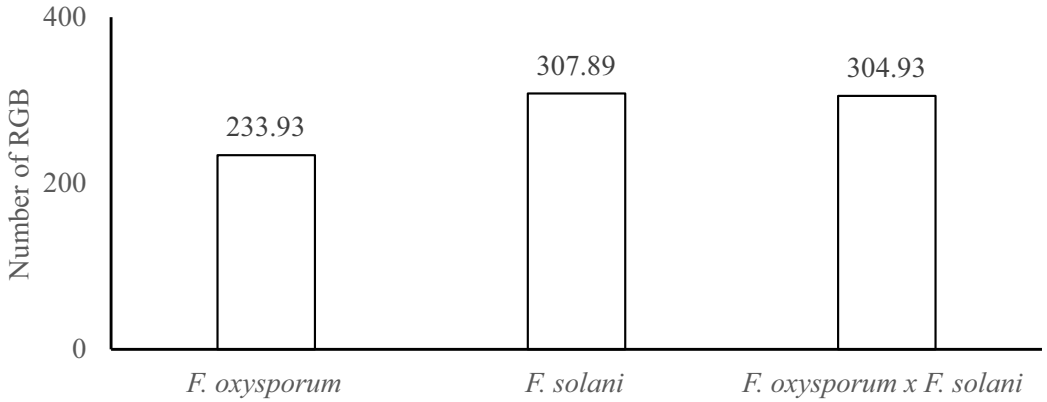

Figure 4 The RGB value of *Gyrinops versteegii* induced by *Fusarium oxysporum*, *F. Solani*, and *F. oxysporum* × *F. solani* in three agroforestry practices.

significant than that of 1, 3, and 6 months after induction. Furthermore, the RGB value of inoculated *G. versteegii* wood decreases when the time of induction increases. The RGB value correlates to the wood color of *G. versteegii*. The higher the RGB value, the lighter the wood color, and vice versa.

The induction technique affected the RGB value. The infusion technique gave the lowest RGB value of 273.04, followed by the infection technique and control, which had RGB values of 325.93 and 411.37, respectively.

Discoloration area Based on the variance analysis (Table 2), the discoloration area of inoculated *G. versteegii* wood in the multi-layer garden was significantly affected by the sig induction technique (p-value = 0.04). In the taungya system, the discoloration area was significantly affected by both hole position with sig values (p-value = 0.001) and induction technique with sig. (p-value = 0.000). In the home garden, the discoloration area was affected by a hole position with sig (p-value = 0.005), induction technique with sig (p-value = 0.000), and a combination of hole position and induction technique (p-value = 0.033).

Table 3 shows that combining the infusion technique's induction technique and the top position's hole position gives the most expansive discoloration area. In contrast, the injection technique and the bottom hole position gave the lowest discoloration area. The discoloration area of all the treatments was relatively more significant than the control. Moreover, the discoloration area of inoculated *G. versteegii* wood in the taungya system was relatively broader than in the home and multi-layer tree gardens.

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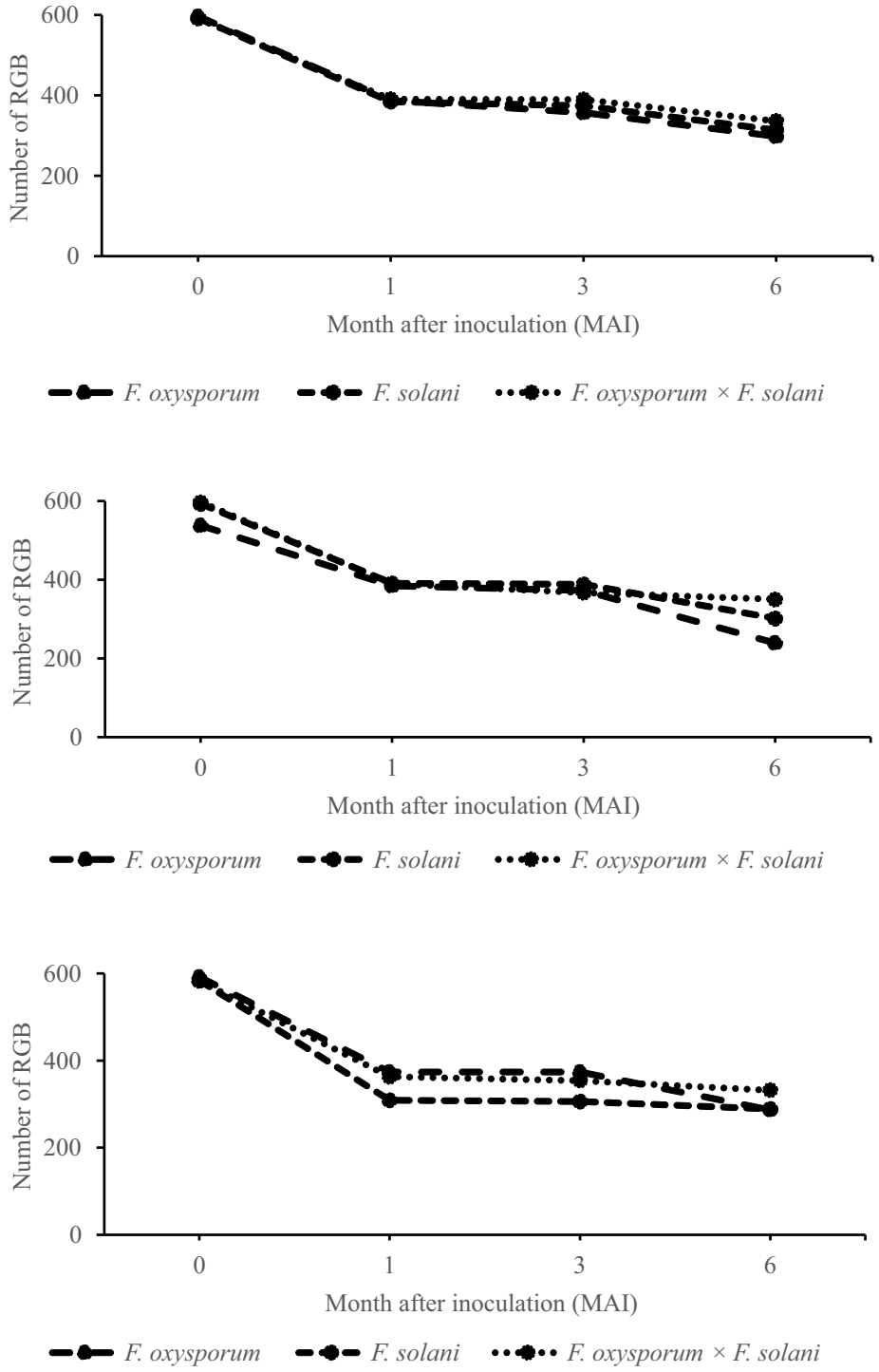

Figure 5 The trend of RGB values of 0, 1, 3, and 6 MAI (months after inoculation) with *Fusarium oxysporum*, *F. solani,* and *F. oxysporum* × *F. solani* (a) multi-layer garden, (b) taungya, and (c) home garden.

Wood aroma Table 4 shows that in the multi-layer tree garden, the wood aroma of inoculated *G. versteegii* wood was significantly affected by all of the treatments, fungal species with sig (*p*-value = 0.001), hole position treatment with sig (*p*-value = 0.000), and induction techniques with sig (*p*-value = 0.001) and their combinations.

The combination between the fungal species and hole position with sig *p*-value = 0.000, the combination between the fungal species with induction technique with sig *p*-value = 0.000, and the combination of three treatments with sig *p*-value = 0.002. According to the variance analysis, in the taungya system, the wood aroma was affected by the fungal species' treatment with sig (*p*-value = 0.007), the induction technique with a significance of 0.000, and the combination of treatments of hole position and induction technique with sig *p*-value = 0.035. Furthermore, the home garden system's

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Table 2 The analysis of variance of fungal species, hole positions, and induction techniques of *Gyrinops versteegii* in the multi-layer garden, taungya, and home garden

Sources of variation	Df	Sum of square (SS)	Mean of square (MS)	*F*-value	Pr>F
		Multi-layer tree garden			
Main plot					
Group (K)	2	0.620	0.310	0.014	0.986
Fungi (M)	2	64.223	32.112	1.467	0.333 ns
Error (m)	4	87.536	21.884	5.528	0.096
Sub plot					
Hole position (P)	2	14.271	7.135	0.824	0.462 ns
MP	4	26.008	6.502	0.751	0.576 ns
Error (p)	12	103.894	8.658	1.427	0.199
Sub-sub plot					
Technique (T)	2	80.349	40.174	6.621	0.004 **
MT	4	37.984	9.496	1.565	0.205 ns
PT	4	14.569	3.642	0.600	0.665 ns
MPT	8	30.344	3.793	0.625	0.751 ns
Error (t)	36	218.448	6.068		
Total	80	678.246			
		Taungya			
Main plot					
Group (K)	2	54.489	27.244	0.282	0.768
Fungi (M)	2	209.681	104.841	1.084	0.421 ns
Error (m)	4	386.919	96.730	11.875	0.000
Sub plot					
Hole position (P)	2	229.678	114.839	14.098	0.001 **
MP	4	91.330	22.833	2.803	0.074 ns
Error (p)	12	97.746	8.145	0.307	0.984
Sub-sub plot					
Technique (T)	2	1215.282	607.641	22.895	0.000 **
MT	4	269.998	67.500	2.543	0.056 ns
PT	4	166.464	41.616	1.568	0.204 ns
MPT	8	228.740	28.547	1.076	0.402 ns
Error (t)	36	955.455	26.540		
Total	80	3905.782			
		Home garden			
Main plot					
Group (K)	2	18.324	9.162	4.508	0.094
Fungi (M)	2	4.624	2.312	1.138	0.406 ns
Error (m)	4	8.129	2.032	2.248	0.125
Sub plot					
Hole position (P)	2	15.356	7.678	8.495	0.005 **
MP	4	4.098	1.025	1.134	0.387
Error (p)	12	10.847	0.904	1.519	0.163
Sub-sub plot					
Technique (T)	2	281.761	140.881	236.738	0.000 **
MT	4	2.820	0.705	1.185	0.334 ns
PT	4	7.033	1.758	2.954	0.033 *
MPT	8	1.995	0.249	0.419	0.902 ns
Error (t)	36	21.423	0.595		
Total	80	376.410			

Note: ns = not significantly different; * = significantly different at the test level of 5%; ** = significantly different at 1% test level.

wood aroma was significantly affected by the hole position, the induction technique, and the combination of the fungal species treatment and induction technique with sig 0.020.

Table 5 shows that the combination of three treatments, *F. oxysporum*, the top-hole position, and the infusion technique, gives the highest wood aroma with a score of 4.68. Furthermore, these combinations were not significantly different from the three mixed *F. oxysporum* × *F. solani* treatments. The top-hole position and the infusion technique had a wood aroma score of 4.45 at a test level of 5%. All of the wood aroma value was up to the control value.

Terpenoid content According to the GCMS analysis, the sesquiterpene compounds of *G. versteegii* in the taungya

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Table 3 Duncan's test of discoloration area of inoculated *Gyrinops versteegii* wood of 6-month after induction

Hole position	Induction technique		Control
	Infusion	Injection	
Top	8.06 [a]	5.06 [d]	2.72 [e]
Middle	7.24 [b]	4.97 [d]	2.89 [e]
Bottom	6.29 [c]	4.72 [d]	2.14 [e]

The same superscript word after the numbers do not look significantly different at the test level 5%.

Table 4 The analysis of variance of the wood aroma of inoculated *Gyrinops versteegii* tree in the multi-layer tree garden, taungya system, and home garden

Sources of variation	Df	Sum of square (SS)	Mean of square (MS)	*F*-value	Pr>F
Multi-layer tree garden					
Main plot					
Group (K)	2	0.460	0.230	6.311	0.058
Fungi (M)	2	4.047	2.023	55.510	0.001 **
Error (m)	4	0.146	0.036	0.471	0.757
Sub plot					
Hole position (P)	2	4.199	2.099	27.108	0.000 **
MP	4	2.377	0.594	7.674	0.003 **
Error (p)	12	0.929	0.077	1.510	0.166 ns
Sub-sub plot					
Technique (T)	2	124.045	62.023	1209.472	0.000 **
MT	4	2.115	0.529	10.310	0.000 **
PT	4	2.833	0.708	13.812	0.000 **
MPT	8	1.610	0.201	3.925	0.002 **
Error (t)	36	1.846	0.051		
Total	80	144.607			
Taungya					
Main plot					
Group (K)	2	0.114	0.057	1.477	0.331
Fungi (M)	2	1.715	0.857	22.316	0.007 **
Error (m)	4	0.154	0.038	0.136	0.966
Sub plot					
Hole (P)	2	1.943	0.971	3.430	0.066 ns
MP	4	2.274	0.569	2.007	0.157 ns
Error (p)	12	3.399	0.283	0.819	0.630
Sub-sub plot					
Technique (T)	2	156.810	78.405	226.733	0.000 **
MT	4	1.223	0.306	0.884	0.483 ns
PT	4	4.011	1.003	2.900	0.035 *
MPT	8	5.510	0.689	1.992	0.076 ns
Error (t)	36	12.449	0.346		
Total	80	189.602			
Home garden					
Main plot					
Group (K)	2	0.296	0.148	0.719	0.541
Fungi (M)	2	0.422	0.211	1.026	0.437 ns
Error (m)	4	0.822	0.206	2.565	0.092
Sub plot					
Hole (P)	2	1.206	0.603	7.525	0.008 **
MP	4	0.822	0.205	2.563	0.093 ns
Error (p)	12	0.962	0.080	0.990	0.477
Sub-sub plot					
Technique (T)	2	156.309	78.155	965.432	0.000 **
MT	4	0.981	0.245	3.030	0.030 *
PT	4	0.819	0.205	2.528	0.057 ns
MPT	8	1.017	0.127	1.570	0.169
Error (t)	36	2.914	0.081		
Total	80	166.570			

Note: ns = not significantly different; * = significantly different at the test level of 5%; ** = significantly different at 1% test level.

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system were *allo-aromadendrene*, *aromadendrene*, *α-bulnesene*, *α-cendrene*, *α-caryophyllene*, *α-copaene*, *α-guaiene*, *β-gurjunene*, *α-muurolene*, and *α-selinene.* Furthermore, in the multi-layer tree garden, the sesquiterpene compounds were composed of 9 compounds, viz. *allo-aromadendrene*, *aromadendrene*, *α-bulnesene*, *α-cendrene*, *α-caryophyllene*, *α-guaiene*, *β-gurjunene*, *α-muurolene*, dan *α-selinene*. Also, in the home garden, the wood of *G. versteegii was allo-aromadendrene*, *aromadendrene, α-bulnesene*, *α-cendrene*, *α-caryophyllene*, *α-copaene*, *α-guaiene*, *β-gurjunene*, *α- muurolene*, and *α-selinene.* The retention time of agarwood compound is presented in Table 6. Figure 6 shows that *G. versteegii* inoculated using the fungi *F. oxysporum, F. solani* and *F. oxysporum* × *F. solani* produced terpenoids that were deposited in the included phloem, vascular tissue, and ray parenchyma.

The number of sesquiterpene compounds found in the taungya system was more the sesquiterpene compounds (10 compounds) than in the home garden (9 compounds) and multi-layer tree garden (9 compounds). Never was the *trans-α-bergamotene* compound found both in the taungya system and the others. In the taungya system was the *α*-copaene compound merely found.

Discussion

After injection, indicators forming agarwood in the *G. versteegii* tree are a wood color, a discoloration area, a wood aroma, and terpenoid contents.

Wood color Wood color is one of the indicators of agarwood quality. The darker the wood color, the higher the agarwood quality. This research showed that treating Fusarium fungus, hole position, and the inoculation technique influenced the change in the wood color surrounding the inoculation hole. Furthermore, the time of induction affects the wood color as well. One month after injection, the color of the *G. versteegii* wood surrounding the hole inoculation was brownish white. When the induction was three months old, *G. versteegii* wood's color was darker than the 1-month induction. Besides, in the 6-month inoculation, the *G. versteegii* wood was blackish brown.

F. solani, F. oxysporum, and mixed *F. solani* × *F. xysporum* can markedly induce the formation of agarwood in the *G. versteegii* tree, indicated by the wood color surrounding the inoculation hole. The *F. solani* fungus can successfully achieve the blackish-brown indicated by an RGB value of 233.93 than *F. oxysporum* and mixed *F. oxysporum* × *F. solani* with RGB values 304.94 and 307.89, respectively.

Table 5 Duncan's test of the wood aroma of inoculated *Gyrinops versteegii* at six months after induction

Fungal species	Hole position	Induction technique		
		I	J	K
O	A	4.68 ^{a}	4.17 ^{bc}	1.07 ^{k}
	T	4.25 ^{bc}	3.50 ^{efg}	1.00 ^{k}
	B	3.85 ^{cde}	3.25 ^{fghi}	1.10 ^{k}
S	A	3.94 ^{cd}	3.71 ^{def}	1.00 ^{k}
	T	4.15 ^{bc}	3.36 ^{efg}	1.00 ^{k}
	B	3.42	3.49	1.00 ^{k}
OS	A	4.45 ^{ab}	3.55 ^{def}	1.00 ^{k}
	T	3.00 ^{hi}	2.00 ^{j}	1.00 ^{k}
	B	3.08 ^{ghi}	2.92 ^{i}	1.00 ^{k}

Note: O = *Fusarium oxysporum*; S = *F. solani*; OS = *F. oxysporum* × *F. solani*; A = top; T = middle; B = bottom; I = infusion; J = injection; K = control; the same superscript word after the numbers not significantly different at test level of 5%.

Table 6 Retention time, initial time, final time, and percentage of the area of the ten main compounds that compose agarwood oil

No	Peak	Retention time	Initial time	Final time	Area (%)			Compound
					KC	TG	PK	
1	12	25,463	25,358	25,658	0.12	0.12	0.11	*Allo-Aromadendrene*
2	13	26,882	26,767	27,100	0.53	0.23	0.13	*Aromadendree*
3	17	28,023	27,925	28,167	0.48	0.66	0.43	*α-bulnesene*
4	18	28,308	28,167	28,350	1.30	1.69	1.20	*α-caryophyllene*
5	32	29,394	29,250	29,542	0.18	0.59	0.21	*α-selinene*
6	33	31,415	31,300	31,467	0.81	0.79	0.69	*α-guaiene*
7	36	31,825	31,758	31,842	0.04	0.07	0.02	*α-muurolene*
8	39	32,588	32,242	32,800	0.56	0.73	0.35	*β-gurjunene*
9	52	33,590	33,442	33,658	0.11	0.20	0.17	*α-cedrene*
10	62	35,091	35,042	35,200	0.00	0.03	0.00	*α-copane*

Note: KC = multi-layer-tree garden; TG = taungya; PK = home garden

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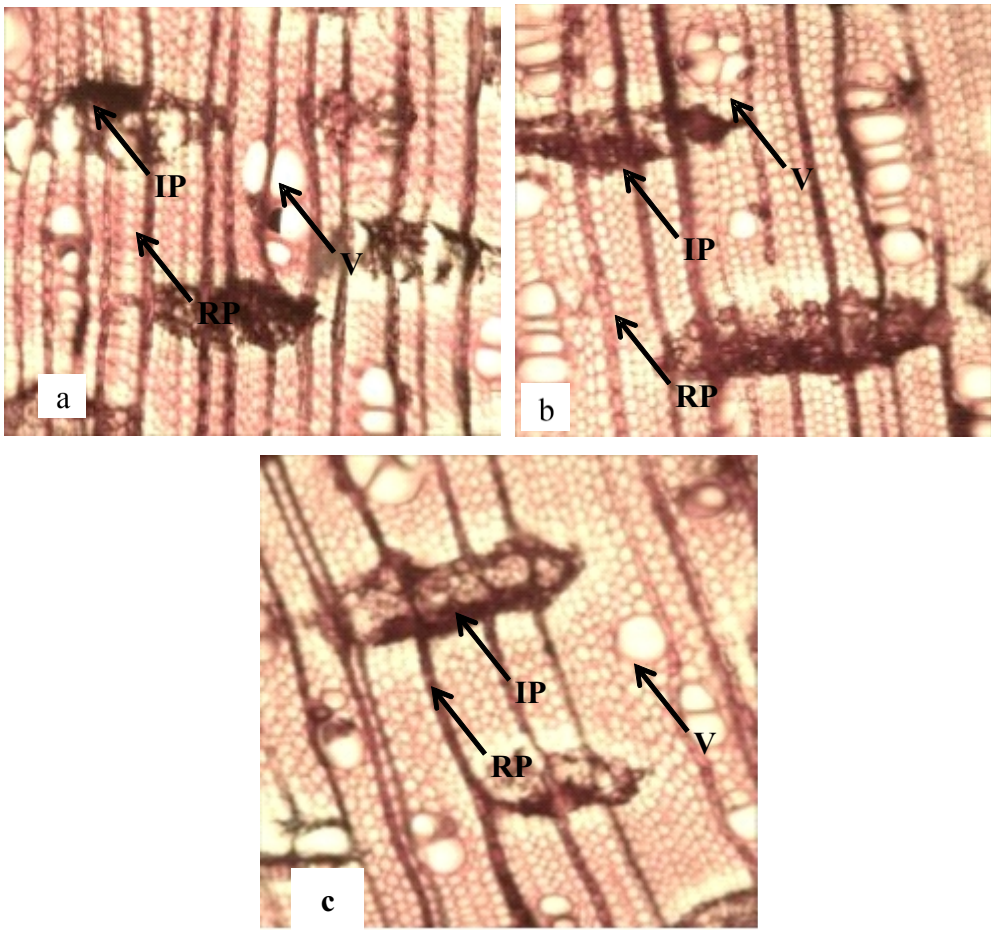

Figure 6 Cross-section of *Gyrinops versteegii* inoculated by fungal species (a) *Fusarium oxysporum*, (b) *F. solani*, (c) *F. oxysporum* × *F. Solani*. Note: IP = included phloem; V = vessel; RP = ray parenchyma.

Similarly, according to Iskandar and Suhendra, *Aquilaria beccariana* is deliberately induced by *Fusarium* sp., changing the wood color and becoming more blackish brown (Iskandar & Suhendra, 2013). The 4-year-old of *A. mallacensis* tree inoculated by *Fusarium* sp. fungus in the Pontianak City, West Kalimantan Province, can distinctly change the wood color from brownish white to blackish brown (Vantompan et al., 2015). After being inoculated by *Fusarium* sp., the *A. microcarpa* wood changes obviously from brownish white to blackish brown.

The darkest wood, such as *G. versteegii, A. beccariana*, and *A. microcarpa,* is caused by phytoalexins, as the antidote substance coloring the brown, specifically exudated by alkaloid cells (Mega et al., 2012). Also, in the Tenggarong Kutai Kertanegara District, the inoculation of *A. microcarpa* tree applies the *F. oxysporum* fungus yielding the darkest wood than using the *F. solani* (Akhsan et al., 2015).

The fungal kinds or inoculation techniques greatly affected the wood color. The infusion technique can effectively obtain the darkest wood of the *G. versteegii* tree than the injection technique. The inoculation technique is related to the number and availability of inoculants employed to induce the agarwood in the *G. versteegii* tree. The infusion technique is better to obtain both quantity and quality of agarwood of *G. versteegii* than the others because it abundantly provides much inoculant.

Discoloration area The discoloration area is one of the indicators of the succeeding inoculation process due to the formatted agarwood quantity. In the mixed garden, this parameter is pronouncedly affected by inoculation technique parameters. The discoloration area is significantly affected by the hole position in the stem and the inoculation treatment technique in the taungya system. Furthermore, in the home garden system, the discoloration area was markedly influenced by the hole position, inoculation technique, and the combination of two parameters. Combining the upper hole position and infusion inoculation technique has the largest discoloration area than other treatment combinations. The hole position in three sites shows that the upper hole position has the largest discoloration area.

F. oxysporum attacks the wood vessel tissues and grows and develops in it (Cheikowski, 2002). This fungus stimulates the formation of tylosis when the parenchymal cell xylem membrane grows. A tylosis resists the wood tissue and interferes with moving water in the plant. It says that *F. oxysporum,* as a pathogenic fungus of the specific plant causing the damping-off disease, exudates a phytoalexin to penetrate inside the plant tissue, causing the xylem tissue dysfunction to transport the nutrients due to being covered by

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gel, composed of the kind of sugar (Leslie & Summerell, 2006). The pathogen's infection rate is influenced by the pathogens' species and determined by the number of individual pathogens (Stevens, 1939). As the upper section stem has more moisture than the bottom section stem, the *Fusarium* fungus infection in the upper section stem is higher than its disease in the bottom. The wood's moisture markedly influences the development of pathogens (Zobel & van Buijtenen, 1989).

The wood aroma ANOVA analysis shows that the wood aroma of *G. versteegii* wood in the mixed garden, taungya, and the home garden system showed different influences. In the multi-layer garden, the wood aroma is significantly affected by all the treatments: a) the fungus species, b) the hole position, c) the inoculation technique, and d) the treatment combination of the three treatments. In the taungya system, the wood aroma is affected by the fungus species, inoculation technique, and the treatment combination of three treatments. Furthermore, the wood color is influenced by the hole position, inoculation technique, and treatment combination of the hole position and inoculation technique treatment in the home garden system.

The best wood aroma rises through the treatment combination of fungus species of *F. oxysporum,* the hole position in the upper part stem, and the inoculation technique of infusion inoculation technique. The results of this study are in accordance with the research conducted by Iskandar and Suhendra (2012) and Akhsan et al. (2017) that agarwood inoculated using *Fusarium* sp. fungus can produce a distinctive agarwood aroma. The burned-wood fragrant of an inoculated *G. versteegii* tree caused by the accumulation of terpenoid compounds deposited in the plant tissue is the secondary metabolite induced by *Fusarium* sp. The three species of *F. oxysporum, F. solani,* and mixed *F. oxysporum* × *F. solani* can cause agarwood to form, in the *G. versteegii* tree the mixed garden, taungya system, and home garden system. *F. oxysporum* can elicit the best agarwood than the others. It states that *F. oxysporum* has the highest pathogenesis to the *G. versteegii* tree growing in the mixed garden, taungya, and home garden system. Both the species recognition and the pathogen-host compatibility significantly influenced the pathogenetic rate of *Fusarium* fungus.

It states that when a pathogen penetrates the plant host, the host cells can recognize the pathogens through specific chemical compounds secreted by the pathogen with particular receptor cells and recognize physical and chemical traits due to the host (Gullino & Bonants, 2014). The wood properties of the upper section stem are known to compose a juvenile tissue, a lower specific gravity, a lower radial and tangential toughness, a higher radial and tangential shrinkage, a longer vessel, and tracheid cells than those of the bottom section stem (Zobel & Buijtenen, 1989). The wood on the top of the stem is relatively susceptible to pests and disease. Moreover, as the photosynthesis process results, photosynthate is distributed by the mesophyll in the leaves to all the plant tissues. In other words, photosynthate will translocate from the tree crown to the roots. Thus, the upper part stem can early get the photosynthate before the bottom section stem. It states photosynthetic acceleration is 1–15 g h^{-1} cm^{-2} (Taiz & Zeiger, 2002). As the upper part of the stem is available for photosynthate, it is susceptible to pathogen attack as an energy resource for the pathogen's life cycle.

Terpenoid content A terpenoid compound is one of the indicators of agarwood quality. The main composition of oil agarwood contains *allo-aromadendrene*, *aromadendrene*, *α-bulnesene*, *α-cedrene*, *α-caryophyllene*, *α-copaene*, *α-guaiene*, *β-gurjunene*, *α-muurolene*, *α-selinene*, and *trans-α-bergamotene* (Mohamed, 2016). The compounds that compose agarwood found in this study are *allo-aromadendrene*, *aromadendrene*, *α-bulnesene*, *α-cedrene*, α-*caryophyllene*, *α-copaene*, *α-guaiene*, *β-gurjunene*, *α-muurolene*, and *α-selinene*. Meanwhile, Tajuddin (2016) found that the compounds that make up agarwood are *allo-aromadendrene*, *aromadendrene*, *α-bulnesene*, *α-cedrene*, *α-caryophyllene*, *α-copaene*, α-*guaiene*, β-*gurjunene*, α-*muurolene*, *α-selinene*, and *trans-α-bergamotene*. The number of compounds that make up agarwood found at the research location was lower compared to the number of compounds found by Mohamed (2016) and Tajudin (2016). The *G. versteegii* growing in the taungya system is relatively more terpenoid than in the mixed and home garden systems. The fungus species, the hole position at the stem, and the inoculation technique markedly affect the terpenoid content of the *G. versteegii* wood.

The upper stem has more terpenoid content than the middle and bottom stems. This condition is related to the process of forming agarwood by the fungus as a pathogen. As a pathogen, *Fusarium* sp. needs adequate nutrition for its life span and a suitable habitat to grow. Generally, the upper wood traits are composed of softly young wood tissue provided by available nutrition from photosynthesis due to the closer distance from leaves. Both habitat compatibility and nutritional overflow cause the Fusarium to grow vastly and viciously invade the *G. versteegii* tree–the more the attack of pathogens, Fusarium, the more terpenoid product as an antibody. Similarly, when pathogens invade the *Picea excelsa* tree, its top is more destroyed than the bottom stem. Thus, the top is more pentosan production, a mechanical compound excluded by particular plants for self-defense mechanism, than the bottom stem (Zobel & Buijtenen, 1989).

Containing the *G. versteegii* wood's chemical compound, extracted by smetana, planted in the mixed garden, taungya, and home garden, is equal except for the α-copaene compound found merely in the taungya system. This compound gives a specific scent of agarwood. Indeed, the exciting fragrance of agarwood determines the distinct location. Terpenoid compounds secreted by tree-producing agarwood are secondary metabolites plants use as a defense mechanism for pathogen attack. Microscopically, the deposit of these compounds includes phloem, vessel compound, and rays. Composed by the live parenchyma cells, the included-phloem tissue produces the terpenoid compounds. However, they are deposited in the included phloem tissue and deposited in the vessel tissue and ray cells.

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Conclusion

The agarwood quality resulted from the inoculated *G. versteegii* tree characterized by the wood aroma, the discoloration area, wood aroma, and terpenoid content, is significantly affected by the wood position in the stem, the fungal species, and the inoculation technique. The upper stem results in better agarwood than the bottom stem. Using the inoculant of the *F. oxysporum* can achieve better agarwood than the inoculant of *F. solani* and mixed *F. solani* × *F. oxysporum*. Furthermore, the infusion technique gains a better agarwood result than others.

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