AGARWOOD PRODUCING FUNGAL INOCULANT FORMULATION IN KETIMUNAN TREE (*Gyrinops versteegii* DOMKE)

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ABSTRACT

This study aimed to find out the agarwood producing fungal inoculant formulation that infect Ketimunan tree (*Gyrinops versteegii* DOMKE) effectively and resulted in high quality of agarwood. Randomized Completely Block Design was applied in this study with formulation of *Fusarium solani* and *Rhizopus sp* inoculant in liquid and solid form were combined as treatments. Treatments were: solid *Fusarium solani* inoculant, solid *Rhizopus sp* inoculant, mixture of solid *Fusarium solani* inoculant and solid *Rhizopus sp* inoculant, liquid *Fusarium solani* inoculant, liquid *Rhizopus sp* inoculant, mixture of liquid *Fusarium solani* inoculant and liquid *Rhizopus sp* inoculant, and mixture of solid and liquid of *Fusarium solani* and *Rhizopus sp* inoculants. There are three replicates for each treatment. Parameters measured were level of fragrance, agarwood color, and its resin content. The result showed that all formulations affected resin content significantly compared to control of agarwood. Mixture of liquid *Fusarium solani* and liquid *Rhizopus sp* inoculant showed the best quality agarwood with quality characteristics: brownish black or agarwood with black and brownish-black color, very strong fragrance, and with 13.58% resin content.

Key words: agarwood forming fungi, *Gyrinops versteegii*

INTRODUCTION

Agarwood is one of economically valuable forestry products and also as an export commodity. According to Indonesian Forestry Department that since 1997 until September 2001, Indonesian agarwood export volume tends to decreased from 328,496 tons in 1997, 313,308 tons in 1998; 308,186 tons in 1999; 144,852 tons in 2000, and 184,069 tons in 2001. Agarwood selling price is very attractive, recently reached 50 million IDR per kilogram in international market. Furthermore, the selling price of agarwood continuously increases due to increasing demand of agarwood around the world. In Saudi Arabia, for example, agarwood become the requirement of every household (Paimin, 2004). Agarwood has a lot of uses in human life such as 1) industrial raw material of perfumes, fragrances and cosmetics, 2) material for religious ritual activities, 3) as raw material of many kinds of natural healing medicines namely: anti-asthmatics, anti-microbe, nerve stimulant, stomach ache medicine, sex stimulant, pain killer, cancer drug, renal medicine, relieving stress, liver drug, malaria and diarrhea medicine (Sumarna, 2002). Based on the result of phytochemical analysis, it is known that agar wood leaves containing phenol, flavonoid and steroid (Mega and Swastini, 2010).

Agarwood export from Indonesia was increasing until year 2000, however then agarwood production has declined

afterward till the end of year 2002 which its production only reached an average of about 45 tons per year. Decreased production is mainly due to high exploitation; especially high quality agarwood has collected without any conservation measure. Most of exported agarwood from Indonesia was taken from Indonesian tropical rain forest. Agarwood high demand and high selling price resulting in inappropriate harvesting for agarwood has been done and so resulted in agarwood-producing trees getting lower and became rare. The scarcity of agarwood-producing plants is declared by Indonesian Ministry of Forestry Appendix II as a protected plant (Parman, 2004).

In anticipating the scarcity of agarwood producing plant and to meet agarwood sustainable export in the future, plants producing agarwood cultivation is necessary. Plant producing agarwood cultivation are as follow : a) vigorous free disease seedling provision, b) suitable planting area, c) appropriate planting technique, d) proper fertilization, e) precise plant pest and diseases control, and f) agarwood harvesting in a sustainable manner. Inoculating fungi producing agarwood is critical step of this work in producing high quality agarwood. In this matter, suitable fungi inoculant that could infect plant producing agarwood should be properly considered. Each plant producing agarwood cultivated in certain place needs certain kind of fungi so difference plant producing agarwood in difference geographic and climate condition need difference species of fungi producing agarwood.

According to the study result of Mega and Phabiola (2010), *Fusarium solani* and *Rhizopus sp* could infect plant producing agarwood and producing resinous heartwood in Ketimunan plant (*Gyrinops versteegii* DOMKE). Mucharromah (in Syarieva, 2009) mention that *Fusarium cylindriscorpum* and *Fusarium oxysporum* were two best fungi for producing agar wood in *Aquilaria malacensis*. Since 2003 in Tabanan Regency, west part of Bali, 36,750 Ketimunan plant has been planted (Tabanan Plantation and Forestry Agency, 2009). Furthermore, Ketimunan plant has cultivated more than 75,000 plants approximately and recently people in Bali continuously grow more Ketimunan trees. Producing agarwood require to apply fungi to induce the formation of agarwood. Until now, effective formulation containing fungi producing agarwood is not available yet. In 2009, a research concerning kinds of fungi infecting *Gyrinops versteegii* was conducted by Mega and Phabiola. The research result showed that *Fusarium solani* and *Rhizopus sp* could infect Ketimunan plant and resulted in to agarwood formation however its quality is still rather low. Therefore, further research is needed with the aim to find out the effective inoculant formulation to infect plant producing agarwood efficiently and then resulted in high quality of agarwood. Through this research high quality agarwood yield is expected and finally could increase farmer's income and welfare.

MATERIALS AND METHODS

This research was conducted from June 2012 until October 2013. Fungi producing agarwood inoculant and its formulation were made in Soil Biology laboratory, Agriculture Faculty, Udayana University, Denpasar. Fungi formulation injection to plant producing agarwood was conducted in Marga Village, Marga District, Tabanan Regency, Bali Province. Randomized Block Design was applied in this research, and there were seven

formulations were tested as follow:

A. Formulation containing solid *Fusarium solani* inoculant,
B. Formulation containing solid *Rhizopus sp* inoculant,
C. Formulation containing mixture of solid *Fusarium solani* and *Rhizopus sp* inoculant,
D. Formulation containing liquid *Fusarium solani* inoculant,
E. Formulation containing liquid *Rhizopus sp* inoculant,
F. Formulation containing mixture of liquid *Fusarium solani* and liquid *Rhizopus sp* inoculants, and,
G. Formulation containing mixture of solid and liquid of *Fusarium solani* and *Rhizopus sp* inoculants.

Inoculant formations were treated into 4-6 years old Ketimunan plant using inoculation equipment and each treatment repeated three times. Twenty one Ketimunan plants were treated all together. Quantitative data of agarwood color, level of agarwood fragrance, and agarwood resin content were analysis statistically using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DRMT) at P=0.05. Qualitative data were compared to SNI (Indonesian National Standard) of agarwood criteria. For determining resin content, some chemical analysis was conducted to determine resin content through acetone extraction.

RESULT AND DISCUSSION

Agarwood Color

The results showed in Table 1 indicated that agarwood color of each treatment varies such as brown, blackish brown, brownish black, and black. The darkish color was black to brownish black produced by F treatment (mix of liquid *Fusarium solani* and liquid *Rhizopus sp*) and G treatment (Combination liquid and solid of *Fusarium solani* and *Rhizopus sp*). Treatment B and D also produced dark color agarwood, however less dark compare to treatment F and G. Treatment A, C, and E produced relatively brown agarwood.

Agarwood Fragrance Level

The statistically analysis of the result on agarwood fragrance level of Ketimunan tree is presented in Table 2. The results indicated that inoculant formulation treatment affects level of agarwood fragrance were not significant, however, the highest fragrance level was produced by F (4,73) treatment (Combination liquid of *Fusariumsolani* and *Rhizopussp*) in value 4.67, catagorized is a very strong fragrance, and then respectively followed by G treatment (4,67), D (4,53), A (4,37), B (4.17), C (3,97) and the lowest was observed from E treatment (3,95)

Table 1. Treatment Effects Agarwood Color

Treatments	*Replication*		
	I	*II*	*III*
A	Blackish Brown	Blackish Brown	Blackish Brown
B	Brown	Brownish Black	Brownish Black
C	Blackish Brown	Blackish Brown	Brown
D	Brownish Black	Brownish Black	Brownish Black
E	Brownish Black	Brown	Brownish Black
F	Brownish Black	Black	Black
G	Black	Black	Blackish Brown

Table 2. Treatment Effects on Agar Wood Fragrance Level

Treatments	*Values*	*Fragrance level*
A	4,37 a	Very Strong
B	4,17 a	Very Strong
C	3,97 a	Strong
D	4,53 a	Very Strong
E	3,95 a	Strong
F	4,73 a	Very Strong
G	4,67 a	Very Strong

Note : Values followed by the same letters are not significantly different based on Duncan's Multiple Range Test at p= 5%

Table 3. Treatment Effect on Resin Content (%)

Treatment	*Resin Content (%)*
A	9.70 abc
B	10.39 abc
C	8.17 a
D	11.63 abc
E	8.46 ab
F	13.59 c
G	12.86 abc

Note : Values followed by the same letters are not significantly different based on Duncan's Multiple Range Test at p= 5%

Resin Content Analysis

The statistically analysis of the resin contents (Table 3) indicated that treatments were affected significantly to resin contents. The resin content G (12.85%) was not significantly different with treatments D (11.63%), B (10.39 %), A (9.70%), E (8.46%), and C (8.17%) , however significant to treatment F and treatment C.

The quality of agarwood is determined by size, color, shape, fiber condition, weight, resin content and its fragrance. In this study, the quality was examined for duty colors, fragrance and resin content. Those three components are closely related. Qualitatively, agarwood colors in this study were obtained from brown, blackish brown, brownish black and black. The color of agar wood closely related to its fragrance and its resin content. According to Indonesian National Standardization Agency (BSN, 1999), brownish black agar wood color is classified into class I (first class quality) and class II (second class quality). Black color agar wood classified to U class. U class is a super quality.

Inoculant treatment with inoculant formulation affected significantly on fragrance level and resin content of agarwood. Resin content of agar wood has very close relationship to fragrance level. The highest resin content was obtained from F treatment (13.58%) and highest fragrance

level as well, in value 4.73 classified into very strong category. Mega *et al*, (2012) mention that inoculant treatment affected agarwood resin content significantly and the highest percentage was 7.69% obtained from G treatment.

Inoculation time of agarwood formulating fungi in Ketimunan plant obviously affects resin content. The longer inoculation time the resin content will be higher. Resin content comparison between result of this research (16 months) and previous one (5 months) (Mega *et al*, 2012) showed that resin content increase of treatment A, B, C, D, E, F and G were 82.33%, 98.28%, 59.57%, 120.68%, 75.86% and 67.23% respectively.

Generally the darker agarwood color means that fungi infection was stronger resulted in higher fragrance level (Anonymous, 2008). Based on Indonesian National Standardized Agency (BSN, 1999; Chaidir, 2009), agarwood quality in relation to fragrance level stated that agarwood quality obtained from treatment A, B, C, D, and E classified into Quality II and agarwood obtained from treatment F and G classified into quality I to U. Brown and blackish brown agarwood classified into quality I and II. Black color agar wood is classifiedinto U quality.

Based on this study, it is obviously can be seen that F treatment, combination of two kinds of fungi gave darker agarwood, with stronger fragrance and higher resin content compare to single fungus species. It might be due to positive synergism between the two kinds of fungi in infecting Ketimunan tree resulted in better quality of agarwood.

CONCLUSION

Based on result and discussion of this research, it is concluded that: F formulation, mixture of liquid *Fusarium solani* and *Rhizopus sp* inoculant is the best formulation in producing high quality of agarwood compare to other formulations. Through using F formulation it was obtained brownish black color of agarwood.

1) This research duration was 22 months and 16 months for agarwood formation. Sixteen months considered rather short time for agarwood formation. Further research should be conducted in longer period in the aim to give more time for plant to produce more and better agarwood.
2) Inoculation should be done using mixture of liquid *Fusariumsolani* and liquid *Rhizopussp.*

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