

The Best Inoculation Technique Applied on *Gyrinops versteegii* Tree Trunk

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Abstract. Agarwood is a non-timber forest product having high economic value, however, its population in nature is getting decrease. *Gyrinops versteegii*, an agarwood-producing plant, has been listed in Appendix II CITES, therefore, exploitation of the agarwood in nature must be reduced. An effort to reduce the agarwood exploitation the nature is by agarwood cultivation including cultivation of agarwood-producing plant and agarwood inoculation. Some of agarwood inoculation methods are simpori and implant techniques. Simpори is a modified inoculation method using porous nail and *Fusarium solani*, meanwhile implant technique uses drill and pieces of wood that is soaked in *F. solani* spores. The present study aimed to determine the best inoculation technique applied to *G. versteegii* tree trunk at 4 to 5m height, comparing simpори and implant techniques. This study used completely randomizes design with two treatments (simpори and implant techniques). The result showed that the averages yield of production agarwood produced by simpори and implant techniques are 0.66 and 0.64%, respectively. Based on SNI 7631:2011, the visual quality of the produced agarwood belongs to ke-medangan TG.C. In conclusion, both simpори and implant technique produced agarwood in similar quality and quantity at six months inoculation, however, simpори is more practical for application in the field. This study provides additional data about agarwood produced by simpори and implant technique and give the alternative methods for agarwood farmers producing their agarwood themselves.

Key words: Agarwood; Implant; Simpори

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INTRODUCTION

Agarwood is a non-timber forest product with high economic value due to its role in perfume production, aromatherapy, medicine, and religious activities (Kakino et al., 2010). The physical wound or pathogen attacks cause the plant produce a resinous compound namely agarwood. (Karlinasari et al., 2015). Agarwood is produced by plant from the family of Thymelaeaceae with 58 genus, including *Aquilaria* and *Gyrinops* as a main agarwood-producing plant (Compton & Ishihara, 2004; Subasinghe et al., 2012). *Gyrinops versteegii* is a species of agarwood-producing plant in Nusa Tenggara Barat, Nusa Tenggara Timur, and Papua (Roemantyo & Partomihardjo, 2010), particularly in Lombok island. The over exploitation of natural agarwood has affected the availability of agarwood-producing species in their natural habitat. All *Gyrinops* species have been listed in Appendix II of CITES (the Convention on the International Trade in Endangered Species of Wild Flora and Fauna) to prevent its excessive exploitation and to regulated its trade (Resti Wahyuni et al., 2020).

A number of technologies have been developed to find the most efficient technique of agarwood production to meet the high demand and could decrease exploitation of the agarwood trees in the natural habitat (Faizal et al., 2017). Furthermore, cultivation of

agarwood is required to reduce the agarwood exploitation in nature (Resti Wahyuni et al., 2018). The agarwood cultivation includes cultivation of agarwood-producing plant and agarwood inoculation. Many agarwood inoculation techniques have been developed, and the techniques are divided into two groups, conventional and modification techniques. Conventional technique is conducted by nailing and wounding the trees. The disadvantage of the technique is requiring a long time and generally inadequate and show low quality in agarwood production (Li et al., 2015; Van Thanh et al., 2015). Meanwhile, modification technique uses chemical, microorganism, and tools in the application, which is more complex compared to conventional technique. Commonly, the chemical used in agarwood inoculation are salicylic acid, jasmonic acid, sodium chloride, and hydrogen peroxide (Okudera & Ito, 2009). However, this technique is becoming less preferable due to side effects of the chemicals which are harmful to the environment. Other modification technique applied *Deuteromycetes*, *Ascomycetes*, *Fusarium solani*, *F. oxysporum*, dan *F. ambrosium* for inoculation (Mohamed, Jong, & Kamziah, 2014; Nurbaya et al., 2014). Furthermore, Faizal et al. (2017) combined the technique by using microorganism equipped with tools including drills.

Agarwood is generally formed associated with the wounding, fungal infection or pathogen attacks in the heartwood trees of agarwood-producing plant (Karlinasari et al., 2015; Liu et al., 2013; Mohamed, Jong, & Kamziah, 2014; Wong et al., 2013). Prior to infection, a healthy wood is light, pale coloured, and having no odour. When an injury or infections occur, the tree responds it with producing a self-defence material, a dark aromatic resin, deposited around the infected area (Xu et al., 2013). As the infection progress, the resin are accumulated and eventually forms agarwood (Subasinghe & Hettiarachchi, 2013). The resin increases the mass and density of the infected wood, changing its colour from a pale beige to yellow, orange, red, dark brown or black and having an odour after burned (Abdullah et al., 2007). Generally, tissues were more turns dark closer to the inoculation hole and lighter as the distance increased. Agarwood tissue colour were darker and resin content was higher closer to the inoculation holes/sites, which could be due to the impact of high growth of fungi in that area (Subasinghe et al., 2019) Therefore, the evaluation of agarwood or the success of agarwood production might be approached through the quantity and quality of the agarwood produced.

Non-Timber Forest Product Technology Research and Development Institute (NTFPT-RDI) have developed inoculation technique named simpori and implant techniques. Simpори is a modified inoculation method using porous nails and *Fusarium solani*. Meanwhile, implant technique uses drill and pieces of wood which are soaked in *F. solani* spores. Simpори is commonly applied in agarwood tree with diameter approximately 15cm or until 4 m in height. Meanwhile, implant technique initially used in a lower diameter or at branches of agarwood tree, however, it is also necessary to be applied in the tree trunk. Therefore, to optimize the production of agarwood, it is required to investigate the effective inoculation to the agarwood tree trunk at above 4 m in height. Based on these reasons, the present study aimed to investigate the best inoculation technique applied to *G. versteegii* tree trunk at 4 to 5m height, comparing simpори and implant techniques. This research may also provide alternative method of agarwood production for using by agarwood farmer.

METHODS

The research was conducted from May until November 2018 in Gangga, North Lombok applying simpори and implant techniques. The materials used were *G. versteegii* tree, porous nail, *F. solani*, Potato Dextrose Broth (PDB) medium, plastic pipette, generator, drill, piece of wood, *F. solani* spore, and plastic straw.

Simpори technique

The porous nails were applied in the tree trunk from 4 to 5 m height. The vertical distance between nails was 20 cm, meanwhile the horizontal distance was 1/3 tree diameter. The *F. solani* in PDB medium was inserted in porous nail with dosage of 3 ml/nail (R Wahyuni et al., 2018). The porous nails were left in the trunk until harvesting time. The inoculation hole in the nails was covered with silicon to avoid contamination from insects and water (rain).

Implant technique

The tree trunks were drilled at 4 to 5 m height. The distance of the holes was arranged similar to simpори technique. Pieces of agarwood sticks were prepared in small size according to the hole of inoculation. The prepared agarwood sticks were soaked in *F. solani* spore overnight, and then were inserted into the hole of the tree equipped with plastic straw (Anggadhania et al., 2014).

Agarwood harvesting

The agarwood was harvested after 6 months inoculation. The inoculated trees were cut and the inoculated trunks were weighed. The inoculated trunks were then carved to obtain the agarwood. The produced agarwood was collected and weighed. Moreover, the quality of produced agarwood was observed based on SNI 7631:2011. The parameters required in the analysis are colour, weight, and the odour after burning. The yield production of agarwood was calculated using the following formula:

$$Y = \frac{\text{Output}}{\text{Input}} \times 100\%$$

Y = yield of agarwood production (%)

Input = log of wood before carving (kg)

Output = agarwood production after carving (kg)

Data analysis

A completely randomized design with two treatments and nine replications were applied in the study. The yield was further analysed using T-test analysis with SPSS 15 software.

RESULTS AND DISCUSSION

The quantity of agarwood produced in the study after 6 months inoculation is shown in Table 1. Both simpори and implant techniques produced agarwood in similar quantity with $0.66 \pm 0.4\%$ and $0.64 \pm 0.3\%$, respectively. The results mean that in every 100 kg of inoculated wood, approximately 640 or 660 g agarwood were produced. The result showed a low quantity of agarwood, however, both simpори and implant techniques succeed to produce agarwood in

every hole. It indicated that both methods are effective to produce agarwood at tree height of 4 to 5 m.

Table 1. The average yields of agarwood production

Treatment	Yield (%)
Simpori technique	0.66 ± 0.4 ^a
Implant technique	0.64 ± 0.3 ^a

Note: numbers which are followed by the same letter on the same column represent no significant difference ($p < 0.05$)

Quality of agarwood is graded based on amount of resin content, wood colour, fragrance, size, species, country of origin, density, product purity, weight, age, location, etc. (Amin et al., 2012; Subasinghe et al., 2019). Furthermore, quality grading of agarwood has been a major concern in the agarwood business (Azah et al., 2013). In Indonesia, the quality of agarwood generally graded using colour, weight, aroma, and

resin content. In this study, the quality of agarwood was analysed based on SNI 7631:2011. A darker colour on the agarwood indicated higher content of agarwood fragrant. Evaluation of the weight of agarwood is conducted by sinking it in the water. The more sinking indicated the heavier chip. It means the quality of agarwood is better. The agarwood fragrance was evaluated by cutting agarwood into chip and then burn the chip. The content of agarwood fragrant after burning can be indicated if the chip is melted and are emitted strong scent of agarwood fragrant. The more fragrance represents the higher quality of agarwood.

The results showed that based on the analysis, the quality of agarwood produced both from simpori and implant techniques belong to kemedangan TG.C (Table 2). It is in line with (Mucharromah, 2010) that the quality of agarwood that was harvested after 6 months inoculation belongs to kemedangan TG.C. Moreover, the visual quality of the agarwood is shown in Figure 1. Kemedangan TG.C is agarwood grading in the middle class of kemedangan quality (Table 3).

Table 2. Quality of the agarwood produced by simpori and implant technique

Grade	Colour	Weight	Odour (burned)
Agarwood (Simpori)	TG.C	Brown striped wide white	Floating fragrant
Agarwood (Implant)	TG.C	Brown striped wide white	Floating fragrant

Table 3. Grading of kemedangan quality based on SNI 7631:2011

Grade	Colour	Weight	Odour (burned)
Kemedangan	Sabah	Blackish brown	Suspending fragrant
	Kemedangan A	Brown striped black	Suspending fragrant
	Kemedangan B	Brown striped white	Suspending fragrant
	TG.C	Brown striped wide white	Floating fragrant
	Green kemedangan	Brown striped green	Suspending fragrant
	White kemedangan	Grayish white striped black	Floating pungent



Figure 1. Agarwood produced after 6 months inoculation by: (A) simpori and (B) implant technique

Kemedangan TG.C is the agarwood quality in the middle class by SNI 7631:2011. Classification of agarwood using SNI 7631:2011 is applied in Indonesia. In kemedangan TG.C, agarwood has brown colour with thin white stripes, floating, and emitted agarwood fragrant. Agarwood produced by inoculation technique and harvested in 3 – 6 months after

inoculation usually reach kemedangan TG.C quality (Mucharromah, 2010). Furthermore, (Mohamed, Jong, & Irdayu, 2014) have reported that at 6 months of post wounding (inoculation), *Aquilaria malaccensis* wood became blackish brown and the discoloration was more widespread. In their research, the healthy wood that was initially creamy white in color,

had turned into light yellow at two days after wounding, changing to light brown and then brown after 5 days and 2 weeks, respectively (Mohamed, Jong, & Irdayu, 2014).

Agarwood produced in this study was emitted agarwood fragrant (both simpori and implant technique). The fragrant of agarwood varies among the grade of agarwood quality. Agarwood fragrant is an effect of different stressors which depend on the growth stages of the plant, genetic, and its interaction with biotic and abiotic environment (Naziz et al., 2019). In this study, fragrant of agarwood produced by simpori and implant technique is like incense.

Agarwood aroma also plays an important role in agarwood quality grading due to its function as ingredients for perfume and incense. The incenses and perfumes that are produced from agarwood has a high value from a long time ago and used by many cultures for spiritual, opulent, and aphrodisiac purposes (López-Sampson & Page, 2018). Based on that reason, agarwood has different market share for different agarwood grade or quality. For example, in Japan, the market preference of agarwood derived from *Aquilaria crassna* (Compton & Ishihara, 2004). While in Middle East, both agarwood smoke and oil are customarily used as perfume (Compton & Ishihara, 2004). In India, there are *attar* oil that made of various grade of agarwood from *Aquilaria* species (Akter et al., 2013; López-Sampson & Page, 2018). *Attar* oil is a water-based perfume containing agarwood oil, which is traditionally used by Muslim in their prayer clothes (Akter et al., 2013).

The fragrant of agarwood is influenced by chemical content in the resin. Fragrant compounds of agarwood are known to be sesquiterpenoid and chromone derivatives, which are the main source of agarwood particular odour (Subasinghe et al., 2012). In this study, the data of chemical content in agarwood resin is not available. The chemical compounds analysis of the produced agarwood to evaluate the content of agarwood fragrances is required for further investigation.

According to the same grade of agarwood produced by both techniques in the present study, it can be considered that simpori and implant techniques applied in tree height of 4 to 5 m give similar effect on the agarwood production. However, practically simpori is simpler than implant technique. It is due to simpori technique does not require drill and generator. Furthermore, simpori can be applied at any time and at various field conditions.

In the process of agarwood formation, there are some influential factors such as environmental conditions, differences in the tree caused by seasonal variation, the age of the tree, and genetic variation (Akter et al., 2013). Moreover, (López-Sampson & Page,

2018) stated that the aromatic qualities of agarwood are influenced by the species, geographic location, branch, trunk and root origin, length of time since inoculation, and methods of harvesting or processing. In addition, the quantity and quality of agarwood were also influenced by the purity of microorganism used as isolates (Mucharromah, 2010). Still, dark colour intensity was directly proportional to time after wounding, suggesting that prolonged exposure to the environment produces darker wood (Mohamed, Jong, & Irdayu, 2014). As a consideration, a longer harvesting time might be necessary for the study.

This study gives additional data about agarwood produced by simpori and implant techniques as a modification technique using certain microorganism and tools. From this study, we hope that farmers can get an alternative method for producing their agarwood themselves. By using simpori technique, farmer can do their agarwood production any time and at various field conditions.

CONCLUSION

The quality of agarwood produced both from simpori and implant techniques belong to kemedangan TG.C based on SNI 7631:2011. The yields of agarwood production after 6 months inoculation from both techniques are not significantly different. Both simpori and implant techniques produced agarwood in similar quality and quantity after six months inoculation, however, simpori is more practical for application in the field.

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