Agarwood Production of Aquilaria malaccensis Using Various Inoculants and Induction Techniques

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Abstract

Agarwood, the fragrant resinous wood of Aquilaria species, is precious and widely used in medicine, religion, and perfume. Aquilaria malaccensis cultivation and inoculation techniques have been successfully developed in Malaysian plantations to protect endangered Aquilaria species. Several inoculation techniques have been explored in local plantations to stimulate the agarwood resin in a short period. Therefore, in this paper, five inoculants and four methods of inductions were analysed and compared for their effectiveness. Two phases of agarwood harvesting were

executed after 18 months and 24 months of the incubation period. The results showed that inoculant IGB711 had effectively stimulated the agarwood formation during 24 months of incubation with mean yield 25.76 kg/tree. Meanwhile, the RAHE Probio inoculant, in combination with the bamboo stick and dripping method, was effective in producing high-quality agarwood. With the latest induction technologies developed in Malaysia plantations, it is now possible to cultivate high-value agarwood in young plantation trees.

Keywords: *Agarwood, resinous wood, Aquilaria malaccensis, inoculant, plantation*

Introduction

Agarwood is usually referred to as the fragrant resinous tree *Aquilaria*, a valuable non-timber forest product worldwide. For the past hundred years, it has been used for medicine, religion, and primary material to produce perfumes[1]. A previous study reported about nine species of *Aquilaria* known to have agarwood, including *A. beccariana, A. crassna, A. filaria, A. hirta, A. khasiana, A. malaccensis, A. microcarpa, A. rostrata* and *A.sinensis[2]*. Agarwood is also known by several names, such as *gaharu* (in Malaysia and Indonesia), *jin-koh* (Japan), *chenxiang* (China), agar (India), oud (Middle East), *chim-hyuang* (Korea) and *kritsana noi* (Thailand)[2, 3]. The agarwood resin's unique and pleasant odour was believed to contribute from a particular chemical compound, especially the sesquiterpene group and chromones[4-6]. The first grade of agarwood is the most valuable raw material globally and usually traded in woodchips, wood pieces, powder, dust, oil, incense ingredients, and perfume [7]. The grade of agarwood usually depends on its physical properties such as resin content, specific gravity, colour, and odour. For example, *gaharu* and *kalambak* are the most popular agarwood grade in Malaysia, meanwhile in Japan, *kananko*h and *jinkoh* refer to the highest and lowest grade, respectively[8].

In nature, agarwood usually takes decades to form when exposed to external factors, such as lightning strikes, animal grazing, insect attack, or microbial invasion, within injured trunk parts[6, 9]. On the other hand, agarwood formation was hard to be detected inside the trunk, causing unauthorized harvesters to cut down the whole tree to collect the agarwood[10]. Thus, the exploitation by illegal harvesters led to a substantial population loss, impaired natural availability, and insufficient market supply for agarwood species. Consequently, *Aquilaria* species were listed on the International Union for Conservation of Nature (IUCN) red list as endangered species and listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)[9-11].

Since the demand for agarwood has risen significantly; therefore, *Aquilaria* species' plantation was grown to a large scale with combination induction techniques to sustain the agarwood production [10, 12, 13]. Agarwood resin formation in the *Aquilaria* tree is thought to be linked to plant distress and offensive responding to wounding and fungal infection[12]. Therefore, two paths were considered in developing proven methodologies, including the technique and superior inductors in microbes, chemicals, or both[3]. Hence, several physical approaches in finding the most effective agarwood-production techniques to satisfy the high demand have been introduced, including chopping, nailing, holing, and trunk breaking[10, 14]. Besides, chemical methods such as *methyl-jasmonate*, soybean oil, brown sugar, *sodium chloride*, *formic acid*, hydrogen, and biological approach, for example, several fungal strains, were frequently used to stimulate agarwood formation[15].

Aquilaria cultivation in Malaysia is an alternative source of agarwood production. In Peninsular Malaysia, there are five *Aquilaria* species cultivated, including local species, *A. malaccensis*, *A. hirta*, as well as introduced species, *A. subintegra* (Thailand), *A. sinensis* (China) and *A. crassna* (Thailand, Cambodia, Laos and Vietnam[10, 16]. *A. malaccensis*, locally known as the karas tree, is a large, evergreen tree up to 40 m tall and 1.5 to 2.5 m wide. It has shining leaves surface, pointy, and

oval like Spanish Cherry[17, 18]. A prior study indicated that *A. malaccensis* is one of the best-known species that produce agarwood[8]. Other than that, *A. malaccencis* has been recorded as the most planted species and accredited as the major manufacturer of agarwood in Malaysia[12, 18].

Sustainable plantation and maintenance of agarwood by induction of resin formation have been carried out and resulted in a ready supply of various parts of the agarwood plant, allowing for different value-added products[19]. Therefore, the present study addressed the comparison of inoculation techniques to the induced formation of agarwood. Thus, this paper will give a platform to review the potential inoculation technology in improving the production of high-quality agarwood in Malaysia plantation.

Materials and Method

A) Study Location

Location of the research was at Empangan Jus, Jasin Melaka. There were 50 hectares of *Aquilaria* trees planted in 2009 by MTIB-UPEN Melaka and Malacencis Sdn Bhd, with 40,000 matured *Aquilaria* trees. The species of *Aquilaria* tree has been verified as *A. malaccensis* species through registration number AQ 0117 by the Malaysian Timber Industry Board (MTIB).

B) Inoculation Process

75 *A. malaccensis* trees of the same size (20 cm diameter at breast height) and same age (eight years old) were selected. The selection of five companies was based on the Malaysian Timber Industry Board (MTIB) previous inoculation research in Kuala Lipis, 2012. The name of companies and inoculant types are shown in Table 1.

Each company was given 15 trees, and the inoculation techniques were observed and recorded. Four methods were introduced by each company, such as pressurized injection (*chemjet*), drip bag method, bamboo stick and dripping, and direct infuse system, respectively.The detail of these methods as described in the latter section.

Table 1

The five selected companies and their inoculant types.

	Name of company	Inoculant	Technique
1.	Bio-Benua Teknoloji Sdn Bhd	Black Gold Bio Booster (BGBB)	Pressurized Injection (Chemjet)
2.	Sansen Agarwood Sdn Bhd	TW Destang	Drip Bag Method
3.	Redclaw Aqua and Herbs (RAHE) Sdn Bhd	RAHE Probio	Bamboo Stick and Dripping
4.	Inokulum Gaharu Biotech Sdn Bhd	IGB711	Direct - Infuse System
5.	Etlingera Sdn Bhd	UPMV16	Direct - Infuse System

Inoculation Technique

In nature, *Aquilaria* trees produce agarwood naturally are tremendously low, where can be found on pathogenically infected or wounded trees[20]. Therefore, some inoculation techniques were developed to find the most reliable method for enhancing agarwood formation[7]. A small physical wound is required on the trunk before the inducer is applied, and it can be liquid, semi-solid or solid according to its application[10].

Several types of inducers are currently available in the market, such as chemical inducer and biological inducer. Up till now, several kinds of chemical induction have been developed and practised, such as cultivated agarwood kit (CA-kit), the whole-tree agarwood inducing technique (Agar-Wit) and biologically agarwood-inducing technique (Agar-bit)[20]. On the other hand, inoculums or biological inducers may take a long incubation period to produce darker and better agarwood before harvesting[10].

Thus, 75 trees of *A. malaccensis* and five commercial inoculants have been chosen in the present study. Each commercial inoculant was used in a different method to stimulate the formation of agarwood. Those inoculated trees were observed in their agarwood formation and harvested in two phases, 18 and 24 months. A range of the harvesting period in this study is based on few preliminary investigations showing that high-quality agarwood is similar to wild agarwood obtained with 18 to 20 months of the incubation period[2, 12, 15].

Pressurized Injection (Chemjet)

Biobenua Teknoloji Sdn Bhd introduced the inoculation technique, namely *chemjet* (pressurized injection). This treatment using inoculant Black Gold Bio Booster (BGBB) (Figure 1a). This treatment uses a spiral technique to make many holes in the trunk and ensure it has to withstand wind and rain during the incubation period lasting for one or more years[10]. The inoculant, namely Black Gold Bio Booster, was delivered from a container through tubing into the drilled hole. About 3 to 40 holes were drilled using 4 mm for each tree and inserted 20 ml of inoculant. The cost of this inoculant is RM280/litre, and the price for each tree, including workmanship, total up to RM320.

Drip Bag Method

Sansen Agarwood Sdn Bhd developed the drip bag method using their inoculant which is TW Destang (Figure 1b). This technique is performed by hanging the drip bag upside down, filled with inoculant, as the inoculant dripped through the inserted hose into the wounded tree trunk. This treatment was operated similarly to the previous study[5]. An 8 mm drill size was used to make holes in the *Aquilaria* trees (spiral technique) with ten bags of inoculant for each tree and 3 litres of inoculant for each bag. The cost of the inoculant is RM25/litre, and the price for each tree, including workmanship, is RM750.

Bamboo Stick and Dripping

Another treatment of inoculation is the bamboo stick and dripping method (Figure 1c). This method was applied by Redclaw Aqua and Herbs Sdn Bhd, using their inoculant, namely RAHE Probio. The inoculation process happened using a bamboo stick as a device to make a small hole in the trunk. The inoculant was dipped with a bamboo stick before it was put inside the holes. This process is similar to the previously reported method, involving a device inserted into the wound to keep the pores from healing, establish prolonged infection, and allow the tree to respond with physical and chemical defensive mechanisms[7, 9]. They used a 6 mm drill size to make holes in the *Aquilaria* trees with 180-300 holes for each tree and approximately 10 ml inoculant in each hole. The cost of the inoculant is RM70/litre, and the price for each tree, including workmanship, is RM210.

Direct - Infuse System

The other inoculation performance is a direct-infused system. This treatment was using spiral techniques and a bottle filled with inoculant (Figure 1d). This treatment has been used by Inokulan Gaharu Biotech (IGB) Sdn Bhd and Etlingera Sdn Bhd using their inoculant, namely IGB711 and UPMV16, respectively. IGB Sdn Bhd used a 16 mm drill size to make four holes for each tree and 100 ml of inoculant in each hole. The cost of the inoculant is RM100/litre, and the price for each tree, including workmanship, is RM40. The latter used a 10 mm drill size to make six holes in each tree and 60 ml of inoculant in each hole. The cost of the inoculant is RM150 for each tree, including workmanship.

(a) (b) (c) (d)

Figure 1: Illustration of inoculation techniques, (a) pressurized injection (chemjet), (b) drip bag, (c) bamboo stick and dripping, and (d) direct-infuse system method.

C) Statistical Analysis

The data were presented as a mean with a standard deviation (SD). A non-parametric Kruskal-Wallis Test via Minitab software was used in the statistical analysis to determine whether there are statistically differences between two or more groups of an independent variables, proceed with Mood's median test to compare the medians of the two independent treatment groups. The values were considered significantly different when the *p*-value was less than 0.05 ($p < 0.05$). Then, Games-Howell Post-hoc test was used for multiple non-parametric comparisons between all possible combinations of the treatment groups.

Results and Discussion

Resin Content

Agarwood resin contains distinctive colours regarding countries and regions, including green, dark green, yellow, golden, red-black, brown, and white. Usually, the darker colour comprises rich in resin content[21]. The latter is also responsible for the colour strength, which also contributes to the grade level.[10]. However, the system grading of agarwood is subjective based on individual perception and experience[22].

The agarwood resin has been extracted from the white wood and sculpted to produce the agarwood part. Since the positions and areas of agarwood wounding sites vary between inoculation approaches, the appearances of induced agarwood vary[23]. The classification of agarwood grade of these five samples has been summarized in Table 2 based on their physical characteristics. Inoculant RAHE Probio has produced a high grade of agarwood resin and resembled the wild agarwood. Meanwhile, inoculants TW Destang and IGB711 grew less quality agarwood with light yellow colour and thin formation. Thus, morphological observations of agarwood resin stimulated from each inoculant are shown in Figure 2. The morphology represented of each agarwood resin resulted from varied inoculant techniques; (a) using the chemjet technique, (b) via drip bag method, (c) using a bamboo stick and dripping, (d), and (e) through the direct-infuse system.

Table 2

The grade and characteristics of five agarwood resins produce by different inoculant

Resin produced by inoculant	Grade	Characteristic
(a) Black Gold Bio Booster (BGBB)	AB	Light brown with thick agarwood formation
(b) TW Destang	B	Light yellow with thin agarwood formation
(c) RAHE Probio	A	Brownish agarwood with a formation similar to wild agarwood
(d) IGB711	B	Light yellow with thin agarwood formation
(e) UPMV16	B	Light yellow with formation almost similar to wild agarwood

(a) Black Gold Bio Booster

(b) TW Destang

(c) RAHE Probio

(d) IGB711

(e) UPMV16

Figure 2*:* Morphological observations of agarwood resin produced by different kinds of inoculants and techniques.

Twenty-five trees were analysed and weighed with the agarwood resin after an 18-month inoculation process. Meanwhile, ten trees were harvested after 24 months for each inoculant for yield estimation. There is no control group in our experiment in this study since the stimulation of agarwood production is through the inoculum response itself in the tree rather than using water (control group). This study performed a statistical analysis using the non-parametric Kruskal-Wallis test for accumulation agarwood yield in 18 and 24 months of incubation.

The inoculant Black Gold Bio Booster stimulated agarwood formation most effectively in 18 months inoculation period. The same situation also happened in inoculant TW Destang. Thus, our finding suggested that the best period for harvesting agarwood stimulated by both inoculants Black Gold Bio Booster and TW Destang is

18 months. However, compared to the cost operation and yield of agarwood resin, the inoculant Black Gold Bio Booster is preferred. On the other hand, the rest of the inoculant produced less accumulation of agarwood formation. Therefore, our finding suggested that inoculant**s** RAHE Probio, IGB711, and UPMV16 did not wholly or effectively stimulate agarwood formation in less than two years of incubation. Despite this, the Kruskal-Wallis and Mood's median test results in Figure 3 showed there was no significant difference (*p-value* > 0.05) in agarwood production harvested at 18 months for all inoculant**s**. The evidence could be showed in graphical presentation as the plotted graph showed that there was no significant difference in agarwood production harvested for all inoculants, as the plotted line graph seems overlapped to each other for all groups.

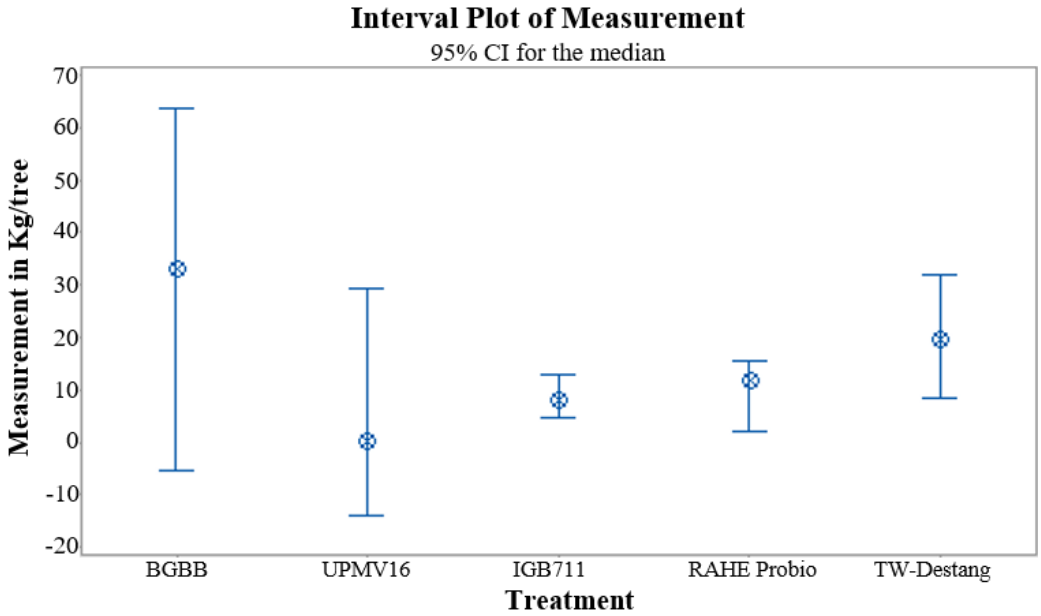

Figure 3: Statistical analysis of median yield of agarwood harvested after 18 months incubation periods. Individual standard deviations are used to calculate the interval.

Meanwhile, inoculant IGB711 harvested the most excellent production of agarwood resin after 24 months, with a mean accumulation of 25.76 kg/tree. They were then followed by inoculant RAHA Probio, with produced mean of agarwood yield, 22.56 kg/tree. However, inoculant UPMV16 had extremely low agarwood resin in both periods of harvesting. Even though the inoculation technique for inoculant IGB711 and UPMV16 were the same. It could have happened perhaps due to their secret formulation of both inoculants being diverse. The inconsistency of the accumulation of agarwood yield can be suggested caused by human error during the inoculation process. The non-parametric Kruskal-Wallis test's shows a significant difference (*p*-value < 0.05) in agarwood production harvested at 24 months. The result of Mood's Median Test also synchronizes with the finding in the Kruskal-Wallis test that there is a significant difference in agarwood production harvested at 24 months for at least one inoculant is different. The plotted graph in Figure 4 showed Games-Howell test for all possible combinations of treatment group differences in agarwood production harvested, as the plotted line graph which does not contain zero implies there is a significant difference between the treatment groups. As for the example, the comparison between inoculant IGB711 and UPMV16 shows that there are greater agarwood production harvested at 24 months using inoculant IGB711 rather than using inoculant UPMV16, as shown in Figure 4.

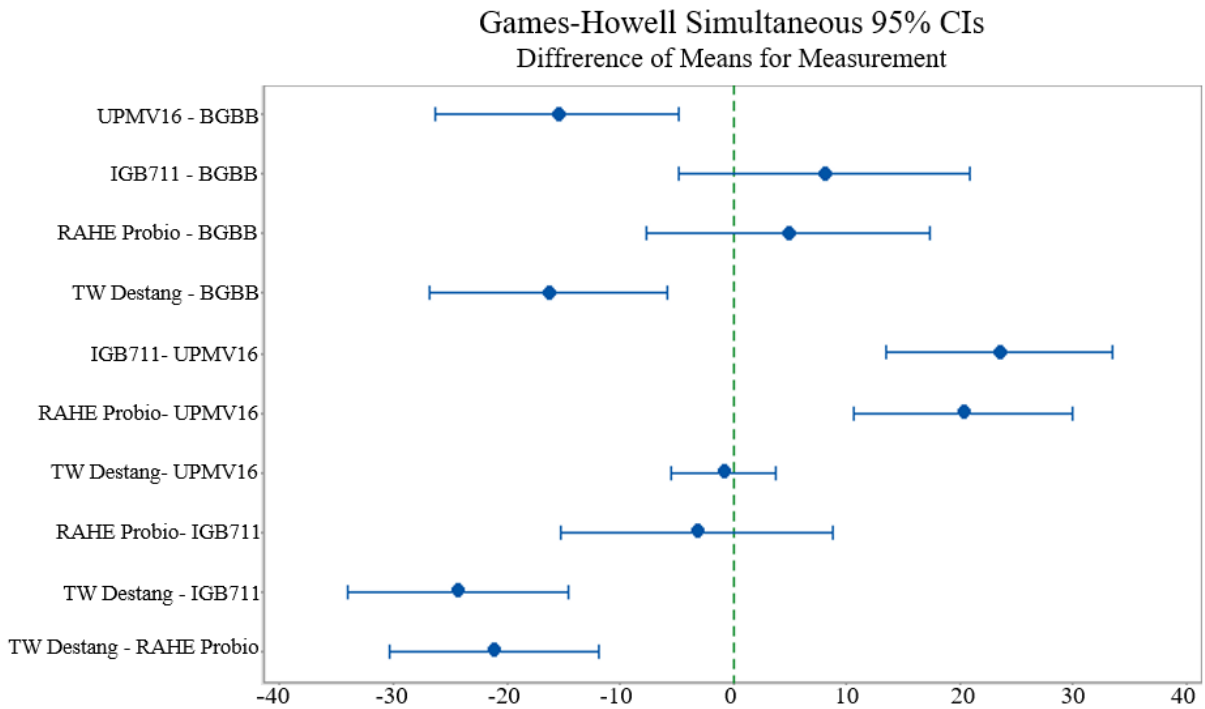

Figure 4*:* Statistical analysis of mean agarwood yield for each inoculant through Kruskal-Wallis and Games-Howell Simultaneous 95% CIs. *If an interval does not contain zero, the corresponding mean is significantly different.

In addition, the effectiveness of stimulation of agarwood resin is also related to their formulation of inoculants. For example, inoculant TW Destang was effective at 18 months incubation. However, when harvested at 24 months, the yield of agarwood is meager. Therefore, this study suggested that after 18 months, the tree's wound slowly healed on its own. Nevertheless, as far our concerned, none of the previous reported has been done to favour this hypothesis.

Our findings indicated that inoculant IGB711 is the best stimulation agarwood production among the assessed inoculant**s**. Nevertheless, the inoculant RAHE Probio was excellent in producing high agarwood resin due to its refined appearance and darker resin and resembled the wild agarwood. In addition, this result suggested that the bamboo stick and dripping method might be one of the suitable treatments to stimulate agarwood formation since it is easy to inoculate, locally available, and has a slightly low cost of operation[15]. Thus, the bamboo stick and dripping technique, similar to the aeration method, was remarkable, stimulating higher agarwood yield than the existing wounding method[19].

Conclusion

Conclusively, in the present study, four techniques and five inoculants were analyzed and compared. This study discovered that inoculant IGB711 could stimulate the highest agarwood production for 24 months incubation period. However, based on the cost-effective assessment, agarwood production using the inoculant RAHE Probio in combination with a bamboo stick and dripping technique would be more economically profitable for rural agarwood tree growers. Henceforward, the induction technique and responsiveness of *Aquilaria* trees to stimulation play a decisive role in improvising production of agarwood resin. Therefore, more research on this topic needs to be undertaken before the inoculant and induction technique association is more clearly understood.

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