EFFECTS OF *TRICHODERMA HARZIANUM* AND *FUSARIUM SOLANI* INOCULANT ON THE RESIN CONTENT OF AGARWOOD (*GYRINOPS VERSTEEGII* (GILG.) DOMKE)

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ABSTRACT

This study aimed to determine which fungal inoculants, *Trichoderma harzianum* or *Fusarium solani* in a solid or liquid form, produced the highest resin content in agarwood trees. This experimental study used a randomized completely block design (RCBD) with seven treatments in triplicates: solid *Trichoderma harzianum* inoculants in pellets(A) and capsules (B), liquid *T. harzianum* inoculants (C), solid *Fusarium solani* inoculants in pellets (D) and capsules (E), liquid *F. solani* inoculants (F), and control or without inoculants (G). Parameters observed were the color and aroma of the infected agarwood and the resin content of the harvested sapwood. Quantitative data obtained from observations/measurements were analyzed statistically with analysis of variance and a subsequent LSD test at a 5% significance level following a significant result. The results showed that treatments using different fungal inoculants had no significant effects on the resin content of agarwood. However, inoculants in pellets and capsules tended to increase the resin content. The resin content of agarwood ranged from 15.97% to 21.53%, and the highest level was obtained from treatment A (solid *T. harzianum* inoculants in a pellet form).

Keywords: agarwood, fungal inoculant, resin content

INTRODUCTION

Resin is a secondary metabolite produced by agarwood trees due to injury or microbial infection. The agarwood resin produced from the inoculation with *Trichoderma harzianum* contains compounds like hexanedioic acid, dioctyl ester; 1-octadecanoic acid, methyl ester; cyclopropanedodecanoic acid, 2-octyl-, methyl ester; 9-octadecenoic acid, methyl ester; octadecanoic acid, 9.10-dihydroxy-, methyl ester; cyclopropanepentanoic acid, 2- undecyl-, methyl ester; pentadecanoic acid, 14-methyl-, methyl ester; and non-ester compounds, namely 2-tridecen-I-ol; 2-(2', 4', 4', 6', 6', 8', 8'

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Heptamethyltetrasiloxan -2'; 3-Hydroxybutanamide. 8-methoxy-2- (2 (Mega and Kartini, 2020). In agarwood trees, sapwood contains lumps of aromatic substances between wood cells with various distinctive shapes and colors and resin contents (Syukur and Muda, 2016). There are various factors influencing the sapwood formation, including the genetics of agarwood trees, microbial inducers, environments, and the length of the sapwood formation process. Sapwood is formed when specific pathogens infect agarwood trees, which responds by releasing secondary metabolites or resins that produce a unique fragrance when burned (Sitepu *et al*., 2011). The four Fusarium species known to induce agarwood formation in *Aquilaria spp* are *F. Solani* (Mart) Appel & Wollenw, *F. Lateritium* Ness, *F. Tricinotium* (Corda), and *F. Moniliformae* Sheldon (Budi *et al*., 2010)

Gyrinops versteegii is an agarwood tree species growing naturally on Bali Island, Indonesia. However, the current technology is considered inadequate to improve the quantity and quality of sapwood production by this tree. According to Mega *et al*. (2013), the resin contents of the sapwood produced after the infection or inoculation by the fungi *Fusarium solani* and Rhizopus sp. for five consecutive months are 5.32% and 5.24%, respectively. The mixture of liquid inoculants from *Fusarium solani* and Rhizopus spp. proves successful in inoculating *G. versteegii* for 16 months, producing sapwood with 13.58% resin content. (Mega *et al*., 2015). Three fungi that can assist in the sapwood formation in *Gyrinops versteegii* are *Fusarium solani,* Rhizopus sp., and Trichoderma sp. (Mega and Nuarsa, 2018). Sapwood is identified from changes in the wood color from white to brown to blackish-brown, and its resin content can be, on average, 5.24% (inoculation by Rhizopus sp.), 5.31% (*Fusarium solani*), and 7.92% (Trichoderma sp.). Furthermore, based on molecular formulas, Mega *et al*. (2020) identified the three fungi responsible for sapwood formation in *G. versteegii*, namely *Fusarium solani, Rhizopus microsporus,* and *Trichoderma harzianum*. Although studies have found various fungi to inoculate the agarwood trees, their effectiveness and efficiency remain unknown. This information will affect not only the inoculation technique (how it is performed) but also the safe and sterile distribution of inoculants between regions.

Based on the ~~above~~ problems ~~above~~, research~~ing~~ about different forms of fungal inoculants for their effectiveness and efficiency in the formation of resin-rich sapwood becomes necessary.

MATERIALS AND METHODS

Materials and tools

The materials used in this study were agarwood *(Gyrinops versteegii*) from the agarwood farms in Marga Dauhpuri Village (Marga, Tabanan, Bali Province), two isolates of Fusarium and Trichoderma, Potato Dextrose (PD) media, solid media (rice bran, corn bran, sawdust), distilled water, methanol, and capsules. The tools included a bottle clock, measuring cup, petri dish, autoclave, laminar air flow, wood drill, digital scale, bath, oven, and color book.

Experimental Design

This study used a randomized completely block design with seven treatments prepared in triplicates, namely:

A. Solid *Trichoderma harzianum* inoculants in a pellet form
B. Solid *T. harzianum* inoculants in a capsule form
C. Liquid *T. harzianum* inoculants
D. Solid *Fusarium solani* inoculants in a pellet form
E. Solid *F. solani* inoculants in a capsule form
F. Liquid *F.* inoculants
G. Control (without inoculants)

The parameters observed were the color, aroma, and resin content of the sapwood. The quantitative data obtained from observations were analyzed statistically using analysis of variance (variance fingerprint) and, if a significant result was obtained, followed by the LSD test at a 5% significance level.

Research Implementation

The research was conducted in four stages: preparation, inoculation, observation and analysis, and resin content calculation. At the preparation stage, the research tools and materials were prepared. First, the fungal inoculants were prepared at the Soil Biology Laboratory, Faculty of Agriculture, Udayana University, Denpasar. Each type of fungal isolate was cultured on PDA and inoculant media (e.g., sawdust, rice bran). Second, agarwood trees aged 3–4 years with the same diameter of 10–15 cm were selected from a field of *Gyrinops versteegii* in Marga Dauhpuri Village (Marga, Tabanan, Bali Province). Third, the tools for fungal inoculation, including wood drill, inoculation needle, spatula, plasticine, and alcohol, were prepared.

Then, at the next stage, the fungal inoculant was inoculated into selected agarwood trees to stimulate sapwood formation. The inoculated trees had to be 3–4 years old or had a stem diameter of more than 3–6 cm. Several holes measuring 0.6 cm in diameter and 2 cm in depth were made on the stems using a wood drill. These holes were drilled at least 20 cm from the ground and 10 cm

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apart in a circular direction at an angle of 30^o. Afterward, 0.8 cm^3 of the fungal inoculant was placed into each hole using a sleeve (the funnel), and the hole was covered with plasticine to avoid contamination by other materials or organisms.

At the third research stage, observations and analyses were conducted after eight weeks of the inoculation. Sapwood, the brown (infected) parts of the agarwood trees, was harvested by prying using a chisel, stored in a plastic bag, and then chemically analyzed. Part of the plants (wood/ agarwood) was dried for analysis in the laboratory to determine the resin content. Finally, at the last stage, each sapwood sample was extracted with 150 ml of methanol for 3 hours. The resin yield (%) was calculated by dividing the weight of the extracted resin by the weight of the extracted sapwood times 100 (Pasaribu *et al*., 2013). The color of the infected agarwood was determined using the Munsell color chart, and the aroma was determined organoleptically.

RESULTS AND DISCUSSION

RESULTS

Agarwood color

Figure 1 and Table 1 describe the color of the infected agarwood stem with different inoculants or treatments. The color varied from light brown (whitish-brown) to brown. The brown infected stems were obtained in treatments A (solid *Trichoderma harzianum* inoculants in a pellet form) and E (solid *Fusarium solani* inoculants in a capsule form). The light-brown to brown infected stems were produced in treatments B, C, D, and E, while the light brown stems were found in the control (G).

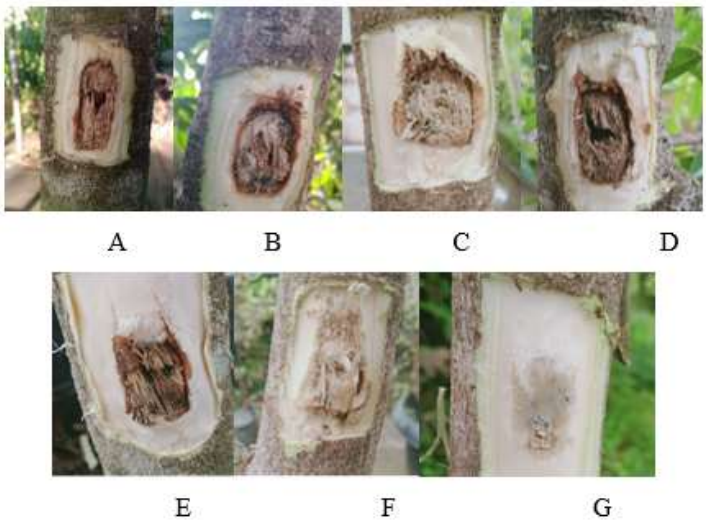

Figure 1. Colors of the agarwood stems infected with different fungal inoculants

Table 1. Colors of the infected agarwood stems by treatment and repetition

Treatment	Repetition		
	I	II	III
A*	Brown	Brown	Brown
B*	Light brown	Brown	Brown
C*	Light brown	Brown	Brown
D*	Brown	Brown	Light brown
E*	Brown	Brown	Brown
F*	Light brown	Brown	Light brown
G*	Light brown	Light brown	Light brown

*Notes:
A = Trichoderma pellet
B = Trichoderma capsule
C = Trichoderma liquid
D = Fusarium pellet
E = Fusarium capsule
F = Fusarium liquid
G = control (no inoculants)

Agarwood fragrance

The organoleptic measurements of the infected stems detected different fragrance levels, from slightly-fragrant to fragrant (Table 2). The fragrant aroma was obtained in treatments A (solid *T. harzianum* inoculants in a pellet form) and E (solid *F. solani* inoculants in a capsule form). The slightly fragrant to fragrant aroma was produced in treatments B, C, D, and E, while the slightly fragrant aroma was in the control (G).

Table 2. Aroma of the infected agarwood stems by treatment and repetition

Treatment	Repetition		
	I	II	III
A*	fragrant	fragrant	fragrant
B*	slightly fragrant	fragrant	fragrant
C*	slightly fragrant	fragrant	fragrant
D*	fragrant	fragrant	slightly fragrant
E*	fragrant	fragrant	fragrant
F*	slightly fragrant	fragrant	slightly fragrant
G*	slightly fragrant	slightly fragrant	slightly fragrant

*Notes:
A = Trichoderma pellet
B = Trichoderma capsule
C = Trichoderma liquid
D = Fusarium pellet
E = Fusarium capsule
F = Fusarium liquid
G = control (no inoculants)

Resin Content

The statistical analysis showed that fungal inoculants had no significant effects on the resin content, although resin levels tended to increase in the treatment groups (A-F) compared with the control (G). The

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highest resin content of 21.53% was found in treatment A (Trichoderma pellet), which was higher by 34.82 percentage points than that of the control (15.97%). The relationship between resin contents and fungal inoculants is presented in Figure 2.

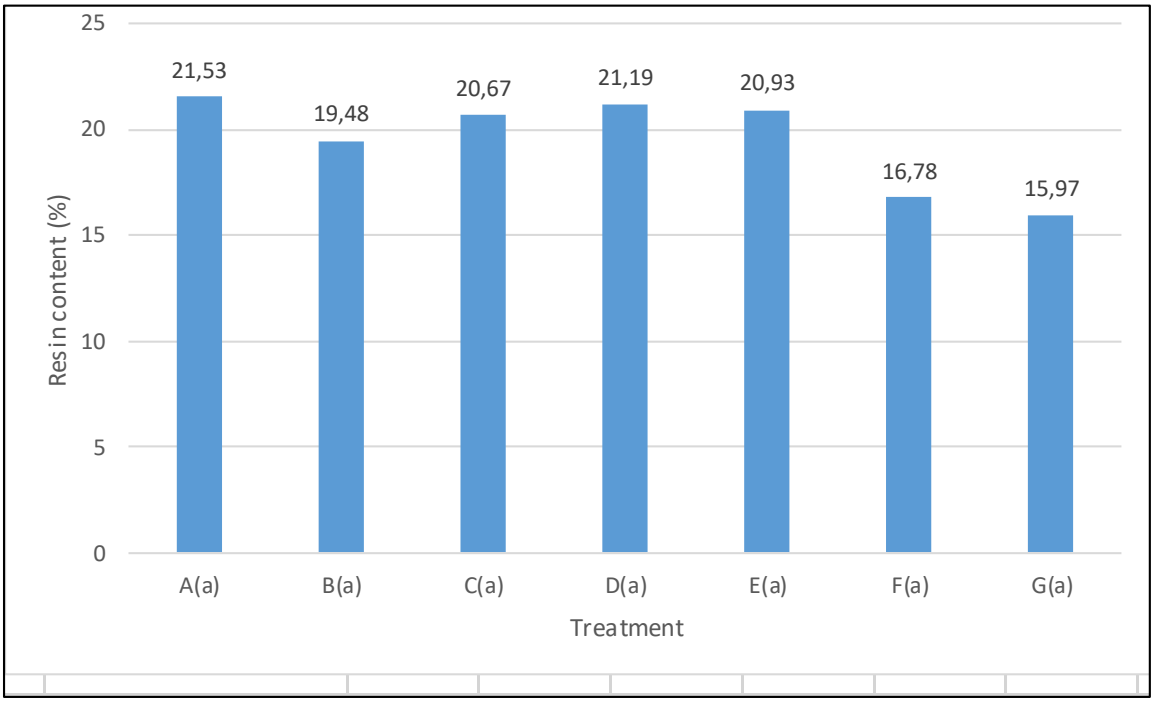

Figure 2. The relationship between the resin content of the sapwood and the preselected fungal inoculants

DISCUSSION

Based on the observation results and the statistical tests, the fungal inoculants had no significant effect on the resin content of the harvested sapwood. The infected agarwood stems (i.e., sapwood) have various color and aroma, from light brown to brown and from less fragrant to fragrant. Although the resin content is not significantly dependent on fungal inoculants, it tends to be higher in percentage than the control. Relative to the control, treatment A (Trichoderma pellet) produces the highest resin content (i.e., 34.82 percent-point higher), followed by treatments D, E, C, B, and F (32.69, 31.06, 29.98, and 5.07 percentage points, respectively). The insignificant effect is potentially attributed to the too-short resin formation time of 60 days.

The agarwood trees respond to these pathogenic infections by producing secondary metabolites or resin compounds that release a fragrant aroma when burned (Sitepu, *et al*., 2011). Resin is a phytoalexin or a defense compound against pathogenic

attacks. The accumulation of resin compounds on the infected plant stems will form sapwood with various color and aroma.

Based on the data obtained in this study, it turns out that there is a relationship between the color, aroma, and resin content of agarwood. A darker or browner agarwood has a stronger aroma (fragrance) and a higher resin content. It is evident from the color, aroma, and resin content of the control, treatment A, and treatment E (light brown-brown-brown, less fragrant, and 15.97% - 21.19% - 21.53%). The results correspond to Mega and Nuarsa (2019), which stated that the darker or the more darkish-brown the agarwood stem, the stronger the aroma (fragrance) and the higher the resin content. These assessments are relative to the control (without inoculants), JF (*Fusarium solani* inoculation), and JT (*Trichoderma harzianum* inoculation), which each produced light brown, brown, and blackish-brown sapwood with less fragrant and 5.25, 5.31, and 7.92% resin content. On the other hand, an increase in the fragrance level is not necessarily in line with an increase in the sapwood's color intensity because fragrance is linked to sesquiterpene compounds. A higher fragrance level is probably caused by a higher concentration of sesquiterpene compounds, while its decrease is due to the loss of these compounds, which are very volatile (Rahayu, 2009).

CONCLUSION

First, the form of fungal inoculation has no significant effect on the sapwood's resin content, but the latter tends to be higher in the treatment groups than the control group. Second, treatments A (*Trichoderma harzianum* pellet) and D (*Fusarium solani* pellet) increase the resin content by 34.82 and 32.69 percentage points relative to the control (G, without fungal inoculation).

Despite the less significant effect on resin contents, using fungal inoculants in the form of pellets is still highly suggested so as to produce the most resin from agarwood trees. The similar results between different forms of fungal inoculants mean that pellets are not the only option to obtain the most favorable sapwood color and aroma and the highest resin levels. It is necessary to test these forms in different locations.

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