# EVALUATION OF DIFFERENT INOCULUM TYPES AND THEIR IMPACT ON AGARWOOD YIELD AND QUALITY IN *AQUILARIA MALACCENSIS*

**By**

**MOHD ZAMAKHSYARY MUSTAPA**

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**Chairman : Associate Prof. Mohamad Azani bin Alias, , PhD**
**Faculty : Forestry and Environment**

**Agarwood**, known locally as Karas or Gaharu and elsewhere as Aloeswood and Eaglewood, originates from the genus ***Aquilaria***, within the family **Thymelaeaceae**. Agarwood is a fragrant resin produced by Aquilaria trees when they are attacked by fungi or certain insects. According to Abd Majid et al. (2018), the essential oil extracted from Agarwood is used in medicine, while the scented wood is highly prized for incense, perfumes, and cosmetics. However, these resources are under threat due to unsustainable production methods, including direct harvesting and indiscriminate felling of trees from natural forests. To alleviate pressure on natural resources and meet market demands, establishing plantations is proposed as a viable solution. This study investigates the methods of stimulating **Agarwood production in plantation trees**, focusing on identifying the best inoculum and methods. The research evaluates the quantity of Agarwood produced from each ***Aquilaria malaccensis*** tree and grades it based on established standards. Additionally, the marketability of the produced Agarwood is assessed. The inoculation process was conducted by five companies at the Karas Plantation located at Empangan Jus, Jasin Melaka. The inoculum materials

were tested for their chemical content using **Gas Chromatography Mass Spectrometer (GCMS).** This study builds on research conducted by MTIB in Kuala Lipis in 2012, involving the following companies: Bio-Benua Teknoloji Sdn Bhd, Sansen Agarwood Sdn Bhd, Redclaw Aqua and Herbs Sdn Bhd, Inokulum Gaharu Biotech Sdn Bhd, and Etlingera Sdn Bhd. The inoculated trees were harvested after 18 and 24 months. The Agarwood was then carved into woodchips and pieces, recorded, weighed, and processed into essential oil. The essential oil was tested for heavy-metal content and active compounds before being graded and traded to study its marketability across **Asia, the Middle East, Europe, and Southeast Asia**. **Five inoculums** were analyzed and compared: Black Gold BioBooster (Bio-Benua Teknoloji Sdn Bhd), TW Destang (Sansen Agarwood Sdn Bhd), RAHE Probio (Redclaw Aqua and Herbs Sdn Bhd), IGB711 (Inokulan Gaharu Biotech Sdn Bhd), and UPMV16 (Etlingera Sdn Bhd). Four inoculation techniques were demonstrated: Chemjet, Dripping Bag, Bamboo Stick, and Infuse System (Bottle).

The study found that the primary chemical responsible for boosting Agarwood production belongs to the **Fatty Acid Ethyl Ester (FAEE)** group. Except for UPMV16, which showed a higher concentration of Copper (Cu), all inoculums were free of heavy metal hazards. The findings also revealed that Bio-Benua Teknoloji Sdn Bhd produced the most Agarwood resin in the shortest time, whereas Inokulan Gaharu Biotech Sdn Bhd produced the most resin after a 24-month inoculation process. Redclaw Aqua and Herbs Sdn Bhd effectively produced Agarwood essential oil, yielding about 2.1 ml per kilogram of wood chips. The study concluded that all five Agarwood oils contained two major compounds, 10-epi-γ-eudesmol and α-cadinol.

**Keywords:** Aquilaria, Aquilaria Malaccensis, Agarwood Production in Plantation Trees, Agarwood, Thymelaeaceae

**SDG:** GOAL 8: Decent Work and Economic Growth, GOAL 9: Industry, Innovation and Infrastructure

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENILAIAN KEPADA PERBEZAAN JENIS INOKULUM DAN IMPAK KEPADA JUMLAH PENGHASILAN GAHARU DAN KUALITI BAGI SPESIE*S Aquilaria Malaccensis***

Oleh

**MOHD ZAMAKHSYARY MUSTAPA**

**Jun 2024**

**Perngerusi : Prof. Madya Mohamad Azani bin Alias, PhD**
**Fakulti : Perhutanan dan Alam Sekitar**

**Gaharu,** secara umumnya dikenali sebagai Karas, Agarwood, Aloeswood dan Eaglewood berasal daripada genus ***Aquilaria*** di dalam famili **Thymelaeceae**. Gaharu merupakan resin yang terhasil daripada pokok *Aquilaria* yang diserang oleh Fungus atau serangga tertentu. Mengikut Abd Majid et al. (2018), Pati MInyak yang terhasil daripada Gaharu ini digunakan dalam perubatan, manakala kayu beraroma wangi ini sangat bernilai untuk dibakar, kegunaan dalam minyak wangi dan juga kosmetik. Walaubagaimanapun, sumber ini terancam disebabkan kaedah pengeluaran yang tidak mapan, termasuk penuaian langsung dan penebangan pokok secara haram di hutan semula jadi. Bagi mengurangkan tekanan atas sumber semula jadi tersebut dan memenuhi permintaan pasaran, penubuhan ladang telah dicadangkan sebagai satu penyelesaian yang berdaya maju. Kajian ini melihat kaedah terbaik bagi merangsang penghasilan **kayu gaharu dari pokok ladang**, dengan fokus untuk mengenal pasti inokulum serta kaedah yang terbaik. Penyelidikan ini juga menilai jumlah kayu gaharu yang dihasilkan dari setiap pokok **Aquilaria malaccensis** dan menggredkannya

berdasarkan piawaian yang ditetapkan. Selain itu, kebolehpasaran kayu gaharu yang dihasilkan juga turut dinilai. Proses inokulasi telah dijalankan oleh lima syarikat di Ladang Karas yang terletak di Empangan Jus, Jasin Melaka. Bahan inokulum juga diuji untuk kandungan kimia menggunakan kaedah *Gas Chromatography Mass Spectrometer* (GCMS). Kajian ini dilaksanakan berdasarkan penyelidikan yang dijalankan oleh MTIB di Kuala Lipis pada tahun 2012 yang telah melibatkan syarikat Bio-Benua Teknoloji Sdn Bhd, Sansen Agarwood Sdn Bhd, Redclaw Aqua and Herbs Sdn Bhd, Inokulum Gaharu Biotech Sdn Bhd, dan Etlingera Sdn Bhd. Pokok yang diinokulasi tersebut telah dituai selepas 18 dan 24 bulan diinokulasi. Kayu gaharu tersebut kemudiannya diasingkan kepada serpihan dan kepingan kayu dan direkod, ditimbang, serta diproses menjadi Pati Minyak. Pati Minyak tersebut seterusnya diuji untuk kandungan logam berat dan sebatian aktif sebelum digredkan dan didagangkan untuk menilai kebolehpasarannya di Asia, Timur Tengah, Eropah, dan Asia Tenggara. **Lima inokulum** dianalisis dan dibandingkan: Black Gold BioBooster (Bio-Benua Teknoloji Sdn Bhd), TW Destang (Sansen Agarwood Sdn Bhd), RAHE Probio (Redclaw Aqua and Herbs Sdn Bhd), IGB711 (Inokulan Gaharu Biotech Sdn Bhd), dan UPMV16 (Etlingera Sdn Bhd). Manakala empat teknik inokulasi telah ditunjukkan: *Chemjet, Dripping Bag, Bamboo Stick,* dan *Infuse System* (Botol). Kajian ini turut mendapati bahawa bahan kimia utama yang bertanggungjawab untuk meningkatkan pengeluaran kayu gaharu adalah daripada kumpulan *Fatty Acid Ethyl Ester* (FAEE). Namun, inokulum UPMV16, menunjukkan kepekatan sebatian kimia daripada *Copper* (Cu) yang lebih tinggi manakala semua inokulum yang lain bebas daripada bahaya logam berat. Hasil kajian ini juga menunjukkan bahawa Bio-Benua Teknoloji Sdn Bhd menghasilkan resin gaharu paling banyak dalam masa yang paling singkat, manakala Inokulan Gaharu Biotech Sdn Bhd pula menghasilkan resin paling

banyak selepas proses inokulasi selama 24 bulan. Redclaw Aqua and Herbs Sdn Bhd pula berjaya menghasilkan Pati Minyak Gaharu, dengan 2.1ml bagi setiap kilogram serpihan kayu yang digunakan. Kajian ini turut menyimpulkan bahawa kesemua lima Pati Minyak Gaharu daripada Syarikat-syarikat tersebut mengandungi dua sebatian utama, iaitu 10-epi-γ-eudesmol dan α-cadinol.

**Kata Kunci:** Gaharu, Aquilaria, *Aquilaria malaccensis*, Kayu Gaharu Dari Pokok Ladang, Thymelaeaceae

**SDG:** MATLAMAT 8: Pekerjaan Sesuai and Penbangunan Ekonomi, MATLAMAT 9: Industri Inovasi and Prasarana

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Forest Management and Ecosystem Sciences. The members of the Supervisory Committee were as follows:

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Agarwood, also locally known as karas or Gaharu, Aloeswood, and Eaglewood elsewhere, is from the genus *Aquilaria*, a family of Thymelaeaceae (Abd Majid et al., 2018). Aquilaria trees produce Agarwood, a fragrant resin, when a fungus or certain insects attack an injured or wounded tree. Agarwood serves as a raw material for producing many aromatic medicinal products, such as stimulants, tonics, and carminative medicines. According to Chakrabarty (1994), the essential oil extracted from the wood also serves as a constituent for medicine, while the scented wood is highly sought after for incense. It is also highly sought after for perfumes and cosmetics.

Overall, there are 22 species of *Aquilaria* worldwide. According to Olfield et al. (1998), Bangladesh, Bhutan, India, Indonesia, Iran, Myanmar, the Philippines, Singapore, Thailand, and Malaysia are the countries where these species can be found. In Malaysia itself, there are five species of *Aquilaria*, namely *A. beccariana* Van Tiegh., *A. hirta* Ridl., *A. malaccensis* Lam., *A. microcarpa* Baill., and *A. rostrata* Ridl. (Whitmore, 1972).

The high demand for this forest product has resulted in the injudicious and rampant felling of trees deep in the forests. While this results in significant tax losses for the

country, it also significantly reduces the biodiversity of the rainforest. Due to the illegal harvests, the Aquilaria population in the forests of Southeast Asia is dwindling, prompting it to be categorised as an endangered species and placed in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) (Barden *et. al.* 2000). As stated in Appendix II, trade must be controlled in order to shun utilisation that is incompatible with the survival of the species.

## 1.2 Problem Statement

Karas is an Agarwood-producing tree from the species of Aquilaria. These Agarwood resources are under threat due to the unsustainable production of Gaharu by direct harvesting and the indiscriminate felling of trees from natural forests (Abd Majid et al., 2018). Thus, to lift pressure on natural resources and meet market demands, plantations are the best solution. In order to minimise the exploitation of Agarwood from natural forests, it shall be important to explore methods of inducing Agarwood formation in plantation trees, with particular emphasis on the methods and inoculum that work best.

In Malaysia, various inoculation products were used to stimulate Agarwood production. However, they have yet to establish any proven track records with a scientific database detailing the production of Agarwood per tree (Abd Majid et al., 2018). Most inoculators will presume Agarwood's presence within a few months after inoculation. Thus, this research was conducted to collect all pertinent data, providing a complete overview of the Inoculum and Inoculation Process for Agarwood Planters.

Malaysia is heading towards a zero quota of Agarwood from natural forests. Thus, in the future, Agarwood production from plantations will be essential for the industry. This research will provide scientific data to the relevant Management Authority and Agarwood Planters so that they can choose the best inoculum and techniques.

## 1.3 Research Objective

### 1.3.1 General Objectives

To identify the inoculation techniques and types of inoculum used in the production of Agarwood in *Aquilaria malaccensis*.

### 1.3.2 Specific Objectives

1. To identify the different types of inoculum applied to *Aquilaria malaccensis* trees.
2. To evaluate the yield of Agarwood produced from *Aquilaria malaccensis* trees based on the different types of inoculum.
3. To grade the Agarwood produced through the inoculation process.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Background

A member of the family Thymelaeaceae, *Aquilaria* is a relatively slow-growing, medium-sized tree, on average 15–25 m tall; some of the more than 15 species (e.g., *A. microcarpa*) reach heights of as much as 40 m. Having a moderately straight stem, it can achieve a diameter (dbh) of up to 250 cm, although some species remain considerably smaller and more shrub-like, e.g., *A. khasiana.* Most *Aquilaria* species have smooth, thin, white bark with dense, dark foliage of shiny, elliptical to oblong leaves (7.5-12 cm long by 2.5 - 5.5 cm wide) (Ding Hou, 1960). The small, pale blooms flowering in clusters on the short stalks of the leaf stems produce 3-5 cm long, bi-valved fruit capsules in July.

*Aquilaria* regenerates freely under natural conditions as seedlings around the mother tree or sprouts from the stumps of harvested trees. However, mother trees are becoming scarce in many areas because of overexploitation (Beniwal, 1989; Paoli et al., 1994; Hasnida et al., 2001; Soehartono & Newton, 2002). Although this condition may not lead to the local extinction of the species, it may severely affect the availability of the product and, thus, the local ‘gaharu’ economy.

A shade-tolerant tree, *Aquilaria* is an understory tree of mature evergreen and semi-evergreen forest occurring at low to medium altitudes, generally up to 1000 m asl

depending on the species. In Indonesia, it also occurs as an emergent (Soehartono & Mardiastuti, 1998). The existence of the tree itself does not guarantee the presence of resin. Scientists estimate that only 10% of the *Aquilaria* trees in the forest may contain *gaharu* (Gibson, 1977). The resin forms in many parts of the trees, including the bark, roots, and heartwood, following wounding and subsequent fungal infection (Jalaluddin, 1977). Under natural conditions, trees of about 20 years or older are more likely to contain the resin, with those over 50 years old reportedly exhibiting the highest concentration (Sadgopol, 1959). A "good" tree may yield several kilograms of the valuable dark, heavy, resinous wood with the characteristic 'kemenyan'-like scent.

Distributed broadly through Southeast Asia, the genus *Aquilaria* has been found from Bhutan and northeastern India across to southern China and then south as far as the island of New Guinea (Burkill, 1966; Whitemore, 1972). Little detailed information exists, however, on its distribution, exploitation, or use in the southeastern edge of its range. In some of the earlier exploited areas, several species are thought to be extremely rare if not extinct in the wild, for example, in Bangladesh and Java (Chakrabarty, 1994).

## 2.2 Production of Agarwood

Three hypotheses exist regarding Agarwood formation, namely that it is the result of pathological, wounding, or pathological and non-pathological processes (Ng et al., 1997). According to Ng et al. (1997), studies have not provided conclusive evidence for any of these hypotheses. Oldfield et al. (1998) stated that resin production is in response to fungal infection. On the other hand, Heuveling van Beek (1999) affirmed

that Agarwood formation is in response to wounding. He added that a fungal infection can trigger an increase in resin production in the host as a response to the increased damage caused by fungal growth.

*Aquilaria* trees are naturally infected by a variety of fungi, including; *Aspergillus* spp., *Botryodiplodia* spp., and *Diplodia* spp. (Anon, 1998). The ecological interaction between the host tree and the wound in order to produce Agarwood is poorly understood. Other factors such as the age of the tree, differences in the tree caused by seasonal variation, environmental variation, and genetic variation of *Aquilaria* spp. may also play an important role in Agarwood formation (Ng et al., 1997).

## 2.3 Status of Agarwood Industry in Malaysia

Trade of Agarwood is governed under CITES regulation. Malaysian Timber Industry Board (MTIB) acts as a Management Authority (M.A.) in Peninsular Malaysia and the Federal Territories of Kuala Lumpur, Putrajaya, and Labuan. While the Sabah Forest Department is for Sabah, the Sarawak Forest Department is for Sarawak. The function of this M.A. is to monitor and facilitate the trade of Agarwood products in the corresponding states. The trade of gaharu is subject to quota, and for 2022, only 25,000 kg is allowed for export annually.

On the other hand, the establishment of Karas plantations is an economic strategy to address the issue of resource depletion from natural forests. As of December 31, 2023, there are about 2,741 hectares of Karas plantations in Malaysia with almost 2.6 million planted trees (CITES, MTIB). However, compared to neighbouring countries such as

Thailand, Vietnam, Cambodia, and Indonesia, Malaysia is still lacking in terms of Karas plantation establishment and gaharu production. Meanwhile, the value of Agarwood products exported in 2023 increased to RM8.74 million from RM3.87 million in 2022.

## 2.4 Characteristics of the Species in Its Ecosystem

*Aquilaria* species have adapted to live in various habitats, including those that are rocky, sandy, or calcareous; well-drained slopes and ridges; and land near swamps. This shade-tolerant plant, when young, may regenerate in almost pure patches underneath mother trees. Pattern and seedling distribution indicate that few seeds are distributed more than a few meters from the adult tree. The species are never found in a single dominant stand but mostly in uneven dispersal throughout the habitat. Therefore, finding the tree (by collectors) would probably require special experience.

## 2.5 Species Description

### 2.5.1 *Aquilaria beccariana* Van. Tiegh.

This species is considered a medium tree, measuring 15-25 meters tall. The spot character of the species is the secondary veins that are strongly raised above and can be seen clearly. The size of the leaves differs from other species of *Aquilaria*. It could be up to 15 cm long and 5 cm wide compared to other *Aquilaria* spp.

The leaf of all *Aquilaria* is almost similar to cellulose-like strands when torn. The leaves are greenish-grey in colour with a dull, non-shiny surface. Its bark and the inner bark of the tree are white. The flower's characteristics are a green pedicel, pale yellow corolla tube, white petal, and yellow stamen.

The fruit's colour is green. *A. beccariana* could only be found in the primary lowland forest of south Johor, Sabah, and Sarawak up to the 825m a.s.l. Figure 2.1 and 2.2 below shows the *A. beccariana* leaves and stem that have been wounded.


**Figure 2.1: Leaves of *A. Beccariana***

**Figure 2.2: Wounded Stem and the Infectious Bacteria (Gunung Panti, Johor)**

### 2.5.2 Aquilaria Hirta Ridl.

Morphologically, this species is also considered a small tree (around 15-25 meters). Buds are silky. The leaves have an oblong-ovate shape and are covered in tomentose beneath the leaf surfaces. The fruits, flowers, and petioles are considered to be hairy. The petioles are thick and long. It can be found on the east coast of Peninsular Malaysia (Terengganu, Kelantan, Pahang, and Johor), Sabah, and Sarawak.

Figure 2.3 below shows the SYNTYPE collection (First Collection of the Species) from the Herbarium of Botanical Garden, Singapore (SING). On the other hand, Figure 2.4 illustrates the log of the *A. hirta* trees being cut down.


**Figure 2.3: SYNTYPE Collection from Bukit Penggaram, Johor**

**Figure 2.4**: **Felled Trees of *A. hirta* Gunung Panti, Johor**

### 2.5.3 Aquilaria Malaccensis Lamk

This species is stated to be a big tree with a height of 15-30 meters tall. The leaf's description is chartaceous, glabrous, shining, and oblong-lanceolate. The flowers are white. *A. malaccensis* are listed as threatened by the IUCN-Red List Categories because of the major harvesting of these trees. It is believed that this tree could produce a first-quality 'gaharu' (Jantan, 1990).

These species are widely distributed within Malaysia. All states of Malaysia, with the exception of Perlis, easily hosts this species. It grew in lowland forest and sometimes on the hillsides and ridges up to 750m a.s.l. Figure 2.5 below shows the tree of *A. malaccensis,* and Figure 2.6 shows the exploitation of the trees in searching for the 'gaharu.'

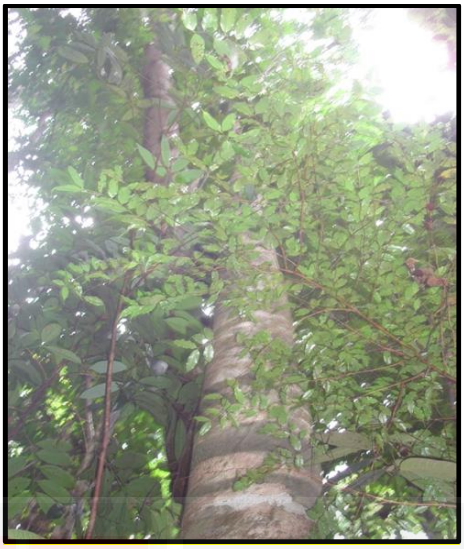

**Figure 2.5**: **Trees of *A. Malaccensis***

**Figure 2.6: Exploitation to the MTrees Found in Ayer Keroh, Melaka**

### 2.5.4 *Aquilaria Microcarpa* Baill

*A. microcarpa* is considered a big tree, measuring 25-36 meters tall. The fruit is heart-shaped and small. It is called 'microcarpa' because of the size of the fruits, which are smaller compared to all other *Aquilaria* spp. It is distributed in the south of Johore and is commonly found in Sabah and Sarawak.

Figure 2.7 below shows a sample collected from the Sepilok Forest Reserved in Sandakan. This is the herbarium collection from SAR (Sarawak Forestry Corporation).

**Figure 2.7: Herbarium Collection of *A. Microcarpa***

### 2.5.5 *Aquilaria Rostrata* Ridl

These species of *Aquilaria* are considered montane species. It was found in 1890 in Kemaman and later in 1911 at the Wrays Camp, Gunung Tahan, by H.N. Ridley. Its branchlet is a bit hairy and more in line with *A. malaccensis* characteristics.

Figure 2.8 below shows the SYNTYPE collection from the herbarium of the Botanical Garden of Singapore (SING). This is the specimen collected in 1911 by H.N. Ridley.

**Figure 2.8: *A. Rostrata* Endemic in Gunung Tahan**

## 2.6 Agarwood Industry in Malaysia

Agarwood is an important non-timber forest product. For centuries, it has been used for medicine and as a basic material to produce perfumes. Today, the trading scenario is rapidly changing and involves almost 18 countries, with trade worth millions of US dollars annually. Indeed, due to the growth in the population and affluence of Agarwood-consuming markets, the demand for Agarwood has risen significantly over the past 30 years. Since 2005, this industry has seen significant growth with the increasing number of Karas plantations in Malaysia. Table 2.1 below shows a summary of the Agarwood industry in Malaysia (December 2023).

**Table 2.1: Summary of Agarwood Industry in Malaysia**

| NO. | ITEMS | TOTAL |
|---|---|---|
| 1 | Registered Planters under the CITES Act 686 | **304 co. / Individual** |
| 2 | Total land size of Karas plantation registered under the CITES ACT686 | **2,741 hectares** |
| 3 | Total number of Karas trees registered under the CITES ACT 686 | **2.6 million trees** |
| 4 | Exporter/Importer of Agarwood registered under the ACT105 | **32 companies** |
| 5 | Export of Agarwood Products (2023) | **RM8.74 million** |
| 6 | Agarwood Essential Oil Producer | **22 co. / Individual** |
| 7 | Total land size of Karas nursery registered under the CITES ACT686 | **6.052 hectare** |
| 8 | Agarwood products producer in Malaysia | **> 50 companies** |

(Source: CITES, MTIB, and FDPM - December 2023)

# CHAPTER 3

# METHODOLOGY

## 3.1 Introduction

This chapter explains the research method that has been conducted. This method includes study location, tree selection for inoculation, harvesting process, chemical analysis and statistical analysis and sampling.

## 3.2 Study Location

The research was conducted at Empangan Jus in Jasin, Melaka, where 50 hectares of *Aquilaria trees* were planted in 2009 by MTIB-UPEN Melaka and Malacensis Sdn Bhd. The site contains a total of 40,000 mature Karas trees.

## 3.3 Tree Selection for Inoculation

The process of selecting 100 trees of the same size and age was done with the criteria of 20 cm dbh and 8-year-old trees. The species of *Aquilaria malacensis* were selected for the research. On the other hand, the selection of five companies was based on MTIB's previous inoculation research in Kuala Lipis (2012). These companies were:

**a) Biobenua Teknoloji Sdn Bhd**

Biobenua Teknoloji Sdn Bhd (BB) is using the inoculum named by BlackGold BioBooster. The inoculation techniques used in the study were spiral and Chemjet (pressurised injections). They used a 4 mm drill size to make holes in the *A. malaccensis* trees, with 30-40 holes per tree and 20 ml of inoculum in each hole. The cost of the inoculum was RM280/litre, and the cost for each tree, including workmanship, was RM320. Figure 3.1 illustrates the inoculation techniques they used.

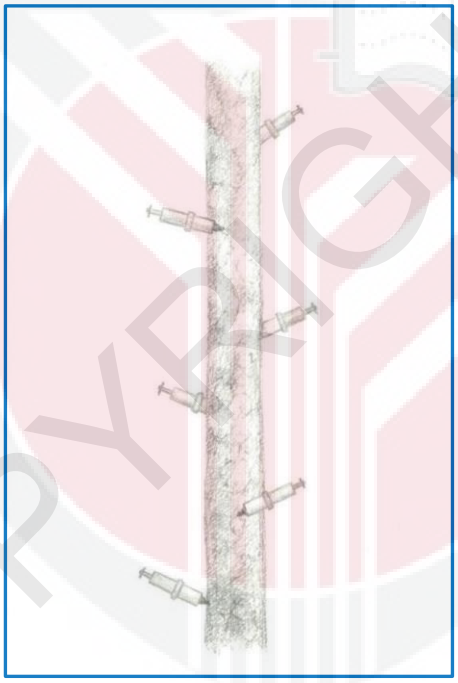

**Figure 3.1: Illustration of Inoculation Techniques by BioBenua Teknoloji Sdn. Bhd.**

**b) Sansen Agarwood Sdn. Bhd.**

Sansen Agarwood Sdn. Bhd. (SS) utilised the inoculum named by TW Destang. The inoculation techniques that they used in the study were spiral techniques and using a drip bag. They used an 8 mm drill size to make holes in the *A. malaccensis* trees, with

ten bags of inoculum for each tree and three litres for each bag. The cost of the inoculum was RM25/litre, and the cost for each tree, including workmanship, was RM750. Figure 3.2 illustrates the inoculation techniques they used.

**Figure 3.2: Illustration of Inoculation Techniques by Sansen Agarwood Sdn Bhd.**

**c) Redclaw Aqua and Herbs Sdn. Bhd.**

Redclaw Aqua and Herbs Sdn. Bhd. (RAHE) utilised an inoculum named by RAHE Probio. The study employed inoculation techniques using small holes with bamboo sticks. The inoculum was dipped with a bamboo stick before it was put inside the holes. They used a 6 mm drill size to make 180-300 holes in each *A. malaccensis* tree, inserting approximately 10 ml of inoculum into each hole. The cost of the inoculum

was RM70/litre, and the cost for each tree, including workmanship, was RM210. Figure 3.3 shows the inoculation techniques they employed.

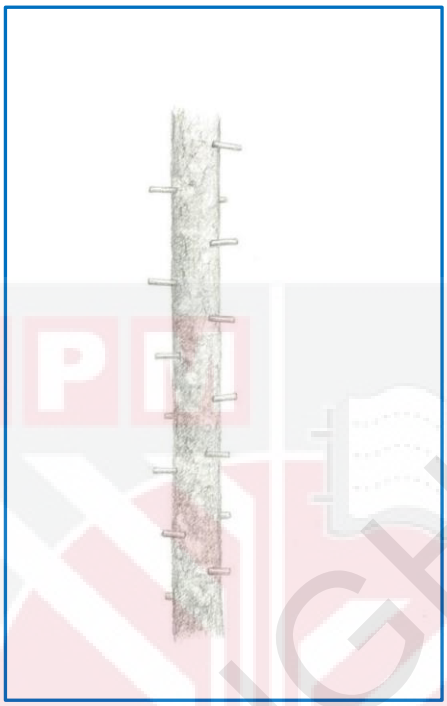

**Figure 3.3: Illustration of Inoculation Techniques by Redclaw Aqua and Herbs Sdn. Bhd.**

**d) Inokulan Gaharu Biotech Sdn. Bhd.**

Inokulan Gaharu Biotech Sdn. Bhd. (IGB) used the inoculum named by IGB711. The inoculation techniques, which they used in the study, were spiral techniques via infuse systems and bottles. They used a 16 mm drill size to create four holes in each *A. malaccensis* tree, each containing 100 ml of inoculum. The cost of the inoculum was RM100/litre, and the cost for each tree, including workmanship, was RM40. Figure 3.4 illustrates the inoculation techniques they employed.

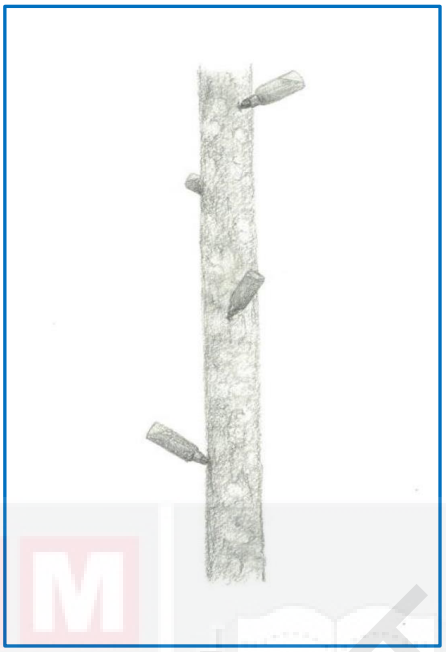

**Figure 3.4: Illustration of Inoculation Techniques by Inokulan Gaharu Biotech Sdn. Bhd.**

**e) Etlingera Sdn. Bhd.**

Etlingera Sdn. Bhd. (ELG) used the inoculum named UPMV16. The inoculation techniques they used in the study were spiral techniques via infusion systems and bottles. They used a 10 mm drill size to make holes in the *A. malaccensis* trees with six holes for each tree and 60 ml of inoculum in each hole. The cost of the inoculum was RM150 for each tree, including workmanship. Figure 3.5 illustrates the inoculation techniques they employed.

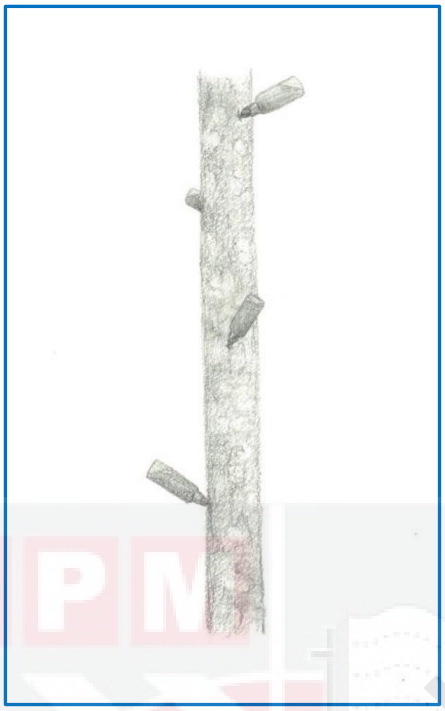

**Figure 3.5: Illustration of Inoculation Tby Etlingera Sdn. Bhd.**

Each company showcased its unique inoculum and inoculation techniques, and documents the inoculation process. Subsequently, all inoculums were tested for heavy metals using Gas Chromatography-Mass Spectrometry (GC-MS). The chemical compounds detected through GC-MS readings were then be analysed.

The entire inoculation process by five companies was observed for a maximum of twenty-four months. Each company used a different inoculation technique depending on the size and preparation of the wound-inflicting holes. Holes with 4 to 16 mm were prepared to make the distribution of inoculum denser, respectively. The distance between the holes varies, ranging from 3 to 8 inches, depending on the respective company. A maximum of 300 holes could be prepared for the tree stem. Additionally, a number of inoculation formulations with innovative delivery methods had been

developed, including the chemjet, dripping bag, bamboo stick, and infuse system using a bottle.

As a result, different concentrations of inoculum spread throughout the trunk tree via xylem transportation, triggering Agarwood formation throughout the whole trunk (Du et al., 2022). Producing Agarwood starts when bacteria that caused disease invade woody tissue (Suharti et al., 2011). For instance, bottle dripping and chemJet techniques use pressurised injections to deliver 20 mL and 3 L of inoculum gradually to wounds, respectively (Mustapa et al., 2022; Ngadiran et al., 2023). Due to their mechanical qualities, bamboo sticks from RAHE were utilised to deliver chemical inducers during the aeration process (Ngadiran et al., 2023). Previous studies indicated that the bamboo stick and dripping method was a suitable treatment for stimulating Agarwood formation, as it is easy to inoculate, locally available, and has a slightly low cost of operation (Chowdhury et al., 2017). Similar to the aeration method, these methods stimulated higher Agarwood yield than the existing wounding method (Adhikari et al., 2021). The following Table 3.1, provides a summary of the differences between the various inoculum providers, as well as a summary of the features of each inoculum.

**Table 3.1: Summary of Inoculation Techniques in This Study**

| NO | INOCULATION METHOD | COMPANY NAME | PRICE PER LITRE (RM) | PRICE PER TREE (RM) | DISTANCE BETWEEN HOLES | DRILL SIZE | INOCULUM QUANTITY PER HOLES |
|---|---|---|---|---|---|---|---|
| **1** | Black Gold BioBooster (Chemjet) | BIOBENUA TEKNOLOJI SDN BHD | RM280 | RM320 | 3 inches x 4 inches (30-40 holes / tree) | 4mm | 20ml |
| **2** | TW Destang (Dripping Bag) | SANSEN AGARWOOD (M) SDN BHD | RM25 (1 bag = 3 liter) | RM750 (10 beg /tree) | Depends on tree size | 8mm | 3liter |
| **3** | RAHE Pro-Bio (Bamboo Stick) | REDCLAW AQUA AND HERBS SDN BHD | RM70 | RM210 | 6inch x 6inch (Diamond Shape) 180-300 holes / tree | 6mm | 10ml |
| **4** | IGB Inoculum 711 (Infuse System (Bottle)) | INOKULAN GAHARU BIOTECH (IGB) 711 | RM100 | `RM40 | 4 ft. x 4 ft. (4 holes per tree) | 16mm | 100ml |
| **5** | UPM V16 (Infuse System (Bottle)) | ETLINGERA SDN BHD | N/A | RM150 | 18inch | 10mm | 60ml |

### 3.4. Harvesting Process

Following the inoculation process, the *Aquilaria* trees were closely monitored every two months for Agarwood production. This regular observation ensured that Agarwood continues to form, as *Aquilaria* trees tend to recover after inoculation (Yahya, A. 2009). The harvesting would take place in two phases: 18 months and 24 months post-inoculation, during which the entire tree would be felled. Figures 3.6 and 3.7 illustrate the tree selection process for harvesting.

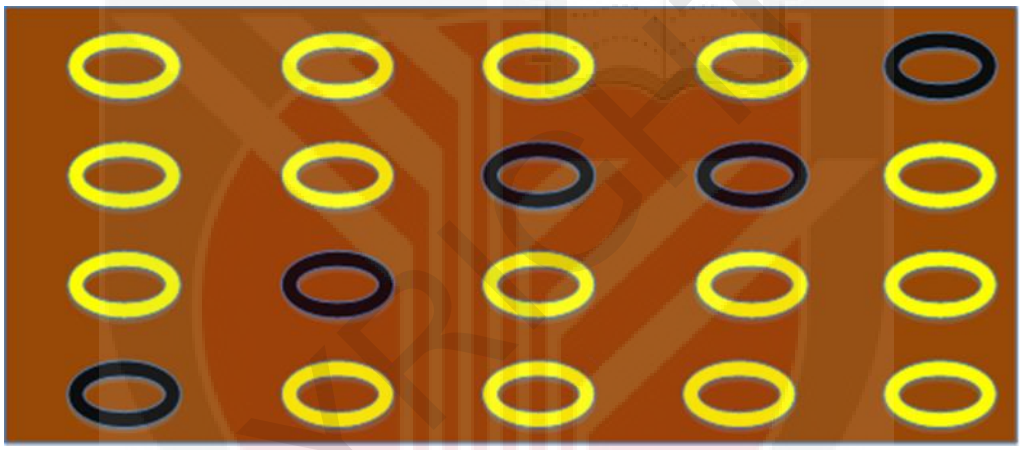

**Figure 3.6: Harvested Tree After 18 Months Inoculated (*Black-circle-colour*)**

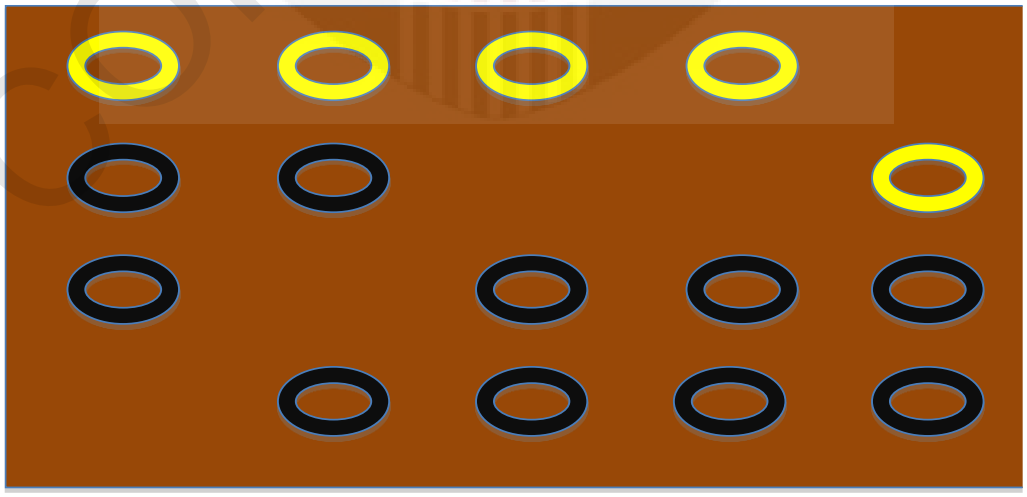

**Figure 3.7: Harvested Tree After 24 Months Inoculated (*Black-circle-colour*)**

### 3.5 Separating *Aquilaria* and Agarwood

The weight of the fresh *Aquilaria* tree would be recorded, and the logs would be air-dried in the shade for four weeks before being weighed again. The separation of *Aquilaria* and Agarwood would then be carried out manually using specialised carving tools, as shown in Figure 3.8.

**Figure 3.8: Process of Isolating *Aquilaria* and Agarwood into Woodchips / Woodpiece Forms**

The Agarwood produce would then be graded according to colour and smell and either in woodchip/woodpiece forms. The darker colour of the Agarwood means that it had a higher price and grade. Meanwhile, the lower grade of Agarwood would be turned into Agarwood Essential Oil.

### 3.6 Agarwood Essential Oil Process

The next process was separating the Agarwood with a lower grade (light-brown colour) for the extraction of the Agarwood essential oil. The extraction would be using the Hydro-Distillation procedure, as shown in Figure 3.9. The processing of the Agarwood essential oil would take place in the Oudh Agarwood Sdn Bhd Factory in Jeli, Kelantan. Next, the oil produced would be tested for heavy metals using Gas Chromatography Mass Spectrometer (GCMS). Each chemical compound presence based on the GCMS reading would then be analysed

.

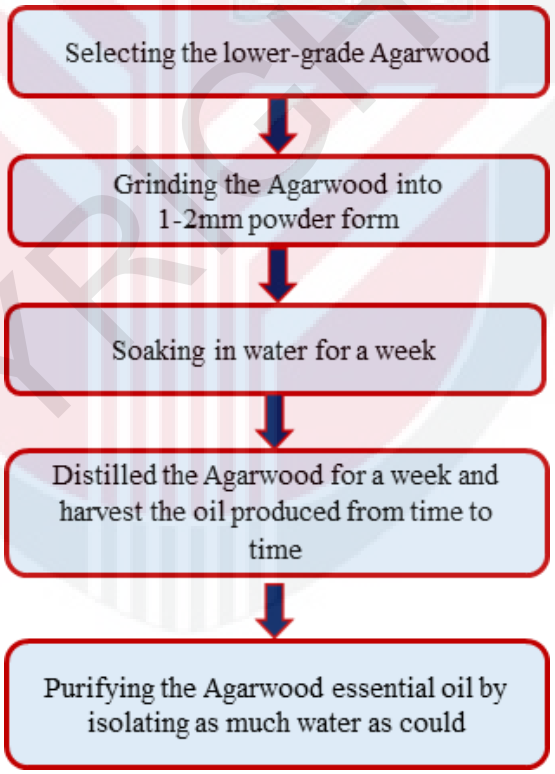


**Figure 3.9**: **Hydro-Distillation of the Agarwood Process**

## 3.7 Chemical Analysis

### 3.7.1 Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis was performed using an Agilent G3170A Network System gas chromatograph, attached to a mass spectrometer (Agilent 5975C) and fitted with a capillary column (HP-5 MS, 30 m × 0.25 mm I.D.; 0.25 µm film thickness). The oven temperature was programmed for 100°C for 5 min, then ramped at 5°C/min to 350°C and held for 5 min. Injector inlet and detector temperatures were set at 250°C. Each sample was diluted in hexane and then injected in 1 µl volume in the splitless mode, using helium as the carrier gas (1.0 ml/min). The analysis parameter is summarised in Table 3.1.

**Table 3.2: The GC-MS Parameter of Inoculant Analysis**

| **Gas Chromatography-Mass Spectrometer** | **Agilent G3170A** |
|---|---|
| **Capillary Column** | HP-5 MS (Non-Polar) |
| **Oven Program** | |
| Initial Temperature | 100$^{o}$C |
| Initial Time | 5 min |
| Rate | 5$^{o}$C/min |
| Final Temperature | 350$^{o}$C |
| Final Time | 5 min |
| **Carrier Gas** | Helium |
| Flow Rate | 1.0 mL/min |
| **Injection Volume** | 1.0 µL |

Each component's relative amount (%) was calculated by comparing its average peak area to the total area. The resulting chromatograms were integrated and aligned according to their groups. The chemical components were identified based on comparing their retention time and authentic mass spectral data with the existing NIST05 library.

### 3.8 ICP-OES Analysis

Elemental analyses were conducted on an inductively coupled plasma optical emission spectrometer (700 Series ICP-OES, Agilent Technologies). The parameters of instrumental conditions, analytical lines, and linear range for each element are given in Table 3.2.

**Table 3.3: Analytical Lines Used for Each Element and the Instrumental Conditions in ICP–OES Apparatus**

| **Parameter** | |
|---|---|
| Radiofrequency power | 1.20 kW |
| Plasma gas flow rate | 15.0 L/min |
| Auxiliary argon flow rate | 1.5 L/min |
| Nebulizer pressure | 200 kPa |
| Replicate read time | 5 s |
| Pump rate | 15 rpm |
| Replicates | 3 |
| Sample uptake delay | 30 s |
| Rinse time | 10 s |

The calibration solutions were prepared using a commercial multi-element calibration standard containing 14 elements at concentrations of 0.5, 1.0, 3.0, 5.0 and 10.0 g/mL. The emission lines that were employed were Al (396.152), As (188.980), Ba (455.403), Cd (214.439), Co (238.892), Cr (267.716), Cu (327.395), Mn (257.610), Mo (202.032), Ni (231.604), Pb (220.353), Se (196.026), Sr (407.771), Zn (202.548), and Zn (213.857). All analyses were conducted in triplicate, and blank experiments were prepared in each batch following the same procedure used for samples.

### 3.9 Statistical Analysis

The data were presented as a mean with a standard deviation (SD). One-way ANOVA was used in the statistical analysis, preceded by Tukey's test for multiple parametric comparisons between the control and treatment classes. The values were considered significantly different when the p-value was less than 0.05 ($p < 0.05$).

# CHAPTER 4

# RESULTS AND FINDINGS

## 4.1 Chemical Analysis of Inoculum

The objective of using artificial inoculum is to enhance Agarwood formation in a shorter time (Chowdhury et al., 2016). The artificial inoculum might be either a chemical inducer or a biological inducer, yet inoculums or biological inducers may take a long incubation period to produce darker and better Agarwood before harvesting (Azren et al., 2019). Chemical liquids produce bacterial or fungal infections that will wound the tree, which can affect the oil produced and cause human skin to itch. The chemicals' content should also depend on the tree size and antibody level (Nasardin et al., 2018). Chemical inducers, such as phytohormones, salts, minerals, and biological substances, shorten the time-consuming holding process by delivering inducers through transpiration through fewer induction sites (Tan et al., 2019).

Thus, in this study, different companies stimulated the yield of Agarwood resin, resulting in the chemically produced five commercial inoculums. Nevertheless, no previous report on the active chemical constituents of each inoculum had been done. Therefore, this study's chemical profiling for each inoculum was generated using GC-MS analysis.

## 4.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for Commercial Inoculum

All five commercial inoculums were subjected to GC-MS analysis to determine the presence of active chemical constituents contributing to the development of Agarwood resin. A chemical profiling for each inoculum was analyzed. Twenty-five compounds were obtained from all samples of inoculums.

### 4.2.1 Biobenua Teknoloji Sdn. Bhd.

Biobenua Teknoloji Sdn. Bhd. produces an inoculum, namely Black Gold BioBooster. The GC-MS analysis of this inoculum exhibited only four active chemical compounds, including ethyl palmitate, 1-tetradecyne, ethyl E-11-hexadecenoate, and 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin. The major compound was identified as ethyl E-11-hexadecenoate (2.22%).

### 4.2.2 Sansen Agarwood Sdn. Bhd.

Company Sansen Agarwood Sdn. Bhd. has created Inoculum TW Destang. The chemical analysis carried out by GC-MS revealed only one active compound, namely ethyl decanoate.

### 4.2.3 Redclaw Aqua and Herbs Sdn. Bhd.

The investigation of chemical constituents in inoculum RAHE Pro-Bio from Redclaw Aqua and Herbs Sdn. Bhd. was also carried out via GC-MS analysis. Twenty active

compounds were found in this inoculum. Several major compounds were identified as *N*-methoxy-*N*-methylacetamide, 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone, 5-hydroxymethylfurfural, 2-ethyl-2-hexenal, ethyl alpha-d-glucopyranoside and ethyl palmitate.

### 4.2.4 Inokulan Gaharu Biotech Sdn. Bhd.

Another inoculum tested in this study was IGB Inoculum 711, from the company Inokulan Gaharu Biotech Sdn. Bhd. The result of GC-MS analysis showed four active compounds present in this inoculum, viz., ethyl palmitate, 1-nonyne, ethyl linoleate and ethyl E-11-hexadecenoate.

### 4.2.5 Etlingera Sdn. Bhd.

The GC-MS analysis of the fifth inoculum was done on the inoculum UPM V16 from Etlingera Sdn. Bhd. Nevertheless, only one compound was identified, namely 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin.

### 4.2.6 Summary of GC-MS Analysis

The contribution of active compounds for each inoculum is summarised in Table 4.1 The finding showed that inoculum RAHE Pro-Bio had the highest presence of active compounds, contributing 78.12%. Meanwhile, inoculum Black Gold BioBooster contributed the least percentage, 5.39%. In addition, the sole active compound was obtained in inoculum TW Destang and UPM V16, identified as ethyl decanoate

(21.94%) and 7-hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin (20.63%), accordingly.

Inoculum RAHE Pro-Bio showed the presence of 20 compounds, with 5-hydroxymethylfurfural (36.76%) as the major compound. On the other hand, Inoculum Black Gold BioBooster and IGB Inoculum 711 obtained four compounds each. The analysis also showed the major compound contributions were ethyl E-11-hexadecenoate (2.22%) and ethyl linoleate (11.34%), respectively.

**Table 4.1: Chemical Composition Assessed by GC-MS of Five Inoculums**

| No | Chemical constituents | RT | RA % | | | | |
|---|---|---|---|---|---|---|---|
| | | | BB | SS | RAHE | IGB | ELG |
| 1. | 3-(Dimethylamino) acrylonitrile | 3.90 | | | 0.60 | | |
| 2. | 1,4-Dimethylpyrazole | 4.07 | | | 0.83 | | |
| 3. | 2-furanmethanol | 4.92 | | | 1.49 | | |
| 4. | 2-pyrimidinol | 5.34 | | | 0.22 | | |
| 5. | 3,4-Dihydro-2H-pyran | 6.12 | | | 0.53 | | |
| 6. | Methylcyclohexane | 6.52 | | | 0.37 | | |
| 7. | 5-Methylfurfural | 7.21 | | | 0.51 | | |
| 8. | 3-Methylcyclopentane-1,2-dione | 9.81 | | | 0.57 | | |
| 9. | Furaneol | 11.77 | | | 1.10 | | |
| 10. | *N*-Methoxy-*N*-methyl acetamide | 13.93 | | | 6.77 | | |
| 11. | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyrone | 14.29 | | | 7.91 | | |

**Table 4.1: Continued**

| No | Chemical constituents | RT | RA % | | | | |
|---|---|---|---|---|---|---|---|
| | | | BB | SS | RAHE | IGB | ELG |
| 12. | **5-Hydroxymethylfurfural** | **17.55** | | | **36.76** | | |
| 13. | 2-Ethyl-2-hexenal | 17.77 | | | 2.42 | | |
| 14. | Ethyl alpha-d-glucopyranoside | 30.94 | | | 9.15 | | |
| 15. | Ethyl 4-methylpentanoate | 32.44 | | | 0.41 | | |
| 16. | n-hexadecanoic acid | 37.15 | | | 0.48 | | |
| 17. | **Ethyl decanoate** | **37.21** | | **21.94** | | | |
| 18. | Ethyl palmitate | 37.23 | 1.34 | | 2.76 | 2.98 | |
| 19. | 1-nonyne | 40.53 | | | | 1.95 | |
| 20. | 1-tetradecyne | 40.62 | 0.68 | | | | |
| 21. | **Ethyl linoleate** | 40.70 | | | 2.16 | **11.34** | |
| 22. | **Ethyl *E*-11-hexadecenoate** | 40.74 | **2.22** | | | 5.50 | |
| 23. | Ethyl oleate | 40.84 | | | 2.59 | | |
| 24. | Ethyl stearate | 41.30 | | | 0.49 | | |
| 25. | **7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin** | 60.09 | 1.15 | | | | **20.63** |
| | **Total** | | **5.39** | **21.94** | **78.12** | **21.77** | **20.63** |

*RT: retention time; RA%: Relative area in total compounds; Identification by comparison of MS with the NIST05 library. BB- Biobenua Teknoloji Sdn. Bhd.; SS – Sansen Agarwood Sdn. Bhd.; RAHE- Redclaw Aqua and Herbs Sdn. Bhd.; IGB- Inokulan Gaharu Biotech Sdn. Bhd.; ELG- Etlingera Sdn. Bhd.*

In addition to this finding, the fatty acid ethyl ester (FAEE) group was the chemical that made the most significant contribution. Table 4.2 tabulates the percentage contribution of FAEE for each inoculum. The FAEE contributed the most to

inoculum TW Destang (21.94%), followed by IGB Inoculum 711 (11.34%) and 8.0% to inoculum RAHE Pro-Bio. Inoculum Gold BioBooster has an FAEE contribution of 2.22%, whereas inoculum UPM V16 is completely devoid of FAEE. Therefore, FAEE is believed to be the most important inoculant for stimulating Agarwood production. Previous research has shown that organic chemical inductors in Agarwood are safe, but further testing is required to establish the remaining chemical concentration (Rasool & Mohamed, 2016). Yet, comparing this finding with a prior study is challenging due to the need for more data on inoculant constituents and the unclear mechanism behind Agarwood formation (Faizal et al., 2017).

**Table 4.2: Percentage (%) of Fatty Acid Ethyl Ester (FAEE) in Each Inoculum**

| TYPE OF INOCULUM | FAEE (%) |
|---|---|
| Black Gold BioBooster | 2.22 |
| TW Destang | 21.94 |
| RAHE Pro-Bio | 8.00 |
| IGB Inoculum 711 | 11.34 |
| UPM V16 | Not detected |

## 4.3 Elemental Analysis of Inoculum Using ICP-OES

In addition, the present study also examined the presence of any metal components in the inoculum samples. Elemental analyses were conducted on an inductively coupled plasma optical emission spectrometer (ICP-OES). About 14 elements were analysed: Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sr, and Zn. From the ICP-OES result in Table 4.3, four inoculums did not show any detected hazardous element inside. However, inoculum UPM V16 has detected copper (Cu) elements. The concentration

of Cu was 16.5855 g/mL, higher than the WHO-approved level from 2004. This concentration may pose a risk to human health, as copper concentrations greater than 0.418 g/m³ in the air and 1.3 mg/L in water are considered harmful to the environment and human health.

Inoculum UPM V16 has levels of Cu exceeding the WHO's recommended upper limit, potentially causing severe health consequences, such as anaemia and damage to the pancreas, liver, and kidneys. The remaining inoculant was expected to be free from metal threats, but concerns were raised about exposing the consumer to long-term hazardous effects.

**Table 4.3: The Concentration of Trace Elements in Five Commercial Inoculums**

| METAL ELEMENTS | | INOCULUM | | | | |
|---|---|---|---|---|---|---|
| | | **Black Gold Bio Booster** | **TW Destang** | **RAHE Pro-Bio** | **IGB Inoculum 711** | **UPM V16** |
| 1. | Al | (not detected) | - | - | - | - |
| 2. | As | - | - | - | - | - |
| 3. | Ba | - | - | - | - | - |
| 4. | Cd | - | - | - | - | - |
| 5. | Co | - | - | - | - | - |
| 6. | Cr | - | - | - | - | - |
| 7. | Cu | - | - | - | - | 16.5855 g/mL |
| 8. | Mn | - | - | - | - | - |
| 9. | Mo | - | - | - | - | - |
| 10. | Ni | - | - | - | - | - |
| 11. | Pb | - | - | - | - | - |
| 12. | Se | - | - | - | - | - |
| 13. | Sr | - | - | - | - | - |
| 14. | Zn | - | - | - | - | - |

## 4.4 Production of Agarwood Resin

This study showed the importance of inoculating *A. malaccensis* trees in order to boost the formation of Agarwood resin in the trees. Non-inoculated *A. malaccensis* trees usually produce around 5-10% Agarwood naturally, while inoculation will help increase the percentage yield to 70-80%. This study specifically documented the Agarwood production of each *A. malaccensis* tree according to the inoculum and the techniques applied, respectively.

Natural infection, chemical induction, and artificial screw procedures that wound the trunk are all feasible methods for stimulating resin production (Hashim et al., 2014). Chemically, terpene compounds in *A. malacce*nsis xylem accumulate due to wounds and infections. Diterpenes act as a nontoxic waterproofing layer, while monoterpenes and sesquiterpenes are toxic chemical agents (Chong et al., 2014).

The shades of colour in Agarwood resin depend on geographical origin and may vary from green to dark green, yellow to golden, and red-black to brown to white. The darker colour has a higher percentage of resin content, which helps contribute to the grade level (Liu et al., 2017; Azren et al., 2019). The Agarwood component was crafted by removing the resin from white wood and shaping it into the desired form. Since the inoculation methods use different locations and sizes for their wounding sites, the induced Agarwood might have vastly different visual appearances (Yan et al., 2019).

### 4.4.1 Biobenua Teknoloji Sdn. Bhd.

Biobenua Teknoloji Sdn. Bhd. inoculated fifteen trees with Black Gold BioBooster inoculum. Five trees were harvested after 18 months of enticement, while the remaining trees were harvested after 24 months. Figure 16 depicts cross sections of induced trees after 18 and 24 months of incubation. A thick layer of resin was evident in the middle of the trunk after 18 months (Figure 4.2 & 4.3), indicating that the Agarwood inducement solution had induced the resin to exude actively. Nonetheless, less Agarwood was formed when the resin content widened after 24 months of harvesting (Figure 4.2).

**Figure 4.1: The Cross Sections of the Trees for 18 months Induced by Inoculum Black Gold Bio Booster**

**Figure 4.2: The Cross Sections of the Trees for 24 months Induced by Inoculum Black Gold Bio Booster**

Table 4.4 shows the Agarwood resin production across the 18- and 24-month inducement periods. Inconsistencies in Agarwood production were discovered in each tree (A1 to A5). The highest Agarwood production was from tree A4, with 68.10 kg; nevertheless, resin production was low after a 24-month incubation period, implying that Black Gold Bio Booster is less efficient for long-term incubation periods.

**Table 4.4: The Production of Agarwood Resin after an 18- to 24-month Inducement Period (Black Gold Bio Booster)**

| Treatment | Agarwood resin (in kg) per tree | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 months | A1 | A2 | A3 | A4 | A5 | | | | | |
| | 1.9 | 206 | 39.70 | 68.10 | 32.90 | | | | | |
| 24 months | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 |
| | 0.0 | 12.4 | 13.9 | 15.7 | 30.8 | 9.31 | 27.31 | 14.64 | 24.05 | 28.83 |

### 4.4.2 Sansen Agarwood Sdn. Bhd.

TW Destang inoculum from Sansen Agarwood Sdn. Bhd. was used to induce fifteen trees, and observations of the resin production of these trees were made twice, at 18 and 24 months, respectively. Figure 4.3 and 4.4 exhibits the cross-section of trees harvested after 18 and 24 months and resin content. The result showed that the resin's colour was deeper after 18 months of harvesting (Figure 4.3), as opposed to 24 months when it was more brownish (Figure 4.4). The distribution of resin accumulated for each tree (A1 to A5) and B1 to B10 is shown in Table 4.5. The production of Agarwood resin was greatest during the 18-month induction period, whereas resin accumulation was lowest during the 24 months. The result indicated that over an 18-

month incubation period, the inoculum TW Destang's efficacy was diminished. Therefore, our finding implies that TW Destang is most effective within a short incubation time frame.

**Figure 4.3: The cross sections of the trees for 18, months induce by inoculum TW Destang**

**Figure 4.4: The cross sections of the trees for 24 months induce by inoculum TW Destang**

**Table 4.5: The Production of Agarwood Resin after an 18- to 24-Month Inducement Period (TW Destang)**

| Treatment | Agarwood resin (in kg) per tree | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 months | **A1** | **A2** | **A3** | **A4** | **A5** | | | | | |
| | 8.5 | 14.10 | 28.60 | 30.60 | 19.30 | | | | | |
| 24 months | **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** | **B8** | **B9** | **B10** |
| | 0.48 | 0.54 | 0.39 | 0.43 | 0.53 | 0.55 | 5.6 | 2.1 | 3.2 | 0.42 |

### 4.4.3 Redclaw Aqua and Herbs Sdn. Bhd.

Redclaw Aqua and Herbs Sdn. Bhd. inoculated fifteen trees with RAHE Pro-Bio inoculum. Five trees were harvested after 18 months, and the remaining trees were harvested following a 24-month inducement period. Tree cross sections and resin formation are shown in Figure 4.5 and 4.6 for trees stimulated for 18 and 24 months. After both periods, a thick layer of resin was seen in the middle of the trunk, indicating that the Agarwood inducement solution had caused the resin to be actively secreted (Peng et al., 2021). The colour of the resin was a little light, indicating that Agarwood did not stimulate the inoculum vigorously. The distribution of Agarwood resin for each tree is shown in Table 4.6, with average accumulations of 8.9 and 22.6 kg for incubation times of 18 and 24 months, respectively.

**Figure 4.5: The Cross Sof the Trees for 18 Months Induce by Inoculum RAHE Pro-Bio**

**Figure 4.6: The cross sections of the trees for 24 months induce by inoculum RAHE Pro-Bio**

**Table 4.6: The Production of Agarwood Resin after an 18- to 24-Month Inducement Period (RAHE Pro-Bio)**

| **Treatment** | **Agarwood resin (in kg) per tree** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **18 months** | **A1** | **A2** | **A3** | **A4** | **A5** | | | | | |
| | 11.7 | 13.5 | 7.8 | 0.0 | 11.60 | | | | | |
| **24 months** | **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** | **B8** | **B9** | **B10** |
| | 19.4 | 6.55 | 28.30 | 19.1 | 19.6 | 40.0 | 27.0 | 24.50 | 24.50 | 17.20 |

### 4.4.4 Inokulan Gaharu Biotech Sdn. Bhd.

Inokulan Gaharu Biotech Sdn. Bhd. inoculated 15 trees with IGB Inoculum 711. Figure 4.7 and 4.8 depicts the cross sections of the 18-month and 24-month induced trees. Table 4.7 provides information on the resin production for both periods following inoculum inducement. After 24 months, the analysis revealed a thick layer of resin in the middle of the trunk (Figure 4.8), which suggests improved outcomes in average yield. The Agarwood's absorption mechanism may be responsible for differences in size and colour. The findings indicate that IGB Inoculum 711 was a slow-release system. As a result, the resin developed and accumulated along the xylem, getting larger and more intense over the lengthy period of inducement.

**Figure 4.7: The cross sections of the trees for (a)18 months induce by inoculum IGB Inoculum 711**

**Figure 4.8**: The cross sections of the trees for 24 months induce by inoculum IGB Inoculum 711

**Table 4.7**: The Production of Agarwood Resin after an 18- to 24-Month Inducement Period (IGB Inoculum 711)

| **Treatment** | **Agarwood resin (in kg) per tree** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **18 months** | **A1** | **A2** | **A3** | **A4** | **A5** | | | | | |
| | 13.4 | 6.2 | 10.7 | 5.8 | 7.9 | | | | | |
| | 0 | | 0 | | | | | | | |
| **24 months** | **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** | **B8** | **B9** | **B10** |
| | 25.2 | 21.0 | 24.1 | 24.0 | 21.0 | 24.1 | 44.0 | 15.0 | 19.03 | 40.0 |
| | 5 | 3 | 2 | 2 | 0 | 6 | 2 | 0 | | 0 |

### 4.4.5 Etlingera Sdn. Bhd.

Etlingera Sdn. Bhd. inoculated the tree with UPM V16 inoculum. Five trees were harvested after 18 months during the inducement period, while the remaining trees were taken after 24 months. Figure 4.9 and 4.10 depicts the cross-sections of trees

induced for 18 and 24 months, revealing a thin layer of light brown Agarwood resin. Table 4.8 shows how much Agarwood resin each tree made during the two inducement times. Only three A1 produced Agarwood resin among the five trees induced with UPM V16 for 18 months, whereas the others did not. After a 24-month induction period, ten trees were also found to have a diminished Agarwood yield. Therefore, the UPM V16 inoculum has weaker penetration through the plant cell and fails to proliferate within the tree.

**Figure 4.9**: The cross sections of the trees for 18 months induced by inoculum UPM V16

**Figure 4.10**: The cross sections of the trees for 24 months induced by inoculum UPM V16

**Table 4.8: The Production of Agarwood Resin after an 18- to 24-Month Inducement Period (UPM V16)**

| Treatment | Agarwood resin (in kg) per tree | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 months | A1 | A2 | A3 | A4 | A5 | | | | | |
| | 38.9 | 0.00 | 0.00 | 0.00 | 0.00 | | | | | |
| 24 months | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 |
| | 11.2 | 9.40 | 0.20 | 0.25 | 0.00 | 0.25 | 0.35 | 0.45 | 0.00 | 0.25 |

### 4.4.6 Average Accumulation of Agarwood Resin

This study analysed and compared the Agarwood production of five inoculums. Figure 4.11 depicts each inoculant's mean Agarwood harvesting yield after 18 and 24 months of incubation.

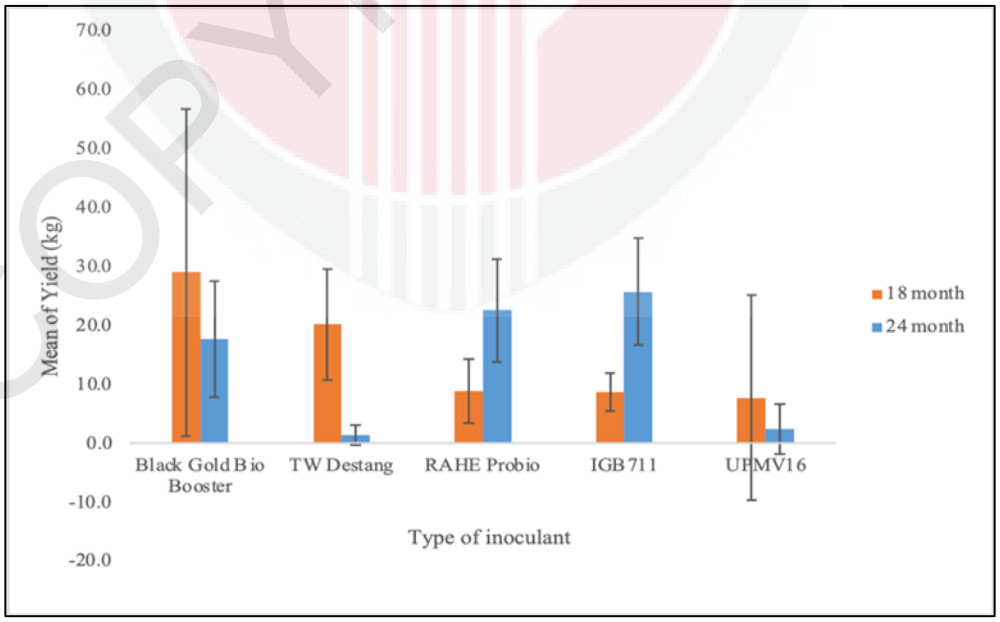


**Figure 4.11: Mean of Agarwood Yield Harvested in 18 and 24 Months. Data are shown as Mean ± s.d. * The Mean Ddifference is Significant at $p < 0.05$ Level; [a] n=5, [b] n=10.**

The results demonstrated that the RAHE Probio and IGB711 inoculums, when incubated for 24 months, produced a high yield of Agarwood resin compared to the 18-month incubation period. The RAHE Probio produced an average of 22.6 kg of Agarwood, while IGB711 produced an average of 25.8kg. Statistical data (one-way ANOVA) showed significant differences in the production of Agarwood for 24 months between RAHE Probio and IGB711 as compared to others ($p<0.05$). Despite this, there was no substantial difference between inoculant IGB711 and RAHE Probio, as their Agarwood production was nearly comparable.

The study found that inoculum Black Gold BioBooster was the most effective in stimulating Agarwood formation in the 18-month inoculation period, with a mean yield of 29.0 kg. TW Destang produced an average of 20.2 kg of Agarwood resin. The best period for harvesting Agarwood stimulated by both inoculums was 18 months, but Black Gold BioBooster was preferred due to its cost-effectiveness and yield of Agarwood resin. On the other hand, inoculum UPMV16 yielded the lowest amount of Agarwood resin in both periods compared to other inoculums. Despite the fact that inoculants IGB711 and UPMV16 used the identical inoculation method, it might have occurred because the two inoculants' formulations were different. The results suggest the inoculants RAHE Probio, IGB711, and UPMV16 only partially or efficiently drive Agarwood production in less than two years of incubation. Yet, the one-way ANOVA statistical results showed no significant difference ($p > 0.05$) in the amount of Agarwood harvested at 18 months for any of the inoculums.

Conclusively, among the evaluated inoculants, RAHE Probio had the most potential in stimulating Agarwood production. This inoculant yielded an excellent quality of Agarwood resin due to its refined appearance and darker resin.

## 4.5 Grading of Agarwood Resin

Agarwood's market value is determined by its quality, which trained human graders grade based on colour, odour, fixative, and consumer perception. Different countries have different labels for Agarwood quality, such as 'high quality' and 'low quality,' or categorising it into groups A, B, C, or D (Ngadiran et al., 2023; Shivanand et al., 2022). The ABC system in Malaysia heavily determines Agarwood grades by its appearance. There are various classification schemes available to compare harvested wild or synthetic Agarwood. Malaysia has no specific system for grading Agarwood resin, but it heavily depends on its appearance.

The ABC system is used, with the highest grade identified as A, followed by AB, B, BC, and C, and the lowest grade as CD. The resin content is categorised into Grade A, which is brown/black wood; Grade B, which is yellow with flecks of brown/black; and Grade C, which is white with some yellow/brown flecks. In addition, as mentioned in the previous study, Grade A is called kalambak, while lower grades are called gaharu (Liu et al., 2017). According to Mazlan and Dahlan (2010), the colour of resin is Grade A Black turning into grey, AB Black turning into brown, C grey, D grey, and whitish.

Table 4.9 shows the various grades of Agarwood resin (A, AB, BC, C, and CD) acquired through the inoculation method from each company. Figure 4.12, 4.13 and

4.14 depict the Agarwood resin's structure, with different thicknesses and patterns reflecting specific physical characteristics.

After an 18-month inoculation period, this research demonstrated that the highest grade produced by all of the chosen companies was an AB, followed by grades of BC, C, and C. 30% and 40% of AB grade (thick) were produced by Biobenua Teknoloji Sdn. Bhd. and Redclaw Aqua and Herbs Sdn. Bhd., respectively (hence referred to as BB and RAHE). The results also showed that Inokulan Gaharu Biotech Sdn. Bhd. (IGB) generated 30% of the BC (thick) grade of Agarwood, compared to Sansen Agarwood Sdn. Bhd. (SS), which only produced 2% of the BC (thin) grade. The second-lowest grade of Agarwood, C grade (5%) produced by Etlingera Sdn. Bhd. (ELG), showed that ELG's inoculum was ineffective in stimulating the finest grade of Agarwood. The output of Agarwood oil from these companies ranged from 40% to 98%, with SS producing the maximum proportion of it. In comparison, BB only produced 40% since it generated more Agarwood chips with various grades.

After a 24-month inoculation period, the highest grade of Agarwood resin was determined to be A, followed by AB, BC, and grade C. Agarwood grade A was produced by Company SS and RAHE to a respective extent of 32% and 60%. In contrast, BB and IGB were responsible for producing 30% and 100% grade C Agarwood resin, respectively. According to these results, RAHE produced the highest quality Agarwood resin among the other companies.

Agarwood quality is determined by resin content and other components; nevertheless, Agarwood scents do not always reflect quality. Thus, the Malaysian Timber Industry

Board (MTIB) proposed a classification system for Agarwood products to provide a standardised grade and indicative price list (Mohamed & Lee, 2016).

**Table 4.9: The Grades of Agarwood Resin (A, AB, BC, C, and CD) Acquired through the Inoculation Method from Each Company**

| Company | 18 Months | | 24 Months | |
|---|---|---|---|---|
| | Grade | Yield (%) | Grade | Yield (%) |
| **Biobenua Teknoloji Sdn. Bhd.** | AB (Thick) | 30 | C (Thin) | 30 |
| | BC (Pattern) | 10 | Oil (Good) | 70 |
| | BC (Thin) | 5 | | |
| | BC (Thick) | 5 | | |
| | C (Pattern) | 10 | | |
| | Oil (Good) | 40 | | |
| **Sansen Agarwood Sdn. Bhd.** | BC (Thin) | 2 | A (Patern) | 1 |
| | Oil (Good) | 98 | A (Thin) | 1 |
| | | | A (Thick) | 30 |
| | | | AB (Pattern) | 1 |
| | | | AB (Thin) | 1 |
| | | | AB (Thick) | 5 |
| | | | BC (Thin) | 1 |
| | | | BC (Thick) | 5 |
| | | | Oil (Good) | 55 |
| **Redclaw Aqua and Herbs Sdn. Bhd.** | AB (Thick) | 40 | A (Pattern) | 30 |
| | BC (Thin) | 20 | A (Thin) | 20 |
| | C (Pattern) | 5 | A (Thick) | 10 |
| | C (Thin) | 10 | AB (Pattern) | 20 |
| | CD (Thick) | 5 | AB (Thin) | 5 |
| | Oil (Good) | 20 | Oil (Good) | 15 |
| **Inokulan Gaharu Biotech Sdn. Bhd.** | BC (Thick) | 30 | C (Thin) | 40 |
| | Oil (Good) | 70 | C (Thick) | 60 |
| **Etlingera Sdn. Bhd.** | C (Thin) | 5 | AB (Pattern) | 5 |
| | Oil (Good) | 95 | BC (Thin) | 5 |
| | | | BC (Thick) | 5 |
| | | | Oil (Good) | 85 |

**Figure 4.12:** Grade Pattern

**Figure 4.13:** Grade Thin

**Figure 4.14**: Physical Properties of Grade of Agarwood Resin Formation

## 4.6 Production of Agarwood Oil

The oil is typically extracted from Agarwood with low oil concentration by the process of distillation. It is often used as a prominent base note for perfumes owing to its rich aroma, low volatility, and extended fragrance longevity (Ampson & Page, 2018).

The lower grade of Agarwood was subjected to hydrodistillation to produce Agarwood oil. The oil extracted was then collected prior to analysis. The chemical analysis of Agarwood oil was undertaken by gas chromatography-mass spectrometry (GC-MS). Compound identification was done by comparing the HPCH2205.L and Wiley7Nist05.L library data of the peaks with those reported in literature and mass spectra of the peaks with literature data.

### 4.6.1 Biobenua Teknoloji Sdn. Bhd.

Biobenua Teknoloji Sdn. Bhd. produces 1.7 ml of Agarwood essential oil for each kilogram of Agarwood processed. The GC-MS analysis revealed 21 compounds: one monoterpene, seven sesquiterpenes, eleven oxygenated sesquiterpene and two diterpenes. The oxygenated sesquiterpene is the most prevalent, accounting for 46.5% of the total. The major compound contributors were 10-epi-γ-eudesmol (10.51%) and α-cadinol (19.20%). Other oxygenated sesquiterpene contributions included α-calocarene, elemol, 1-epi-cubenol, γ-eudesmol, agarospirol, β-eudesmol, dihydroeudesmol, cadalene, and dihydrocolumellarin. Seven sesquiterpene hydrocarbons were obtained, namely α-cedrene, allo-aromadendrene, γ-amorphene, α-

muurolene, γ-cadinene, δ-cadinene, and α-cadinene. Other compounds discovered were 4-phenyl-2-butanone, dehydroabietol, and cis-ferruginol acetate.

### 4.6.2 Sansen Agarwood Sdn. Bhd.

Sansen Agarwood Sdn. Bhd. produces 1.2 ml of Agarwood oil for every kilogram of Agarwood processed. The Agarwood oil was then subjected to GC-MS analysis and obtained 24 chemical constituents. The major group of compounds was oxygenated sesquiterpenes, with thirteen compounds identified, viz., α-calocarene, elemol, 10-epi-γ-eudesmol, 1-epi-cubenol, γ-eudesmol, agarospirol, β-eudesmol, α-cadinol, dihydroeudesmol, β-bisabolol, lippifoli-1(6)-en-5-one, cyclocolorenone, and dihydrocolumellarin. Other compounds identified were eight sesquiterpene hydrocarbons, one monoterpene hydrocarbon, and two diterpene compounds. The major compounds were identified as 10-epi-γ-eudesmol (9.70%), α-cadinol (19.32%), dihydroeudesmol (5.05%), and β-eudesmol (4.93%).

### 4.6.3 Redclaw Aqua and Herbs Sdn. Bhd.

Each kilogram of Agarwood inoculated from Redclaw Aqua and Herbs Sdn. Bhd. produced 2.1 ml of Agarwood essential oil. The chemical profiling showed the presence of 23 compounds. These compounds include 4-phenyl-2-butanone, α-cedrene, β-agarofuran, α-muurolene, γ-cadinene, δ-cadinene, α-calocarene, elemol, cis-isolongifolanone, 10-epi-γ-eudesmol, 1-epi-cubenol, γ-eudesmol, agarospirol, β-eudesmol, α-eudesmol, α-cadinol, dihydroeudesmol, β-bisabolol, lippifoli-1(6)-en-5-one, cyclocolorenone, dihydrocolumellarin, dehydro abietol, and cis-ferruginol

acetate. The major compounds were found as oxygenated sesquiterpenes, such as 10-epi-γ-eudesmol (**7**.79%) and α-cadinol (11.27%).

### 4.6.4 Inokulan Gaharu Biotech Sdn. Bhd.

1.5 ml of Agarwood essential oil was produced from every one kilogram of lower-grade Agarwood. A total of 21 compounds were obtained, consisting of an abundance of the major group of oxygenated sesquiterpenes (52.94%), followed by sesquiterpene hydrocarbons (12.11%), diterpenes (2.81%), and monoterpene hydrocarbons (1.44%). 4-phenyl-2-butanone, α-cedrene, allo-aromadendrene, γ-amorphene, α-muurolene, γ-cadinene, δ-cadinene, α-cadinene, α-calocarene, elemol, 10-epi-γ-eudesmol, 1-epi-cubenol, γ-eudesmol, β-eudesmol, γ-muurolene, α-cadinol, dihydroeudesmol, β-bisabolol, dihydrocolumellarin, dehydro abietol, and cis-ferruginol acetate.

### 4.6.5 Etlingera Sdn. Bhd.

1.5 ml of Agarwood essential oil was produced from one kg of Agarwood from Etlingera Sdn Bhd's inoculum. 4-phenyl-2-butanone, α-cedrene, allo-aromadendrene, γ-amorphene, α-muurolene, γ-cadinene, δ-cadinene, α-cadinene, α-calocarene, elemol, 10-epi-γ-eudesmol, 1-epi-cubenol, γ-eudesmol, β-eudesmol, α-eudesmol, α-cadinol, dihydroeudesmol, dihydrocolumellarin, dehydro abietol, and cis-ferruginol acetate. Two major compounds were identified from oxygenated sesquiterpene, namely 10-epi-γ-eudesmol and α-cadinol.

## 4.7 Quality of Agarwood Essential Oil

In summary, twenty-eight compounds were discovered: eight sesquiterpene hydrocarbons and 17 oxygenated sesquiterpenes, together with one monoterpene and two diterpenes. Figure 4.15 displays the percentage contribution of each terpene group for all Agarwood essential oil (EO) samples. Based on the terpene compounds found in the Agarwood EOs, the Agarwood oil from BB contained 46.50% of oxygenated sesquiterpenes, 11.07% of sesquiterpene hydrocarbons, 1.57% of monoterpene hydrocarbons, and 3.49% of diterpenes. Meanwhile, the Agarwood oil induced by the SS contained 49.53% of oxygenated sesquiterpenes, 12.45% of sesquiterpene hydrocarbons, 1.58% of diterpenes, and 1.34% of monoterpenes. The Agarwood oil induced by the RAHE, IGB, and E methods contained 45.75, 52.94, and 49.12% of oxygenated sesquiterpenes; 11.08, 12.11, and 11.74% of sesquiterpene hydrocarbons; 1.91, 2.81, and 4.53% of diterpenes; and 1.34, 1.44, and 1.64% of monoterpenes, respectively.

The result showed that most terpene groups in all Agarwood EOs were oxygenated sesquiterpenes and sesquiterpene hydrocarbons, as well as monoterpenes and diterpenes (Chong et al., 2015; Haron et al., 2020; Zhang et al., 2012). The Agarwood EO chemical profile is a complex mixture of sesquiterpenes and their chromone derivatives, with pattern variation indicating the behaviour of chemical compounds (Ismail et al., 2013). The quality of the Agarwood EO corresponds directly to the amount of sesquiterpenes that it contains (Ngadiran et al., 2023; Shivanand et al., 2022). The main composition of Agarwood EO has been discovered as oxygenated sesquiterpene through this analysis, which was identified as a major constituent in luxury perfume (Latib et al., 2018). Also, this result is consistent with previous studies

and suggests that sesquiterpenes prominently influence the wood aroma and quality of Agarwood (Abd Majid et al., 2018). Thus, these significant compounds from sesquiterpene are expected to yield a pleasant odour and aroma of Agarwood (Hashim et al., 2014).

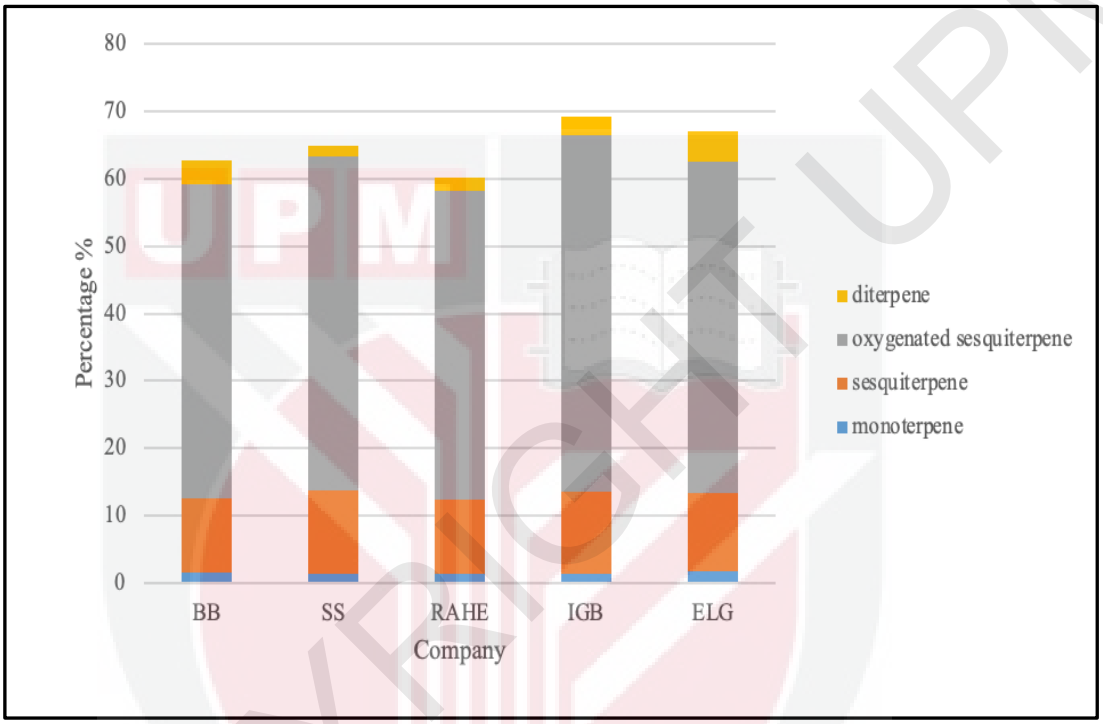


**Figure 4.15**: The percentage contribution of each terpene group for five Agarwood EO. Note: BB- Biobenua Teknoloji Sdn Bhd; SS – Sansen Agarwood Sdn Bhd; RAHE- Redclaw Aqua and Herbs Sdn Bhd; IGB- Inokulan Gaharu Biotech Sdn Bhd; ELG- Etlingera Sdn Bhd

Table 4.10 tabulates the relative percentages of every volatile compound from the terpene group in the Agarwood EO of five inoculums. Agarwood EO from SS exhibited the highest number of chemical compounds; meanwhile, the least chemical compounds were present in Agarwood EO from ELG. The most common volatile compounds observed in all tested Agarwood EOs were known as 4-phenyl-2-butanone, α-cedrene, α-muurolene, γ-cadinene, δ-cadinene, α-calocarene, elemol, 10-epi-γ-eudesmol, 1-epi-cubenol, γ-eudesmol, β-eudesmol, α-cadinol, dihydroeudesmol, dihydrocollumellarin, dehydro abietol, and *cis*-ferruginol acetate,

some of which were discovered in previous studies (Chong et al., 2015; Haron et al., 2020; Tajuddin & Yusoff, 2010). Few major compounds were detected in all tested essential oils, such as 10-epi-γ-eudesmol, α-cadinol, δ-cadinene, β-eudesmol, and dihydroeudesmol. Nevertheless, some common compounds of essential oil only appeared in a particular sample, such as β-agarofuran in SS, and RC, agarospirol in BB, SS and RAHE; α-eudesmol in RAHE and ELG; γ-muurolene in IGB; and β-bisabolol in SS, RAHE, and IGB.

**Table 4.10**: Chemical Composition using GC-MS Analysis of Agarwood EO Inoculated with Commercial Inoculum from Five Companies

| No | Compounds | Peak Area (%) | | | | |
|---|---|---|---|---|---|---|
| | | BB | SS | RAHE | IGB | ELG |
| ***Monoterpene hydrocarbon*** | | | | | | |
| 1 | 4-phenyl-2-butanone | 1.57 | 1.34 | 1.34 | 1.44 | 1.64 |
| ***Sesquiterpene hydrocarbons*** | | | | | | |
| 2 | α-cedrene | 0.66 | 0.61 | 0.47 | 0.71 | 0.68 |
| 3 | Allo-aromadendrene | 0.30 | 0.45 | - | 0.53 | 0.52 |
| 4 | β-agarofuran | - | 1.98 | 4.48 | - | - |
| 5 | γ-amorphene | 1.06 | 0.91 | - | 1.06 | 1.05 |
| 6 | α-muurolene | 1.77 | 1.74 | 1.18 | 1.87 | 1.85 |
| 7 | γ-cadinene | 1.97 | 1.85 | 1.47 | 2.12 | 2.05 |
| 8 | δ-cadinene | 4.92 | 4.51 | 3.48 | 5.31 | 5.14 |
| 9 | α-cadinene | 0.39 | 0.40 | - | 0.51 | 0.45 |
| ***Oxygenated sesquiterpene*** | | | | | | |
| 10 | α-calocarene | 0.62 | 0.69 | 0.44 | 0.63 | 0.65 |
| 11 | elemol | 1.97 | 1.85 | 1.43 | 2.16 | 2.09 |
| 12 | *cis*-isolongifolanone | - | - | 0.96 | - | - |

**Table 4.13**: **Continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | **10-epi-γ-eudesmol** | **10.51** | **9.70** | **7.79** | **11.21** | **10.93** |
| 14 | 1-epi-cubenol | 1.01 | 0.59 | 1.67 | 0.43 | 1.03 |
| 15 | γ-eudesmol | 0.67 | 0.60 | 0.51 | 0.65 | 0.70 |
| 16 | Agarospirol | 2.94 | 1.01 | 1.91 | - | - |
| 17 | β-eudesmol | 5.21 | 4.93 | 1.50 | 4.68 | 2.82 |
| 18 | α-eudesmol | - | - | 4.75 | - | 5.35 |
| 19 | γ-muurolene | - | - | - | 2.28 | - |
| 20 | **α-cadinol** | **19.20** | **19.32** | **11.27** | ***20.59** | **20.04** |
| 21 | Dihydroeudesmol | 3.22 | 5.05 | 4.90 | 5.43 | 4.73 |
| 22 | β-bisabolol | - | 3.05 | 2.43 | 4.18 | - |
| 23 | Cadalene | 0.41 | - | - | - | - |
| 24 | Lippifoli-1(6)-en-5-one | - | 0.94 | 2.53 | - | - |
| 25 | Cyclocolorenone | - | 0.74 | 2.02 | - | - |
| 26 | Dihydrocolumellarin | 0.74 | 1.06 | 1.64 | 0.70 | 0.78 |
| ***Diterpenes*** | | | | | | |
| 27 | Dehydro abietol | 1.77 | 1.07 | 0.85 | 1.32 | 3.17 |
| 28 | *Cis*-ferruginol acetate | 1.72 | 0.51 | 1.06 | 1.49 | 1.36 |
| **Monoterpene Hydrocarbon** | | **1.57** | **1.34** | **1.34** | **1.44** | **1.64** |
| **Sesquiterpene Hydrocarbon** | | **11.07** | **12.45** | **11.08** | **12.11** | **11.74** |
| **Oxygenated Sesquiterpene** | | **46.5** | **49.53** | **45.75** | **52.94** | **49.12** |
| **Diterpene** | | **3.49** | **1.58** | **1.91** | **2.81** | **4.53** |
| **Total area (%)** | | **62.63** | **64.9** | **60.08** | **69.3** | **67.03** |

*Note: BB- Biobenua Teknoloji Sdn Bhd; SS – Sansen Agarwood Sdn Bhd; RAHE- Redclaw Aqua and Herbs Sdn Bhd; IGB- Inokulan Gaharu Biotech Sdn Bhd; ELG- Etlingera Sdn Bhd.; '-': not identified; '*': highest abundance calculated by comparing its average peak area to the total areas. Identification by comparison of MS with those of the HPCH2205.L, and Wiley7Nist05.L library*.

The major compounds identified in all five samples, which are 10-epi-γ-eudesmol (7.79 - 11.21%), α-cadinol (11.27-20.59%), and δ-cadinene (3.48-5.31%), were commonly found in essential oil from Malaysian *A. malaccensis* (Ahmaed & Kulkarni, 2017). Together, 4-phenyl-2-butanone and agarospirol were also consistently present in *A. malaccensis* Agarwood samples (Jong et al., 2014). However, 4-phenyl-2-butanone has shown the lowest amount in all samples (1.34%-1.64%) compared to the previous study reported as the dominant compound in Agarwood (Ismail et al., 2013; Nor Azah et al., 2015; Tajuddin & Yusoff, 2010). In addition, 4-phenyl-2-butanone may play a role in plant interaction with each other, means for survival, attracting pollinators, and predators to the pathogen and helping affected trees nearby (Yusof et al., 2018).

In addition, the Agarwood marker compounds, including agarospirol, agarofuran, and α-eudesmol, are found in specific essential oils like BB, SS, and RC. High concentrations of these compounds indicate high-grade Agarwood. High-quality Agarwood is determined by the abundance of these compounds, as well as the presence of important compounds like 4-phenyl-2-butanone, β-selinene, α-bulnesene, 10-epi-γ-eudesmol, aromadendrene, β-agarofuran, α- agarofuran, and γ-eudesmol. (Chong et al., 2015; Nor Azah et al., 2013; Yusof et al., 2018). Following study using artificial intelligence techniques indicated that β-agarofuran, α-agarofuran, and 10-epi-γ-eudesmol are also consistently found in high-quality oil (Haron et al., 2020). Figure 4.16 shows some of the marker compounds obtained in Agarwood essential oil.

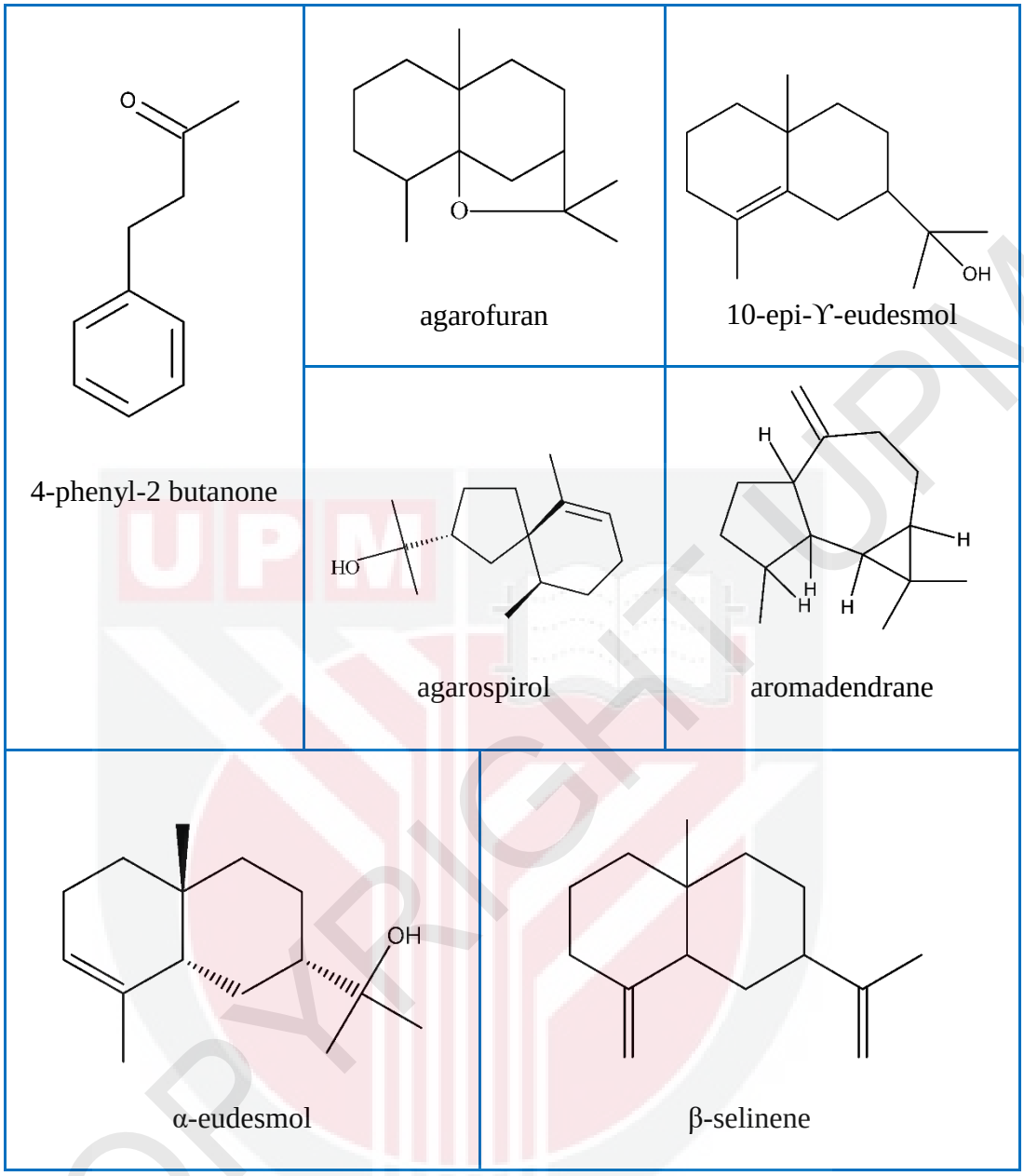


**Figure 4.16: Chemical Structure of Marker Compounds in Agarwood Essential Oil**

Previous studies have summarised man-treated and wild Agarwood oil quality, focusing on chemical compositions and host-pathogen relationships. Inoculum composition, natural variants, and host-pathogen relationships significantly influence resin production in Agarwood (Hashim et al., 2014). The differences in aromatic compounds among essential oils may be due to the resin production technique and the inoculum composition, which affects Agarwood resin quality (Chen et al., 2011;

Hashim et al., 2014). However, there is no significant difference in each essential oil's abundance of marker compounds. This is likely due to the hydrodistillation technique used in all essential oils, resulting in similar compounds (Hashim et al., 2016). Studying chemical composition in essential oils can help develop controlled grading and quality for commercial use (Hashim et al., 2016; Hashim et al., 2014; Yusof et al., 2018). Generating GC-MS profiling of Agarwood could develop marker compounds for grading and potentially serve as a parameter for measuring Agarwood quality in Malaysian plantations (Abd Majid et al., 2018).

## 4.8 Economic Value of Agarwood

Agarwood products, including wood pieces, chips, powder, oil, dust, incense, and perfumes, have a market value of several thousand billion USD globally (Shivanand et al., 2022). Since Agarwood is a key component of perfume and incense and is prized for its spiritual, luxurious, and aphrodisiac properties, Agarwood scent is essential to grading Agarwood quality. The market has varying proportions for various quality levels (Wahyuni & Prihantini, 2020). The market varies for different quality levels, with first-grade Agarwood being highly valued at several thousand US dollars per kilogram (Chowdhury et al., 2017).

**Table 4.11: Agarwood Resin for Each Company and Its Estimation Market Value**

| Inoculum/Company | Agarwood resin | Price (estimated) per kg |
|---|---|---|
| Black Gold BioBooster/ Biobenua Teknoloji Sdn. Bhd. |  | USD170 |
| TW Destang/Sansen Agarwood Sdn. Bhd. |  | USD150 |
| RAHE Pro-Bio/Redclaw Aqua and Herbs Sdn. Bhd. |  | USD200 |
| IGB Inoculum 711/ Inokulan Gaharu Biotech Sdn. Bhd. |  | USD170 |
| UPM V16/ Etlingera Sdn. Bhd. |  | USD180 |

Each company gave an average range from 2.4 to 29.0 kg of resinous wood. The estimated value of Agarwood resin in the international market ranges, reflecting its quality (Table 4.11). Company RAHE may produce the finest quality, as its highest market value is USD 200 per kg. Meanwhile, Company SS's result was the average resin quality; its estimated value was USD 150 per kg. When Agarwood chips are processed into oil, the Agarwood oil is sold at USD 100 per tola. This finding also showed that company RAHE produces the highest yield, 2.1 ml of Agarwood essential oil for every kilogram of Agarwood chip.

Therefore, based on the cost-effective assessment, it could be concluded that Agarwood production from company RAHE would be more economically profitable for the rural Agarwood tree growers in Malaysia.

# CHAPTER 5

# CONCLUSION

## 5.1 Introduction

Conclusively, in the present study, five inoculums were analysed and compared, namely Black Gold BioBooster from Biobenua Teknoloji Sdn. Bhd., TW Destang (Sansen Agarwood Sdn. Bhd.), RAHE Probio (Redclaw Aqua and Herbs Sdn. Bhd.), IGB711 (Inokulan Gaharu Biotech Sdn. Bhd.), and UPMV16 (Etlingera Sdn. Bhd.). Four techniques were demonstrated via chemjet, dripping bag, bamboo stick, and infusion system (bottle).

This study examined the efficacy of five inoculums in promoting the development of artificial Agarwood. The GC-MS analysis disclosed the chemically active constituents of each inoculum for the first time. The most active chemicals were found in the inoculum RAHE Pro-Bio, which provided 78.12%. Additionally, it was shown that the chemical mainly responsible for boosting Agarwood production belongs to the fatty acid ethyl ester (FAEE) group. On the other hand, these inoculums are free of any metal hazards, except UPM V16, which includes a greater concentration of Cu, which may be detrimental in prolonged use. The findings of the chemical analysis of each inoculum are summarised in Table 5.1.

**Table 5.1: Summary of Chemical Analysis of Each Inoculum via GC-MS and ICP-OES**

| Inoculum | Chemical Constituents Analysis | Metal Element Analysis |
|---|---|---|
| Black Gold BioBooster | 4 compounds (5.39%)<br>FAEE (2.22%) | Not detected |
| TW Destang | 1 compound (21.94%)<br>FAEE (21.94%) | Not detected |
| RAHE Pro-Bio | 20 compounds (78.12%)<br>FAEE (8.00%) | Not detected |
| IGB Inoculum 711 | 4 compounds (21.77%)<br>FAEE (11.34%) | Not detected |
| UPM V16 | 1 compound (20.63%)<br>FAEE (not detected) | Cu (16.5855 g/mL) |

The formation of Agarwood resin was observed in two phases: an 18-month and a 24-month incubation time. Agarwood resin was extracted and processed into woodchips. Meanwhile, hydro distillation turned the lower-grade Agarwood chips into essential oil (EO). Table 5.2 provides an overview of the average Agarwood resin production between 18 and 24 months and the yield of Agarwood EO for each company engaged.

The findings demonstrated that company BB produced the most Agarwood resin in the shortest time, whereas company IGB produced the most resin after a 24-month inoculation process. At the same time, the company RAHE effectively produces Agarwood EO, which is about 2.1 ml for every kg of Agarwood woodchips.

**Table 5.2: Summary of Agarwood Resin and Agarwood Oil Production**

| Company | Average Agarwood resin (in kg) | | Agarwood Oil Yield (per kg) |
|---|---|---|---|
| | 18 months | 24 months | |
| Biobenua Teknoloji Sdn. Bhd. | 29.040 | 17.714 | 1.7 ml |
| Sansen Agarwood Sdn. Bhd. | 20.220 | 1.424 | 1.2 ml |
| Redclaw Aqua and Herbs Sdn. Bhd. | 8.920 | 22.565 | 2.1 ml |
| Inokulan Gaharu Biotech Sdn. Bhd. | 8.800 | 25.763 | 1.5 ml |
| Etlingera Sdn. Bhd. | 7.780 | 2.415 | 1.5 ml |

The ABC grading system indicated that grade A Agarwood resin was obtained after a 24-month inoculation process, with SS and RAHE yielding 30% and 60%, respectively. The highest grade, only an AB grade, was produced during the 18-month inoculation period, with BB and RAHE producing 30% and 40% of the grade, respectively. Therefore, based on this result, it can be concluded that during the 18- and 24-month inoculation periods, company RAHE produced the best possible quality of Agarwood resin.

Hydro distillation of each kilogram of lower-grade Agarwood chips yielded 1.2 ml to 2.1 ml of Agarwood oil for each company. The chemical components of Agarwood essential oil were examined using GC-MS, and twenty-eight compounds were discovered. The main fraction was found to be oxygenated sesquiterpenes, with IGB contributing the most (52.94%) and BB contributing the least (46.50%).

Sesquiterpene, diterpene, and monoterpene hydrocarbons were the next subgroups of the terpene group. The marker compounds of Agarwood oil were 4-phenyl-2-butanone, α-cadinol, δ-cadinene, α-bulnesene, 10-epi-γ-eudesmol, agarospirol, β-agarofuran, and α-eudesmol. These compounds are responsible for the woody and pleasant aroma of Agarwood. Table 5.3 summarises the presence of marker compounds in each respective Agarwood oil with comparisons to the previous study. This study found that all five respective Agarwood oils showed that two major compounds contributed the most, namely 10-epi-γ-eudesmol and α-cadinol.

**Table 5.3: Marker Compounds in Individual Agarwood Oil with Comparisons to Previous Study**

| Compound | BB | SS | RAHE | IGB | ELG | (Tajuddin & Yusoff, 2010) |
|---|---|---|---|---|---|---|
| 4-phenyl-2-butanone | √ | √ | √ | √ | √ | √ |
| α-cadinol | √ | √ | √ | √ | √ | √ |
| δ-cadinene | √ | √ | √ | √ | √ | |
| 10-epi-γ-eudesmol | √ | √ | √ | √ | √ | √ |
| agarospirol | √ | √ | √ | | | √ |
| β -agarofuran | | √ | √ | | | √ |
| α-eudesmol | | | √ | | √ | √ |

## 5.2 Recommendations for Future Studies

The present study makes several noteworthy contributions to extending knowledge about inoculums and inoculation techniques in producing artificial Agarwood from the *A. malaccensis* tree. However, this work has several drawbacks, including the absence of suitable previously reported data on the chemical contents of the inoculum and the uncertainty surrounding the mechanism behind Agarwood development. Since this study has concentrated on the chemical analysis of inoculums, other researchers and Agarwood farmers will be able to develop a chemical reference profile in the future.

As Agarwood resin is still graded using traditional methods, we also recommend that, using the findings from this and previous reports, a systematised grading mechanism be developed based on scientific evidence. This system will improve the transparency and efficiency of Agarwood trade activities while assisting authorised bodies in preventing fraud and price manipulation.

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## BIODATA OF STUDENT

Mohd Zamakhsyary Mustapa a local Klang-Born student has a strong background in the forestry and timber industry. He graduated Degree of Forestry from University Putra Malaysia (UPM) in 2007 and started to work with Malaysian Timber Industry Board in 2010. He continued this Master of Forest Management and Ecosystem Science in 2018. He has been actively involved in promoting sustainable forestry practices and enhancing the production of high-quality timber products in Malaysia. His work has significantly contributed to the development and management of forest plantations in the country. He has also been involved in research related to the agarwood industry, which is known for producing valuable resin used in perfumes, medicinal products, and other applications. His efforts have helped address the challenges of over-exploitation and conservation of agarwood-producing species.

## PUBLICATIONS

Mustapa, M. Z., Alias, M. A., Syed Abdul Azziz, S. S., Wong, C. F., Ibrahim, M., Yahaya, R., Bakri, Y. M., & Rajak, N. A. (2022). Agarwood Production of Aquilaria malaccensis Using Various Inoculants and Induction Techniques. Central Asia & the Caucasus (14046091), 23(1), 3042–3051. https://doi.org/10.37178/ca-c.21.5.085

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