THE EFFECTIVENESS OF AGARWOOD INDUCTION BY FUNGAL INOCULATION ON *Gyrinops versteegii*

(*Efektivitas Induksi Gaharu dengan Inokulasi Fungi pada* Gyrinops versteegii)

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ABSTRAK

Terjadinya peningkatan perdagangan gaharu disebabkan karena nilai pasarnya yang tinggi. Pembentukan gaharu secara alami membutuhkan waktu yang sangat lama, sehingga tidak dapat memenuhi permintaan pasar. Berbagai pendekatan untuk mempelajari pertumbuhan dan inokulasi gaharu telah berkembang. Penelitian ini bertujuan untuk mengetahui efektivitas induksi gaharu dengan inokulasi fungi asal Nusa Tenggara Barat pada Gyrinops versteegii *di Lombok. Metodologi yang digunakan dalam penelitian ini adalah pengeboran pada batang pohon* Gyrinops versteegii. *Inokulan gaharu kemudian diinokulasikan pada bagian yang berlubang dan kemudian ditutup dengan parafin. Inokulan yang digunakan dalam penelitian ini adalah jamur yang diisolasi dari pohon gaharu* G. verstegii *yang ditemukan di Nusa Tenggara Barat. Inokulan fungi diinokulasi kembali pada spesies pohon gaharu sama dan pada lingkungan yang mirip dengan asal isolat. Uji coba inokulasi gaharu ini diharapkan mampu meningkatkan produksi gaharu yang berkualitas. Inokulasi gaharu dengan isolat yang diisolasi dari jenis pohon gaharu yang sama mampu meningkatkan kompabilitas isolat dan pohon penghasil gaharu. Setelah 6 bulan inokulasi, temuan menunjukkan bahwa area rata-rata dengan infeksi tinggi pada isolat fungi dengan kode LT adalah 2728 mm2, sedangkan kode M, kode ALS, dan kontrol memiliki nilai masing-masing 543 mm2, 281,3 mm2, dan 166 mm2. Karakteristik penciuman dan warna masing-masing bahan isolator tidak menunjukkan perbedaan yang signifikan. Efikasi isolat jamur LT adalah 73%, tetapi efektifitas isolat jamur ALS dan M masing-masing adalah 7% dan 14%. Efektivitas (p≤0,05) ditunjukkan oleh parameter luas.*

***Kata kunci: efektifitas induksi, fungi, gaharu,* Gyrinops versteegii**

ABSTRACT

Agarwood commerce increases as a result of its high market value. Due to the length of time required for agarwood to develop in nature, the supply cannot meet market demand. A variety of methods for growing and inoculating agarwood were being developed. This research aimed to determine the efficacy of inoculating *Gyrinops versteegii* with agarwood-producing fungus from West Nusa Tenggara in Lombok. The methodology used in this study is bio-induction with drilling on the stems of *Gyrinops versteegii* trees. The agarwood inoculant was then applied to the perforated and then covered with paraffin. Inoculants used in this study were fungi isolated from the agarwood tree *G. verstegii* found in West Nusa Tenggara. The fungal inoculants were re-inoculated on identical agarwood trees in a similar environment to the origin of the isolate. This agarwood inoculation study is intended to improve agarwood's overall quality. Inoculating agarwood with isolates obtained from the same species of agarwood tree improved the compatibility between the isolates and agarwood-producing plants. After 6 months of inoculation, the findings revealed that the mean area with high infection in fungal isolates code LT was 2728 mm2, while code M, code ALS, and the control isolates had respective values of 543 mm2, 281.3 mm2, and 166 mm2. The olfactory and color characteristics of each isolating agent showed no significant differences. The efficacy of LT fungal isolates is 73%, but the effectiveness of ALS and M fungal isolates is 7% and 14%, respectively. The effectiveness ($p \leq 0.05$) is shown by the area parameter.

Keywords: agarwood, fungi, *Gyrinops verstegii*, the effectiveness of induction

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I. INTRODUCTION

Agarwood is a reaction that can be caused by natural or artificial injuries, such as thin cuts, burning, punching, cutting, inserting nails, or artificial fungus/inoculation (injecting fungi into the stems and roots of agarwood-producing trees) of *Aqualaria* sp. and *Gyrinops* sp., which leads to the production of resin and aromatic compounds (Li *et al*., 2015) This substance has value as a scent, traditional medicine, religious activity aid, aromatherapy, and perfume ingredient (Chen *et al*., 2011; Liao *et al*., 2017; López-Sampson & Page, 2018; Hashim, Kerr, Abbas, & Mohd Salleh, 2016). *Aquilaria* is a genus that is capable of producing high-quality agarwood (Lee & Mohamed, 2016). Due to its considerable monetary worth, agarwood is a regularly traded commodity (Azren, Lee, Emang, & Mohamed, 2019).

Local residents regard agarwood as a profitable source of money, leading to a growth in agarwood commerce. As a consequence of excessive exploitation of natural agarwood to fulfill high market demand, all genera of Aquilaria and other agarwood-producing species are listed on CITES Appendix II as endangered species, and their trading is governed by law (CITES, 2017). Agarwood cultivation is one way to safeguard the population of agarwood-producing trees such as *Aquilaria* and *Gyrinops* that are on the verge of extinction (Sukenti *et al*., 2021; Tian *et al*., 2021).

Several induction techniques for agarwood formation research were also established. Among them are Pojanagaroon & Kaewrak (2005) studies on physical damage procedures that might stimulate the development of agarwood resin, particularly the way of producing 1.27 cm-diameter holes using screws. The lengthy formation of agarwood is a shortcoming of this technique. Then, (Rahayu, Santoso, & Wulandari, 2010) developed mechanical procedures by inoculation using a pellet form. The findings demonstrated that single and double inoculants of *Acremonium* and *Fusarium* were more successful than other induction techniques in encouraging the development of agarwood symptoms. The combination of mechanical and biological approaches may boost the rate of agarwood production and decrease the time required for agarwood formation. In 2011, Santoso & Turjaman, developed a bio-induction approach including a drilling depth of one-third the tree's diameter and the addition of liquid inoculants with a success rate of 40 to 60 percent.

This study is an application of the bioinduction technology, which has a high success rate as claimed. This work used fungus isolated from agarwood-producing plants native to West Nusa Tenggara, namely *G.*

versteegii. When inoculated on the same species of agarwood-producing trees, it is expected that the study's findings would yield agarwood of excellent quality since the identified isolates have a high level of compatibility.

II. MATERIALS AND METHODS

A. Materials

A methodology devised by Santoso and Turjaman (Santoso & Turjaman, 2011) was modified and used as the basis for the inoculation procedure in this study. The used drill bit is a 3 mm diameter screw drill. In the last phase, silicon is used to seal the inoculation hole. This study was done between 2013 and 2014. In this study, twelve agarwood-producing *G. verstegii* trees were used. The utilized isolates were obtained by isolating agarwood-producing fungus from the *G. verstegii* tree in West Nusa Tenggara. The isolates were confirmed to be *Fusarium solani* (Nugraheni *et al*., 2015). PDA and PDB were used for the cultivation of isolates.

B. Procedures

This study uses a completely randomized design with three replications. We treated the tree sample with three distinct fungal isolates (LT, ALS, and M) and with no isolate as control with three replicates. Isolate codes LT, LB and ALS have been identified as *Fusarium solani* (Nugraheni *et al*., 2015). Mix fungus isolates (M) were created by combining four West Nusa Tenggara fungal cultures (isolates from North Lombok (LU), West Lombok (LB), East Lombok (LT), and Alas (ALS)). This isolate is part of the Non-Timber Forest Products Technology Research Institute's (BPTHHBK) collection of aloes-forming fungus isolated from *G. versteegii* in NTB.

In this study we used twelve sample trees. The inoculation process was initiated by penetrating the stem surface with a 3 mm drill bit. Each point is drilled at a slope of 45 degrees with 50 cm spacing. The drilling depth is one-third of the diameter of the tree. Using 6 mL pipettes, liquid isolates were injected onto the agarwood host tree. In addition, the hole is sealed with paraffin to prevent the introduction of rainwater and other irritants.

The next stage was observation. The observations were made in the first, third, and sixth months after inoculation. The data parameters used are the infection area, the color of the infected area, and the odor of agarwood. The infection area was measured by tracing the pattern of infection with transparent plastic. The image on the plastic was transferred into a millimeter block. The area infected was then tallied.

Figure 1 depicts the agarwood formation color standard. The color standard was developed by imagining several types of agarwood on the market and classifying them according to their pricing. Scores were made based on the agarwood's color and structure, from lightest to darkest hues.

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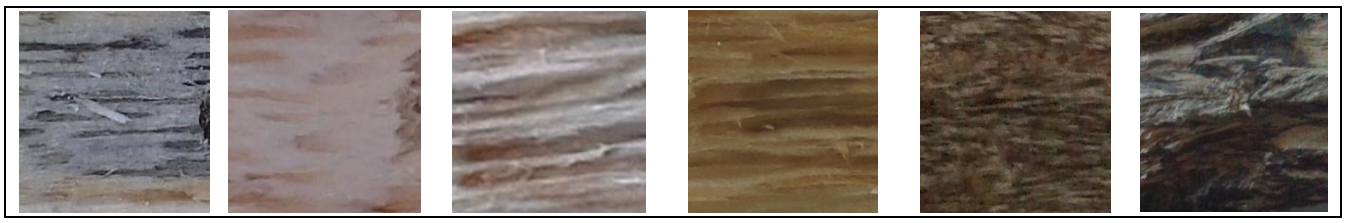


Figure (*Gambar*) 1. Color criterion for determining the color of agarwood formation (*Kriteria warna dalam penentuan standar warna dari gaharu yang terbentuk*).

The aroma of agarwood was rated using a scoring system. The agarwood sample was burnt and then sniffed. The standard score for agarwood aroma is 1 for no agarwood aroma, 2 for fragrant, and 3 for very fragrant.

1. Preparation of fungi isolates

Three fungus isolates were utilized in this study. East Lombok (LT), Alas (ALS), and mixed fungus isolates (M) were used. A medium inoculated with no isolate is used as the control treatment. Each isolate was planted on PDA (potato dextrose agar) medium before being cultured for 7 days. The isolate was placed in a liquid medium, PDB (potato dextrose broth), and shaken for three weeks.

2. Inoculation

The research was done in Sidemen, in the village of Kekait, a subdistrict of Gunung Sari, West Lombok Regency. The study's tree is about 10 years old. Sample trees are less than 14 cm in diameter, have straight trunks, are healthy, and have never been inoculated. In general, this village is 500 meters above sea level and gets 1500 millimeters of rain per year.

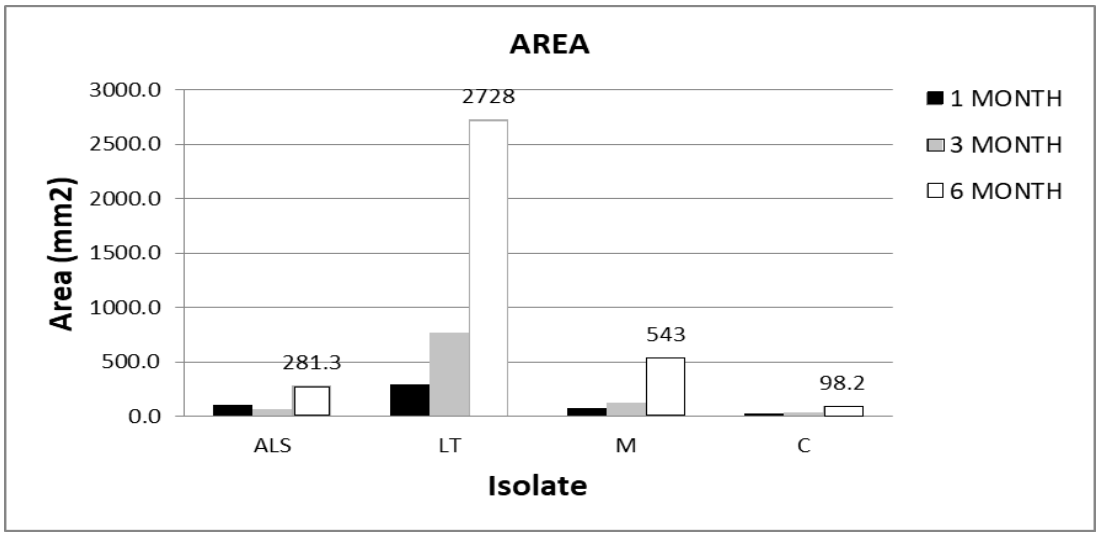
C. Data Analysis

The significance of the collected data from observations in the first, third, and sixth months after inoculation are then evaluated using ANOVA with the SAS program.

III. RESULT AND DISCUSSION

A. Result

The agarwood development based on area infection of three isolates of fungi forming agarwood origin NTB in West Lombok was presented in Figure 2. In Figure 2, the greatest area is clearly visible in the LT isolation, especially after 6 months following inoculation, whereas the control shows the formation of the lowest area, as demonstrated in Figure 3.



Remarks (*Keterangan*) : ALS (Alas); LT (East Lombok/*Lombok Timur*); M (Mix/*campuran*) : C (control/*kontrol*)

Figure (*Gambar*) 2. Agarwood development based on area infection of three isolates of fungi forming agarwood origin NTB in West Lombok (*Perkembangan pembentukan gaharu berdasarkan pada luasan area infeksi dari tiga isolate pembentuk gaharu asal Nusa Tenggara Barat di Lombok Barat*).

Figure (*Gambar*) 3. The establishment of the isolate ALS (A), LT (B), M (C), and Control (D) reaction areas (1, 3, and 6 months after inoculation from left to right) (*Pembentukan luas area isolate ALS (A), LT (B), M (C) dan Kontrol (D) (1, 3, dan 6 bulan setelah inokulasi)). ALS (Alas); LT* (East Lombok/Lombok Timur); M (Mix/*campuran*): C (control/*kontrol*).

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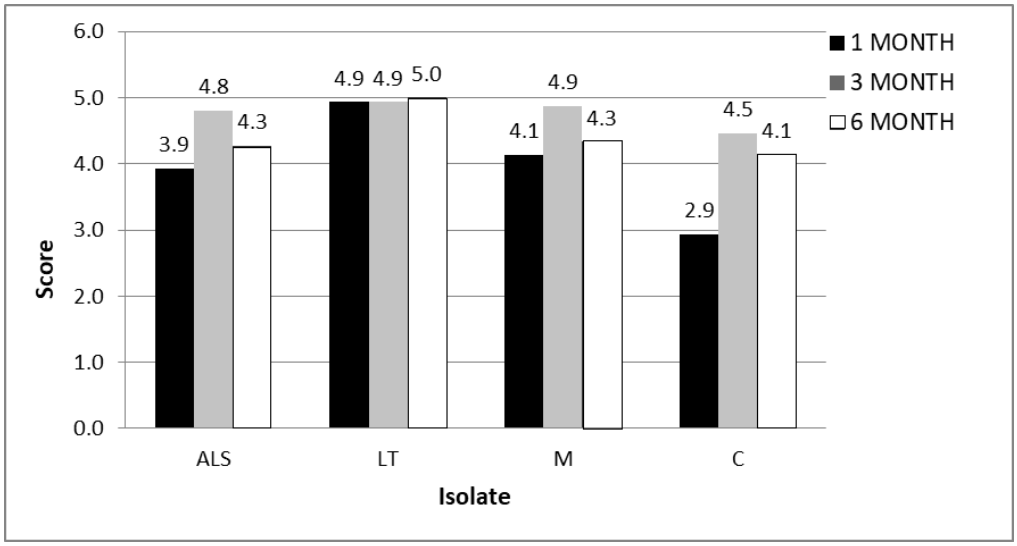
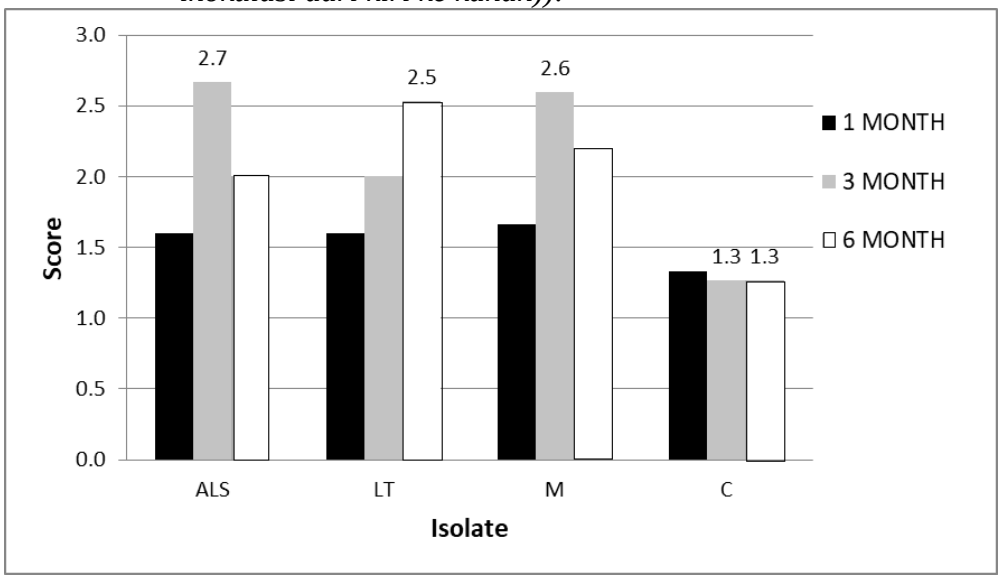
Figure 4 shows that the color formation produced by each isolate was not too dissimilar. The following figure shows that the fragrance produced by fungal inoculation was not significantly different amongst isolates. However, the control treatment had the lowest scent score.



Remarks (*Keterangan*) : ALS (Alas); LT (East Lombok/*Lombok Timur*); M (Mix/*campuran*) : C (control/*kontrol*)

Figure (*Gambar*) 4. The establishment of the ALS, LT, M, and C reaction color (1, 3, and 6 months after inoculation from left to right) (*Pembentukan reaksi warna ALS (A), LT (B), M (C), dan Kontrol (D) (1, 3, dan 6 bulan setelah inokulasi dari kiri ke kanan))*.



Remarks (*Keterangan*) : ALS (Alas); LT (East Lombok/*Lombok Timur*); M (Mix/*campuran*) : C (control/*kontrol*)

Figure (*Gambar*) 5. The establishment of the isolate ALS, LT, M, and C reaction scent (1, 3, and 6 months after inoculation from left to right) (*Pembentukan reaksi aroma isolat ALS, LT, M, dan C (1, 3, dan 6 bulan setelah inokulasi dari kiri ke kanan*)).

B. Discussion

These data demonstrate that agarwood is produced upon inoculation. The findings indicated that agarwood formation had developed to some degree through the expansion of the reaction area, the change in color, and the intensification of the agarwood odor from the first to the sixth month after inoculation. The presence of brown patches around the inoculation site, which, when burnt, have a pleasant odor. This trait suggests the existence of agarwood resin. This research used an inoculant discovered in West Nusa Tenggara and derived from an agarwood infection of *G. versteegii*.

This study demonstrates that inoculation with LT fungus isolate increases efficiency by 16 times compared to control. ALS and M isolates increased efficacy by one and two times, respectively, in comparison to the control. This research demonstrated that LT fungus isolates were much more successful at producing agarwood. It is shown statistically by the p value ≤ 0.05.

In addition, the development of the reaction area might suggest that LT fungal isolates have high virulence and strong host-fungus compatibility. Due to the ferocity of the LT fungus's attack, a somewhat large reaction area is affected. In the research of (Mohamed, Jong, & Kamziah, 2014), the induction of fungus in *Aquilaria malaccensis* species had no influence on the lengthening of discolored patches. It is thought that environmental conditions and the species and genetics of agarwood-producing trees are responsible for the disparity in outcomes (Akter, Islam, Zulkefeli, & Khan, 2013; Rasool, & Mohamed, 2016).

Observing the efficiency of an agarwood-forming fungus isolate in West Lombok is possible by analyzing the agarwood's color and scent (Figures 4 and 5). Figure 4 demonstrates that the color of infected agarwood by LT fungus isolates tends to darken. The color of the resin is a significant aspect in determining the aroma of burnt agarwood (Liu, Wei, Gao, Zhang, & Lyu, 2017; Subasinghe, Hitihamu, & Fernando, 2019). Changes in color are caused by a pathological response to the introduction of fungus, which results in the deposition of resin in tree cells (Akter, Islam, Zulkefeli, & Khan, 2013; Karlinasari, Danu, Nandika, Tujaman, 2017). The graphs of the ALS and M fungus isolates 3 months after inoculation are remarkably identical (Figure 4). This suggests that the color intensity of agarwood from both isolates responded similarly.

Based on the third criteria (Figure 5) of agarwood creation, the degree of agarwood's fragrance, all three isolates had a favorable response in the presence of fragrance when agarwood was burnt. In general, statistical analysis ($p \leq 0.05$) indicates that color and odor characteristics were not substantially

different.

In addition to the color change, the response of the isolate inoculation causes the synthesis of aromatic molecules, resulting in the odor. According to various research, the fragrant compounds created include sesquiterpene, terpenoid, and pheniletyl chromone, which are the most significant metabolites in agarwood (Huang, Liao, Chen, & Zhang, 2017; Liao et al., 2017; and Li et al., 2014; Zhang, Li, Cui, & Xu, 2022). In this work, isolates obtained from *G. versteegii* in Lombok were reinoculated into *G. versteegii* in Lombok under identical habitat circumstances. With this treatment (carrying out inoculation on the site of its original habitat), it is possible to manufacture more suitable agarwood, and the outcomes (efficacy) may be observed in the results produced.

In general, the color and odor of agarwood will influence the price and grade or quality of the agarwood produced. There is no worldwide standard for evaluating the quality of agarwood. In certain reaction areas, users of agarwood utilize color and aroma as a price indication. Agarwood has several uses, such as a perfume ingredient, a craft item (sculpture, beads), and an air freshener/body spray. Agarwood may be collected at various ages, depending on its intended use. If the crop is meant for oil, it may be harvested six months after inoculation. The maximum output of agarwood oil is also affected by the process of extraction (Jok, Radzi, & Hamid, 2015; Kusuma, Putri, Triesty, & Mahfud, 2019; Samadi, Abidin, Yunus, Biak, Yoshida, & Lok, 2017).

IV. CONCLUSION

The fungus *Fusarium solani* codes LT, ALS, and M from West Nusa Tenggara were all capable of promoting the production of agarwood to the same extent. These isolates might potentially be produced as agarwood-inducing fungus isolates for the *G. verstegii* species.

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