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# RECENT ADVANCEMENT IN AGARWOOD INDUCTION TECHNOLOGY: A COMPREHENSIVE REVIEW FOR THE TRANSFORMATION OF ARTIFICIAL AGAR RESIN INDUCTION METHODS

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## ABSTRACT

*Agarwood is a high valued resinous wood containing aromatic constituents of sesquiterpenes and phenyl-ethylchromones. They are typically found in the wood tissue of Thymalaeaceae family trees such as Aquilaria and Gyrinops species once they have been physically or chemically damaged or diseased by microbial pathogens. However, the natural occurrence based agarwood production is inadequate to fulfill the worldwide market demand as it never reach to the potential yield. As a result, recent advancements in artificial agarwood induction technology have led to the efficient production of agarwood resin, surpassing conventional methods. These technologies include mechanical, biological, and chemical methods, as well as combinations of these techniques such as fungal inoculation, nailing, drilling, partly trunk pruning, aeration method, agar-wit, C-A kits, Agar-sit, Bottle dipping, ChemJet, Pinhole infusion, Automated transfusion, Agar-Bit and Bamboo stick method. While interdisciplinary approaches have pros and cons, chemical inducers have shown rapid invasion inside particular tree species trunks to produce superior agarwood resin at consistent rate. Therefore, diverse forms of inducers can be utilized to develop this finest fragrance resinous wood in cultivated trees within a short period of time compared to the natural approach and almost similar in the quality compared to the natural agarwood formations. The objective of this review paper was to presents a comprehensive collection of agarwood resin inducing methods and their potential to enhance the total production and the quality of agarwood in the final harvest. The application of these techniques has significant implications to the agarwood industry as it seeks to meet the growing global demand for this highly sought-after and valuable product leading to a high profitable business. Therefore, this review article serves as a valuable collection of resource for the researchers and industry professionals who are ambitious to develop their agarwood industry to a new level.*

***Keywords: Agarwood, Aquilaria species, Artificial methods, Fragrance industry, Resin induction***

## INTRODUCTION

Out of the forest products in the world, Agarwood is one of the most luxurious and expensive commodities that has a higher market demand via ranked as the most valuable number one product in the world out of the available plant-based aromatics (Kanazawa, 2017; López-Sampson and Page, 2018). This fragrance resin is formed due to a self-defense mechanism in the genera Aquilaria and Gyrinops, that in the family Thymalaeaceae. However not each species of this genus produces high-quality agarwood, and not all release the desired aromatic odor. In the natural habitat, around 10% of the agarwood producing Aquilaria plants may contain this resinous wood (Ngadiran *et al.,* 2023). Agarwood formation will be initiated when the self-defense mechanism activates inside the tree and the sickness of the trees are responsible for this resin formation. (Zhang *et al.,* 2012). The trees are particularly responsive to physical damage, chemicals, and microbial infections and produce agarwood. There are natural methods and artificial methods for agarwood induction. Agarwood resinous develops naturally only when trees are exposed to particular environmental conditions. For example, wildfires, grazing, attacks stem boring insects, lightning strikes, or microbial activities (Liu *et al.,* 2019; Fitriasari *et al.,* 2021). Agarwood resin formation in a natural manner is a very rare phenomenon in the wild up to the expected severity and when considering commercial agarwood production, artificial inoculation methods should be practiced. Without the inoculation process, only 1-2% yields can be expected from the full potential, and the productivity of the trees is not enough for profit gaining. Furthermore, natural development takes time more than artificial methods. Generally, this takes around 25 to 30 years (Chowdhury *et al.,* 2016; Wu *et al.,* 2017).

In fact, the low rate of agarwood availability in the natural habitat leads to insufficient supply to reach the market demand (Chhipa and Kaushik, 2017). Therefore, these species are dangerously close to going extinct as a direct result of the illegal harvesting of trees in the wild and the limited availability of agarwood development inside those plants (Lee and Mohamed, 2016). Therefore, cultivating the agarwood-producing trees became the trend and established the industry in a legal manner. These farmers use a different kind of techniques to initiate the formation of agarwood as well as get a productive harvest from those methods. Some of them are conventional methods and some are novel techniques that have been developed by going through scientific research. Indigenous methods such as placing nails, chopping the tree by using an axe, or making pinholes, burning, trunk splitting, and extensive removal of the outer bark were used commonly by small-scale farmers and wild agarwood hunters (Tian *et al.,* 2013; Yan *et al.,* 2019). There also some techniques that the farmers were used to develop the agarwood inside the trees by using beneficial insects and snails by providing a desirable environment for them in the trees. Such as drilling the main branches, trunk, and roots of older trees facilitate a desirable environment for snails and insects like ants within the trees (Akter *et al.,* 2013).

Conventional methods for agarwood development are more affordable, but those methods are labor-intensive and time-consuming. Furthermore, the amount of agarwood production is low and cannot predict the quality of the resinous wood and the extracted oil (Tan *et al.,* 2019). Also, as a result of some methods that under conventional methods badly influence the tree by which frequently inhibiting the tree's growth, in some cases the tree died after practicing these methods. (Liu *et al.,* 2013). Because of this, the agarwood industry always seeking novel and efficient methods to induce resinous production in order to protect wild resources from such declines. Many artificial methods for inducing agarwood resin have been developed to produce agarwood that has higher quality and is similar to agarwood that were in natural habitats.

Nowadays, the industry utilizes combination methods that are based on physical damages and use different kinds of chemical inducers as well as bioinoculants (Faizal *et al.,* 2020). The invention of a synthetic technique for agarwood production has a twofold objective: enhancing both the yield and the quality of both the raw agarwood resinous wood and the extracting essential oil. Nonetheless, these distinct strategies have their own set of pros and cons. In this article, we have endeavored to provide a detailed and up-to-date account of the various technologies employed for agarwood production. Our review encompasses a broad range of methods and techniques, including both traditional and modern approaches.

### *Agarwood Formation*

Agarwood is formed after the wound infection of healthy plants, and better healthy sound trees never produce agarwood (Rasool and Mohamed, 2016). Initially, it remains in tylose or gel form after initiating the formation. Agarwood's antimicrobial and anti-disease properties are due to sesquiterpenes (Xu *et al.,* 2013). The development of Agarwood within trees is unrelated to the diameter and height of the plants, as well as the volume of timber. However, some resin characteristics and aroma properties can be influenced by species, environmental variations, genetic variation of species (Ngadiran *et al.,* 2023), and geographical distribution, as well as the inoculation method (Subasinghe and Hettiarachchi, 2015). Furthermore, seasonal changes and rainy weather can hasten this formation faster than other seasons (Chowdhury *et al.,* 2016). Previous studies show factors like soil fertility, temperature, light intensity, and humidity influence this formation process. When it comes to yield, plants grown in poor soil produce more agarwood than trees grown in rich soil. However, when soil conditions are in a good manner, trees develop well and produce resin for their self-defense throughout the inoculation period, contributing to high-quality agarwood (Hamdan *et al.,* 2021). Plants are more susceptible to infections that are cultivated at higher elevations (Turjaman *et al.,* 2016). Also, the ability to produce agarwood in juvenile trees is higher than in matured trees (Rasool and Mohamed, 2016).

Agarwood formation takes several years, and older trees respond to inoculation more slowly than younger trees, implying that agarwood formation in older trees is uncommon (Liu *et al.,* 2013). In contrast, even three-year-old cultivated trees can produce agarwood after artificial treatment (Rasool and Mohamed, 2016). It was confirmed through a chemical analysis by Rainforest Project (TRP) in Vietnam (Ngadiran *et al.,* 2023).

The agarwood formation is linked to starch and sugar metabolism and electron microscopic observations of *A.sinensis* confirmed this. The healthy wood contained a large number of starch granules, which were degraded after the wounds were placed and the Agarwood accumulated (Xu *et al.,* 2013). When it comes to agarwood formation, living parenchyma cells are the most valuable component because they perform the biosynthetic process of resinous substances (Rasool and Mohamed, 2016).

### *Inoculation methods*

The inoculation process is critical for the formation of agarwood within the Thymelaeaceae family species. Because the tree's self-defense mechanism should be activated, and resins should be produced because of the tree's illness. The theory behind this inducing is that the trees are being intentionally damaged (Zhang *et al.,* 2012).

There are two ways to inoculate the trees. Those are natural and artificial. Inoculation of trees occurs rarely naturally, and when comes to commercial agarwood production, artificial inoculation methods should be used. The productivity of the trees is insufficient for profit without a synthetic inoculation process. When all these methods and inducers are considered, the chemical composition and quality of the outcome can be varied (Lee and Mohamed, 2016).

### *Natural formation*

Gnawing ants and other insect damages, as well as other natural damages to trunks or branches, are commonly responsible for these damages. Bacterial infections, physical damage from wind, and lightning strikes may also be factors in the formation of agarwood inside trees (Xu *et al.,* 2013). Some caterpillars also bore the trunk, causing agarwood to form (Turjaman *et al.,* 2016). It has been observed that these things have a very low probability of occurring and naturally inducing the agarwood (Subasinghe and Hettiarachchi, 2013). It around 7% of trees are produce agarwood in their natural habitat (Chowdhury *et al.,* 2016). When comes to *Aquilaria spp.,* it's about 10% (Liu *et al.,* 2013).

### *Artificial inoculation techniques*

Artificial agarwood induction is a successful approach for obtaining a fruitful agarwood harvest from commercial agarwood plantations (Yin *et al.,* 2016). The traditional method of inducing agarwood is to make wounds in the tree with sharpened tools. Chemical methods are also used for this, particularly in large commercial-scale agarwood plantations. It is the most recent method used in this industry (Subasinghe and Hettiarachchi, 2013). Among these artificial methods, small-scale and large-scale farmers commonly use nailing, drilling, aeration, agar-wit, partially-trunk pruning, burning-chisel drilling, fungal inoculation, CA kits, agar-sit, agar-bit, bamboo stick method, etc. (Liu *et al.,* 2013). The chemical-inducing approaches are more effective and cost-effective, and they are also easier to practice than others. Moreover, results are also provided in a timely manner (Rasool and Mohamed, 2016). However, in some countries, this method has become unpopular due to the environmental impact of these chemicals leaking into the water and causing soil pollution (Turjaman *et al.,* 2016). Indigenous techniques, such as peeling off the bark and promoting infection, are also used in some countries to induce this process. There could be other reliable artificial methods for producing agarwood (Chowdhury *et al.,* 2016). The method of inoculation can have an impact on the agarwood's quality. For example, axe chopping, holing, and nailing yield low-grade agarwood. According to studies, the resin and oil content of chemically induced agarwood is higher than that of wild agarwood (Zhang *et al.,* 2012).

According to research, pure water cannot induce the formation of agarwood within trees (Liu *et al.,* 2013). If the trees are heavily attacked by insects such as *Heortia vitessoides*, the endophytic fungal inoculation process is not recommended because the trees are already sick and may die. (Turjaman *et al.,* 2016).


**Fig. 1.** Extracted agarwood A) Naturally formed resinous wood extracted from *G. walla* B) Artificially induced resinous wood extracted from *A. crassna*

### *Fungal inoculation technique*

The basic principle of this method is the development of fragrance resin inside the tree by making wounds and inoculating the tree with beneficial fungi species (Ngadiran *et al.,* 2023).

To produce this artificial infection, pure or mixed fungus strains can be utilized in the fungal inoculation procedures. The wood gets a richer or dark crimson color after a fungal infection (Elias *et al.,* 2017). An infection may enhance resin synthesis when the host responds to the rise in infection induced by fungal growth. The variety of sesquiterpenes retrieved from mycological metabolites is related to these compounds' strategies for cooperating with other species and defending themselves, culminating in the creation of resinous wood (Rohlfs and Churchill, 2011).

The fungi are inoculated into 8 cm deep holes drilled in the tree trunk. The bores in the tree trunk will begin 50 cm above ground level. Holes should be 20 cm apart in the vertical distance, with about 2-3 holes in a horizontal line around the perimeter (Liu *et al.,* 2013). After the holes have been drilled, the inoculation can be carried out using the culture medium used for fungi growth. After inserting the culture into the hole, it should be wrapped in a rubberized fabric (Chowdhury *et al.,* 2016).

**Fig. 2.** Fungal inoculation by drilling and injecting inoculum

Generally, natural inoculation can be possible by *Aspergillus spp., Botryodyplodia spp., Diplodia spp., Fusarium bulbiferum, F.oxysporum, F.laterium, F.solani, Penicillium spp.*, and *Phythium spp*. like species. And these can benefit from the immunization process (Ngadiran *et al.,* 2023). *Cunninghamella, Curvularia, Lasiodiplodia*, and *Trichoderma* are also used for this purpose in some cases. However, *Fusaruim solani, Cunninghamella bainieri,* and *Lasiodiplodia theobromae* are commonly used in this Inoculation process (Rasool and Mohamed, 2016). *Fusarium solani* is the most effective agarwood-forming agent (Turjaman *et al.,* 2016). Tunstall first used this fungi inoculation technique in 1929. (Liu *et al.,* 2013).

In addition, to use a single fungus, some studies used a combination of species to inoculate *Aquilaria spp*. and examined formulations (Justin *et al.,* 2020; Ma *et al.,* 2021). In addition to *Aquilaria spp.*, and *Gyrinops versteegii,* Domke has been infected with a variety of fungal inoculants, including *F. solani, Rhizopus spp*., and *Trichoderma spp* (Mega and Nuarsa, 2019). *F. solani* strains, Gorontalo and Jambi were inoculated into *G. versteegii* trees in another study and resulted in agarwood formation (Faizal *et al.,* 2020)

When practicing this method high humidity, as well as the carbon sources availability and energy sources, can promote fungal growth. However, not all fungi can initiate the agarwood induction process. When using this method, triangle factors play an important role in agarwood formation in these trees. In that case, the tree as the host, Endophytic fungi as the inoculating agent, and the Environmental are the three triangle factors (Turjaman *et al.,* 2016).

***Drilling method***

An electrical hand drill is typically used to place drill holes in the tree trunk, limbs, and main branches (Akter *et al.,* 2013; Faizal *et al.,* 2017). The drilled pores were spirally placed from the ground up to the crown. Drill holes were spaced 3 to 5 cm apart and then infected with agarwood-inducing powder or remaining open to allow natural agents easier access (Chowdhury *et al.,* 2016). This is a strategy for attracting insects to the tree to infect it. To speed up the infection, the liquid syrup can be added to pores and attract natural agents such as beneficial insects to it. Every 2-3 months, pores are examined and wounded again (Ngadiran *et al.,* 2023).

***Nailing method***

In the past, the most common method was nailing. Hammering nails into the trunk is used in this method. However, the quality of the agarwood produced by this method is not as desired, and it has a low market value (Persoon, 2007). In some cases, when the nails were installed, they were partially inserted into the trunk (Chowdhury *et al.,* 2016). When using this method, a grown tree required an average of 20 kg of nails, which were placed every 10 cm along the tree's length (Turjaman *et al.,* 2016).

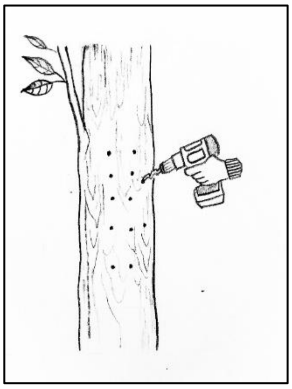

**Fig. 3.** Drilling method

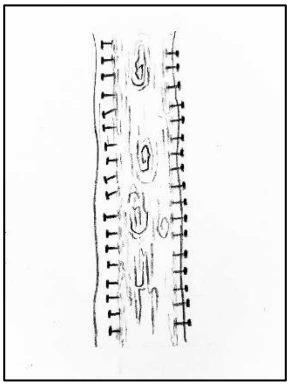

**Fig. 4.** Nailing method

***Burning chisel drilling technique***

The Burning-Chisel Drilling (BCD) method is also based on drilling the tree trunk at a higher temperature. The basic principle of this also creates wounds in the tree and activates its self-defense mechanism. The iron chisel or a drill bit used here is heated to Red-hot around $600^0$C and is 1.2 cm in diameter. The holes created by the drilling will be approximately 20cm apart. Following that, the holes should be instantly sealed by using sterilized paraffin wax and it avoids contamination by detrimental microbes (Chowdhury *et al.,* 2016). It is best to avoid making holes up to 50 cm from the ground (Liu *et al.,* 2013).

***Partly trunk pruning technique***

Partly trunk pruning is practiced by sawing along a side of the tree trunk of the tree and placing cuts that are wide around 2-4 cm and 3-5 cm in depth. The lowest cut should be approximately 50 cm above the ground level. The distance between each pair of cuts should be about 20 cm. It is comparable to axing (Liu *et al.,* 2013). A study shows that the characterization of *A. sinensis* wounded tree trunks induced by BCD and the Agar-Wit method is similar to that of formed resinous wood by PTP. Moreover, the chemical composition, as well as vessel-occlusion formation, are similar to those methods (Zhang *et al.,* 2014). Chinese farmers have increasingly used both burning chisel drilling and partial trunk pruning in recent decades (Liu *et al.,* 2013)

In some areas, indigenous agarwood hunters remove the bark by peeling it to stimulate infection and harvest wood chips from live trees seasonally (Pojanagaroon and Kaewrak, 2003). Agarwood hunters in Papua New Guinea wounded agarwood inducing tree species and initiate agarwood production inside them, and around three years later hunters were able to harvest resinous wood of average quality (Gunn *et al.,* 2003).

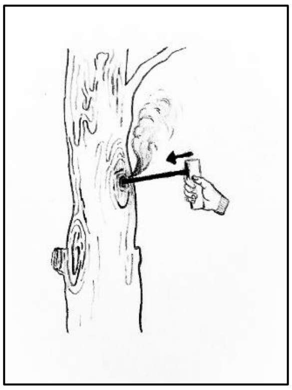

**Fig. 5.** Burning-Chisel Drilling method

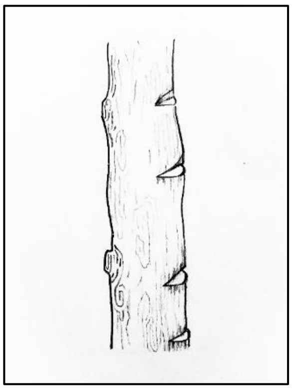

**Fig. 6.** Partly trunk pruning technique

***Aeration method***

The basic principle of this method is introducing a foreign material into a wound that is created artificially and prevents pores from healing and maintaining a long-term infection (Liu *et al.*, 2013). This therapy induces the tree's self-defense mechanisms to respond. The introduced aeration device may have holes in it or grooves on its outside surface or both. Introduce device could be made of plastic, bamboo, proffered wood, or another organic substance, or it could be made of metal, such as iron. Mostly this device is with 2cm diameter. When implanting this, it may extend 2 to 15 cm from the tree's exterior.

A resin-inducing substance may also be applied to the cells around the wound in this approach. It has the potential to kill the parenchyma cells in the vicinity of the xylem's injured region. It could be sodium bisulfate, iron powder, NaCl, chitin, ferrous chloride, ferric chloride, cellobiose, or yeast extract, for example. Also, sodium bisulfate solution, Difco yeast extract, or iron powder can use as a 1:1:3 ratio (Ngadiran *et al.,* 2023). Also, the inducing agent for agarwood formation could be an organism, such as an insect, microbe, or a fungus like *Deuteromyota sp*., *Ascomycota sp*., or *Basidiomycota sp*. This method has the ability to produce agarwood ten times faster than natural agarwood production (Chowdhury *et al.*, 2016).

***Agar-wit method***

Whole tree agarwood induction (Agar-wit) is a high-quality agarwood-inducing method that has been available since 2013. (Liu *et al.,* 2013). This is a chemical-producing method used in industry (Rasool and Mohamed, 2016). This method can produce agarwood within 20 months of applying the treatment to the plant (Zhang *et al.,* 2012). The inoculation process will involve drilling holes in the trunks of the trees up to the xylem and injecting inducers into these holes using a transfusion set. The 5 mm holes will be placed in the tree by avoiding 50 cm of trunk from the ground level. Electrical drills are typically used for drilling.

The benefit of this method is that the natural flow of water in the xylem transports the inducer throughout the tree due to transpiration. As a result, Agar-wit can produce systemic agarwood from the stem of the entire tree. Within a few months, the resin will be produced around the wound (Liu *et al.,* 2013). Low-pH substances such as formic acid, as well as high-pH substances, can be used in this method to induce agarwood inside the trees by killing the live cells (Chowdhury *et al.,* 2016). This method produces superior-quality agarwood with a high resin content as well as ethanol-soluble extract content. When compared to other methods, this method produces a consistent agarwood yield (Liu *et al.,* 2013) in a short

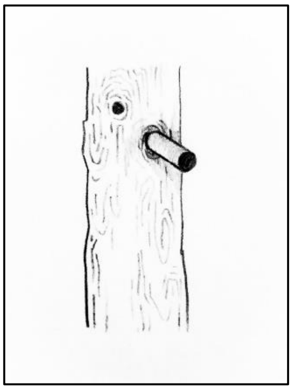

**Fig. 7.** Aeration method

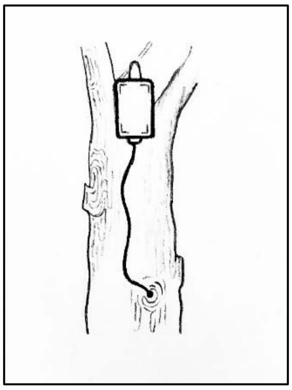

**Fig. 8.** Agar-wit method

period of time (Zhang *et al.,* 2012).

***Cultivated agarwood kits***

The technology comprises a Cultivated Agarwood Kit (CA Kit) for each tree treatment, which contains specific tubes and capsules containing a non-hazardous chemical substance (Blanchette and Chowdhury, 2009). Blanchette from the University of Minnesota in Vietnam invented CA-Kits. To establish CA-Kits in trees, numerous holes should be placed in tree trunks, placing the holes from the base of the stem to the upper end of the stem. As a result, using the CA-Kit, agarwood can be induced and produced near the holes. The basic theory behind this method is to keep the incision open with a small plastic pipe, and then inoculate it with different chemical media into the prepared wound. This chemical therapy is far superior to previous physical wounding treatments since it allows for easy examination of the discolored region (Rasool and Mohamed, 2016).

***Agar-sit method***

To obtain agarwood development meanwhile avoiding the cutting down of a whole tree for harvesting agarwood and using fungal inoculant for agarwood production, an effective approach known as the “Trunk Surface Agarwood-Inducing Technique” or “Surface Inducement Technique” (Agar-sit) was developed. This technique First, roughly 50 cm of bark was removed to reveal a rectangular xylem surface. The bark remained attached to the tree trunk for future coverage. Using a knife, grids (2cm×2cm) 1.5±2.0 cm deep were created. One tree yielded several rectangular barks. between the upper and lower rectangular surface, the distance will be maintained at 20 cm, while the distance between opposite ones on opposite sides of the tree trunk was maintained at 5 cm. The inoculant liquid that contains beneficial fungus was sprayed into the grids by using a watering container or applying with a brush. The infected xylem was afterward enclosed by the original bark. Then six months later, the surface degradation layer was removed, and the agarwood created was severed, leaving the xylem open for another round of agarwood induction.

This strategy protects trees while also making agarwood collection more convenient. Meanwhile, there was a large output and high quality of agarwood available, as well as the possibility of recurrent agarwood creation from the same tree, and this method is well-known among farmers who cultivate agarwood for oil production. This strategy, however, is strongly recommended for trees older than 10 years (Chen *et al.*, 2018).

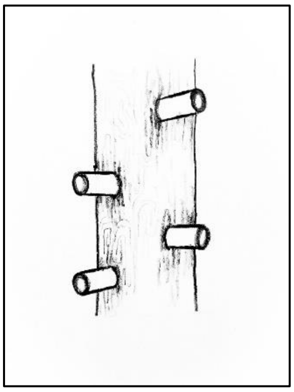

**Fig. 9.** CA Kit method

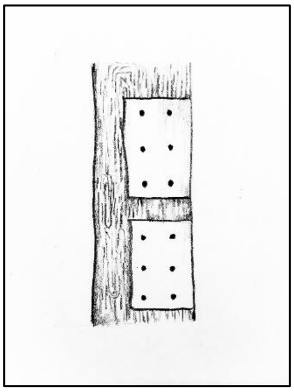

**Fig. 10.** Agar-sit method

### *Bamboo stick method*

In general, the bamboo stick method is also known as the knocking method (KM), and it is practiced alone on agarwood producing trees as well as in combination with the injection method. In this method mainly bamboo sticks or culms are utilized to convey a chemical agarwood inducer as well as a part of the maintaining the aeration process. Because of the microfibrils that surround the epidermis, parenchyma cells, also vascular bundles, it has outstanding mechanical capabilities for this work (Habibi and Lu, 2014). When bamboo sticks or culms are introduced into a tree's trunk, it slowly releases the agarwood inducer into the surrounding tissues of the tree trunk, resulting in a protracted period of fighting between the tree's defense mechanism and then the stress created by the inoculant. The dynamic interplay of trees and constant stress results in the formation of agarwood. A layer of resinous wood is gradually created, and if the ideal atmosphere is fulfilled, this agarwood creation thickens. If the tree is in good health and the tree's defensive mechanism wins, the treated bark will most likely end up in white tissue, where the wounded bark has recovered. An external force from a bamboo stick, on the other hand, can cause major harm, even death, if the treated tree is weak. Farmers can often gain about 5-10 grams of agarwood per hole after about a year and a half of successful treatment using this method. However, a longer time period is preferable for a healthy yield. Furthermore, the harvest quality is comparable to natural Agarwood.

Independently performing the knocking method (KM) as follows, an electric impact drill bit was used to drill two holes per 10 cm2 region of stem, 5 cm deep into the phloem or xylem, in a spiral pattern from the ground of the tree trunk. Bamboo sticks are soaked overnight in the agarwood inducement solution and were placed into each hole. Those sticks are 6 cm long and 1 cm in diameter (Peng et al. 2021). Agarwood inducement solution will be made by using acetic acid, sodium chloride, as well as fruit enzymes (Ngadiran *et al.*, 2023). After that, the bamboo sticks will be placed into each hole.

The knocking method, which is combined with the injecting method, is accomplished in the same manner as the previous. After that Using the injecting and knocking methods alternately, the agarwood inducement solution was applied to each hole.

In terms of quality, the procedure effectively produced agarwood that nearly resembled natural agarwood. 2021 (Peng et al. 2021).

### *Bottle dipping method*

Bottle dipping is a procedure that involves placing the bottle to gradually drip the agarwood inducer into the holes for inoculating the trees. Parafilm will be used to prevent inoculant leaking and connect the bottle to the wound via the hose, and close it by using clay (Justin *et al.*, 2020). By using this method of bottle dripping, approximately 10 mL to 20 mL inoculants can be delivered in each hole, and a total of 1 to 3 L of inoculant delivered per tree (Mustapa *et al.*, 2022).

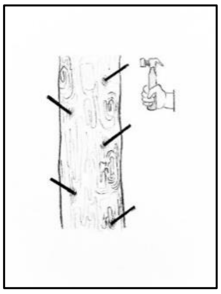

**Fig. 11.** Bamboo stick method

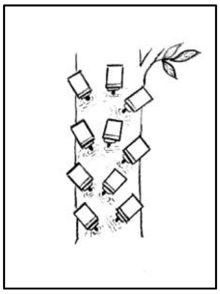

**Fig. 11.** Bottle dipping method

### *ChemJet method*

The ChemJet approach is similar to the bottle dipping in that the pressurized injection is placed upside down into the hole and dripped gently into the lesion. In this case, also, the same amount of discharge can be delivered to the holes of the tree and a total of 1-3 liters of inoculum will be delivered into a single tree (Mustapa *et al.*, 2022).

### *Pinhole infusion method*

To practice the pinhole-infusion technique, deep holes of 4 to 5 cm with a width of 0.5 cm are drilled into the trunk. For softening the tissues of the wood formic acid treatment is introduced to the drill holes. This procedure was conducted to promote the absorption and dissemination of the fungus solution into the near cell, and the inoculated hole was then covered with tape (Tian *et al.*, 2013).

Moreover, Agar-Sit is a similar procedure that has been attempted with very slight variations from the pinhole-infusion method (Chen *et al.*, 2018).

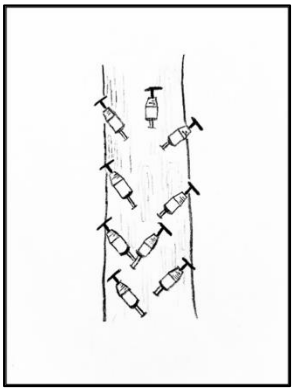

**Fig. 12.** Bottle dipping method

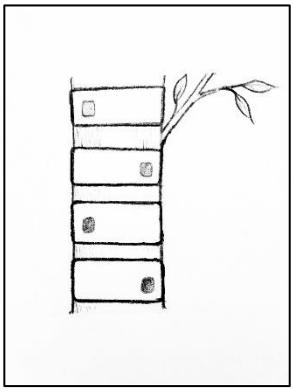

**Fig. 13.** Pinhole infusion method

### *Automated transfusion set method*

An automated transfusion set is a sophisticated transfusion method that introduces to the agarwood industry. In this system, a magnetic sensor container, a DC water pump, and a transfusion outlet are all included. This system was based on an Arduino, so the Arduino controller was used to control all the modules of the kit. The setup program is developed to determine the discharge volume of the inoculant solution with maintaining consistent pressure during the operation. As a result, both operating time and labor costs are reduced by this application (Roslee *et al.*,2018). The inoculum is very similar to other methods, such as the agar-wit technique. However, the application technique is at the next level (Ngadiran *et al.*, 2023).

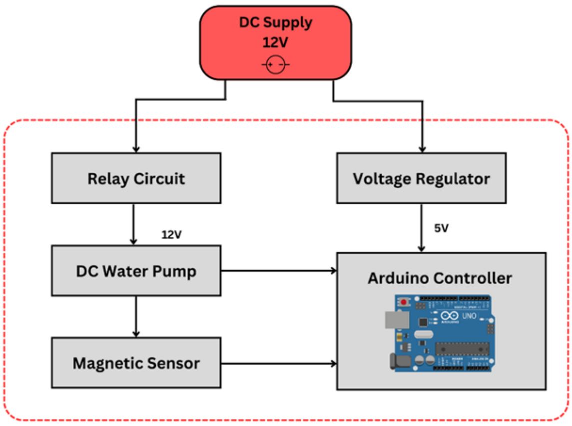


**Fig. 14.** Diagram of the hardware arrangement for the automated transfusion set

### *Biological method*

Biological inoculation also practices introducing fungi to plants. It is done by vectors like weevils and termites but controlling the insect population should be strictly caring and preventing an outbreak is a challenge. If not, it negatively affects the trees and other species (Turjaman *et al.*, 2016).

### *Agar-Bit method*

This method contains both chemical and biological inducers. Biologically agarwood-inducing technology (Agar-Bit) in which a combination of plant hormones, and bacteria were injected into the stems 300 cm from the ground. Moreover, a penetration enhancer is used for successful inoculation. Approaching six months after inoculation, it was observed that this method produced high-quality agarwood with a promising output of essential oil and chromone content compared with the burning nails and BCD methods (Wu *et al.*, 2017, 2020). Another study shows, within three months, the combination of nitrogen fertilizer and *F. solani* enhanced the production of agarwood in *A. malaccensis* compared to the use of these treatments alone (Wahyuni *et al.*, 2018). In addition, using a mixture of formic acid and *Fusarium spp.*, resulted in the content of accumulated chromones increasing significantly as in other related studies and this has resulted after 12 months of inoculation (Chen *et al.*, 2017).

### *Challenges in the Agarwood Industry and the Agarwood Inoculation Process*

The agarwood industry encounters various challenges in both the agarwood inoculation process and the identification of agarwood-producing trees. In the inoculation process, factors such as the age of the tree, the inoculation method, and environmental conditions contribute to the alteration of resin quality and composition. This inconsistency poses difficulties in meeting market demands and maintaining product standards. Additionally, accurately identifying agarwood-producing trees is crucial for sustainable production practices and resource preservation. These challenges require focused efforts to ensure consistent resin quality, meet market demands, and promote responsible practices in the agarwood industry.

Agarwood trees are an integral part of forest ecosystems, supporting biodiversity and environmental balance. However, the agarwood industry must address the potential environmental impacts associated with unsustainable practices and the cultivation of agarwood plantations (Persoon and van Beek, 2008). Uncontrolled or excessive harvesting of agarwood trees from natural forests can lead to deforestation and habitat degradation. This can disrupt ecosystems, endanger wildlife, and reduce biodiversity. Unsustainable practices can also result in soil erosion, water pollution, and the depletion of natural resources (Xu *et al.,* 2013). To mitigate these environmental impacts, sustainable cultivation methods, such as agroforestry systems or plantation establishment, can be implemented. These approaches allow for the cultivation of agarwood trees while maintaining forest cover (Desa *et al.,* 2021).

Rapid depletion resulting from overharvesting poses a significant problem for agarwood production (Persoon and van Beek, 2008). The misidentification of agarwood formation and lack of knowledge about the subject contribute to this issue, leading to illegal and unsustainable harvesting practices that negatively impact natural agarwood sources worldwide. The current production is insufficient to meet the demand due to the low natural growth rate of agarwood species (Yin *et al.,* 2016). Unfortunately, the majority of Aquilaria and Gyrinops forests have already vanished (Xu *et al.,* 2013).

Deforestation is also a major concern for the depletion of agarwood sources. Many traders and consumers do not prioritize sustainable production practices, engaging in the industry solely for quick profits. This has led to a phenomenon known as "gold fever," where the news about agarwood spreads rapidly within society, driving further unsustainable harvesting. To address these issues, the cultivation of agarwood should be emphasized for production purposes, while harvesting from the wild should be avoided (Persoon and van Beek, 2008). Consequently, agarwood trading is now subject to strict controls by authorities worldwide.

In terms of commercial cultivation, farmers often lack the necessary knowledge to select the appropriate species for cultivation on their lands. Their choice of species is typically influenced by the providers of planting materials (Lee and Mohamed, 2016). As a result, farmers may invest 6-7 years in maintaining plantations only to face setbacks due to ineffective techniques. Additionally, certain internal secrets and patented knowledge remain inaccessible to the public, limiting the dissemination of comprehensive information. Workshops, seminars, and meetings often provide only a fraction of the patented findings (Turjaman *et al.,* 2016)

The agarwood industry encounters challenges related to the uncertainty of resin quality after the inoculation process. The quality of agarwood resin can vary in terms of aroma, color, and potency. Several factors contribute to this inconsistency, including the age of the tree, the inoculation method used, and environmental conditions (Najib *et al.,* 2011).

The age of the tree plays a crucial role in determining resin quality. Younger trees tend to produce resin of lesser quality compared to mature trees. Consequently, achieving consistent resin quality becomes challenging as the age of the trees used for inoculation varies (Hidayat *et al.,* 2010). Additionally, the method of inoculation, whether through drilling holes or making incisions, can influence the resin's quality. Variations in the depth, size, and location of the incisions or holes can lead to inconsistent resin formation (Chowdhury *et al.,* 2016). Environmental conditions also impact resin quality. Factors such as temperature, humidity, soil quality, and sunlight exposure influence the resin's chemical composition. Agarwood trees grown in different regions or under varying climatic conditions may produce resin with different qualities, posing a challenge for maintaining consistent standards (Najib *et al.,* 2011).

To address these challenges, researchers and producers are exploring innovative methods such as controlled environment cultivation and optimized inoculation techniques. These approaches aim to create more favorable conditions for resin formation and enhance the overall quality of agarwood.

Agarwood resin is valued for its unique chemical composition, which contributes to its distinct fragrance and therapeutic properties. However, the inoculation process can alter the composition of the resin, leading to variations in its chemical profile. This challenge raises concerns regarding the authenticity and reliability of agarwood products (Tajuddin *et al.,* 2016).

During the inoculation process, the introduction of fungal inoculum triggers a response in the tree, resulting in resin formation. However, this response can lead to changes in the resin's chemical composition (Lee and Mohamed, 2016). The presence of different fungal species and their interactions with the tree's defense mechanisms can influence the production of specific compounds responsible for the resin's fragrance and therapeutic properties (Yin *et al.,* 2016).

The alteration in resin composition poses challenges for producers who strive to maintain consistent product quality and meet market demands. Consumers expect agarwood products to exhibit specific aroma profiles and therapeutic benefits. Any significant deviations in the chemical composition may affect the desired properties and reduce consumer confidence in the product's authenticity (Ngadiran *et al.,* 2023).

The agarwood industry heavily relies on market demand and consumer perception. Any changes in resin quality or composition can significantly impact consumer preferences and purchasing decisions, presenting a challenge for the industry. Inconsistent resin quality resulting from variations in the inoculation process can lead to reduced market demand. Consumers seeking high-quality agarwood products expect consistent sensory experiences, including specific aromas and therapeutic properties (Zich and Compton, 2001). When resin quality varies significantly, it becomes difficult to meet these expectations consistently, resulting in a loss of consumer trust and reduced demand (Mohamed and Lee, 2016).

Moreover, consumer perception plays a vital role in determining market demand. If consumers perceive that the agarwood industry lacks transparency or if authenticity concerns arise due to altered resin composition, it can have a negative impact on market demand. Educating consumers about the complexities of the agarwood inoculation process, the factors influencing resin quality, and efforts to ensure consistency can help alleviate these concerns and maintain market demand.

To address these challenges, producers and stakeholders in the agarwood industry must prioritize quality control and adopt standardized practices throughout the inoculation process. Implementing certification systems and promoting transparency in sourcing, production, and processing can help build consumer trust and support market growth (Adhikari *et al.,* 2021).

The agarwood inoculation process requires careful consideration of environmental factors and completion of the disease triangle, which involves the interaction of a susceptible host, a virulent pathogen, and a conducive environment. Failure to manage these factors properly can result in ineffective inoculation, decreased resin production, and increased susceptibility to diseases (Rohlfs and Churchill, 2011).

Environmental factors, such as temperature, humidity, and soil conditions, play a crucial role in the success of agarwood inoculation. Deviations from optimal conditions can hinder resin formation and negatively impact the overall yield. Adequate moisture levels in the soil, appropriate temperature ranges, and proper sunlight exposure are essential to promote healthy tree growth and resin production (Rabgay *et al.,* 2020). Additionally, the completion of the disease triangle is critical in fungal inoculations. Agarwood trees are vulnerable to various diseases caused by pathogenic fungi. When making incisions or drilling holes for inoculation, there is a risk of introducing pathogens to the tree, which can lead to infections. These infections can weaken the tree's immune system, reduce resin production, and even cause tree mortality (Rohlfs and Churchill, 2011).

To mitigate the risk of disease outbreaks, producers should implement effective disease management strategies. This includes using disease-resistant agarwood tree varieties, maintaining optimal environmental conditions, employing proper hygiene practices during the inoculation process, and monitoring tree health regularly. Integrated pest management techniques and timely interventions can help minimize the risk of disease and maintain a healthy agarwood plantation (Syazwan *et al.,* 2019).

Inoculating agarwood trees by physical interventions to introduce the fungal inoculum, these procedures can potentially damage the trees if not performed with precision and care. Improper inoculation techniques or inadequate post-inoculation care may lead to irreversible harm, including infections, stunted growth, or even tree mortality. Drilling holes or making incisions in the wrong locations or at incorrect depths can cause unnecessary damage to the tree. Careful consideration of tree anatomy, growth patterns, and proper techniques are crucial to minimize harm. Additionally, inadequate post-inoculation care, such as failure to prevent infections or provide suitable environmental conditions, can further exacerbate plant damage (Akter *et al.,* 2013; Faizal *et al.*, 2017).

Proper training and education of agarwood producers, along with the dissemination of best practices, can help reduce the risk of plant damage. Emphasizing precision, hygiene, and monitoring tree health after inoculation are essential steps in preventing long-term harm to agarwood trees (Ngadiran *et al.,* 2023).

The challenges encountered in the agarwood industry, including the agarwood inoculation process and the identification of agarwood-producing trees, have far-reaching implications for market demand, sustainability, and the preservation of natural resources. However, recent advancements in agarwood induction technology offer promising solutions to overcome these obstacles and transform artificial agar resin induction methods. These advancements encompass a range of innovative techniques, including controlled environment cultivation, optimized inoculation methods, disease management strategies, and precision-based practices. By embracing and implementing these advancements, the agarwood industry can significantly enhance resin quality, effectively meet market demands, promote sustainable production practices, and ensure the long-term viability of this invaluable resource. It is of utmost importance for stakeholders to remain updated on the latest advancements in agarwood induction technology, as these developments hold the key to the continued growth and development of the industry while addressing its inherent challenges.

## CONCLUSIONS

The present global demand for agarwood, the high-valued resinous fragrant of the wood, cannot be met by natural harvests from the forests alone. The natural causes of invasions present in the environment is limited and inefficient to induce the production of this precious resource inside trees up to its full potential. Fortunately, recent advancements in artificial agarwood induction technology have led the production of agarwood resin be efficient by surpassing conventional methods. These technologies include mechanical, biological, and chemical methods, as well as combinations of these techniques. Chemical inducers, in particular, have shown rapid invasion inside particular tree species trunks to produce superior agarwood resin at highly efficient and consistent rate. However, all such methods are being practiced in the industry or in the research level, have their own merits and limitations.

Therefore, to get the utmost benefit from this industry, the most appropriate induction method should be selected by the stakeholders by considering their own specific circumstances. In such instances, the feasibility, safety, replicability, targeted market, cost-effectiveness and the financial stability of each method should among the most priorities if the industry is to be transformed in to a high profit oriented business.

Furthermore, it is crucial to acknowledge and address the multitude of challenges encountered in the agarwood industry. These challenges include potential alterations in resin quality and composition, market demand fluctuations, completion of the disease triangle in fungal inoculations, risk of permanent plant damage, environmental impacts, and increased vulnerability to pest and disease outbreaks. By proactively addressing these challenges through sustainable practices, responsible sourcing, and effective regulations, the agarwood industry can achieve thriving growth while upholding the delicate equilibrium between economic prosperity and environmental preservation.

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