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Production of Agarwood Resin in *Aquilaria beccariana* Using Inducement Technology

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ABSTRACT

Agarwood is a type of resin impregnated wood produced from the wounded *Aquilaria* trees. This agarwood gives a pleasant fragrant when it is burned. It becomes high-priced and increase demanded in the world due to the depletion of wild agarwood in the forest caused by illegal poaching activities. Agarwood resin can only be produced by injuring caused by lightning or wounded by animals under natural conditions. However, the natural process of resin accumulation is uncertain and time-consuming. Therefore, we developed an agarwood inducement technique that served as the alternative way to induce the agarwood formation in a short time. Three inducement techniques, including the injecting method, knocking method and combination of injecting & knocking method were applied to induce resin formation. In this study, we evaluated the technique for producing agarwood in species *Aquilaria beccariana,* which is native and only can be found in Borneo Malaysia. For *A. beccariana* trees treated with the inducement technique, resin formed and spread throughout the cell in the trunk. The evaluation results showed that the agarwood yield per tree reached around 5 to 7 kilogram. Furthermore, this agarwood derived from the induction was found to have a similar quality with the wild agarwood. This indicates the inducement technology had successfully produced agarwood resin in *A. beccariana* with a grade similar to the wild agarwood.

Keywords: Agarospirol, Agarwood, *Aquilaria beccariana*, Inducement technology, Resin formation

INTRODUCTION

Aquilaria spp. is a tropical woody tree grown well in South East Asia and the main source of agarwood (Rogers, 2009). Agarwood is produced from the resin deposits which are found in tree species of the genera *Aquilaria* and *Gyrinops*. This scented agarwood contains resin known as oud in Arabic. Agarwood becomes expensive and highly demanded in the world due to the depletion of wild agarwood in the forest caused by illegal poaching activities. The agarwood is mainly for the cultural, medicinal and religious needs of societies from the Middle East countries right across Asia to China, Korea and Japan. Agarwood has been widely used in the production of high-grade incense and perfumes (Lim & Noorainie, 2010). However, the demand for agarwood currently far exceeds the available supply, which is naturally restricted owing to the nature of its formation. The agarwood

is only found in a small portion of *Aquilaria* or *Gyrinops* trees of those species known to produce it (TRAFFIC, 2000). Moreover, the conventional methods to produce resin in *Aquilaria* tree do not give a promising result due to the less understanding of the plant response system (Barden, 2007).

Four species of Aquilaria are native to Malaysia included *Aquilaria malaccensis, Aquilaria microcarpa, Aquilaria beccariana* and *Aquilaria hirta* (CITES, 2004) (CITES, 2005a & 2005b). Other species such as *Aquilaria crassna, Aquilaria subintergra and Aquilaria sinensis* are brought in from Thailand, Vietnam, Cambodia and Hainan. Among the native species, *A. beccariana* is especially found only in Borneo Malaysia. Agarwood resin can only be produced by injuring caused by lightning or wounded by animals under natural conditions. However, the resin produced was very low in yield and only accumulated at certain spots where the injuries took place (Pojanagaroon & Kaewrak, 2006). Moreover, the natural process of resin accumulation is a time consuming process, it usually takes 10 to 20 years to accumulate enough resin for harvesting. To overcome the problem, the agarwood inducement technology has been developed to enhance the resin production and incorporated with a special technique, which maximized the resin accumulation throughout the stem and shortened the resin production time to 1 to 2 years.

So far, the agarwood inducement products available in the market now are those imported from other countries such as Thailand, Vietnam, and America-(Blanchette & Heuveling, 2009). Those products have not been tested in Malaysia especially using our native species. As a result, the effectiveness of the imported products remains unknown. We have the experience in developing the agarwood inducement technology as well as in analyzing the performance of the inducement technique, which can help in evaluating and analyzing the currently imported agarwood inducement products. Besides, to educate the public in term of the actual value of *Aquilaria* tree, awareness of the existence of fake agarwood inducement products in the market and also to reduce foreign poachers in our forest and home garden.

Although we have developed the agarwood inducement technology, the studies in agarwood continue to look into the new aspect to produce sustainable resin production in most of the *Aquilaria spp*. From the previous studies, our inducement technology has been studied and tested for a few years in *A. malaccensis* and *A. crassna* but not much study has been conducted in *A. beccariana*. Due to this purpose, a study of the performance of agarwood inducement technology in species *A. beccariana* will be conducted to enhance the understanding of the efficiency of the inducement technology for higher grade agarwood in different species. The mechanism of agarwood formation in *A. beccariana* is believed triggered by the mechanical and chemical inducement that bring the stress to the plant system. This is a novel study in *A. beccariana* related to inducement technology.

MATERIALS AND METHODS

Plant material

The inducement experiment was carried out at Kampung Timbang, Kota Belud, Sabah, Malaysia. Approximately 30 stands of the wild *A. beccariana* trees with 100 to 300 cm in girth and 20 to 30 m in height were selected for this study in the surrounding forest area near Kampung Timbang (Table 1). The inducement experiment was conducted for almost 2 months in the forest area at Kota Belud, Sabah, where especially skilled workers were hired to carry out the inducement techniques according to the experimental design.

Table 1. Specimen *A. beccariana* trees treated with different inducement methods in Kampung Timbang, Kota Belud, Sabah.

Inducement Method	Number of Tree	Average Height (ft)	Average Girth (cm)
Injecting (IM)	10	79.5	191.7
Knocking (KM)	10	84.0	193.6
Injecting+Knocking (IKM)	10	83.5	222.4

Experimental design and statistical analysis

The experimental design used in this study was the randomized complete block design (RCBD), with 3 different inducement treatments and each treatment had 10 replicates. Analysis of variance (ANOVA) was generated, and the agarwood yields were determined. The significances of all treatments were judged statistically by computing the p-value <0.05.

Inducement techniques

Three inducement techniques, including the injecting method, knocking method and combination of injecting & knocking method were applied to induce resin formation. Each inducement technique was tested in ten trees respectively. The pure water treatment was taken as a negative control (NC), and healthy wood as a blank control (BC). The composition of the agarwood inducement solution used in this study contained acetic acid, sodium chloride, and fruit enzymes.

Injecting method (IM)

Two holes were made per 10 cm^2 area of stem, with 5 cm deep into the phloem or xylem were drilled in a spiral from the ground of the main trunk by an electric impact drill (Model GSB 500, Bosch) with drill bit No. 8 (Fig. 1). Approximately 5 ml of agarwood inducement solution was slowly injected into the xylem or phloem tissue through a syringe.

Knocking method (KM)

Two holes were made per 10 cm^2 area of stem, with 5 cm deep into the phloem or xylem were drilled in a spiral from the ground of the main trunk by an electric impact drill (Model GSB 500, Bosch) with drill bit No. 8 (Fig. 1). Each hole was inserted a bamboo stick soaked overnight with the agarwood inducement solution. A bamboo stick with 6 cm in length and 1 cm in diameter was soaked with the agarwood inducement solution for overnight. The bamboo stick was then inserted into each hole.

Injecting with knocking method (IKM)

Two holes were made per 10 cm^2 area of stem, with 5 cm deep into the phloem or xylem were drilled in a spiral from the ground of the main trunk by an electric impact drill (Model GSB 500, Bosch) with drill bit No. 8 (Fig. 1). Each hole was applied with the agarwood inducement solution using injecting method and knocking method alternately.

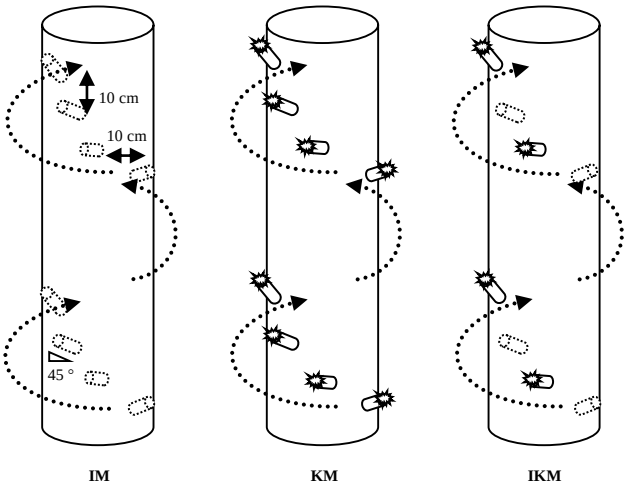


Fig. 1. The diagram of injecting, knocking and injecting+knocking method used in the inducement techniques in *A. beccariana*.

Monitoring and sampling

The inducement performance was monitored 6 and 12 months after inducement and the sampling of the wood chips at 5 different holes were carried out randomly for all the 30 trees.

Harvesting

One of the trees was harvested after 18 months from each inducement technique and was evaluated for the agarwood inducement performance. Agarwood wood chips were crafted from the trees and were used for quantitative and qualitative analysis.

Agarwood yield estimation

As agarwood was formed inside the tree stem, to accurately measure the agarwood yield per tree, we had separated the agarwood resin from the white wood and dried it in a 40°C oven for 15 days. After weighing the dry weight (M_n), the estimated agarwood yield of the tree was calculated using the following formula (Eqn. 1).

$$\text{Estimated Yield (kg)} = \frac{M_1 + M_2 + M_3}{3} \times \frac{L}{5} \qquad \textbf{Eqn. 1}$$

Where M_1, M_2 and M_3 represent the dry weight of resinous wood respectively isolated from the 5 cm thick cross-sections, and *L* is the length of a trunk containing agarwood (Fig. 2). The formula above for estimates of agarwood yield per tree is an ideal model used in the study of the whole-tree agarwood-inducing technique developed by the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College (Liu *et al.*, 2013).

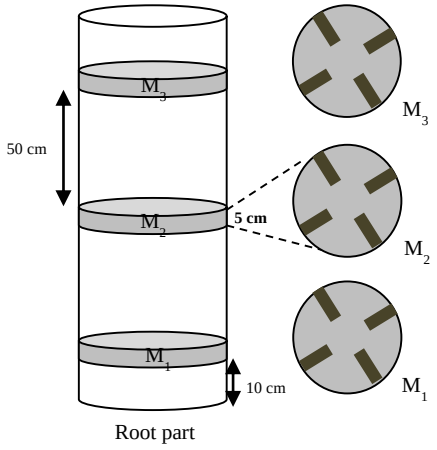


Fig. 2. The diagram of the trunk of an *A. beccariana* tree and the agarwood yield estimation method used in this study (Liu *et al.*, 2013).

Material processing

To evaluate agarwood quality, the resinous wood from each inducement technique, as well as the wild agarwood as control were ground into powder.

Quality analyses

The agarwood oil was extracted using the hydro-distillation unit in Laboratory. Approximately 500 g of the agarwood of IM, KM and IKM were ground into a powder and extracted in water for 8 hours. The agarwood oil was condensed in a collecting tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask. The extracted agarwood oil was collected and stored in a sealed bottle at room temperature for further analysis. The chemical constituents in the agarwood oil were send to the chemical analysis service provider laboratory at Natural Product Division, Forest Research Institute Malaysia (FRIM) to identify using a Gas Chromatography Mass Spectrometer (GC-MS). The GC-MS conditions (Xie *et al.*, 2013) are listed in Table 2.

Table 2. GC-MS conditions used in the study.

Gas chromatography mass spectrometer	Agilent 7890A/5975C
Capillary column	HP-5MS
Oven program	
− Initial temperature	60°C
− Initial time	10 min.
− Rate	3°C/min.
− Final temperature	230°C
− Final time	1 min.

RESULTS AND DISCUSSION

The agarwood inducement solution was applied into the drilled hole of *A. beccariana* trees through different methods. The agarwood inducement solution was spread through the xylem tissue due to water transpiration pressure and induced the agarwood formation (Blanchette, 1992). Agarwood resin formed over several months throughout the trunk of the tree. A dark line from the top to bottom of each hole contained the resin was observed on the sampling samples after 6 months (Fig. 3) and 12 months (Fig. 4) of inducement. The trunk was cut down from 10 cm above the ground at harvesting time. Agarwood was separated from the white wood by carving. The agarwood resin was formed and located in each treated hole throughout the whole tree.

Agarwood resin formed rapidly after induced by the agarwood inducement solution. The resin accumulated in the wood over time appeared as the brown ring in the first 6 months (Fig. 3). A thick resinous layer was observed all over the trunk after 12 months indicates the resin was actively secreted after triggered by the agarwood inducement solution (Fig. 3). The color of the resin formed was correlated to the amount of the resin accumulated. The darker the resin, the more the resin accumulates. After 18 months from the inducement, dark brown resinous wood formed inside the tree.

One of the induced trees with IM, KM, and IKM methods respectively were harvested after 18 months for the yield estimation due to the budget constraints and difficulty working in the deep forest. All of the trees induced with agarwood inducement solution developed a thick layer of dark brown agarwood resin. The resin spread through the trunk and formed pieces of agarwood. The cross sections of the trees induced by the IM, KM, and IKM methods all showed resin formation (Fig. 4). However, the NC remained as white wood, with no resin formation, same as BC, indicating that pure water cannot induce agarwood resin formation. Therefore, agarwood inducement solution plays an important role in inducing the formation of agarwood resin.

Fig. 3. The sampling of treated *A. beccariana* at Kampung Timbang, Kota Belud, Sabah after 6 months and 12 months of inducement. Resin formation was observed from 3 different inducement methods, IM, KM and IKM.

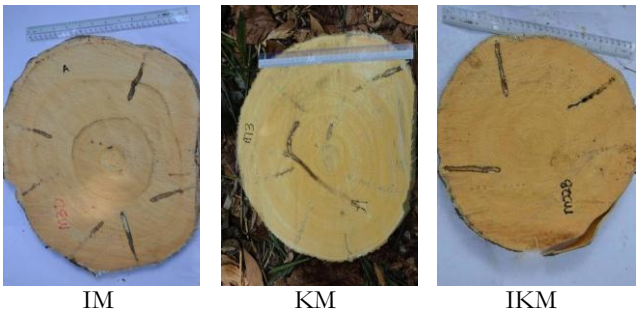


Fig. 4. The cross sections of the trees are induced by the IM, KM, and IKM methods. Results were observed after 18 months of inducement.

In this experiment, to accurately measure the agarwood yield per tree, the agarwood was separated and carved from the white wood to produce the agarwood pieces. The pieces of agarwood produced from each different inducement were weighed and measured. The results showed that the dry weight of agarwood pieces separated from the 30 cross sections for IM, KM and IKM were 0.645, 0.473 and 0.523 kg respectively (Table 3). The One-Way ANOVA statistic results showed that the standard deviation is 0.0054. The *f*-ratio value is 11.18157. The *p*-value is 0.000048. The result is significant at $p < 0.05$.

Table 3. The estimated resin yield was produced by different inducement methods.

Inducement Technique	Total Dry Weight of Agarwood	Estimated Yield of Agarwood Resin/Tree (kg)
Injecting (IM)	0.645	7.095
Injecting+Knocking (IKM)	0.523	5.203
Knocking (KM)	0.473	5.753

After weighing the dry weight (M_n), the estimated agarwood yield of the tree was calculated using the formula given below (Eqn. 2). In this study, 30 cross sections with 5 cm thickness were cut from each tree and the total length of the whole trunk containing agarwood resin was about 1650 cm.

$$\text{Estimated Yield (kg)} = \frac{M_1 + M_2 + \ldots. + M_{30}}{30} \times \frac{1650}{5} \qquad \textbf{Eqn. 2}$$

The results showed differences in estimated yield produced by IM, KM and IKM methods. The agarwood

induced by IM method showed better results in terms of the average yield, roughly 7.1 kg per tree and followed by the KM method with 5.7 kg per tree and the IKM method with 5.2 kg per tree (Table 3). The estimated yield per tree by IM method was 20% higher than the KM method. No agarwood resin was formed in the NC and BC samples.

The result showed all the three inducement methods IM, KM and IKM were able to produce agarwood resin due to the proven agarwood inducement solution was used in this experiment. However, the difference between these three methods is the IM has directly applied the agarwood inducement solution into the tree through each hole without sealed up the hole. Where KM the agarwood inducement solution was absorbed into a bamboo stick overnight before applied the bamboo stick into each hole to seal it. The IKM was the combination of both IM and KM.

The Principe of applied the bamboo stick in this study is to create a slow release system of the agarwood inducement solution into *Aquilaria* tree. Bamboo material has an extraordinary micro-structure that has a high absorptive capacity. The bamboo stick absorbed with the agarwood inducement solution was applied into the hole and sealed to form a closed system. The agarwood inducement solution was released slowly from the bamboo stick and absorbed into the tree. The whole process took around a week to complete compared to the IM method where the agarwood inducement solution was directly injected into the hole and absorbed into the tree within an hour.

The results showed that the agarwood produced by the IM method has slightly bigger in size and lighter in colour compared to the agarwood produced by the KM method. The differences in size and colour are due to the absorption mechanism of agarwood inducement solution in *A. beccariana*. The IM method was a fast release system. The agarwood inducement solution was absorbed directly into the tree and spread through the xylem due to the water transpiration pressure. Therefore, the resin formed and accumulated along the xylem in a longer and bigger shape (~12 cm) (Fig. 5).

On the other hand, the KM method was a slow-release system. The agarwood inducement solution was released slowly and absorbed little by little into the tree. The agarwood inducement solution was not able to spread through the xylem. Therefore, it only managed to induce the cell around the hole and formed resin in a smaller shape (~4 cm). However, the amount of resin accumulated at this site was more compact which indicated by the darker color of agarwood. The localized of resin accumulation due to the continuously induction mechanism caused by the slow release system (Fig. 5).

Fig. 5. The agarwood produced by the IM (A) and KM (B) inducement methods.

Agarwood oil is a complex mixture of aromatic terpene compounds included monoterpenes, sesquiterpenes and diterpenes (Naef, 2011; Chen *et al.*, 2012). The main compounds present in agarwood oil have been identified as the sesquiterpenes. In this study, the agarwood oil extracted from the induced agarwood with IM, KM and IKM were analyzed by GC-MS and compared to the control agarwood oil produced from the local agarwood oil extraction plant, Kedaik Agarwood Sdn. Bhd.

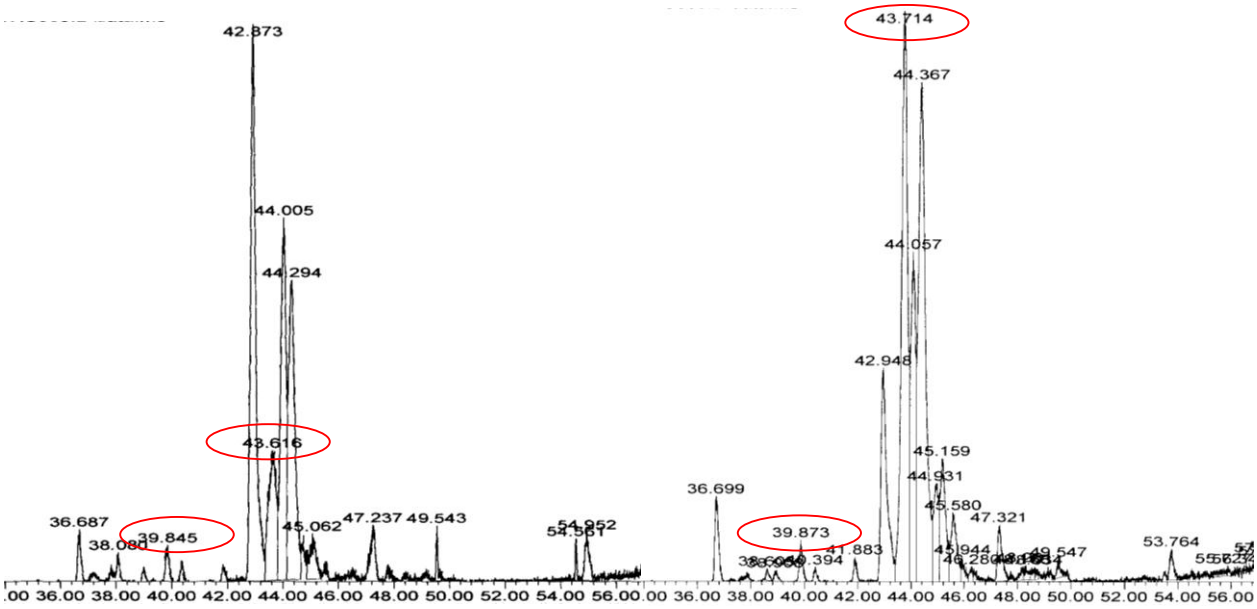
According to the GC-MS analysis data, the agarwood oil marker compounds such as the agarofuran and

agarospirol in the sesquiterpene group had been detected in the agarwood oil (lshihara *et al.*, 1991; lshihara *et al.*, 1993; Näf *et al.*, 1995; Nakanishi *et al.*, 1983; Pant & Rastogi, 1980). The result from the control agarwood oil showed the marker compound agarofuran and agarospirol were found at the retention time (RT) 39.845 and 43.816 respectively. Similar results were also found in the agarwood oil of SF022(IM), SF023(IKM) and SF024(KM) extracted from *A. beccariana.* Agarofuran was detected at RT 39.7-39.8 and agarospirol were detected at RT 43.7 (Table 5).

Table 5. The composition of induced agarwood oil compared to the control agarwood oil from Kedaik Agarwood Sdn. Bhd.

Composition of The Agarwood Oil	**Retention Time**			
	Control	**IM**	**KM**	**IKM**
Agarofuran	39.845	39.873	39.732	39.712
Agarospirol	43.616	43.714	43.735	43.702

The GC-MS results showed the agarwood oil extracted from the *A. beccariana* induced by inducement technology have a similar quality compared to the control agarwood oil which was extracted from the naturally produced agarwood (Fig. 6). In other words, the agarwood oil produced by the inducement technology had achieved the quality of naturally produced agarwood oil.



Control IM

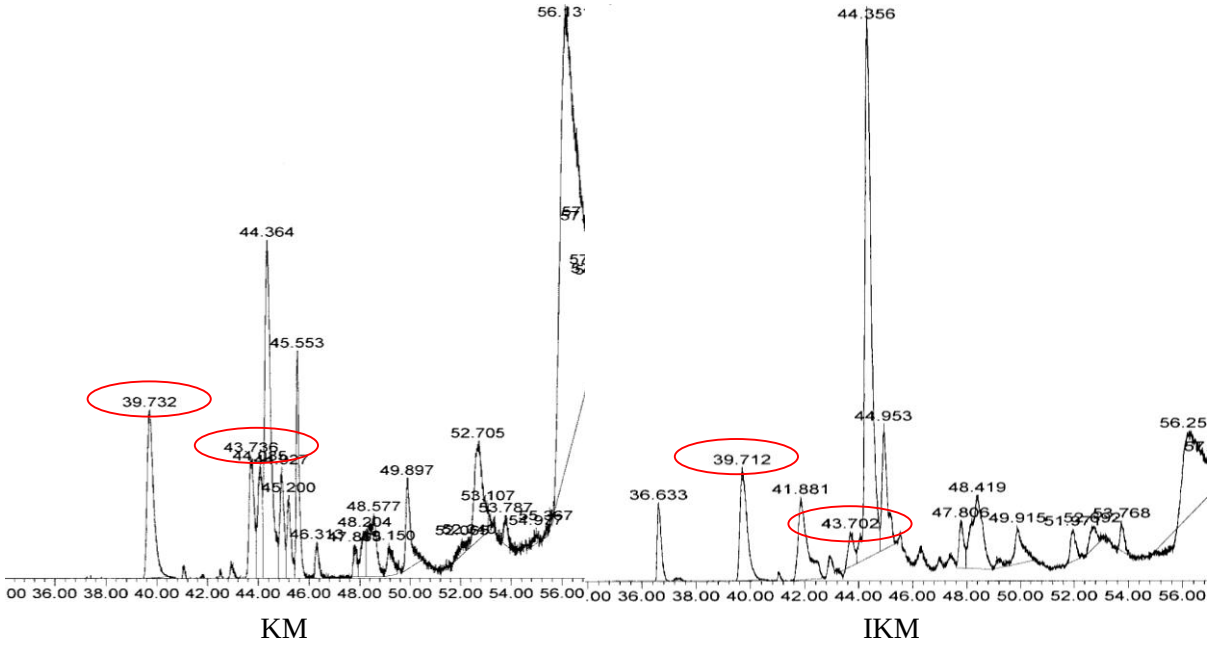


Fig. 6. GC-MS chromatogram for the control agarwood oil and the induced agarwood oil by IM, KM and IKM techniques.

CONCLUSION

The inducement technology used in this study is a proven technology in producing the agarwood in *A. malaccensis* and *A. crassna*. The results from this study showed that once again this inducement technology had successfully produced the agarwood from *A. beccariana*. This inducement technology was able to trigger the plant metabolism system to produce the resin which accumulated in the plant cell. The inducement technique such as the IM and KM showed differences in the yield due to the different methods of applying agarwood inducement solution into the tree. The IM method showed 20% higher than the KM method in terms of yield.

For the quality evaluation, the agarwood oil produced by induced agarwood from *A. beccariana* showed similar quality with the agarwood oil produced naturally. In conclusion, the inducement techniques evaluated in this study had successfully produced the agarwood that closely resembled the wild agarwood in terms of quality in *A. beccariana*.

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REFERENCES

Barden, A., Anak, N. A., Mulliken, T., & Song, M. (2007). *Heart of the matter: agarwood use and trade and CITES implementation for Aquilaria malaccensis*. TRAFFIC Southeast Asia. Petaling Jaya, Selangor, Malaysia. 60 pp.

Blanchette, R. A. (1992). Anatomical responses of xylem to injury and invasion by fungi. p. 76-89. In *Defense Mechanisms of Woody Plants Against Fungi*. Blanchette, R. A. and Biggs, A. R. (eds.). Springer-Verlag, Germany. 81 pp.

Blanchette, R. & Heuveling, V.B.H. (2009). Cultivated agarwood. US 7638145B2.

Chen, H.Q., Wei, J.H., Yang, J.S. Zhang, Z., Yang, Y., Gao, Z.H. Sui, C., & gong, B. (2012). Chemical constituents of agarwood originating from the endemic genus *Aquilaria* plants. *Chemistry & Biodiversity 9*, 236-250.

CITES (2004). Amendments to appendices I and II of CITES. In: *Proceedings of thirteeth meeting of the conference of the parties*. 2 October 2004, Bangkok, Thailand.

CITES (2005a). The trade and use of agarwood in Taiwan, Province of China, http://www.cites.org/common/com/pc/15/x-pc15-07-inf.pdf.

CITES (2005b). The trade and use of agarwood in Japan, http://www.cites.org/common/com/PC/15/X-PC15-06-inf.pdf.

IUCN (2009). 2008 IUCN red list of threatened species, http://www.iucnredlist.org.

Lim, T.W. & Noorainie, A.A. (2010). Wood for trees: A review of the agarwood (gaharu) trade in Malaysia. TRAFFIC Southeast Asia, Petaling Jaya, Selangor, Malaysia.

Liu, Y. Y., Chen, H. Q., Yang, Y., Zhang, Z., Wei, J. H., Meng, H., Chen, W. P., Feng, J. D., Gan, B. C., Chen, X. Y., Gao, Z. H., Huang, J. Q., Chen, B. & Chen, H. J. (2013). Whole-tree Agarwood-Inducing Technique: An efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. *Molecules 18*, 3086-3106.

lshihara, M., Tsuneya, T., & Uneyama, K. (1991). Guaiane sesquiterpenes from agarwood. *Phytochemistry 30,* 3343-3347.

lshihara, M., Tsuneya, T., & Uneyama, K. (1993). Fragrant sesquiterpenes from agarwood. *Phytochemistry 33*, 1147-1155.

Naef, R. (2011). The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: A review. *Flavour and Fragrance Journal 26*, 73-89.

Näf, R., Velluz, A., Brauchli, R., &Thommen, W. (1995). Agarwood oil (*Aquilaria agallocha* Roxb.). Its composition and eight new valencane-, eremophilane- and vetispirane- derivatives. *Flavour and Fragrance Journal 10*, 147-152.

Nakanishi, T., Yamagata, E., Yoneda, K., Miura, I., & Mori, H. (1983). Jinkoh-eremol and Jinkohol II, two new sesquiterpene alcohols from agarwood. *Journal of Chemical Society, Perkin Transaction 1*, 601-604.

Pant, R. & Rastogi, R.P. (1980). Agarol, a new sesquiterpene from *Aquilaria agallocha*. *Phytochemistry 19*, 1869-1870.

Pojanagaroon, S. & Kaewrak, C. (2006). Mechanical methods to stimulate aloes wood formation in *Aquilaria crassna* Pierre ex H Lee (kritsana) trees. *ISHS Acta Horticulturae 676*, 161-166.

Rogers, Z.S. (2009). *A world checklist of Thymelaeacceae* (version 1). Missouri Botanical Garden: St. Louis, MO, USA.

Xie, Z., Liu, Q., Liang, Z., Zhao, M., Yu, X., Yang, D., & Xu, X. (2013). The GC/MS analysis of volatile components extracted by different methods from *Exocarpium Citri Grandis*. *Journal of Analytical Methods in Chemistry* doi:10.1155/2013/918406.

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