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The impact of fungi in increasing essential oils and chemical components of agarwood (*Gyrinops versteegii*)

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[2]Faculty of Agriculture, Universitas Udayana. Jl. P.B. Sudirman, Denpasar 80234, Bali, Indonesia
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Abstract. *Mega IM, Kartini NL, Suranjaya IG, Bhayunagiri IBP, Sumarniasih MS, Adnyana INS. 2024. The impact of fungi in increasing essential oils and chemical components of agarwood (*Gyrinops versteegii*). Biodiversitas 25: 2552-2559.* Essential oil, a key agarwood process product, is the fundamental ingredient in perfume, cosmetics, and drugs. The type of agarwood plant, the induction microbes, and the environmental factors influence the production of essential oil in agarwood. This research, characterized by its rigorous application of a randomized block design (RBD) experimental design and statistical analysis, aims to analyze the agarwood essential oil content and chemical components upon applying three fungal inoculants. The treatments applied are: (i) *Trichoderma harzianum* inoculant, (ii) *Fusarium solani* inoculant, (iii) *Rhizopus microsporus* inoculant, and (iv) Control (without fungal inoculant). Each treatment had 15 repetitions. The parameters observed are agarwood color, scent, essential oil content, and chemical components. The quantitative observation data was statistically analyzed by variance analysis, and if a significant value was found, the analysis was followed by the Least Significant Difference Test (5% level). The results showed that the type of fungal inoculant applied significantly influences the essential oil content in the wood. The highest essential oil content of 0.7413 mL/kg was obtained by applying *Fusarium solani* fungal inoculant. The lowest 0.5249 mL/kg content was obtained in the control treatment (without fungi). The chemical components of essential oils are Butanoic acid, 3,7-dimethyl, 2,6-octa diethyl, Hexadecanoic acid, and methyl ester. Citronelol; Beta Citranelol; 3,7-dimethyl-2,6-octadien-l-ol; Citronellol acetate; Citral; Geranyl acetate; 2,6,10_Dodecatrien-1-ol, 3,7,11-trimethyl (Farnesol); Selina-6-en-4-ol; and (-)-Globulol.

Keywords: *Fusarium solani*, *Gyrinops versteegii*, induction microbes, *Rhizopus microsporus*, *Trichoderma harzianum*

Abbreviations: GC-MS: Gas Chromatography-Mass Spectrophotometer, BLA: Banana Leaf Agar, PCR: Polymerase Chain Reaction, PDA: Potato Dextrose Agar, RBD: Randomized Block Design

INTRODUCTION

More than six genera produced agarwood trees: *Aquilaria, Wikstroemia, Enkleia, Aetoxylon, Gonystylus*, and *Gyrinops* (Lee et al. 2018). Among these six genera, *Aquilaria* and *Gyrinops* are the best agarwood producers. The differences between *Aquilaria* and *Gyrinops* are the leaves (crystal type, number of neighboring cells, shape of upper and lower surface epidermal cells) and the stem (pith radius type and niche shape) (Wangiyana 2019). The quality of natural aloes is determined by the grade of the resin contained in it; the higher the resin content, the better the quality (Liu et al. 2017). The chemical components of *Aquilaria* and *Gyrinops* aloes depend on the class of aloes. The chemical components of the superclass *gaharu* contain more sesquiterpene group compounds than the *kemedangan* class (Nasution et al. 2020).

Along with the knowledge and technological development, agarwood is not only marketed as raw products but can be marketed as processed products such as agarwood essential oil. Agarwood oil is an essential oil obtained from a distillation process. According to Batubara et al. (2021), agarwood oil is beneficial in curing several diseases like stomach acid disease, having anti-rheumatic, anti-asthmatic, anti-microbial, anti-oxidant properties, and so on. With the crucial benefit of agarwood oil, its price in the market is relatively high. For example, agarwood oil from Kalimantan was priced at Rp. 250,000 per 12 mL, and the original Papua agarwood oil was IDR 157,000,000 per liter (Yusoff et al. 2024). In 2020, Indonesia had 89 essential oil exporters spread across the provinces, with a total export value of USD215.81 million (Anggraini 2022).

The production of agarwood oil is influenced by various factors, namely the genetics of agarwood-producing trees, induction microbe, environment, and the length of the agarwood formation process. Agarwood may be formed when a certain pathogen infects the agarwood-producing tree. Putri et al. (2017) showed that agarwood-producing plants inoculated with fungi produced 1.1% higher resin content than uninoculated plants. Furthermore, research by Mega et al. (2015) revealed that a liquid inoculant mixture of *Fusarium solani* and *Rhizopus microsporus* fungi successfully inoculated into agarwood plants (*Gyrinops versteegii*) for 16 months produced agarwood with a resin content of 13.58%. Research from Adnyana et al. (2022) showed that agarwood three in Marga Dauhpuri (Tabanan

District) produced higher essential oil content compared to agarwood three in Klungkung District after being inoculated by *Fusarium* sp. fungi. The chemical composition of the agarwood oil in the research are 9-octadecenoic acid, trans-13-octadecenoic acid, eugenol, 1-nonadecene, propanedioic acid, phenyl-; 2-propanol, 1,1'-oxy bis-; 1-propanol, 2- (2-hydroxypropyl); cyclopentadecanone, 2- hydroxy-dan oleic acid.

According to Yang et al. (2021), the main fragrant compounds in agarwood are the derivatives of sesquiterpene and phenylethyl chromone. The sesquiterpene content varied greatly in high-quality agarwood. Three sesquiterpene compounds with fragrance are α-agarofuran, (-)-10-epi-gammaeudesmol, and oxo-agarospirol. Other than sesquiterpene compound, agarwood from Indonesia, *Aquilaria malacensis*, contains agarwood oil's main component, chromone. When burnt, the chromone compound produces the fragrant smell of agarwood (Yan et al. 2023). Agarwood chemical components contain furan and other ester group compounds that can produce fragrant (Pasaribu et al. 2021).

Research results from Mega and Nuarsa (2019) showed that three types of fungi could help in the formation of agarwood (*Gyrinops versteegii*) wood development: *Fusarium solani*, *Rhizopus* sp., and *Trichoderma* sp., which was indicated by the change in wood color from white to brown and further to blackish brown and the resin content being 5.31%, 5.24%, and 7.92% in the agarwood sapwood. Further, Mega et al. (2020) identified three fungi isolates (*Fusarium*, *Rhizopus*, and *Trichoderma*) that shape agarwood molecularly, and the fungi species are *Fusarium solani*, *Rhizopus microsporus*, and *Trichoderma harzianum.* With three fungi known to help the development of agarwood, whether or not the fungi can increase the essential oil content and the chemical components that produce fragrance and thus increase the agarwood quality need to be researched. Thus, this research aims to analyze the content of essential oil and chemical components in agarwood plants after applying three fungal inoculants.

MATERIALS AND METHODS

Research location

This research is started by developing fungal inoculants in the Soil Biology Laboratory, Faculty of Agriculture, Universitas Udayana, Bali, Indonesia. The fungi inoculation treatment was conducted in agarwood cultivation in Marga Dauhpuri Village, Marga District, Tabanan District, Bali Province, Indonesia. The essential oil distillation was performed in the Phytochemical Laboratory, Pharmaceutical Department, and the identification of chemical components was analyzed in the Integrated Research Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Udayana.

Observation of fungal morphology

The observed fungal morphology must first be rejuvenated in a petri dish. The rejuvenation parent material uses isolates already in the Soil Biology Laboratory in the Faculty of Agriculture, Universitas Udayana, and have been purified. Observation of fungal morphology is carried out with several different work stages, including:

(i). Observing the color of fungal colonies. The method used is to grow each isolate on PDA (potato dextrose agar) media in a petri dish, which is incubated at room temperature (27-28°C) and placed in a room with sufficient lighting. The color and presence or absence of aerial hyphae are determined on the seventh day.

(ii). Observing the characteristic shape of the conidiophore (phyalide). The characteristic shape of the conidiophore can be observed with a slide culture, which is made by taking a small number of fungal colonies on PDA media (approximately 0.3 cm^2 in size), then placed on an object glass and covered with a cover glass. The slide culture is placed on a sterile petri dish that is kept moist by placing tissue/filter paper moistened with sterile distilled water. The slide culture is viewed under a microscope after being incubated for 24-72 hours at room temperature.

(iii). Measuring and observing the shape of macro and microconidia. The shape of macro and microconidia is observed based on the isolates grown on BLA (banana leaf agar). In this natural medium, the structure of the sporodochia and the macroconidia it forms can be observed. BLA is made according to the method of Nelson et al. (1983). The growing fungal colonies are observed under a microscope. Identification of isolates is based on the identification key of Nelson et al. (1983), Leslie and Summerell (2006); the determination of colony color refers to A Mycological Color Chart (Rayner 1970).

Research test design

The design used in this research was Randomized Blocked Design (RBD) with 15 repetitions. The treatments performed were (i) (*Trichoderma harzianum* inoculant, A), (ii) (*Fusarium solani* inoculant, B), (iii) (*Rhizopus microsporus* inoculant, C), and (iv) (control /without fungal inoculant, D).

The parameters observed are agarwood color and scent, essential oil content, and the chemical component in the agarwood. The quantitative observation data was statistically analyzed by analysis of variance, and if a significant value was found, it was further tested with the Least Significant Difference Test (5%).

The research preparation stage

In this stage, the tools and ingredients preparation activities were performed, namely (i) fungi inoculant preparation performed in the Soil Biology Laboratory in the Faculty of Agriculture, Udayana University, Denpasar, where fungal isolate of each fungi type was cultivated in PDA and inoculant media such as sawdust, corn bran, rice bran, and other as the fungal media in the field; (ii) selecting agarwood plants with similar diameter (10-15 cm) aged 8-10 years, and (iii) preparation of tools and ingredients for fungal application, namely wood drill, inoculation syringe, spatula, plasticine, methanol 70%, and others.

The fungal inoculant application on agarwood plant

After the three fungal inoculants are ready, the application is carried out by inoculating (inserting) the

inoculants on trees/branches of agarwood plants on land in Marga Dauhpuri Village, Marga District, Tabanan District, Bali, Indonesia. The plants to be inoculated are 8-10 years or 10-15 cm in diameter. The tree bark was drilled with a wood drill with a diameter of 0.8 cm and a depth of 3 cm. Drilling was performed at a height of more than 50 cm above the ground surface, and several circular holes can be made on one tree at a distance of 10 cm at an angle of 30 degrees; one pellet (1.5-3 cm) of fungal inoculant per hole. The hole is then covered with plasticine (covering material) to prevent contamination by other substances or microbes. For control plants, the tree bark was drilled with a wood drill with a diameter of 0.8 cm and a depth of 3 cm without the fungal inoculant application.

Research observation

Observation and analysis were performed on the harvest products 16 weeks after the fungal inoculant treatment. Harvest was performed by cutting the inoculated part of the plant. The brown-colored plant samples (infected part/ agarwood) were analyzed. The plant sample (agarwood) was dried to be analyzed in the laboratory to determine the essential oil content in the agarwood. Afterward, the essential oil was analyzed by GC-MS to determine its chemical component. The observed and analyzed parameters are the agarwood color, the agarwood fragrance (fragrant level), the agarwood essential oil content, and the chemical component of essential oil.

Essential oil component determination

Essential oil content (%) was determined by distillation. Agarwood oil is distilled using a steam distillation system. Oil content is calculated by the equation of the weight of oil divided by the weight of the material (sapwood) multiplied by 100% or the weight of oil per weight of raw material. The chemical components of essential oils were analyzed using the industry-standard GC-MS (Gas Chromatography-Mass Spectrophotometer). Agarwood color was determined using the Munsell Color Chat color standard, and sapwood aroma was determined using the widely accepted organoleptic method. Quantitative observation data is subjected to a thorough statistical analysis using variance analysis. If a significant value is found, it is used for the Least Significant Difference Test (5% significance level).

RESULTS AND DISCUSSION

The fungi species used in this research are *Trichoderma harzianum*, *Fusarium solani*, and *Rhizopus microspores* based on morphological identification (Figure 1) and molecular identification with PCR (unpublished data). The *T. harzianum* has a green to dark green colony on the PDA medium. Microscopic observation showed a round spore with phialide and partitioned and branching mycelia (Figure 1.A and 1.B). *F. solani* colony is white in the middle and orange on the edge, with tidy mycelium and even growth. The microscopic characteristics are hyalin microconidia, 1-2 cells, ovoid or shaped like a crescent with a slightly curvy tip (Figure 1.C). *R. microspores* have a white colony that gradually turns grey on PDA media. Microscopic observation shows *Rizhopus* fungi-type spore that is round, oval, ellipsis, or cylinder in shape, non-partitioned hyphae (senocytic) which branched and weaved into mycelium (Figure 1.D). *F. solanii* is a pathogenic fungus and decomposer commonly found in soil, trees, stems, and roots (Naranjo-Ortiz and Gabaldón 2019; Perincherry et al. 2019; Piacentini et al. 2019; Ekwomadu et al. 2021; Aris et al. 2023; Janaviciene et al. 2023).), and it also can be found in all agarwood samples. *Fusarium* sp. was identified with characterization according to Faizal et al. (2020), Zhang et al. (2022), and Lukman et al. (2023). The fungi have mostly oval-shaped non-septate microconidia, elongated forms, and occasional septate. Macroconidia can be found on an inoculation point known as sporodochia. The macroconidia are usually straight with some slightly curved shape, separated by 4-5septa with mostly 5 septa, blunt, has an apical cell shape, and a barely notched basal cell.

The agarwood color produced from fungal inoculant treatments varied from light brown, yellowish brown, and dark brown (Table 1; Figure 2). The dark brown to greyish brown agarwood color was obtained from *Trichoderma* (A) and *Fusarium* (B) fungal treatment; yellowish brown was from *Rhizopus* (C) treatment, and no change of color in the control treatment (D/without fungi) (Figure 1). The agarwood color in the three fungal species treatments is still within the brown color level. Agarwood color is generally influenced by the guanene content (sesquiterpene). Dark agarwood color generally contains a large amount of sesquiterpene, influencing the wood density and color (Chen et al. 2020; Mega and Kartini 2020). The brown coloring in agarwood from this research is suspected to be related to the harvesting time three months after inoculations when the formation and accumulation of sesquiterpene were still suboptimal. The dark zone generally appears around the wound/drilling hole and continues to become darker after three months of application (Faizal et al. 2020).

The agarwood scent obtained from fungal inoculant treatment is classified as fragrant (Table 1), while the slightly fragrant agarwood scent was from the control group (D/without fungi). The fragrance of *Gyrinops versteegii* agarwood is related to the chemical compound produced and accumulated inside the sapwood. After being inoculated by three fungi, the agarwood compound was applied with Benzaldehyde and 3,4-dimethoxy. This aligns with Mohamed et al. (2014), who found benzaldehyde and benzenepropanoic acid in the *A. malaccensis* agarwood extract after six months of treatment with *Deuteromycetes*. The chemical compounds in agarwood inoculated by fungi are benzylacetone, benzaldehyde, guaienes, palustrol, anisyl acetone, and cromone derivatives (Jong 2014). Chhipa and Kaushik (2017) reported that *A. sinensis* agarwood inoculated by *Melanotus flavones* for six months contained *benzaldehyde, benzene propanoic* acid, anisyl acetone, and chromone.

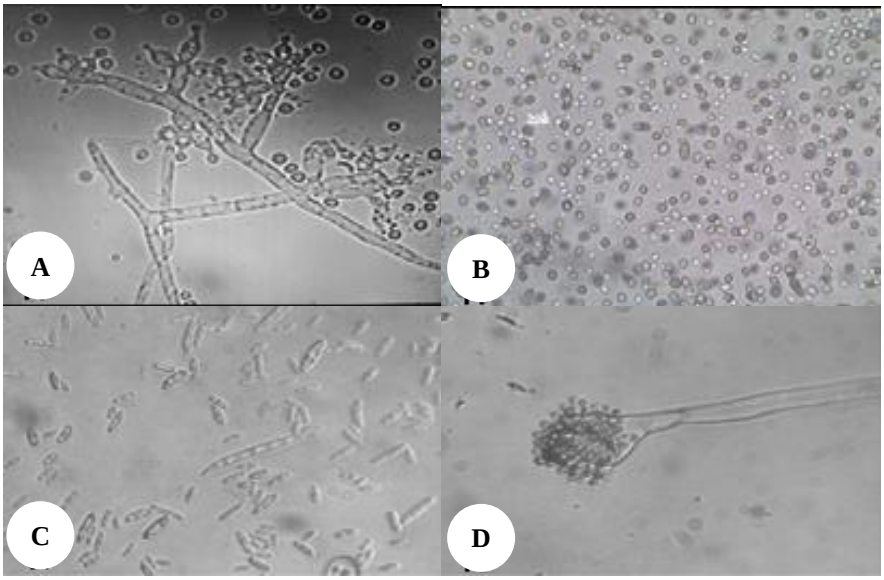

Figure 1. Microscopic morphology of fungi. A. *T. harzianum* mycelium; B. *T. harzianum* spores; C. *F. solani* conidia; D. *R. microsporus* spores and hyphae at 400x magnification

Table 1. Color, aroma, and essential oil content in agarwood after fungi application

Treatments	Agarwood color	Agarwood aroma	Average essential oil content in agarwood (mL/kg)
Trichoderma harzianum	Dark brown, grayish brown	Fragrant	0.6024ab
Fusarium solani	Grayish brown, dark grayish brown	Fragrant	0.7413b
Rhizopus microsporus	Light brownish gray, yellowish brown	Fragrant	0.5532a
Control	Light brown	A bit fragrant	0.5249a

Note: Numbers followed by the same letter in the column show no significant difference in the Least Significant Difference Test (5% level)

The statistical analysis showed that the type of fungi applied significantly influences the agarwood essential oil content (Table 1). The highest essential oil content was found in treatment B (*Fusarium* inoculant) with 0.741300 mL/kg, followed by treatment A (*Trichoderma* inoculant) with 0.6024 mL/kg, treatment C *(Rhizopus* inoculant) with 0.5532 mL/kg, and lowest was found in the control with 0.5249 mL/kg. Treatment A and B results were not significantly different but significantly different from treatment C and D (Table 1). This showed that *Fusarium* and *Trichoderma* fungi stimulate the agarwood plant stronger than *Rhizopus*. The stronger stimulation will produce better agarwood with resin and essential oil contained in the formed agarwood. Agarwood can occur when certain pathogens infect agarwood-producing trees. The tree's response against stimulation or pathogen invasion is producing secondary metabolites or resin compounds which produce fragrance when the agarwood sapwood is burnt (Subasinghe and Hettiarachchi 2013).

Based on the area data in the chromatogram figure of the agarwood essential oil, the chemical compound amount is presented in Table 2. Based on the retention period, the first compound to be present in fungi-inoculated agarwood essential oil is Citronella, followed by 6-octenal, 3,7-dimethyl, Beta-Citranelol, 2,6-Octadienal, 3,7-dimethyl, 2,6-Octadien-1-ol, 3,7-dimethyl, and Citral. The Citronell, 6-Octenal, 3,7-dimethyl, 2,6-Octadienal, 3,7-dimethyl, 2,6-Octadien-1-ol, 3,7-dimethyl act as antimicrobial compound. These compounds are possibly synthesized by *Gyrinops versteegii* to fight against the *F. solani, Trichoderma harzianum*, and *Rhizopus microspores* infection. The Beta-Citranelol and Citral may function as antifungal and scented ingredients or perfume. These aromatic compounds are assumed to play a role in forming agarwood fragrance during the three fungal treatments.

The GC-MS analysis of the agarwood essential oils under the three fungal treatments shows the presence of secondary metabolites belonging to the fatty acid ester and terpenoid compounds. These compounds were found in the retention period range (RT) of 11.67 to 20.89 (Table 2). Fatty acid esters are dominantly found in the essential oils produced from the inoculation of *Fusarium solani* (B) and *Rhizopus microsporus* (C), are: Butanoic acid, 3,7-dimethyl,2,6-octadiethyl; and Hexadecanoic acid, methyl ester. Terpenoid compounds found in essential oil from the inoculation of *Fusarium solani* (B) and *Rhizopus microsporus* (C) are citronelol, beta citranelol, 3,7-dimethyl-2,6-octadien-l-ol, citronellol acetate; citral; geranyl acetate; 2,6,10_dodecatrien-1-ol,3,7,11-trimethyl (farnesol); selina-6-en-4-ol; and (-)-globulol (Table 2). several compounds belonging to the monoterpene group are citronellol: beta citranelol, citronellol acetate, geranyl acetate, citral, and the sesquiterpene group, which are farnesol, selina-6-en-4-ol, and (-)-globulol.

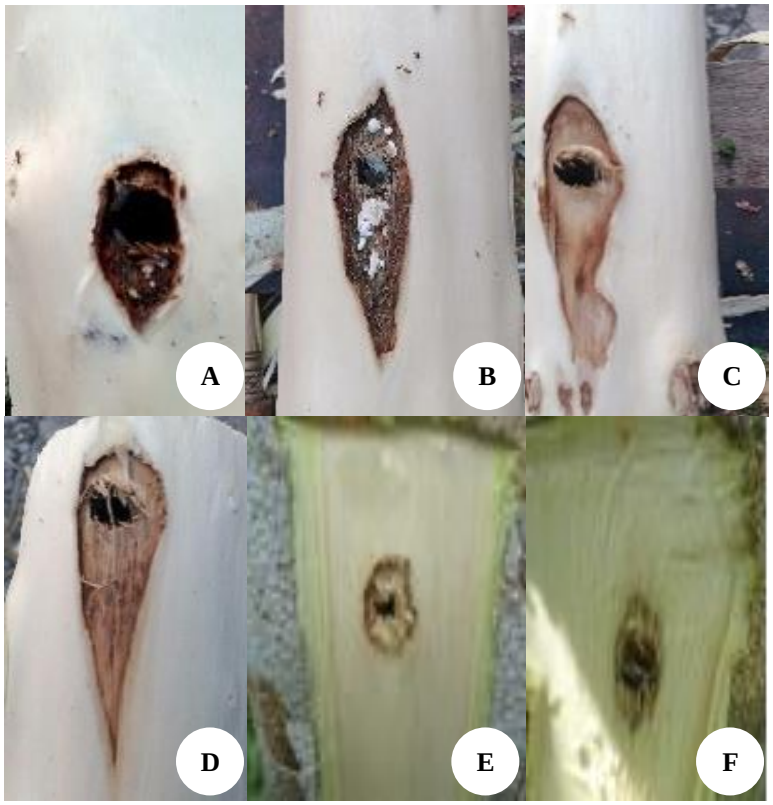

Figure 2. The color of agarwood resulting from Fungi inoculation: A. dark brown agarwood after *T. harzianum* application; B. Grayish brown agarwood after *F. solani* application; C and D. Light brownish gray agarwood after *R. microsporus* application; E and F. Light brown agarwood without application (control)

The fatty acid esters dominantly found in essential oil from the inoculation of *Trichoderma harzianum* (A) are Butanoic acid, 3,7-dimethyl,2,6-octadiethyl; and Hexadecanoic acid, methyl ester. The terpenoid compounds found in essential oil from the inoculation of *Trichoderma harzianum* (A) are 6-Octenal, 3,7-dimethyl; Beta Citranelol; Citral; Geranyl acetate; and Selina-6-en-4-ol (Table 2).

With its unique blend of secondary metabolites, such as fatty acid esters and terpene compounds, Agarwood oil exudes a distinctive aroma. The fatty acid ester compound is the secret behind the fragrant aroma of agarwood (gaharu) sapwood. One of the terpene compounds, the natural monoterpenoid Citronellol, 3,7-dimethyloct-6-en-1-ol, also known as dihydro geraniol, is a $C_{10}H_{20}O$ formulation obtained from citronella oil, a natural resource.

Agarwood and fungi interaction is due to the changes in biological activity in agarwood trees. The fungi's contribution in forming agarwood's unique has been clearer in recent years. The compounds in *Fusarium solani and Rhizopus microsporus-infected* agarwood were 19, while the control application only had 14. This is in line with previous studies which reported that *Fusarium* increases the type of chemical compounds. Compounds accumulated in agarwood *Fusarium* are the most varied (44 compounds) compared to the control groups, the injured and uninjured juvenile agarwood trees, in Assam after three months of induction (Sen et al. 2017). Within the 44 compounds, 11 were found in oil or chip wood agarwood samples, which differs from previous research.

The fungal filtrate used does not kill agarwood cells, a novel finding that sparks excitement. When treated with an elicitor, the agarwood suspension's correct age also influences sesquiterpenes' formation, a discovery that is breaking new ground. The younger the cell culture suspension during co-culture, the higher the new cells will grow and be affected by the toxin in the filtrate, a phenomenon that is a fresh perspective. Induction and differentiation processes are expected to occur in newly formed cells during co-culture, a process that is a new frontier. Auri et al. (2021) reported that the coarse filtrate of elicitor fungi could induce more fragrant agarwood compounds than other elicitors (both in cell walls and cytoplasm) in callous cultured agarwood, a result that is a game-changer.

F. solani infects *G. versteegii* by destroying the pectin network in the stem cell walls of *G. versteegii*. *F. solani* infection causes *G. versteegii* to produce phytoalexin compounds to prevent the growth and spread of *F. solani*. According to Westrick et al. (2021) and Jian et al. (2023), plants infected with pathogens will produce phytoalexin compounds to prevent the growth of the pathogen. *G. versteegii* can survive by continuing to produce phytoalexin compounds. This phytoalexin compound accumulates and forms gaharu (Ramli et al. 2022).

Some of the phytoalexin compounds in *G. versteegii* infected with *F. solani* are aromatic, and some are supportive of changing the color of the wood to dark brown. *F. solani* infection on *G. versteegii* for three months caused stress and induced the appearance of aromatic compounds, but the stem color was still at a dark brown level. Based on this, it is suspected

The formation of the fragrant aroma of agarwood occurs first, while the color formation takes longer. According to Naziz et al. (2019), the formation of the fragrant aroma of agarwood wood is only sometimes followed by a change in wood color. Liu et al. (2017) show the same thing the aroma of agarwood does not correlate with the intensity of the wood color. This shows that high fragrant aroma is only sometimes accompanied by high color intensity, so color and fragrant are criteria that do not influence each other in determining the quality of agarwood. The fragrance in agarwood originates from the terpene ground volatile compounds. The terpenoid metabolism may cause sesquiterpenoid production to be passed in the metabolic pathway when harvested (Mani et al. 2021).

The interaction between fungal pathogens will cause physiological changes in the impacted trees. This may cause visual changes in cells, tissue, or plant organs. Putri (2011) reported that *Fusarium* sp. IPBCC. 08.569 has the same pathogenicity on agarwood trees during the agarwood formation process at the cell and organ level, where the response is in the form of terpenoid compound accumulation, which can be found in the parenchyma network, including phloem, xylem trachea element, and pith; the changes in cellular level cause a wood color change, which spreads outward from the infection side.

Table 2. Chemical compounds in agarwood essential oil resulting from fungal inoculation on *Gyrinops versteegii* plants

Compound name	Retention time (RT)	Treatments			
		Trichoderma harzianum	***Fusarium solani***	***Rhizopus microsporus***	**Control**
o-Methan-8-ol	5.218				+
Alpha-methyl-alpha-(4-methyl-3-pentenyl)	5.796				+
Trans-linalol oxide	6.041				+
1,6-octadien-3-ol-3,7-dimethyl	6.220				+
Linalol oxide	6.801				+
Citronella	7.013		+	+	+
6-Octenal, 3,7-dimethyl	7.018	+			
Beta-Citranelol	8.121	+	+	+	+
2,6-Octadienal, 3,7-dimethyl	8.326		+	+	
2,6-Octadien-1-ol, 3,7-dimethyl	8.523		+++	+	
Citral	8.756	++	++		
Cyclohexanol, 2-(2-Hydroxy-2-propanol)	9.880			+	+
2,6-Octadien-1-ol, 3,7-dimethyl, acetate	10.259		+	+	
Geranyl acetate	10.263	++			
Benzene, 1,2 dimethoxy-4-(2-prophenyl)-	10.582	+			
2-Hydroxy-2-methyl-hept-6-en-3-ol	11.173				+
Cis-methyl-isoeugenol	11.801			+	
Trans-methyl-isoeugenol	11.285		+		
Benzaldehyde, 3,4-dimethoxy	11.680	+	++	+	
Selina-6-en-4-ol	12.179	++	+		
Butanoic acid, 3,7-dimethyl,2,6-octadiethyl	12.570	+		+	
3,4-Dimethoxyphenylacetone	12.791		++	+	
2-(2,5-Dimethoxypheny)-propiona	12.888		+		
2-(2,5-Dimethoxypheny)-propionaldehyde	12.902			+	
Isoelemicin	13.640		+	+	
1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7	14.062		+		
(-)-Globulol	14.072			+	
2,6,10_Dodecatrien-1-ol,3,7,11-trimethyl	14.439		+	+	
(3,4-Dimethoxyphenyl)ethanolamine	14.983		+	+	
Hexadecanoic acid, methyl ester	16.651	+	++	+	
1-n-Pentadecyl-decahydronapth	17.251				+
Citronelyl tiglate	17.739				+
Ethane,1,1-oxybis(2-ethoxy-	17.850				+
9-Octadecanoic acid, methyl ester	18.358	+	+	+	
(S)-(-) Citronellyc acid, methyl ester	18.691				+
8-Mercapto-p-methane-3-one	19.234				+
Hexadecanoic acid, bis(2-ethylhexyl) ester	20.899	+	+	+	
4H-1,3,2-Dioxanborin, 2-ethyl-4-methyl-4,6 dir	21.163		++	+	

Note: +: small area percentage; ++: medium area percentage; +++: large area percentage

In conclusion, the application of fungi inoculant significantly affected the content of agarwood essential oil. The highest content of essential oil is 0.7413 mL/kg obtained from the *Fusarium solani* inoculant fungi treatment, followed by *Trichoderma harzianum* inoculant treatment with 0.60245 mL/kg, and the lowest 0.5249 mL/kg was found in the control group (without fungi). The chemical compounds of agarwood oil inoculated by fungi are secondary metabolites, including fatty acid and terpene groups. The compounds are butanoic acid, 3,7-dimethyl,2,6-octadiethyl; hexadecanoic acid, methyl ester; citronelol; beta citranelol; 3,7-dimethyl-2,6-octadien-l-ol; citronellol acetate; citral; geranyl acetate; 2,6,10_dodecatrien-1-ol,3,7,11-trimethyl (farnesol); selina-6-en-4-ol; and (-)-globulol.

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