



**Faculty of Resource Science and Technology**

**Formulation of Microbial Consortium as an Inoculant for Agarwood  
Induction**

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# Formulation of Microbial Consortium as an Inoculant for Agarwood Induction

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## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

A handwritten signature in black ink, appearing to be 'Sabella', written over a horizontal dotted line.

Signature

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## ABSTRACT

Agarwood or gaharu is well known as the most valuable resinous heartwood that can reach up to US\$100,000 per kilogram in market price due to its superior quality and high purity of materials. Agarwood has a high commercial value due to its fragrance properties and medicinal value. Agarwood commonly occur within the trees of *Aquilaria* species which belong to the family of Thymelaeaceae. The long period of agarwood formation in nature that takes many decades to develop had restrained the supply in the market which leads to overexploitation of gaharu in *Aquilaria* spp. in the forest. Thus, *Aquilaria malaccensis* has been listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Fungi were used to induced agarwood in *Aquilaria* trees. Agarwood is formed due to interaction between fungi and plant defense mechanism. Depletion of wild resource from the jungle leads to the high price of agarwood. Hence, it is the main concern to be handled by the researchers to find alternative ways to induce gaharu formation to meet the global demand for agarwood trading. Therefore, agarwood needs to be artificially induced for a better amount of resin production. This research was conducted to formulate microbial consortia that made up of fungi as fungal inoculants for agarwood induction in *Aquilaria* spp.. The effectiveness of the formulated microbial consortia was observed based on the colour and mean length of infected zone formation after a three month and six month inoculation periods in each wet and dry seasons, respectively. *Aquilaria* spp. was inoculated with three types of formulated fungal inoculants which were Formulation 1 (F1) consisting combination of *Trichoderma* sp. and *Aspergillus* sp., Formulation 2 (F2) consisting combination of *Fusarium* sp. and *Penicillium* sp., whereas Formulation 3 (F3) consisting combination of *Trichoderma* sp., *Lasiodiplodia* sp. and *Curvularia* sp.. Negative control used in this study was sterile distilled water. In this study,

the fungal inoculants were inoculated into the *Aquilaria* trees by using the bottle drip method. Production of secondary metabolites of induced *Aquilaria* trees was analysed by using Gas Chromatography-Mass Spectrometry (GC-MS). A total of 115 chemical compounds were detected in all inoculated trees where inoculation during the dry season produced a relatively greater number of chemical compounds compared to the wet season. Major constituents of the gaharu such as sesquiterpenes, chromones, fatty acid methyl ester, and aromatic compounds were found in inoculated *Aquilaria* trees. New agarwood sesquiterpenes, cryptomeridiol (0.93%) has been detected in F3 inoculated *Aquilaria* tree after six months inoculation during the dry season. Besides, aromatic compound such as 3',5' dimethoxyacetophenone (0.25%), guaia-1 (10), 11-diene (0.45%), octacosanol (0.57%) and heptadecanolide (0.24%) can only be detected in F3 inoculated *Aquilaria* trees. These chemical compounds were known as major constituents that contribute to aromatic properties in agarwood. The findings showed that F3 inoculant (a combination of *Trichoderma* sp., *Lasiodiplodia* sp., and *Curvularia* sp.) is the most potential fungal inoculant in inducing resin in *Aquilaria* tree due to its largest length of resinous zone and the presence of metabolic compound in inoculated *Aquilaria* trees after three and six months inoculations for both wet and dry seasons, respectively. From this study, it is recommended that the formulated microbial consortium can be used as potential agarwood fungal inoculant for future sustainable supply of agarwood.

**Keywords:** Agarwood, bottle drip method, GC-MS, microbial consortium

## **Perumusan Konsortium Mikrob sebagai Inokulan untuk Induksi Gaharu**

### **ABSTRAK**

Gaharu terkenal sebagai kayu jantung resin yang paling berharga yang boleh mencapai sehingga AS\$100,000 sekilogram dalam harga pasaran kerana kualiti unggul dan ketulinan tinggi bahan. Gaharu mempunyai nilai komersil yang tinggi kerana sifat haruman dan nilai perubatannya. Gaharu biasanya berlaku di dalam pokok spesies Aquilaria yang tergolong dalam keluarga Thymelaeaceae. Walaubagaimanapun, tempoh inokulasi gaharu adalah panjang secara semulajadi yang mengambil masa beberapa dekad untuk pembentukan gaharu, telah menghalang bekalan gaharu di pasaran yang membawa kepada lebih eksploitasi gaharu di Aquilaria spp. Oleh itu, Aquilaria malaccensis telah disenaraikan dalam Lampiran II Konvensyen mengenai Perdagangan Antarabangsa Spesies Fauna dan Flora Liar (CITES) yang terancam. Kulat telah digunakan dalam induksi gaharu dalam pokok Aquilaria. Gaharu dibentuk disebabkan interaksi antara kulat dan mekanisme pertahanan tumbuhan. Penurunan sumber gaharu liar dari hutan menyebabkan harga gaharu menjadi tinggi. Oleh itu, ia menjadi kebimbangan utama para penyelidik dalam menangani permintaan dagangan gaharu secara global. Oleh itu, kayu gaharu perlu diinduksi untuk mempertingkatkan jumlah pengeluaran resin. Kajian ini dijalankan untuk merumuskan konsortia mikrobial yang mengandungi kulat sebagai inokulan untuk induksi gaharu dalam spesies Aquilaria. Keberkesanan konsortia mikrobial yang dirumus diperhatikan berdasarkan tempoh dan warna panjang pembentukan zon yang dijangkiti selepas tempoh inokulasi selama tiga dan enam bulan di setiap musim basah dan kering. Aquilaria sp. telah diinokulasi dengan tiga jenis formulasi kulat yang terdiri daripada Formulasi 1 (F1) mengandungi gabungan Trichoderma sp. dan Aspergillus sp., Formulasi 2 (F2) mengandungi gabungan Fusarium

*sp. dan Penicillium sp., dan Formulasi 3 (F3) mengandungi gabungan Trichoderma sp., Lasiodiplodia sp. dan Curvularia sp.. Air suling steril digunakan sebagai kawalan negatif dalam kajian ini. Dalam kajian ini, kulat digunakan untuk menyuntik pokok Aquilaria dengan menggunakan kaedah titisan botol sebagai kaedah inokulasi tiruan. Pengeluaran metabolit sekunder oleh pokok Aquilaria ditentukan dengan menggunakan analisis Kromatografi Gas-Spektrometri Jisim (GC-MS). Sebanyak 115 sebatian kimia dikesan dari semua pokok yang diinokulasi di mana inokulasi pada musim kering mempunyai bilangan sebatian kimia yang lebih banyak berbanding dengan musim basah. Unsur-unsur utama seperti seskuiterpena, kromon, metil ester asid lemak, dan sebatian aromatik telah ditemui dalam pokok-pokok Aquilaria yang diinokulasi. Seskuiterpena gaharu baharu, kriptomeridiol (0.93%) telah dikesan dalam pokok Aquilaria F3 selepas 6 bulan inokulasi pada musim kering. Selain itu, sebatian aromatik seperti 3',5' dimetoksiasetofenon (0.25%), guaia-1 (10),11-diena (0.45%), oktakosanol (0.57%) dan heptadekanolide (0.24%) hanya dapat dikesan dalam pokok Aquilaria F3. Sebatian kimia ini dikenali sebagai unsur utama yang menyumbang kepada sifat aromatik gaharu. Hasil kajian menunjukkan bahawa F3 inokulan (gabungan Trichoderma sp., Lasiodiplodia sp., dan Curvularia sp.) adalah inokulan kulat yang paling berpotensi untuk membentuk resin di dalam pokok Aquilaria kerana zon resinnya yang terbesar dan kehadiran sebatian metabolit pokok-pokok Aquilaria yang diinokulasi selepas tiga dan enam bulan inokulasi masing-masing bagi musim basah dan kering. Dari kajian ini, adalah disarankan bahawa konsortium kulat yang dibangunkan berpotensi digunakan sebagai inokulan untuk pengeluaran bekalan gaharu yang berterusan.*

**Kata kunci:** *Gaharu, kaedah titisan botol, GC-MS, konsortium mikrob*

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## LIST OF ABBREVIATIONS

|        |                                      |
|--------|--------------------------------------|
| cm/sec | Centimetres per second               |
| DMSO   | Dimethyl sulfoxide                   |
| g      | Gram                                 |
| GCMS   | Gas Chromatography Mass Spectrometry |
| kPa    | Kilo Pascal                          |
| L      | Litre                                |
| ml/min | Millilitre per minute                |
| m      | Metre                                |
| mm     | Millimetre                           |
| PDA    | Potato Dextrose Agar                 |
| PDB    | Potato Dextrose Broth                |
| Psi    | Pounds per square inch               |
| RI     | Retention Index                      |
| Spp.   | Species                              |
| TIC    | Total Ion Chromatography             |
| °C     | Degree Celsius                       |
| µl     | Microlitre                           |
| %      | Percentage                           |

# CHAPTER 1

## INTRODUCTION

### 1.1 General Introduction

Agarwood is known as dark resinous heartwood that occurs in trees of *Aquilaria*, *Gonystulus*, and *Gyrinops* spp. which belong to the Thymelaeaceae family. In Malaysia, agarwood is also known as gaharu. These fragrant resinous woods have high value component as it can be used in religious activities such as incense, alternative medicine, and perfume industries. Chen et al. (2011) state that agarwood essential oil has antimicrobial properties.

The world most precious resinous aromatic wood which is known as agarwood can reach up to US\$100,000 per kilogram in the market price for excellent quality (Naef, 2011). Due to increased need of agarwood, *Aquilaria malaccensis* were listed as the most endangered species among all agarwood trees species as stated in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Uncontrol harvest of *Aquilaria* sp. in wild jungle lead to the almost extermination of *Aquilaria* sp. (CITES, 2015).

Generally, agarwood form when the tree is injured either in the main branch or the tree stem (Mohamed et al., 2010). Only 7-10% of the wild agarwood trees in the natural jungle were made by fungi infection (Ng et al., 1997). Fungal endophyte is the common pathogen that infects the wounded tree. Fungal endophytes are microorganisms that commonly involves in producing a distinct group of secondary metabolites (Monggoot et al., 2017). Thus, fungal endophytes were relevant in this study as the infected tree will

produce a volatile organic compound in the response of fungi infection causing the suppression of the fungal growth (Ashwin, 2009). Furthermore, endophytic fungi can synthesize bioactive compounds that found to be useful in the discovery of novel drugs as a plant response against microbial attack (Guo et al., 2008; Yan et al., 2011).

Agarwood needs to be induced artificially to maintain the diversity of *Aquilaria* sp. to increase resin production. To meet the worldwide needs of agarwood, sustainability production of agarwood needs to be increased with large scale plantation of *A. malaccensis* and efforts in the formulation of effective artificial inoculants for agarwood inducement. The cultivation of *Aquilaria* trees has been widely practiced in many countries to meet the need for agarwood.

Based on the previous study, the formation of resinous agarwood inside the tree can takes years as it is exposed to natural or artificial injury, include cutting, pest or insect disturbance, microbial infection, fire and more (Chhipa & Kaushik, 2017). In this study, artificial inoculations were done in inducing agarwood for a short period. Artificial inoculation includes wounding, chemicals and microbes inducing agents have been used in inducing agarwood (Pojanagaroon & Kaewrak, 2005; Zhang et al., 2010). Media for fungi growth, type of fungal strain and injection methods are important factors in artificial induction of agarwood formation (Santoso et al., 1996). It has been widely reported that various fungi have been isolated from infected trees and scientists have research on the tree-fungi interaction related to agarwood formation.

However, the production of agarwood in term of biological interaction between the fungi and the host tree is still poorly understood. The role of fungi in the artificial induction of agarwood is still an unexplored area (Chhipa & Kaushik, 2017). There is not

much good inoculum yet to be discovered for the inoculation of gaharu in term of colour and shorter period of agarwood production. Therefore, an examination of fungi infection on agarwood and analysis of their microbial activity is necessary. This can help researchers to develop effective microbial consortium as a fungal inoculant for artificial agarwood induction.

Thus, this study was carried out to induce the formation of resin within shorter periods of time in term of months as natural agarwood requires a long period which can take up to a decade to produce its resinous compounds (Chen et al., 2017). A new effective microbial consortium needs to be formulated as agarwood inducer to accelerate the resin formation in planted agarwood in comparisons with wild agarwood in the species of *A. malaccensis*. The exploitation of wild agarwood in the natural forest can be overcome through these studies.

The general objective of this research project was to formulate new microbial consortium by fungi isolated from *A. malaccensis* and to identify the potential fungal inoculant as agarwood inducer in *A. malaccensis* based on the presence of chemical constituents in inoculated *Aquilaria* tree. The hypothesis of this study is the formulated inoculant could induce higher resin formation in the gaharu tree within shorter period.

## **1.2 Objectives**

The objectives in this study were:

- i. To develop new formulation of microbial consortium as agarwood inducer.
- ii. To determine effects of selected microbial consortium toward agarwood formation.
- iii. To determine the quality of resin formation between wet and dry seasons.
- iv. To identify the chemical constituents in the inoculated agarwood

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 General overview of agarwood

Agarwood or also well-known as gaharu by locals in Malaysia, is an aromatic dark resinous heartwood present within *Aquilaria* trees belong to the plant family of Thymelaecaceae (Nor Azah et al., 2013). Oudh and eaglewood are also other common names for agarwood. Besides, the etymology of agarwood differs in different cultures. In Hindi, agarwood is well-known as agar by Sanskrit aguru (Bouverie, 1885). In Chinese, agarwood is known as chénxiang. In Japan, agarwood is known as jinko which means “sinking incense” where kyara is the highest grades of jinko (Kiyoko, 1992).

Agarwood cannot form within a healthy normal wood tissue because agarwood only forms in infected or injured *Aquilaria* plants (Okudera & Ito, 2009). In a natural habitat, agarwood can take several years to form within a damaged *Aquilaria* plant (Gerard, 2007). Agarwood formed in *Aquilaria* trees often relates to fungal infection (Mohamed et al., 2014). Ng et al. (1997) mentioned that fungi are the main responsible microbes that infect 7-10% of *Aquilaria* trees in wild jungle. Besides, Ng et al. (1997) also state that factors involved in agarwood formation include genetic background, tree age, variation of seasonal and ecological.

Agarwood can be obtained within the branches, stem and other parts of trees. Agarwood is collected in the form of wood before imported or exported in the form of powder, chips and lumps (Karlinasari et al., 2015). Agarwood is extremely valuable for its aromatic scent in soap production; agarwood oil and smoke are commonly used as an

incense in the Middle East (Chakrabarty et al., 1994). Agarwood oil is a high demand ingredient in the perfume industry due to its warm and distinct balsamic notes with sandalwood-ambergris tonalities (Zviely & Li, 2013). Agarwood can also be used for religious activities. For example, ‘minyak attar’ is a modified agarwood oil as a water-based perfumes that traditionally used to tie at Muslims prayer clothes (Yaacob, 1999).

In terms of soap manufacturers, agarwood essences are used as a scent in shampoos and detergents (Kadir et al., 1997, cited in Schippmann, 1999). Agarwood also has found applied in medication as it is often used as a pain reliever, arrest nausea by warming the abdominal, and asthma reliever in traditional East Asian medicine (Anonymous, 1995). Malaysians have discovered the use of coconut oil mixed with agarwood as an ointment, treating rheumatism and other body discomfort (Burkill, 1966).

Therefore, products made of agarwood have high demand in the worldwide market. Thus, Yusoff et al. (2015) state that the global demand for agarwood products makes agarwood as one of the most valued products in the world. It can be shown in global market that agarwood can be estimated at US\$6 billion to US\$8 billion (Akter et al., 2013). Besides, agarwood oil prices can reach up at US\$50,000 to US\$80,000 per liter (Persoon, 2007).

### **2.1.1 *Aquilaria malaccensis***

*Aquilaria malaccensis* has been well known as the major producer of agarwood in Malaysia (Mohamed et al., 2010). Borneo is a centre of *Aquilaria* sp. and *A. malaccensis* is a common species in Sarawak. *A. malaccensis* is one of 15 tree species in the genus *Aquilaria* (Mabberley, 1997). It also has been discovered that *A. malaccensis* commonly exists in Southeast Asia countries including Myanmar, Malaysia, Borneo Island, Vietnam

and more (Gibson, 1977; Hai et. al., 1999). Figure 2.1 shows a map distribution of gaharu tree species from a study done by Soeharto et al. (2016).

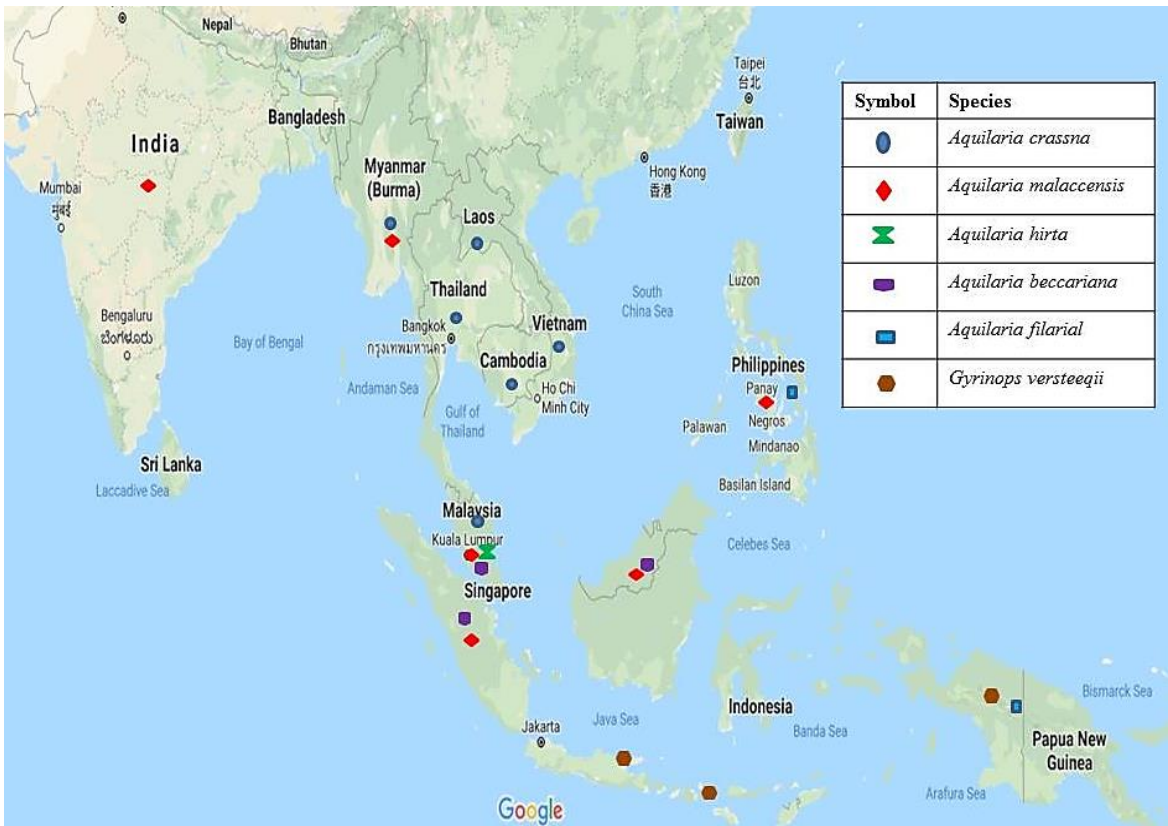


**Figure 2.1:** Distribution of gaharu tree species throughout Asian countries (GoogleMap, 2020)

*A. malaccensis* is described as a large evergreen tree that grows until 15-30 m tall and 1.5- 2.5 m in diameter and has flower in white colour (Chakrabarty et al., 1994). Soeharto et al. (2016) concluded that a mature *A. malaccensis* can grow up to 40 m in height and its flowering and fruiting occurred during the dry season based on observation in Indonesia Bogor Botanical Garden. It can be observed that young bark has fine hairs with light brown in colour while older bark has smooth and whitish in colour. Furthermore,

without the presence of resin within *A. malaccensis*, the wood is described as light, white, and soft, compared to wood that contain resin were heavy, dark, and hard.

Besides, *A. malaccensis* has an alternate, elliptic or lanceolate leaf in which the leaves wide are in 3-3.5 cm, having 12-16 pairs of veins with 6-8 cm long. The flower is described as fragrant, yellowish green or white and it is hermaphroditic with 5 mm long (Figure 2.2). At the age of five to six years of the tree, flowers and fruits were produced where the fruits are described to be green in colour, egg-shaped capsule and has fine hairs of leathery exocarp. The size of the fruit is around 4 cm long and 2.5 cm wide (Irnayuli et al., 2011).



**Figure 2.2:** (a) Yellowish green flower and green colour fruits of *Aquilaria malaccensis*. (b) The egg-shaped capsule of *Aquilaria* fruits showing its seed and elliptic leave of *A. malaccensis*. (Irnayuli et al., 2011)

### 2.1.2 Price and grading of agarwood

Agarwood or gaharu has a high price value in global market trade due to its aromatic resin. Presence of ‘gubal’ (Figure 2.3) within agarwood have high prices in economic value. Gubal is the component located within the sapwood portion of host tree stands that forms after infection of agarwood (Persoon, 2007).



**Figure 2.3:** Dark and black resinous gaharu wood known as “gubal” (Gaharubogor, 2018)

Grade of agarwood affects greatly on the price of agarwood in international trade. Hansen (2000) has stated that around \$1.2 billion value of agarwood has been exported from Singapore alone each year. In Malaysia, agarwood is graded based on their unique scent, colour, density and gaharu formation (Nor Azah et al., 2013). However, some agarwood is graded based on individual perceptions or experiences which has classified their grade according to the origin or its unique name. Nor Azah et al. (2009) stated that the price of premium grade agarwood (grade A) can be priced from RM16,000 to RM20,000 per kg whereas the price of its essential oil may be ranged from RM400-RM2000/tola (1 tola = 12 mL).

Lim et al. (2011) has classified the grade of agarwood according to their physical appearance as shown in Table 2.1. The grade of agarwood is affected by agarwood physical appearance. Table 2.2 shows the market prices of agarwood in year of 2014 in Kelantan, Malaysia based on their grade. Agarwood market price in Kelantan, Malaysia can be used as an example of Malaysia agarwood market trade.

**Table 2.1:** Grade of agarwood based on their physical appearance (Lim et al., 2011)

| Grade | Characteristic                      |
|-------|-------------------------------------|
| A     | Dark, dense, concentrated, heavy    |
| B     | Dark purple, less dense, small hole |
| C     | Dark yellow stripes, dark yellow    |
| D     | Whitish yellow                      |

**Table 2.2:** Prices of agarwood in Kelantan, Malaysia (Year 2014) (Gaharu Online, n.d.)

| Grade              | Market Price (per kg) |
|--------------------|-----------------------|
| Double Super Grade | RM 10,000 – RM 12,000 |
| Super Grade        | RM 8,000 – RM 10,000  |
| Grade A            | RM 4,000 – RM 8,000   |
| Grade B            | RM 3,000 – RM 4,000   |
| Grade C            | RM 1,000 – RM 2,000   |
| Mixed Grade        | RM 60 – RM 250        |
| Essential oil      | RM 19,000 – RM 38,000 |

### 2.1.3 Agarwood induction technique

The natural formation of agarwood takes a long time within 25 to 30 years and less yield may be obtained which leads to difficulties in meeting the demand on agarwood growing market (Zhang et al., 2010). Recovery of natural injured agarwood tree may affect the yield of agarwood. Thus, many efforts had been done on the induction technique of agarwood to induce agarwood more effectively within short periods to meet the high demand for agarwood in the global market. Traditional inoculation which is a physical

method for the induction of agarwood has been implemented for a long time. These physical methods were used to physically wounding the tree by making a hole with screws, removal of barks with hatchets, inflicting the wounds using chisels (Pojanagaroon & Kaewrak, 2005). Chen et al. (2011) observed that the methods of deliberately wounding the tree by using large knives and hammering of nails into the trunks are often used in China and other countries. However, these physical methods produced low yield and poor quality of agarwood that often takes a long time (Liu et al., 2013).

Hence, Professor Robert Blanchette from the University of Minnesota has developed innovative and effective methods known as cultivated agarwood kits (CA-Kits). The principle of this process is to wound the tree by drill holes in the tree trunk and the wound left open by inserted with a small piece of plastic pipe in the hole before the wound is inoculated with different chemical media (Blanchette & Heuveling, 2009; Liu et al., 2013). The common chemical agents used include sodium chloride, methyl jasmonic, sodium bisulfite, ferric chloride, ferrous chloride, formic acid, salicyclic acid, chitin, cellobiose, iron powder or yeast extract were applied to cells surrounding the wound (Blanchette & Heuveling, 2002). These chemical agents would then denature the living parenchyma cells around the wounded region of xylem and eventually stimulates the resin formation in the agarwood tree. According to Ye et al. (2016), the use of chemical methods aids to increase the quantity and quality of agarwood resin formation. However, Chen et al. (2011) stated that the yield of agarwood produced has mixed results in terms of quantity and quality.

Gibson (1977) reported a biological method known as fungi-based inoculation had been introduced. Fungal infection leads to a response of resin production (Oldfield et al., 1998). Thus, fungi were used to induced agarwood in *Aquilaria* trees as the resinous

agarwood formed due to the interaction of fungi with the plant defense mechanism to suppress or retard the fungi growth. Recently, research have been done to find the most effective fungi to accelerate the formation of agarwood intensively. Based on Irnayuli et al. (2011), agarwood has been successfully formed in a four years old agarwood tree in Indonesia after two months inoculate with *Fusarium laterium*. In Sebadu Village, West Kalimantan, Indonesia, had observed the usage of fungal species in inducing the formation of agarwood that includes *Fusarium laseritium*, *Fusarium oxysporum*, *Botryodiplodia* sp., and *Phytium* sp. (Irnayuli et al., 2011). Formation of agarwood is more preferred when fungal infection was used as an agarwood inducing agent.

Nowadays, fungal-based inoculation is more desirable due to its efficiency and more practical (Santoso et al., 2011). It is easier to obtain the result on the agarwood formation due to direct application of fungal inoculant in *Aquilaria* tree. Furthermore, the agarwood formation can be accelerated by high pathogenic type of fungus (Chen et al., 2018). Fungal based inoculation has been practiced by several agarwood producer countries and consequently had yielded more promising quality of agarwood.

To date, an additional method which involves fungi as inducing agents known as bottle drip method had been invented by Tang (2012). These methods have been conducted and been studied to find more effective fungal inoculants to induce agarwood artificially. The method utilizes discarded plastic bottles that filled with fungal inoculant as the cap of bottles were tightened before placed them in an inverted position and channelling of the tubes into a hole of drilled *Aquilaria* tree. Fungal-based inoculation of using bottle drip method is made to increase the effect directly in the artificial wound of *Aquilaria* tree to overcome the previous limitations of agarwood induction. Besides, this practice has shown

to be able to induce agarwood in *Aquilaria* tree as it can stimulate the *Aquilaria* tree self-callus mechanism completely.

#### **2.1.4 Agarwood chemical constituents**

Agarwood formed as the resin is deposited in the wood tissue of *Aquilaria* tree when exposed to a pathogenic infection or tree injury. Cui et al. (2013) studied that artificial inoculation by using fungi can induce the production of chemical characteristics of agarwood. Agarwood is valuable due to its fragrant resin in the heartwood. Hence, presence of a complex mixture of chemical compounds essentially contributes to the aromatic properties of agarwood which make it as one of high cost materials (Naef, 2011; Chen et al., 2012; Chu et al., 2013), that include chemical compounds such as P-methoxycinnamic acids, agarosterol, agarol, agarospirol,  $\alpha$  and  $\beta$  agarofuran, gmelofuran, dihydroagarofuran, 4-hydroxydihydroagarfuran, oxo-nor-agarofuran, cardinanes, eudesmanes, valencanes, eremophyllanes, guanines, prezizanes, vetispiranes, chromones, thialkaloid liriodenine and more other simple volatile aromatic compounds.

Sesquiterpenes and 2-(2-phenylethyl) chromone derivatives are two main active compounds that is commonly found in agarwood (Chen et al., 2011). In agarwood, sesquiterpenes are distributed into several categories based on their skeleton for instance agarofurans, agarospiropanes, guaianes, eudesmanes, eremophilanes and prezizaanes (Chen et al., 2012). Sesquiterpenes have medicinal values as anti-allergy and anti-inflammatory (Ashwin, 2009). More than 40 years ago, Indian chemists had discovered and first characterized the chemical compound in agarwood which is sesquiterpenes (Konishi et al., 2002). In Malaysia, Nor Azah et al. (2008) discovered many sesquiterpenes which includes  $\beta$ -agarofuran,  $\alpha$ -agarofuran, nor-ketoagarofuran, agarospirol, jinkoh-eremol, (-)-10-epi- $\gamma$ -

eudesmol, jinkohol ii,  $\alpha$ -guaiene,  $\beta$ -eudesmol in her research that involve chemical profile in agarwood oils. The following Figure 2.4 below shows the chemical structures of some sesquiterpenes group.

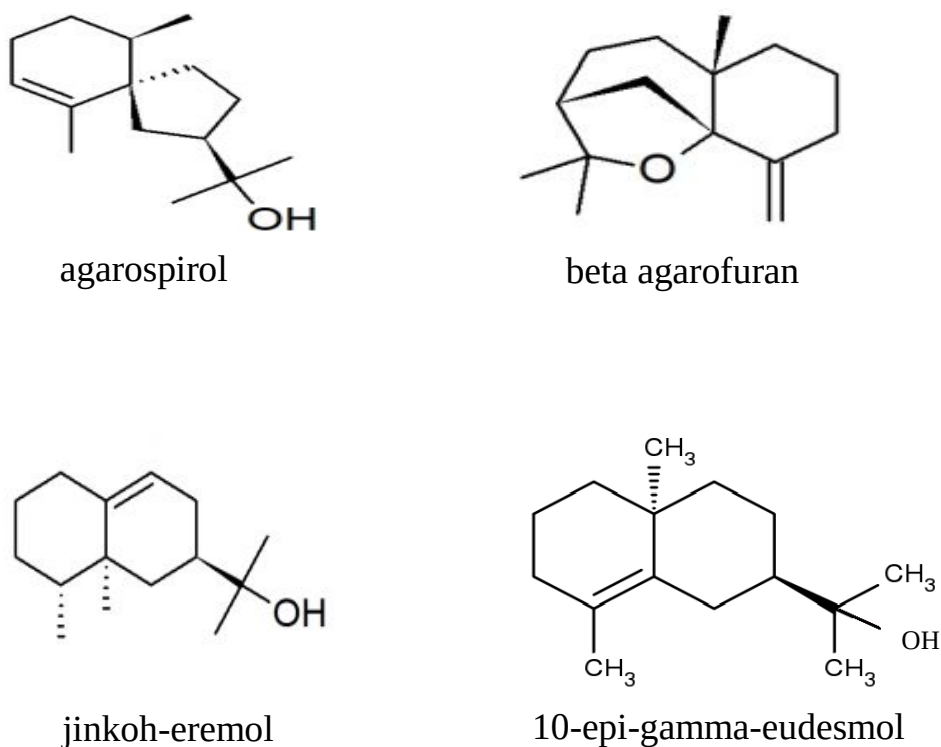


**Figure 2.4:** Chemical structure of sesquiterpenes (Pakamas, 2012)

In 1989 and 1990, scientists from China had discovered another two new chromone derivatives (Konishi et al., 2002). Ahmaed and Kulkarni (2017) concluded that 6-methoxy-2-[2-(4-methoxyphenyl)-ethyl] chromone and 2-[2-(4-methoxyphenyl)-ethyl] chromone is the major chromones that can be found in most species of agarwood tree. Furthermore, Yoshii et al. (1978) had reported the first presence of oxygenated chromone derivative in agarwood. There are many kinds of chemical structures for 2-(2-phenylethyl)-4H-chromen-4-one chromone and their derivative compounds. The molecular formula for 2-

(2-phenylethyl) chromone is  $C_{17}H_{14}O_2$ . The chemical structure for 2-(2-phenylethyl) chromone is shown in Figure 2.5.

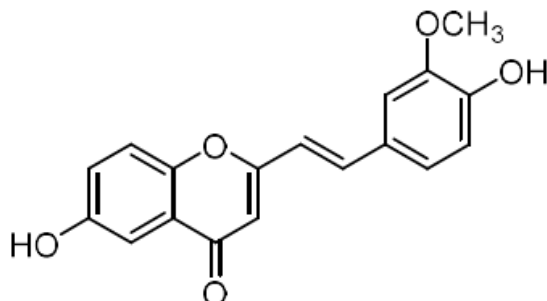


**Figure 2.5:** Chemical structure of 2-(2-phenylethyl) chromone (Wang et al., 2018)

Based on research study by Jong et al. (2014), discovery of chemical compound such as benzaldehyde, 4-phenyl-2-butanone, benzenepropanoic acid, methyl ester, guaiene, palustrol, anisylacetone, 8-naphthol, 1-(benzyloxy)- and 2-(2-phenylethyl) chromone derivative were detected in the fungal elicited young wood of *Aquilaria malaccensis*. These fragrant compounds from the fungal elicited young wood of *A. malaccensis* were found to be almost similar with high quality commercial agarwood and naturally infected agarwood from wild *A. malaccensis*. Besides, this study had shown that the major constituent of Malaysia agarwood was chromone derivative.

### 2.1.5 Agarwood oil extraction process

Agarwood oil which also known as oudh oil has a high demand for its rich fragrant properties and medicinal benefits. Agarwood essential oils can be obtained by extraction. There are various extraction methods that had been used in extracting the agarwood oil. Methods of agarwood oil extraction include hydrodistillation or water distillation, solvent extraction, soxhlet extraction and supercritical fluid extraction (Naef, 2011). These

extraction methods of agarwood oil had been used throughout agarwood producer countries. It is very crucial in choosing the effective agarwood oil extraction method as the extraction method of agarwood can affect the quality and chemical composition of agarwood oil (Hashim et al., 2014). This is because the trading of agarwood oils depends on their quality in which cheap in price for low quality oil and expensive for high quality oil (Nurlaila et al., 2014).

Hydrodistillation is a traditional method of agarwood oil extraction and commonly used in manufacture. Hydrodistillation is also described as the simplest and cheapest extraction method. Hydrodistillation also works best for tough materials such as roots, wood or nuts (Ashwin, 2009). However, Jutarut et al. (2011) reported that extracted essential oil by using hydrodistillation methods yield low efficiency process and have very rare data of chemical analysis. In addition, the extraction processes is not always complete and have unstable control of heat (Yusoff et al., 2015). Liu et al. (2008) state that hydrodistillation extraction is safe to operate and environmentally friendly, but the disadvantage is time consuming with a large amount of plant material required.

Figure 2.6 shows the conventional distillation plant by using wood fired stoves that produce low yield and require high energy consumption with time consuming process (Pakamas, 2012). Based on a study by Pornpunyapat et al. (2011), the different temperature has been used in hydrodistillation methods: 80 °C, 100 °C and 120 °C and it was discovered that maximum oil was produced on higher temperature (120 °C) with better physical properties and attributes.



**Figure 2.6:** Conventional distillation plant with wood fire stoves (Pakamas, 2012)

Next, solvent extraction method can be used in extraction of agarwood essential oil. Solvent extraction works when the mixture is dissolved in solvent to separate the constituents. However, some residual solvents or impurities could remain in the product after solvent extraction process. Ether, ethanol, methanol, hexane, alcohol and petroleum were used as solvent in solvent extraction methods. Hexane is often used as solvent to extract mono- and sesquiterpenoids in essential oils (Bhat et al., 2009). Figure 2.7 shows solvent extraction that combine with distillation process where the essential oils are dissolved by putting a hydrocarbon solvent in the wood dust. Hence, the results solution was then filtered prior going through a distillation process that distillates the oil and eventually lead to final production of resin. Meanwhile, "concrete" will remain as a waste as "concrete" is made up by combination of wax and essential oils (Pakamas, 2012).



**Figure 2.7:** A solvent extraction unit that combined with distillation process for agarwood oils extraction (Pakamas, 2012)

The third method of agarwood oil extraction is Soxhlet extraction. In 1879, Franz von Soxhlet had invented a piece of laboratory apparatus known as Soxhlet extractor which is originally intended to extract lipids from a solid material. Soxhlet extractor commonly used in the process of converting partially soluble components in a solid into the liquid phase.

Soxhlet extractor has three main sections: a percolator (boiler and reflux), a thimble (usually made of thick filter paper) and a siphon mechanism. A percolator circulates the solvent during the extraction process, meanwhile, a thimble retains the solid to be laved, and the siphon mechanism periodically empties the thimble (Castro & Capote, 2010). Soxhlet extraction was also known as an automated batch extractor as its extract drains after reached the maximum volume of the siphon and does not drain continuously into the solvent flask (William, 2007).

The process in Soxhlet extraction methods began by heating the extracting solvent until reflux and it takes place in the lower reservoir as shown in Figure 2.8 (Harvey, 2013).

Then, the vapor phase of the solvent moves upwards until reaches the condenser and consequently condenses back to the liquid state. Furthermore, the solvent was collected in the upper reservoir as it passes through the sample in the filter thimble as shown in Figure 2.8. Meanwhile, the solvent and extracted analyte are siphoned back to the lower reservoir whenever the solvent in the upper reservoir reaches the return tube's upper bend. Hence, this process will be repeated over time and eventually lead to increasing concentration of analytes in the lower reservoir (Harvey, 2013).

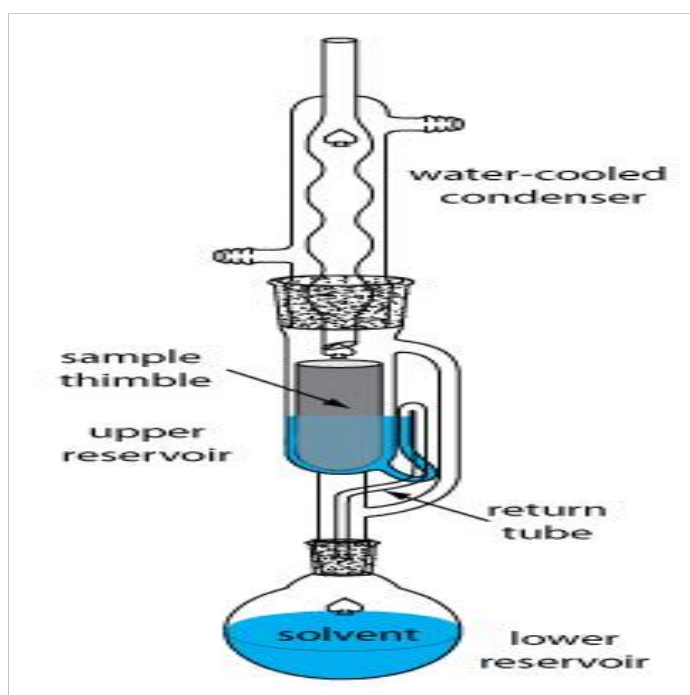


**Figure 2.8:** Soxhlet extraction process by a laboratory apparatus known as Soxhlet extractor invented by Franz von Soxhlet (Harvey, 2013)

After extraction, the extracted compound will be yield by using a rotary evaporator to remove the solvent and the remaining of the extracted solid in the thimble will be discarded.

Supercritical fluid extraction (SFE) is the process involved in the separation of one component (extractant) from another (matrix) by using carbon dioxide ( $\text{CO}_2$ ) as extracting solvent (Sapkale et al., 2010). Less energy consumption, chemically stable, inflammable, and non-toxic were the benefits of using supercritical fluid extraction process. Hence, it gives high yields with faster extraction due to its properties of low viscosity and high diffusivity as a good transporter (Pakamas, 2012).

In addition, above the critical temperature of  $31\text{ }^\circ\text{C}$  and critical pressure of 74 bars is the standard extraction conditions for carbon dioxide ( $\text{CO}_2$ ) as supercritical fluids. Supercritical fluid extraction can be completed within 10 to 60 minutes. Sometimes, Carbon dioxide ( $\text{CO}_2$ ) can be improved with another solvent, for instance, ethanol or methanol to increase its polarity (Sapkale et al., 2010). Figure 2.9 shows the unit of supercritical fluid extraction (SFE).



**Figure 2.9:** Unit of Supercritical fluid extraction (SFE) (Pakamas, 2012)

Figure 2.10 shows the schematic flow of the supercritical fluid extraction method and the process is explained as followed based on Sapkale et al. (2010). The system of SFE

contains carbon dioxide (CO<sub>2</sub>) pump, a pressure cell that contains the sample, and a collecting vessel. The liquid is pumped to the heater, where it is heated to supercritical conditions. Then, it passes into an extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is removed from the extraction cell into a separator at lower pressure, and the extracted material settles out. The carbon dioxide (CO<sub>2</sub>) then cooled, recompressed and recycled or even released to the atmosphere.

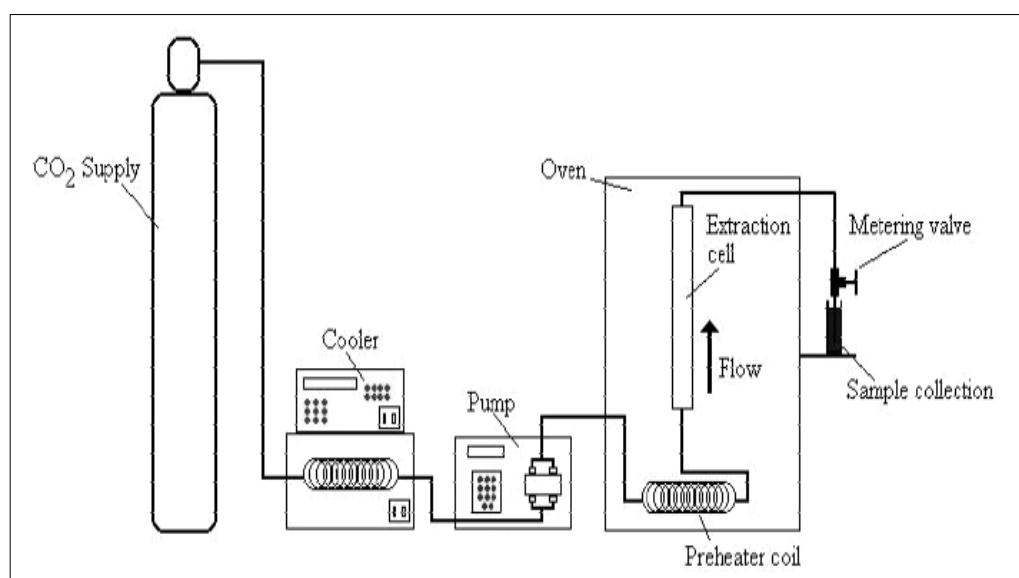


**Figure 2.10:** The schematic flow of Supercritical fluid extraction (SFE) (Sapkale et al., 2010)

## 2.2 Fungi

### 2.2.1 Agarwood endophytic fungal

According to Bhore et al. (2013), endophytes are microorganisms that do not cause any plant disease as it is lived within the plants either its properties of intercellular or intracellular. Endophytic fungi were defined as diverse polyphyletic group that were

primarily ascomycetous fungi which are found within asymptomatic tissue of plants, ferns, mosses, hornworts, lycophytes, equisetopsids, in above ground tissues of liverworts, in seed plants from the arctic to tropics, and from most biotically diverse tropical forest of agricultural fields (Arnold, 2007). Meanwhile, an endophytic fungus is usually affected by growth and colonization that may cause changes in the host by physically. Endophytic fungus on agarwood is a symbiotic microorganism that reside within the plant cell and stimulate the production of secondary metabolite in the plant as a self defense against other pathogenic bacteria (Zulfendi et al., 2019).

Despite agarwood also well-known as traditional medicine, the micro-ecology and bioactivity of endophytic fungi interaction with agarwood is yet unclear, although great progress has obtained in understanding the phytochemical constituents and pharmacological activities of agarwood (Cui et al., 2011). Gaharu or agarwood was derived from the resinous substance produced by the tree in response to the attack by pathogenic fungi (Jalaluddin, 1977; Ng et al., 1997).

Dashtban et al. (2010) have stated that fungi and bacteria were common microorganisms that are capable to degrade other plant cell wall fibers and cellulose. Furthermore, Yusoff et al. (2015) also convinced that fungi can surge the process of “digestibility” in agarwood formation. Besides, the degradation of the agarwood cell wall was required to increase the oil permeability as the plant cell extraction was facilitated. According to Arsat (2008), fungi were used as a technical enzyme in the oil production of agarwood as its extraction gives the highest yield compared to without enzyme pre-treatment of extraction. Cui et al. (2011) discovered that endophytes from agarwood may

also act as a source of antimicrobial and antitumor agents with a different process of action.

Furthermore, Cui et al. (2013) reported the first agarwood formation by a bioactive fungus, *Paraconiothyrium variabile* through *Aquilaria sinensis* inoculation. It was shown that the fungus induced agarwood sample are similar with natural agarwood which can be used as an agarwood substitute. Qi et al. (2005) state that fungus *melanotus flavolives* have been found in natural heartwood where agarwood accumulates.

Fungi can act as elicitors to promote host growth, stimulate the accumulations of plant secondary metabolites, and boost host defenses (Chen et al., 2010; Koffi et al., 2012). As elicitors and partners, fungi are a key in an ecological process in which fungi protect their host against invasions by stimulating the accumulation of secondary metabolites in plant hosts (Selosse et al., 2004). Zhao et al. (2005) studied that stresses and fungal elicitors involve the presence of secondary metabolites in the medicinal plants.

It was discovered that endophytes fungi were isolated from roots, stems and even leaves of *Aquilaria sinensis* (Gong & Guo, 2009; Wang et al., 2009). In the year of 1952, Bhattacharyya has first discovered the role of endophytic fungi in inducing agarwood production in the tree trunk. Then, many scientists were involved in research of several endophytes isolates from the agarwood tree (Chhipa et al., 2017). Few fungal isolates that have been used in artificial infection of agarwood include *Melanotus flavolivens*, *Phytium* sp., *Penicillium* sp., *Diplodia* sp., *Botryodiplodis* sp., *Lasiodiplodia* sp., and *Fusarium* sp. (Gong & Guo, 2009; Zhang et al., 2014). Table 2.3 has shown the isolated species of endophytic fungi from different countries that have been summarized accordingly.

**Table 2.3:** Isolated fungal species from *Aquilaria* trees

| Country                | <i>Aquilaria</i><br>species  | Fungal species                                                                                                                                                                                                                                                                                                                                                   | Reference               |
|------------------------|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| Malaysia               | <i>Aquilaria malaccensis</i> | <i>Fusarium</i> sp., <i>Cochliobolus</i> sp.,<br><i>Cunninghamella</i> sp., <i>Hypocrea</i> <i>lixii</i> ,<br><i>Lasiodiplodia</i> sp.                                                                                                                                                                                                                           | Mohamed<br>et al., 2010 |
| Papua<br>New<br>Guinea | <i>Aquilaria filaria</i>     | <i>Fusarium</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i><br>sp., <i>Trichoderma</i> sp.                                                                                                                                                                                                                                                                | Salosa,<br>2013         |
| China                  | <i>Aquilaria sinensis</i>    | <i>Epicoccum</i> sp., <i>Cladosporium</i> sp., <i>Rhizomucor</i><br>sp., <i>Paraconiothyrium</i> sp., <i>Phaeoacremonium</i><br>sp., <i>Xylaria</i> sp., <i>Fusarium</i> sp., <i>Lasiodiplodia</i><br>sp., <i>Leptosphaerulina</i> sp., <i>Hypocrea</i> <i>lixii</i> ,<br><i>Phoma</i> sp., <i>Coniothyrium</i> sp., <i>Chaetomium</i><br>sp., <i>Pichia</i> sp. | Cui et al.,<br>2011     |

Tamuli et al. (2000) proclaimed that *Fusarium oxysporum* and *Chaetum globosum* had successfully induced agarwood. In Malaysia, *Cunninghamella*, *Curvularia*, *Fusarium*, and *Trichoderma* species are some of the fungal diversity that was found in the stems of agarwood (Mohamed et al., 2010). Meanwhile in Kalimantan and Sumatra, fungus such as *Penicillium* spp, *Epicocum granulatotum*, *Fusarium* spp., *Aspergillus* spp., *Cochiobolus* spp., *Batryodiplodia theobromae*, *Diplodia* spp., and *Phytium* spp. are common fungus found in agarwood tree (Salosa, 2013). Tian et al. (2013) state that a study on the groups of endophytic fungi that isolated from *Aquilaria* trees are crucial for better understanding on agarwood endophytes.

### 2.2.2 Microbial consortiums

The fungal consortium was popular in biotechnology research as it involved a combination of two or more fungi working together within the same environment. Based on ecological selection criteria, microbial consortium is referred to a microbial group of diverse organisms (Jiang et al., 2017). According to Kannahi and Babynisha (2014), a consortium of microorganisms was designed to improve plant yield as it provides different needs of growing plants. Microorganisms in consortia can be modified and exploited to degrade the organic wastes more effectively as the degradation time may be reduced which simultaneously allows the production of products. Based on Kannahi and Babynisha (2014), microbial consortia were used in their research for the degradation of solid organic waste from flower waste. Research involves the screening of microorganisms which were able to produce enzymes such as lipase, protease, amylase, and cellulase.

Presently, not much literature on the microbial consortium that specifically relates to agarwood formation has been studied. According to Maynard-Smith and Szathmary (1997), Herre et al. (1999), and Loftus et al. (2005), the interactions between microbe and host may be influenced by their evolutionary history, symbionts genetic diversity, the ability of co-colonize within individual hosts, their direct and indirect interactions, their pathogenicity that may associate with the genomic architecture or other ecological modes, and also the way they acquired from environment.

Mega et al. (2012) have studied the formulation of fungal inoculant by combining inoculant of *Rhizopus* sp. and *Fusarium solani* in a liquid medium. Then, the research results have shown that *Fusarium* sp. and *Rhizopus* sp. inoculant can cause agarwood formation within *Gyrinops versteegii*. Besides, Gupta et al. (2015) had reviewed several

microbial approaches in perfume and flavoring agent production. In addition, Figure 2.11 shows two different species of fungal grown in a culture media to observe the antagonistic activity of *T. harzianum* and *S. rolfsii* (Chakraborty et al., 2011).

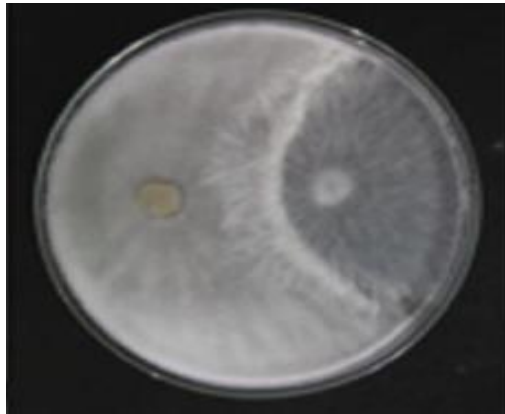


**Figure 2.11:** A microbial consortium made up of two fungal species, *Trichoderma harzianum* and *Sclerotium rolfsii* to test their antagonistic effect against each other. (Chakraborty et al., 2011)

Inoculated fungi are one of the main components in trees that are injured and wounded. When the *Aquilaria* trees are wounded, the fungal inoculated in the wound will eventually stimulate the trees and produce resin from the wounded woody tissue. In addition, the advantage of fungal inoculant includes considered progressive means for the systematic growth of fungal agents that can infect intermittently and cause the continuous formation of agarwood (Novriyanti et al., 2010).

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1 Formulation of microbial consortium from fungi**

##### **3.1.1 Source of endophytic fungi**

Agarwood fungi had been identified and characterized based on the previous studies (Sulang, 2017). The fungal cultures were obtained from stock cultures in Virology Laboratory Faculty Resource Science Technology UNIMAS, Sarawak which includes *Trichoderma* sp., *Curvularia* sp., *Aspergillus* sp., *Fusarium* sp., *Lasioidiplodia* sp., *Whitfordia* sp., and *Penicillium*. The fungal isolates were sub-cultured for further formulation of microbial consortia.

##### **3.1.2 Preparation of culture media**

The assay media used in this research for agarwood fungus cultivation was potato dextrose agar (PDA). Approximately, 39.0 g of PDA was diluted in 1000 mL of distilled water followed by autoclaving for 15-20 min at 15 psi. After autoclaving, the agar was cooled down then poured into petri dishes. After the agar in the plates has been solidified, the agar plates were then used for fungi culture.

##### **3.1.3 Formulation of microbial consortium**

Pure cultures of endophytic fungi used in this study as shown in Table 3.1. The cultures were revived and cultivated on PDA at 28°C for 7-14 days.

**Table 3.1:** Endophytic fungi isolated from agarwood trees by previous study

| Isolate | Species                         | % Identity | Accession no. |
|---------|---------------------------------|------------|---------------|
| G3-B2   | <i>Culvularia affinis</i>       | 99         | EF187909.1    |
| G4-B3   | <i>Aspergillus flavus</i>       | 100        | FJ487932.1    |
| G4-B2   | <i>Trichoderma spirale</i>      | 99         | HQ229947.1    |
| G2-A2   | <i>Trichoderma harzianum</i>    | 99         | JX473716.1    |
| GKS 2   | <i>Fusarium solani</i>          | 99         | JX897000.1    |
| GKS 3   | <i>Lasiodiplodia theobromae</i> | 99         | JQ658976.1    |
| GB 4    | <i>Penicillium citrinum</i>     | 99         | AM745115.1    |

(Source: Sulang, 2017)

Then, fungi mycelia that grow on PDA were cut into small cube with measurement of approximately 1 mm x 1 mm each and was carefully transferred into new PDA plate by using aseptic technique for formulation of microbial consortia. Formulation of microbial consortium were done in a dual culture plate method. There are no specific standard criteria on the type of fungal species in the formulation of the microbial consortium. Hence, several microbial consortia were cultured and formulated by randomly combining an equal proportions of different types species of fungi cultures onto media agar.

Formulations of microbial consortia were done based on the capability of different fungus to grow as culture in the same environment which includes temperature and nutrient supply. The selection of successful microbial consortia were based on the symbiotic relationship of fungal species within the consortium. Parameters include antagonistic effect, growth inhibitory and growth compactness in a microbial consortium were observed. The characteristics to select a successful formulated microbial consortium

includes an equal growth area and growth compactness among the different fungal species in a consortium.

Subsequently, these microbial consortia were incubated in the incubator at 28 °C for 7 days. The fungal growth of each fungal isolates was observed after 7 days of incubation. In a selected formulated microbial consortium, the consortia should fulfil the growth ratio 1:1 for two fungal isolates and 1:1:1 for three fungal isolates. The matrix method of the fungal isolates growth ratio in each formulated microbial consortium were done to select the best three of microbial consortium (Appendix A). The result of the microbial growth is shown in Section 4.1.

### **3.2 Field testing of fungal inoculant**

#### **3.2.1 Study sites of field testing for fungal inoculations**

The study sites for this research were conducted in a local agarwood farm in Serian, Sarawak. Figure 3.1 shows the map location of the agarwood farm as a study sites of this research. A total of 24 samples of *A. malaccensis* trees were used respectively for this research. Besides, the average age of *Aquilaria* trees that were used in this research was 5 years old (Figure 3.2). Triplicate trees were inoculated for each fungal inoculum and negative control. Sterile distilled water was used as a negative control in this study. In this field testing study, Completely Randomized Design (CRD) was used.

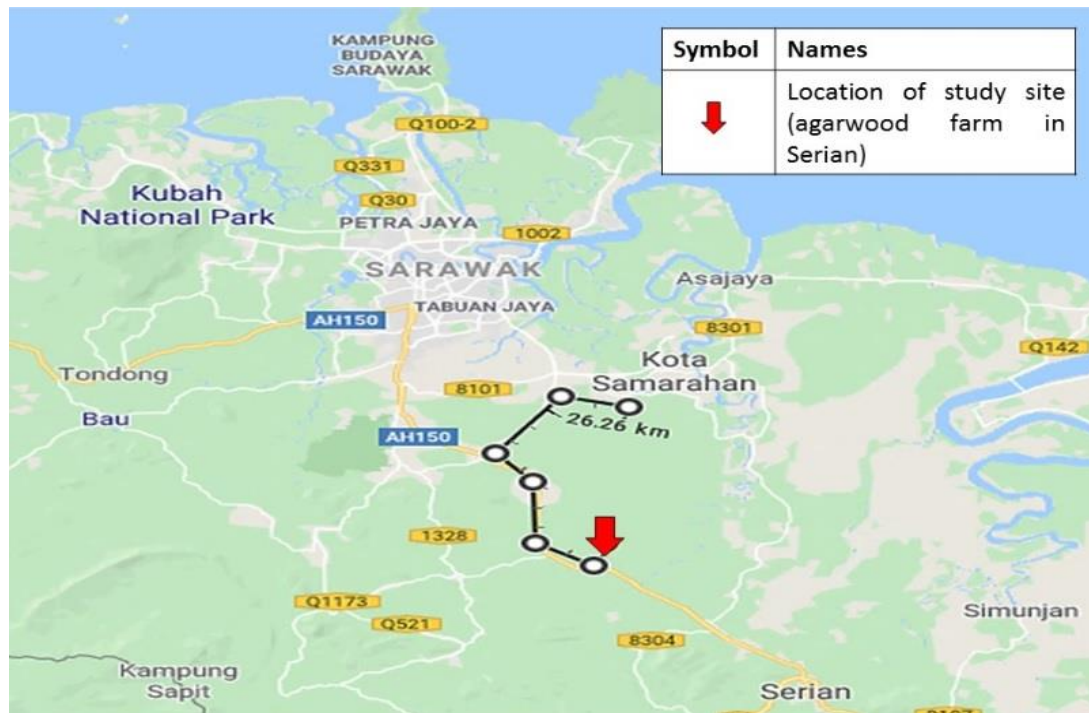


**Figure 3.1:** Map showing the study site. The distance from the study site (Serian Agarwood Farm) to Unimas, Kota Samarahan is approximately 26.26 km.



**Figure 3.2:** Serian Agarwood Farm planted with aged average 5 years old of *Aquilaria* trees. The trunks are small with a diameter average from 48.33 cm to 60 cm, and the leaves are elliptic or lanceolate in shape.

### **3.2.2 Preparation of liquid inoculants**

The method of inoculant media preparation was modified from Santoso et al. (2011). Fungal inoculants were prepared by using Potato Dextrose Broth (PDB) as inoculant media. Therefore, all the selected microbial consortium were cultured on Potato Dextrose Agar (PDA) for a week prior to the preparation of the liquid inoculant. Approximately 1 L of PDB was prepared and autoclaved. The 7-days old mycelia of the fungi in each microbial consortium were then cut into single pieces each and added into PDB. After that, the 1 L of Schott bottle containing fungi isolates were marked with inoculant labels and shaken using the rotary shaker at 120 rpm at room temperature for 7 days. After seven days of incubation, the PDB containing fungi isolate was first blended in blending machine to obtain homogenous inoculum in juice form for the ease of infection into the sample trees via the bottle drip method. Aseptic technique was applied in all stages of the inoculant preparation.

### **3.2.3 Inoculation methods**

Based on previous study, CA Kit and the Taiwan and Pheerapan methods are an examples of novel inducement approaches and tools which are used these days for production of agarwood in short period (Cheng et al., 2011; Mohamed et. al., 2014).

In this study, the method of inoculant media preparation was modified from Santoso et al. (2011). Fungal inoculants were prepared using Potato Dextrose Broth (PDB) as inoculant media. Therefore, all the prepared fungi inoculants were transported aseptically to the locations of field testing study which was Serian Agarwood Farm. The inoculation

process was conducted on two different seasons which were inoculation in wet season in November 2017 and inoculation in the dry season in March 2018.

Before the inoculation process, the perimeter of the stem trees was measured. The sample trees were drilled up to 1/3 of the tree diameter with the electrical driller and a hole were drilled in a position of 45 degrees to each of every sample of the tree as shown in Figure 3.3. Prior to trees inoculation, all equipment was sterilized with 70% ethanol to prevent cross contamination by other microorganisms. The liquid inoculants were stored in 1 L of beverage bottles for the ease of transportation to agarwood farm. The dripping method only conducted once in the first month of each dry and wet season inoculations. The dripping method were observed twice for the third and sixth month of inoculations in each wet and dry season respectively. In the agarwood farm, the stem diameter of the sample tree was measured by using a diameter measuring tape and recorded accordingly. In this study, the bottle drip method was used as an inoculation method that has invented by Tang (2012).



**Figure 3.3:** Physical injury on the stem of *Aquilaria* trees by drilling a hole before application of fungal inoculants.

The agarwood trees were wounded by drilling according to Tang (2012) with modifications. The sample trees were drilled to reach the cambium and phloem which were about 1/3 of the diameter of the tree's stem. Then, the hose that connect to the cap of the plastic bottle were inserted into the hole of tree meanwhile the plastic bottles that contain the fungal inoculant were tied upside down to allow the inoculant to flow through the hose that has inserted into the trees. Hence, 1L of fungal inoculant that stored in waste plastic beverage bottle was slowly dripped into the wounds in the sample trees and the end were sealed with clay as shown in Figure 3.4. Cap of the bottles was sealed with parafilm to avoid any leaking.



**Figure 3.4:** Approximately 1L of liquid inoculant was dripped into holes of sample trees by using bottle drip method according to Tang (2012)

Each sample trees were wounded with a single hole and inserted with each fungal inoculant. Three replicates of trees were made for each selected fungal inoculant. Negative control was also done by dripping 1L of sterile distilled water into the sample trees.

### 3.2.4 Morphological and data analysis of the inoculated trees

Before the observation of the infected zone inside the stem, the tree bark needs to be peeled off by using a ‘parang’ (long knife). The infected zone of the trees’ stem surface was measured longitudinally (L) after three months and six months in each wet and dry season as shown in Figure 3.5. The measurement was recorded in three reading for each of the triplicate treatment trees. These measurements were carried out at the inoculated holes.

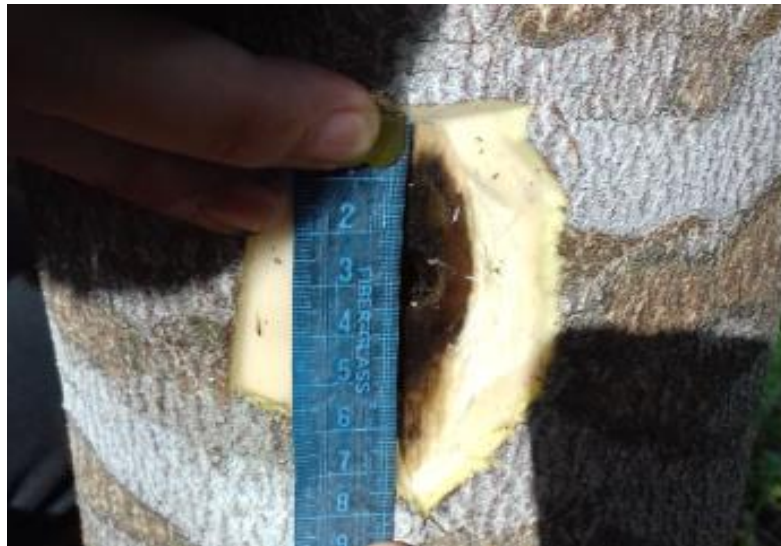


**Figure 3.5:** Longitudinal (L) length of the infected zone in the inoculated tree were measured.

Then, all data obtained were combined and analysed using IBM SPSS Statistics Version 22. Differences in longitudinal measurements of the stem for each fungi treatment were analysed by using one-way ANOVA to test whether there is a significant difference in the treatments. If there were significance, Tukey’s test was performed for further comparisons. In addition, the physical appearance such as colour characteristic of the

infected stems were also observed and recorded accordingly. The result of the infected zone measurement is shown in Section 4.2.

### **3.3 Detection of chemical compound in inoculated agarwood**

#### **3.3.1 Preparation for extraction of chemical compounds**

The samples of inoculated agarwood trees were collected twice which were on third and sixth months of inoculation period in each wet and dry season from agarwood farm Serian (Sarawak), Malaysia. Moreover, the infected sample wood was flamed using a lighter to check for agarwood fragrance. The resinous tissues in the wood sample were carefully extracted by using a knife and a scalpel. The wood samples were air dried and reduced in size by using a scalpel. Then, 100 grams of air dried wood samples were grinded by using a grinder to become an air dried samples powder. Six grams of air dried samples powder was extracted by using Soxhlet extractor approximately for 3 hours until a clear solvent obtained (Jong et al., 2014). A total of 200 ml of methanol solvent was used to extract chemical compound in agarwood for Soxhlet extraction.

After extraction, the solvent was then removed by using a rotary evaporator under reduced pressure to yield the extracted compound. Next, the crude extract was let to be evaporated in the laminar fume hood for a day until it was completely dried. Then, the dried extracts were dissolved in 1 mL of dichloromethane (DCM), transferred into a glass vial and were stored in the refrigerator before proceeding to GC-MS analysis (Jouda, 2013).

### **3.3.2 Gas Chromatography-Mass Spectrometry (GC-MS) analysis**

The extract was further diluted in dichloromethane again to make 10mg/ml of the sample before Gas Chromatography-Mass Spectrometry (GC-MS) analysis. GC-MS analysis was performed with a gas chromatography system; GC-MS QP2010 Plus SHIMADZU with BPX-5 column (30 m × 0.25 mm × 0.25 µm film thickness) (Sulaiman et al., 2015). The column oven temperature was kept at 50 °C for 3 minutes, then increasing to 280 °C at a rate of 15 °C/min and holding for 20 minutes.

The peaks from GC-MS were detected based on the total ion chromatography (TIC) and mass chromatograms; and further identification of the chemical components was based on the comparison of the calculation of their retention indices and authentic mass spectra data with the existing National Institute of Standards and Technology (NIST) 2008 library and comparison with literature (Hashim et al., 2014). The result of GC-MS analysis in this study is shown in Section 4.4.

## CHAPTER 4

### RESULTS

#### 4.1 Selection of microbial consortium

The formulated microbial consortium was selected based on their symbiotic relationship of fungus growth includes the antagonistic effect and growth inhibitory toward each other within the media agar. All the formulated microbial consortium were incubated for 7 days in the incubator at 28 °C. Then, the best 3 types of the formulated microbial consortium were chosen based on their successful characteristics where the different fungal species can grow well together in equal ratio and they did not overgrow against each other. The formulated microbial consortium was chosen based on growth ratio recorded in Appendix A. Table 4.1 shows the origin of the isolates used in the formulated microbial consortium (Sulang, 2017). Table 4.2 shows the list name of fungus species that were used in 3 types of the formulated microbial consortium.

**Table 4.1:** The origin of isolates used in this study (Sulang, 2017)

| Fungi Isolates | Fungi Species        | Origin of the Isolates |
|----------------|----------------------|------------------------|
| G4-B2          | <i>T. spirale</i>    | Baram                  |
| G4-B3          | <i>A. flavus</i>     | Baram                  |
| GKS 2          | <i>F. solani</i>     | Kota Samarahan         |
| GB 4           | <i>P. citrinum</i>   | Baram                  |
| G2-A2          | <i>T. harzianum</i>  | Baram                  |
| GKS 3          | <i>L. theobromae</i> | Kota Samarahan         |
| G3-B2          | <i>C. affinis</i>    | Baram                  |

**Table 4.2:** Formulated microbial consortia that served as inoculants in this study

| Microbial consortium | Fungi Isolates/ Fungi Species                                                           |
|----------------------|-----------------------------------------------------------------------------------------|
| 1                    | <i>T. spirale</i> (G4-B2) and <i>A. flavus</i> (G4-B3)                                  |
| 2                    | <i>F. solani</i> (GKS 2) and <i>P. citrinum</i> (GB 4)                                  |
| 3                    | <i>T. harzianum</i> (G2-A2), <i>L. theobromae</i> (GKS 3) and <i>C. affinis</i> (G3-B2) |

Figure 4.1 and Figure 4.2 show some examples of unselected formulated microbial consortium due to non-symbiotic relationship of fungus species within the media agar. It can be observed some of the fungus species overgrow and inhibit the growth of the other fungus species within the media agar. Hence, unselected microbial consortium has unequal growth ratio. Furthermore, the fungus species in an unselected microbial consortium has antagonistic effect against each other which is not suited as fungal inoculants in this study.

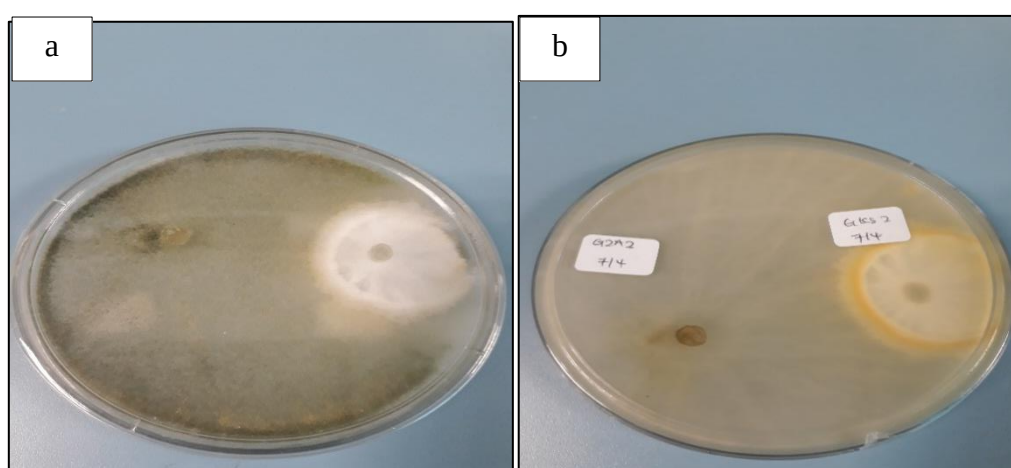


**Figure 4.1:** Morphological characteristic of unselected microbial consortium, *Trichoderma harzianum* (G2-A2) and *Fusarium solani* (GKS 2), (a) front view of cultures on potato dextrose agar and (b) back view of cultures on potato dextrose agar.

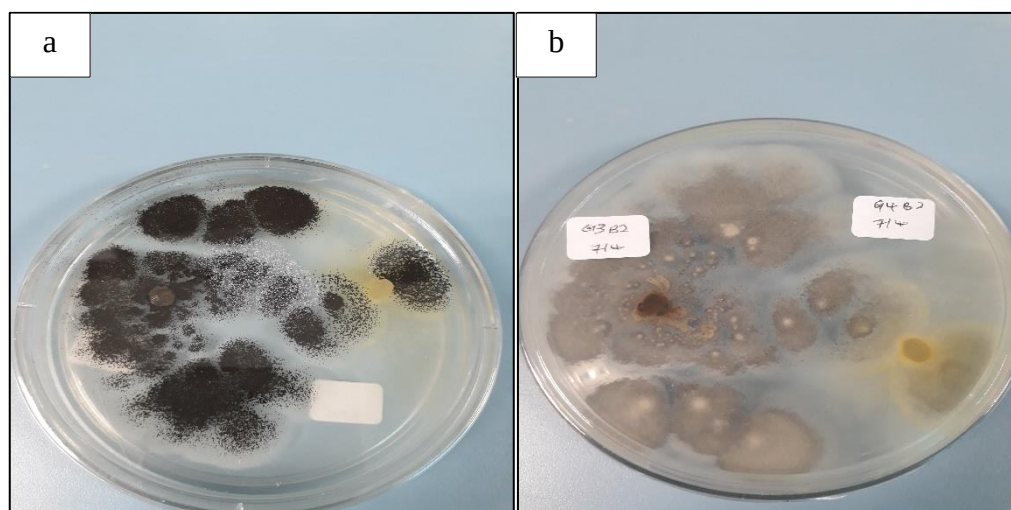


**Figure 4.2:** Morphological characteristic of unselected microbial consortium, *Curvularia affinis* (G3-B2) and *Trichoderma spirale* (G4-B2), (a) front view of cultures on potato dextrose agar and (b) back view of cultures on potato dextrose agar.

Meanwhile, Figure 4.3, Figure 4.4 and Figure 4.5 show the growth of different fungus species in the selected formulated microbial consortia. It can be observed that the fungus species in the selected formulated microbial consortium has a symbiotic relationship with each other within the media agar. The fungus species did not overgrow or inhibit the growth of the other fungus species. Hence, the fungus species in the selected microbial consortium has equal growth compactness and equal growth ratio. Thus, the fungus species in the selected microbial consortium does not have an antagonistic effect against each other. These selected microbial consortia were then further served as fungal inoculants for agarwood inducement.

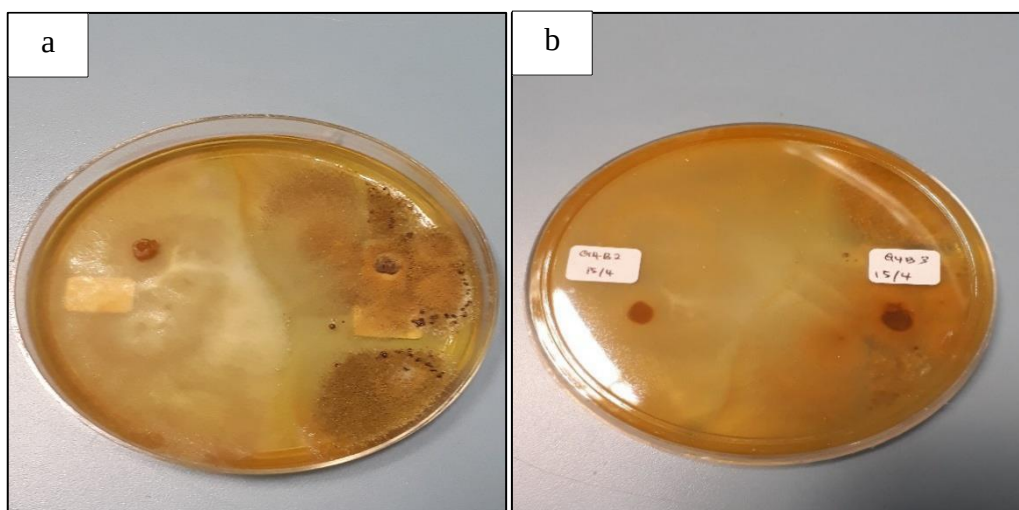


**Figure 4.3:** Morphological characteristic of microbial consortium type 1, *Trichoderma spirale* (G4-B2) and *Aspergillus flavus* (G4-B3), (a) front view of cultures on potato dextrose agar and (b) back view of cultures on potato dextrose agar.

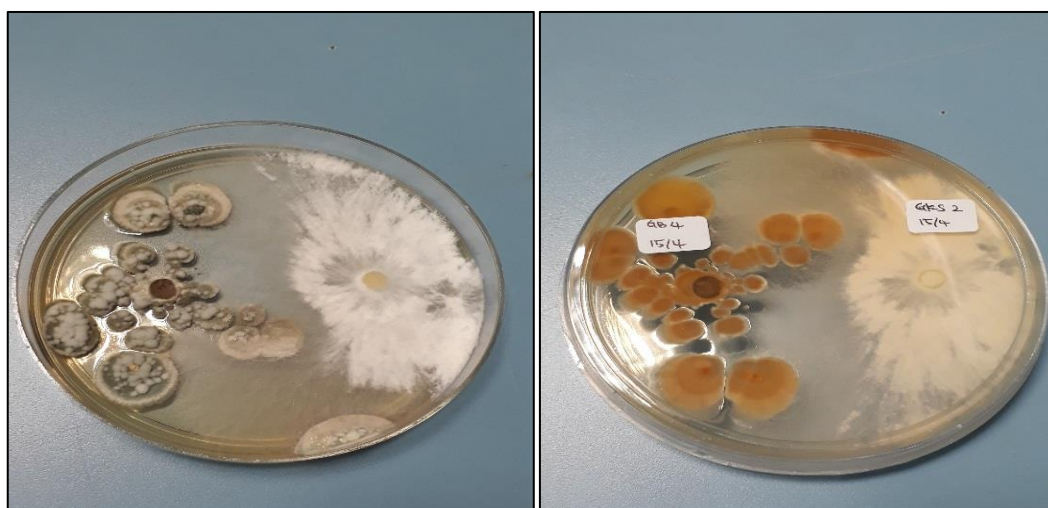


**Figure 4.4:** Morphological characteristic of microbial consortium type 2, *Fusarium solani* (GKS 2) and *Penicillium citrinum* (GB 4), (a) front view of cultures on potato dextrose agar and (b) back view of cultures on potato dextrose agar

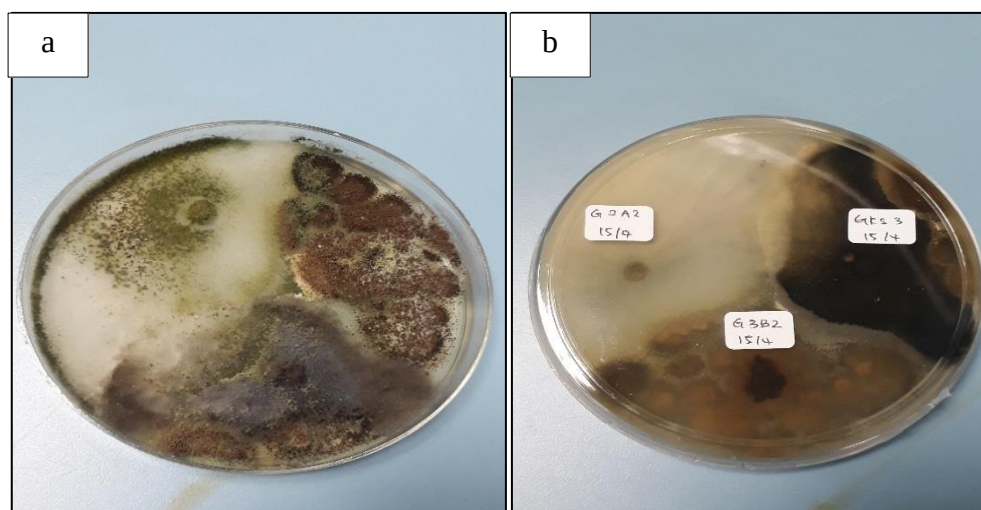


**Figure 4.5:** Morphological characteristic of microbial consortium type 3, *Trichoderma harzianum* (G2-A2), *Lasiodiplodia theobromae* (GKS 3) and *Curvularia affinis* (G3-B2), (a) front view of cultures on potato dextrose agar and (b) back view of cultures on potato dextrose agar

## 4.2 Morphological characteristics of the inoculated trees

The mean value for the perimeter measurement of sample trees used in this study were ranging from 48 cm – 60 cm (Appendix C). Table 4.3 and Table 4.4 shows the colour changes along the inoculated zone within three and six months in wet and dry seasons of the inoculation period, respectively. The whitish fresh stem of *Aquilaria* tree in the earlier stage became either dark brown or black along the inoculated zone after three months of the inoculation. However, only fungi formulation 3 continuously show darker in colour while negative control presented as whitish yellow in the inoculated stem of both wet and dry seasons, respectively (Refer to Table 4.3 and Table 4.4).

**Table 4.3:** Colour changes of inoculated zone for each of the fungal inoculants after three months and six months inoculation for wet season

| Fungal Inoculants/Treatments | Colour of inoculated zone |                |
|------------------------------|---------------------------|----------------|
|                              | 3 Months                  | 6 Months       |
| F1 (G4-B2 + G4-B3)           | Black with stripes        | Dark brown     |
| F2 (GKS 2 + GB 4)            | Brown                     | Pale brown     |
| F3 (G2-A2, GKS 3 + G3-B2)    | Black                     | Black          |
| Negative control             | Whitish yellow            | Whitish yellow |

**Table 4.4:** Colour changes of inoculated zone for each of the fungal inoculants after three months and six months inoculation for dry season

| Fungal Inoculants/Treatments | Colour of inoculated zone |                |
|------------------------------|---------------------------|----------------|
|                              | 3 Months                  | 6 Months       |
| F1 (G4-B2 + G4-B3)           | Black                     | Dark brown     |
| F2 (GKS 2 + GB 4)            | Brown                     | Pale brown     |
| F3 (G2-A2, GKS 3 + G3-B2)    | Black                     | Black          |
| Negative control             | Pale brown                | Whitish yellow |

From the results of the inoculation method, all types of the fungi inoculants gave a positive agarwood production based on the colour and neurosis that occurred within the stem trees. Moreover, the unique scent of the agarwood fragrance can be detected from one of the fungi inoculants which were fungal inoculant type 3 (F3), when it was flamed using a lighter. It was observed that the colour changes of the inoculated zone were more consistent after six months, except for fungal inoculant type 2 (F2), where it showed

recovery of colour from darker to light colour of infected zone in both wet and dry seasons of inoculation, respectively (Table 4.5 and Table 4.6).

Table 4.5 and Table 4.6 show the infected zone formation by its widespread symptoms of each inoculated agarwood trees among different fungal inoculants within the different season of inoculation period (wet season and dry season) respectively. Based on the observation in both wet season and dry season respectively, upon the third month of the inoculation period, all treatments showed less lesion along the inoculated zone whereas, in the sixth month of inoculation period, the widespread symptoms were more spacious or become larger. However, only fungal inoculant type 2 (F2) showed the recovering stage during the sixth month inoculation in both wet and dry season where the wood returned to pale in colour as recovered to its healthy wood condition.

**Table 4.5:** The morphological characteristics of infected zone after inoculation in wet season

| Fungal Inoculant / Treatment | Wet Season                                                                                                                                     |                                                                                                                                                                                        |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                              | 3 months                                                                                                                                       | 6 months                                                                                                                                                                               |
| F1                           |  <p>Black with stripes formed along the inoculated zone</p> |  <p>Colour change to dark brown with stripes and symptoms became more spacious inside the stem</p> |

Table 4.5 continued

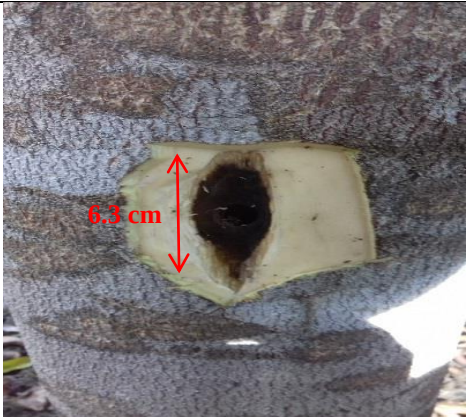
|                  |                                                                                                                                            |                                                                                                                                                                                      |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| F2               |  <p>Brown colour formed along the inoculated zone</p>     |  <p>Colour change to pale brown with stripes and symptoms became more spacious inside the stem</p> |
| F3               |  <p>Black colour formed along the inoculated zone</p>    |  <p>Black symptoms became more spacious inside the stem</p>                                       |
| Negative control |  <p>Whitish yellow formed along the inoculated zone</p> |  <p>Whitish yellow symptoms became more spacious inside the stem</p>                             |

**Table 4.6:** The morphological characteristics of infected zone after inoculation in dry season

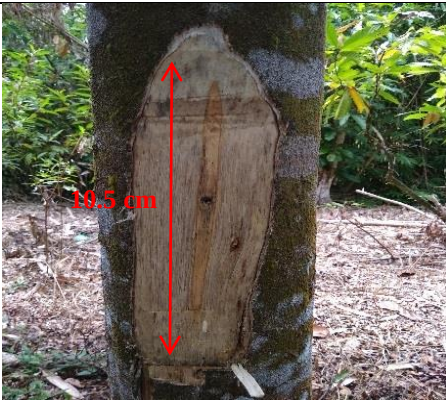
| Fungal Inoculant / Treatment | Dry Season                                                                                                                              |                                                                                                                                                                                       |
|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                              | 3 months                                                                                                                                | 6 months                                                                                                                                                                              |
| F1                           |  <p>Black colour formed along the inoculated zone</p>  |  <p>Colour change to dark brown and symptoms became more spacious inside the stem</p>               |
| F2                           |  <p>Brown colour formed along the inoculated zone</p> |  <p>Colour change to pale brown with stripes and symptoms became more spacious inside the stem</p> |

**Table 4.6** continued

|                  |                                                                                                                                        |                                                                                                                                                              |
|------------------|----------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| F3               |  <p>Black colour formed along the inoculated zone</p> |  <p>Black colour and the symptoms became more spacious inside the stem</p> |
| Negative control |  <p>Pale brown formed along the inoculated zone</p>  |  <p>Pale brown became more spacious inside the stem</p>                   |

Besides, another physical change of inoculated trees was detected throughout the inoculation process as the inoculated trees appearance has disease symptoms such as the leaves became withered and fell on the ground. These symptoms of wilting shows response of fungal attack in *Aquilaria* trees (Sulang, 2017).

### 4.3 Interpretation of Data

The measured mean length of longitudinal length of infected zone were recorded and analysed (Appendix D). In the bar graph as shown in Figure 4.6, the average mean value of infected zone length (L) for three fungi inoculants and negative control in *Aquilaria* stem after three months and six months inoculation were presented.

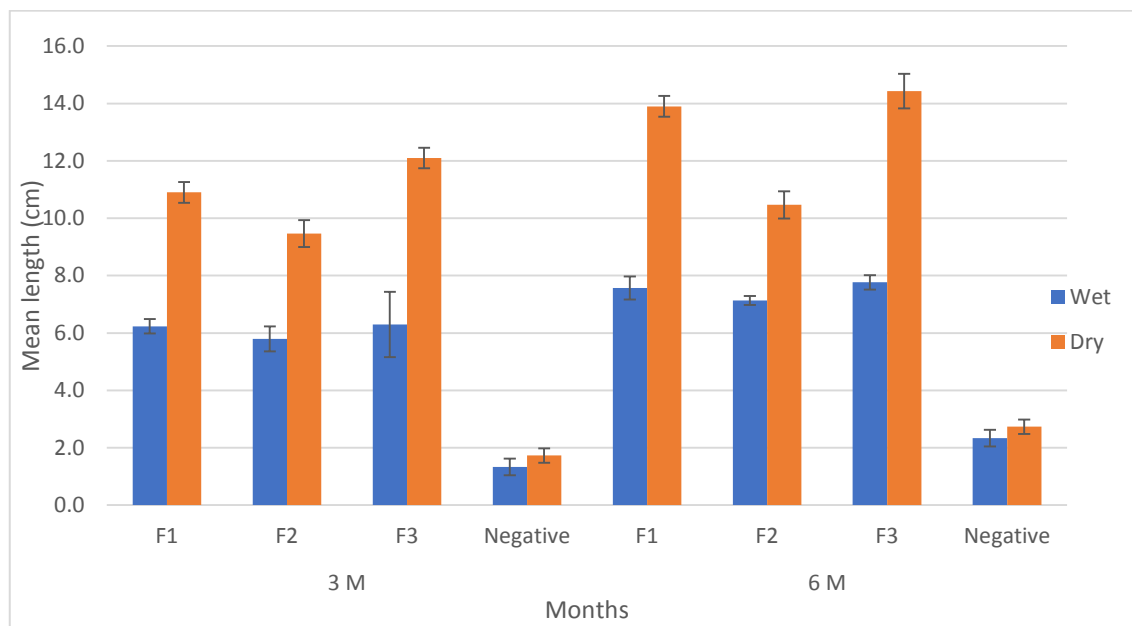


**Figure 4.6:** The average mean value of infected zone length (L) for 3 fungi inoculants and negative control in *Aquilaria* stem after 3 months and 6 months inoculation period for each wet and dry season. Error bar represents standard deviations (n=3).

Within the three months inoculation period, F3 inoculant showed the highest mean value of infected zone length (6.3 cm and 12.1 cm) followed by F1 (6.2 cm and 10.9 cm), F2 (5.8 cm and 9.5 cm), and negative control (1.3 cm and 1.7 cm) in each wet and dry season respectively. The result of the one-way ANOVA showed that the mean length of the infected zone among treatments or fungi inoculants were significantly different

( $P < 0.05$ ). Tukey's test revealed that there was a significant difference in three months and six months for both wet and dry seasons between fungal inoculants and negative control.

In the wet season of three months inoculation, F1, F2, F3, and negative control showed significant differences between them ( $P < 0.05$ ). However, for six months inoculation, there was no significant difference between F1 and F2 ( $P = 0.324$ ), between F1 and F3 ( $P = 0.830$ ), between F2 and F3 ( $P = 0.103$ ).

Meanwhile, in the dry season of three months inoculation, there was significantly difference between F1 and F2 ( $P = 0.006$ ), between F1 and F3 ( $P = 0.017$ ), between F2 and F3 ( $P < 0.05$ ). For six months inoculation, there was a significant difference between F1 and F2 ( $P < 0.05$ ) and between F2 and F3 ( $P < 0.05$ ), but only between F1 and F3 ( $P = 0.491$ ) shows no significant difference between them.

From the observation of mean value length of the infected zone after three months and six months inoculations, all fungi inoculants include negative control has shown increasing in mean value after periods. Further T-test was conducted to determine the significant difference ( $P < 0.05$ ) between different seasons within fungal inoculants. Based on T-test analysis, it shows that all treatments were more effective during the dry season and among the fungal inoculants, F3 inoculant shows the highest mean value compared to other fungal inoculants whether in both wet and dry season.

Thus, F3 inoculant showed the most potential fungi inoculant used to induce agarwood formation in *A. malaccensis* tree. This was because the F3 inoculant gave the darkest colour at the infected zone compared to other inoculants and the mean value for the infected zone length was gradually increased in each inoculation period of both wet and dry seasons.

Besides, F1 inoculant showed a positive result as it also produced an infected symptom towards the *Aquilaria* tree by changing the whitish healthy *Aquilaria* stem tree to dark colour after three months of inoculation in both wet and dry seasons. At the same time, the mean value for the infected zone length was higher compared to F2 inoculants. F2 was presented as the least potential fungi inoculant in this research study as agarwood inducer, due to their smallest mean value in infected zone length compare to other fungal inoculants. Furthermore, F2 was only able to induce the *Aquilaria* tree into brown or light brown, instead of dark brown or black which represents the good quality of agarwood. Hence, the physical quality of agarwood induced by F2 inoculant was not satisfactory.

#### **4.4 Gas Chromatography Mass Spectrometry (GC-MS) data analysis**

A total of 115 chemical compounds were identified in three inoculants and negative control of three months and six months inoculation period in both wet and dry seasons respectively (Table 4.7). From the GC-MS analysis in this study, for both wet and dry season, major agarwood chemical compounds includes sesquiterpenes, fatty acid, fatty acid methyl esters, sterols groups, and aromatic compounds were mainly found in all inoculants and negative control (sterile distilled water) after three months and six months inoculation periods. However, it was observed that dry season inoculations gave a slightly similar number of chemical compounds compared to wet season inoculations (82>79 chemical compounds respectively).

After three months inoculation in the wet season, a steroid compound such as stigmast-4-ene-3-one was detected in F1 inoculated trees (2.82%) and negative control (0.68%). Fatty acid methyl ester compound such as 8,11,14-docosatrienoic acid, methyl ester (0.25%) and 6,9-octadecadiynoic acid, methyl ester (0.49%) can only be detected in

F1 after 6 months inoculation periods in the wet season. Interestingly, 15-octadecenoic acid, methyl ester only present in F2 with a higher amount of 0.96% in the dry season compared to 0.25% in the wet season after three months inoculation periods.

Fatty acid compound such as n-hexadecanoic acid, a saturated long chain fatty acid was a presence for each inoculant and negative control in the wet season which reached 7.92% whereas absence in the dry season for every three months and six months inoculation periods. Dotriacontane (2.13%) and 2-methylhexacosane (10.38%) were absent in all inoculants whereas it can only be found in negative control after six months inoculation period in the dry season.

After six months inoculation in dry season, results of GC-MS analysis detect that aromatic compounds includes crytomeridiol (0.93%), 3',5' dimethoxyacetophenone (0.25%), guaia-1(10), 11-diene (0.45%), octacosanol (0.57%) and heptadecanolide (0.24%) only present in F3 inoculated trees. These chemical compounds contribute an aromatic scent in F3 inoculated trees compare to the other inoculated trees. These aromatic compounds were revealed absent in other inoculants and negative control in both wet and dry season of inoculation periods.

Based on the result in wet season inoculation periods, it was determined that sesquiterpenes includes 2-phenethyl-4H-chromen-4-one (16.48%), and 2-(4-methoxyphenethyl)-4H-chromen-4-one (3.47%) were slightly higher amount compare dry seasons at 2-phenethyl-4H-chromen-4-one (15.21%), and 2-(4-methoxyphenethyl)-4H-chromen-4-one (1.25%). However, 6,7-dimethoxy-2-phenethyl-4H-chromen-4-one (89.17%) and 6-methoxy-2-phenethyl-4H-chromen-4-one (17.79%) in dry season were higher compare to wet seasons of 6,7-dimethoxy-2-phenethyl-4H-chromen-4-one (97.3%)

and 6-methoxy-2-phenethyl-4H-chromen-4-one (15.59%). Overall, these sesquiterpenes were mainly presented in all inoculated trees but certain chemical compounds also presented in the negative control (sterile distilled water) trees.

From these results, it can be observed that most of the major chemical compounds were detected in the inoculated *Aquilaria* trees. Hence, this finding showed positive evidence that these inoculants have induced the agarwood formation in *Aquilaria* tree.

**Table 4.7:** Chemical compounds in each three inoculants and negative control within three months and six months inoculation period (wet and dry seasons)

| No. | Compounds Name                                | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |          |                 |          |                 |          |                 |          |                 |          |                 |          |                 |          |
|-----|-----------------------------------------------|-----------------|------------------------|-----------------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|
|     |                                               |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |          | F2 <sup>b</sup> |          | F2 <sup>c</sup> |          | F3 <sup>b</sup> |          | F3 <sup>c</sup> |          | NC <sup>b</sup> |          | NC <sup>c</sup> |          |
|     |                                               |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M       | 3M              | 6M       | 3M              | 6M       | 3M              | 6M       | 3M              | 6M       | 3M              | 6M       | 3M              | 6M       |
| 1.  | Hexacosane                                    | 2606            | 0.76                   | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        |
| 2.  | Stigmast-4-ene-3-one                          | 2714            | 2.82                   | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | 0.6<br>8        | -        | -               | -        |
| 3.  | Hexadecanoic acid, methyl ester               | 1878            | 3.0                    | 0.52            | -               | 0.3<br>5 | 0.33            | 0.2<br>4 | -               | 0.4<br>1 | 0.55            | 0.4<br>7 | 0.2<br>2        | 1.0<br>1 | 2.5<br>6        | 0.8<br>8 | 0.9<br>6        | -        |
| 4.  | n-hexadecanoic acid                           | 1968            | 1.75                   | 0.71            | -               | -        | -               | 0.8<br>0 | -               | -        | 1.09            | 2.1<br>3 | -               | -        | -               | 1.4<br>4 | -               | -        |
| 5.  | Oxacycloheptadecan-2-one                      | 2264            | 0.47                   | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        |
| 6.  | Hexadecanoic acid, ethyl ester                | 1978            | 1.1                    | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | 0.6<br>6        | -        | -               | -        |
| 7.  | 9, 12, 15-Octadecatrienoic acid, methyl ester | 2093            | 0.42                   | -               | -               | 0.3<br>9 | 0.16            | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        |
| 8.  | 9,12-Octadecanoic acid (Z,Z)-methyl ester     | 2101            | 2.33                   | -               | -               | -        | -               | -        | -               | -        | 1.67            | -        | -               | -        | -               | -        | -               | -        |
| 9.  | Erucic acid                                   | 2572            | 0.74                   | -               | -               | -        | -               | -        | -               | -        | 1.3             | -        | -               | -        | -               | -        | -               | -        |
| 10. | Tetracosane                                   | 2407            | 0.35                   | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | 1        |
| 11. | Tetrapentacontane, 1,54-dibromo-              | 5981            | 1.14                   | -               | 1.3<br>6        | -        | -               | -        | 4.46            | -        | -               | -        | 0.1<br>9        | -        | -               | -        | 4.9<br>7        | 1.8<br>2 |
| 12. | Stigmastane-3,6-dione, (5 $\alpha$ )-         | 2875            | 0.78                   | -               | 2.5<br>7        | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        |
| 13. | Behenic alcohol                               | 2451            | 0.56                   | -               | -               | -        | -               | -        | -               | -        | -               | -        | -               | -        | -               | 0.2<br>9 | -               | -        |
| 14. | Squalene                                      | 2914            | 0.46                   | 0.51            | 2.2<br>7        | 1.4<br>7 | -               | 0.2<br>6 | 2.63            | 1.2<br>3 | 1.61            | 1.4<br>1 | 0.7<br>7        | 1.2<br>4 | -               | 1.3<br>5 | -               | -        |

**Table 4.7** continued

| No. | Compounds Name                                               | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |      |                 |      |                 |      |                 |      |                 |      |                 |      |                 |      |
|-----|--------------------------------------------------------------|-----------------|------------------------|-----------------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|
|     |                                                              |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |      | F2 <sup>b</sup> |      | F2 <sup>c</sup> |      | F3 <sup>b</sup> |      | F3 <sup>c</sup> |      | NC <sup>b</sup> |      | NC <sup>c</sup> |      |
|     |                                                              |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   |
| 15. | Cholest-4-ene-3,6-dione                                      | 2751            | 9.42                   | -               | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | 11.7            | -    | -               | -    |
| 16. | Stigmasterol                                                 | 2739            | 2.07                   | -               | -               | -    | 0.80            | -    | -               | -    | 1.12            | -    | -               | -    | 3.13            | 0.79 | -               | -    |
| 17. | 4,22-Cholestadien-3-one                                      | 2588            | 22.5                   | -               | 1.09            | -    | -               | -    | -               | -    | -               | -    | -               | -    | 6.44            | -    | -               | 2.76 |
| 18. | $\alpha$ -Amyrin                                             | 2873            | 4.88                   | -               | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 19. | Phenol, 3,5-bis(1,1-dimethylethyl)-                          | 1555            | -                      | 0.16            | -               | -    | 0.19            | -    | -               | -    | -               | -    | -               | -    | 1.24            | 0.34 | -               | -    |
| 20. | Cubenol                                                      | 1580            | -                      | 0.25            | -               | -    | -               | -    | -               | -    | -               | -    | -               | 1.28 | -               | -    | -               | -    |
| 21. | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol                | 1653            | -                      | 5.73            | 3.15            | 5.17 | 3.28            | -    | 1.35            | 2.77 | 9.3             | 2.65 | 4.36            | 4.32 | 6.27            | 8.87 | 2.83            | -    |
| 22. | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester      | 1908            | -                      | 0.8             | -               | -    | 1.08            | 0.69 | -               | -    | 1.23            | 0.69 | -               | -    | 3.99            | 1.81 | 0.42            | 7.24 |
| 23. | 4,5-di-epi-aristolochene                                     | 1474            | -                      | 0.62            | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 24. | 4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl- | 1530            | -                      | 0.35            | -               | -    | -               | 0.34 | -               | 0.83 | -               | -    | -               | 0.27 | -               | -    | -               | -    |
| 25. | Sulfurous acid, 2-propyl tetradecyl ester                    | 2270            | -                      | 0.12            | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 26. | Methyl 10-trans,12-cis-octadecadienoate                      | 2093            | -                      | 0.57            | 0.8             | -    | -               | 0.42 | -               | -    | -               | -    | -               | 0.66 | 1.88            | -    | 1.06            | -    |
| 27. | 9-Octadecenoic acid (Z)-, methyl ester                       | 2085            | -                      | 0.49            | -               | 0.48 | -               | 0.21 | -               | -    | -               | -    | -               | 0.86 | -               | 0.52 | 0.68            | -    |

**Table 4.7** continued

| No. | Compounds Name                                               | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |      |                 |      |                 |       |                 |      |                 |      |                 |      |                 |      |
|-----|--------------------------------------------------------------|-----------------|------------------------|-----------------|-----------------|------|-----------------|------|-----------------|-------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|
|     |                                                              |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |      | F2 <sup>b</sup> |      | F2 <sup>c</sup> |       | F3 <sup>b</sup> |      | F3 <sup>c</sup> |      | NC <sup>b</sup> |      | NC <sup>c</sup> |      |
|     |                                                              |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M   | 3M              | 6M   | 3M              | 6M    | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   |
| 28. | 8,11,14-Docosatrienoic acid, methyl ester                    | 2499            | -                      | 0.25            | -               | -    | -               | -    | -               | -     | -               | -    | -               | -    | -               | -    | -               | -    |
| 29. | 6,9-Octadecadiynoic acid, methyl ester                       | 2112            | -                      | 0.49            | -               | -    | -               | -    | -               | -     | -               | -    | -               | -    | -               | -    | -               | -    |
| 30. | Heneicosane                                                  | 2109            | -                      | 1.19            | -               | -    | -               | -    | -               | -     | 1.33            | 1.9  | -               | -    | -               | 0.34 | -               | -    |
| 31. | Nonacosane                                                   | 2904            | -                      | 1.74            | -               | -    | -               | 1.01 | -               | -     | 1.66            | 2.2  | -               | -    | -               | 1.34 | -               | -    |
| 32. | Acetamide, N-(4-benzyloxyphenyl)-2 cyano                     | 2500            | -                      | 3.65            | 5.87            | 6.02 | 5.65            | 4.99 | 5.6             | 8.02  | 3.94            | 4.84 | 6.79            | 5.34 | -               | 2.67 | 1.4             | 0.64 |
| 33. | Tetracosanoic acid, methyl ester                             | 2674            | -                      | 0.16            | -               | -    | -               | -    | -               | -     | -               | -    | 0.3             | -    | 0.98            | -    | 3.08            | -    |
| 34. | Spiromesifen                                                 | 2873            | -                      | 0.27            | -               | -    | -               | -    | -               | -     | -               | -    | -               | -    | -               | -    | -               | -    |
| 35. | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dimethyl-              | 2389            | -                      | 10.98           | 3.64            | 8.1  | 5.74            | 8.88 | 1.97            | 11.02 | 5.12            | 5.54 | 7.86            | 8.45 | -               | 9.79 | 3.71            | -    |
| 36. | p-Acetamidobenzaldehyde 6-methylpyridine-3-carbonylhydrazone | 2902            | -                      | 0.23            | -               | 1.07 | -               | 0.22 | -               | 0.29  | -               | -    | -               | 0.48 | -               | -    | -               | -    |
| 37. | Tetracontane                                                 | 3997            | -                      | 0.77            | -               | -    | -               | 0.87 | -               | 1.06  | -               | -    | -               | -    | -               | -    | -               | -    |

**Table 4.7** continued

| No. | Compounds Name                                    | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |           |                 |           |                 |           |                 |           |                 |           |                 |          |                 |          |
|-----|---------------------------------------------------|-----------------|------------------------|-----------------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|----------|-----------------|----------|
|     |                                                   |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |           | F2 <sup>b</sup> |           | F2 <sup>c</sup> |           | F3 <sup>b</sup> |           | F3 <sup>c</sup> |           | NC <sup>b</sup> |          | NC <sup>c</sup> |          |
|     |                                                   |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M        | 3M              | 6M        | 3M              | 6M        | 3M              | 6M        | 3M              | 6M        | 3M              | 6M       | 3M              | 6M       |
| 38. | 2-[p-Methoxybenzyloxy]-6-methoxy-8-aminoquinoline | 277<br>7        | -                      | 5.2             | 5.7<br>9        | -         | 10.7<br>5       | 10.1<br>8 | 7.03            | 4.68      | 3.16            | 4.21      | 6.51            | -         | -               | 2.1<br>4 | -               | -        |
| 39. | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-one        | 0               | -                      | 29.9<br>5       | 9.1<br>3        | 17.<br>29 | 33.6<br>5       | 25.1<br>3 | 13.6<br>6       | 22.1<br>6 | 15.1<br>1       | 25.9<br>1 | 27.8<br>5       | 20.3<br>3 | -               | 18.<br>4 | 3.3<br>6        | 5.8<br>5 |
| 40. | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-            | 171<br>0        | -                      | 2.91            | -               | -         | -               | 3.03      | -               | -         | -               | 1.21      | -               | -         | -               | 2.7<br>6 | -               | -        |
| 41. | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dimethyl-    | 173<br>2        | -                      | 3.64            | 1.0<br>4        | 6.9<br>3  | 0.52            | 2.72      | -               | 1.15      | 0.98            | 0.17      | 2.49            | 3.38      | -               | 5.0<br>3 | -               | -        |
| 42. | 2-Phenethyl-4H-chromen-4-one                      | 0               | -                      | 1.33            | 1.0<br>1        | 1.6<br>4  | 4.7             | 2.64      | 1.08            | 3.46      | 2.52            | 4.52      | 4.13            | 2.94      | -               | 0.7<br>7 | -               | 0.9<br>5 |
| 43. | cis-5,8,11,14,17-Eicosapentaenoic acid            | 240<br>6        | -                      | -               | -               | -         | 0.6             | -         | -               | -         | -               | -         | -               | -         | -               | -        | -               | -        |
| 44. | 6-Methoxy-2-phenethyl-4H-chromen-4-one            | 0               | -                      | 1.83            | 3               | 2.1<br>5  | 6.29            | 4.17      | 3.14            | 3.9       | 0.84            | 1.77      | 3.2             | 2.4       | -               | 0.6<br>9 | -               | -        |
| 45. | 2-(4-Methoxyphenethyl)-4H-chromen-4-one           | 0               | -                      | 0.32            | 0.7<br>7        | 0.4<br>8  | 1.26            | 0.73      | -               | -         | -               | 0.78      | -               | -         | -               | 0.3<br>8 | -               | -        |
| 46. | cis-Z- $\alpha$ -Bisabolene epoxide               | 153<br>1        | -                      | -               | -               | -         | 0.15            | -         | -               | 0.82      | -               | 1.11      | 0.36            | -         | -               | 0.3<br>8 | -               | -        |
| 47. | Caryophyllene oxide                               | 150<br>7        | -                      | -               | -               | -         | 0.17            | -         | -               | -         | 0.44            | -         | -               | -         | -               | -        | -               | -        |

**Table 4.7** continued

| No. | Compounds Name                                                                                                                    | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |      |                 |      |                 |      |                 |      |                 |      |                 |      |                 |      |
|-----|-----------------------------------------------------------------------------------------------------------------------------------|-----------------|------------------------|-----------------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|
|     |                                                                                                                                   |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |      | F2 <sup>b</sup> |      | F2 <sup>c</sup> |      | F3 <sup>b</sup> |      | F3 <sup>c</sup> |      | NC <sup>b</sup> |      | NC <sup>c</sup> |      |
|     |                                                                                                                                   |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   |
| 48. | 15-Octadecenoic acid, methyl ester                                                                                                | 2085            | -                      | -               | -               | -    | 0.25            | -    | 0.96            | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 49. | trans-Sinapyl alcohol                                                                                                             | 0               | -                      | -               | 1.8             | 4.82 | 1.76            | 2.65 | 1.04            | 2.71 | 4.97            | 3.28 | 3.28            | 4.7  | -               | 6.09 | 1.82            | 3.8  |
| 50. | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1 $\alpha$ ,7 $\beta$ ,8 $\alpha$ . $\alpha$ )]- | 1474            | -                      | -               | -               | -    | 0.21            | -    | 1.03            | -    | -               | 0.36 | -               | -    | -               | 0.68 | -               | -    |
| 51. | Andrographolide                                                                                                                   | 2944            | -                      | -               | -               | -    | 0.76            | -    | -               | 1.27 | -               | 0.84 | -               | 0.56 | -               | -    | -               | -    |
| 52. | Di-n-octyl phthalate                                                                                                              | 2832            | -                      | -               | -               | -    | 0.16            | -    | 0.85            | 0.3  | 0.85            | -    | -               | -    | 0.81            | 0.32 | 0.44            | 1.08 |
| 53. | Decanedioic acid, bis(2-ethylhexyl) ester                                                                                         | 2812            | -                      | -               | 0.88            | -    | 0.48            | -    | 1.00            | -    | -               | -    | -               | -    | 0.51            | -    | -               | 0.72 |
| 54. | Imidazolidin-4-one, 5-benzyl-2-thioxo-3-p-tolyl                                                                                   | 2795            | -                      | -               | -               | -    | 1.04            | 0.47 | -               | 0.89 | -               | 0.58 | -               | -    | -               | -    | -               | -    |
| 55. | Trifluoroacetic acid, 4-benzyloxyphenyl ester                                                                                     | 1757            | -                      | -               | -               | -    | 0.28            | 1.34 | -               | -    | 0.96            | 2.49 | -               | -    | -               | -    | -               | -    |
| 56. | $\gamma$ -Sitosterol                                                                                                              | 2731            | -                      | -               | -               | -    | 1.23            | 0.92 | 2.11            | -    | 3.29            | -    | -               | 1.76 | 6.71            | 3.65 | 1.76            | 1.32 |
| 57. | 4,22-Stigmastadiene-3-one                                                                                                         | 2722            | -                      | -               | -               | -    | 2.31            | 1.08 | -               | -    | 2.49            | 1.15 | 3.24            | 2.18 | -               | 2.35 | 1.28            | -    |
| 58. | 2-Methoxy-4-vinylphenol                                                                                                           | 1293            | -                      | -               | -               | -    | -               | 0.27 | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 59. | Hydrocinnamic acid                                                                                                                | 1349            | -                      | -               | -               | -    | -               | 0.30 | -               | 0.51 | -               | -    | -               | 0.26 | -               | -    | -               | -    |
| 60. | Benzaldehyde, 4-hdroxy-3,5-dimethoxy-                                                                                             | 1581            | -                      | -               | -               | 0.24 | -               | 0.33 | -               | -    | -               | -    | -               | -    | -               | -    | -               | -    |
| 61. | Aromadendrene oxide-(1)                                                                                                           | 1462            | -                      | -               | -               | -    | -               | -    | -               | -    | -               | 4.22 | 0.34            | -    | -               | -    | -               | -    |
| 62. | l-(+)-Ascorbic acid 2,6-dihexadecanoate                                                                                           | 4765            | -                      | -               | -               | -    | -               | -    | -               | -    | -               | -    | 0.29            | -    | 2.48            | -    | -               | -    |
| 63. | Dibutyl phthalate                                                                                                                 | 2037            | -                      | -               | -               | -    | -               | -    | -               | -    | -               | -    | 0.34            | -    | 0.87            | -    | -               | 0.53 |
| 64. | (E)-13-Docosenoic acid                                                                                                            | 2572            | -                      | -               | -               | 0.36 | -               | -    | -               | -    | -               | -    | -               | -    | 0.7             | -    | -               | -    |

**Table 4.7** continued

| No. | Compounds Name                                               | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |      |                 |    |                 |      |                 |    |                 |      |                 |      |                 |      |
|-----|--------------------------------------------------------------|-----------------|------------------------|-----------------|-----------------|------|-----------------|----|-----------------|------|-----------------|----|-----------------|------|-----------------|------|-----------------|------|
|     |                                                              |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |      | F2 <sup>b</sup> |    | F2 <sup>c</sup> |      | F3 <sup>b</sup> |    | F3 <sup>c</sup> |      | NC <sup>b</sup> |      | NC <sup>c</sup> |      |
|     |                                                              |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M   | 3M              | 6M | 3M              | 6M   | 3M              | 6M | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   |
| 65. | 4,8,12,16-Tetramethylheptadecan-4-olide                      | 2258            | -                      | -               | -               | -    | -               | -  | -               | -    | -               | -  | -               | -    | 0.74            | -    | 0.47            | 1.16 |
| 66. | Ethanedioic acid, bis(1-methylpropyl) ester                  | 1222            | -                      | -               | -               | -    | -               | -  | -               | -    | -               | -  | -               | -    | 1.18            | -    | -               | -    |
| 67. | Docosanoic acid, methyl ester                                | 2475            | -                      | -               | 1.36            | -    | -               | -  | 0.89            | -    | -               | -  | -               | -    | 0.79            | -    | -               | -    |
| 68. | Hexacosanoic acid, methyl ester                              | 2872            | -                      | -               | 3.17            | -    | -               | -  | -               | -    | -               | -  | -               | -    | 0.86            | -    | -               | -    |
| 69. | Cholesta-4,6-dien-3-ol, (3 $\beta$ )-                        | 2579            | -                      | -               | -               | -    | -               | -  | -               | -    | -               | -  | -               | -    | 1.31            | 1.17 | -               | -    |
| 70. | Cholestan-3-one                                              | 2569            | -                      | -               | -               | -    | -               | -  | -               | -    | -               | -  | -               | -    | 2.00            | -    | 0.98            | 3.46 |
| 71. | Noscapine                                                    | 3365            | -                      | -               | -               | 1.41 | -               | -  | -               | -    | -               | -  | -               | 1.41 | -               | 1.94 | -               | -    |
| 72. | Diisooctyl phthalate                                         | 2704            | -                      | -               | 1.29            | -    | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 73. | 7-Methyl-Z-tetradecen-1-ol acetate                           | 1822            | -                      | -               | 0.70            | -    | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 74. | Hexacontane                                                  | 5985            | -                      | -               | 3.78            | -    | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | 2.18            | -    |
| 75. | Eicosen-1-ol, cis-9-                                         | 2260            | -                      | -               | 0.87            | -    | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 76. | Tetrapentacontane                                            | 5389            | -                      | -               | 2.36            | -    | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | 6.33            | 1.26 |
| 77. | 5 $\alpha$ -Pregn-9(11)-en-12-one                            | 2111            | -                      | -               | 0.60            | -    | -               | -  | -               | -    | -               | -  | 0.80            | -    | -               | -    | -               | -    |
| 78. | Quinazolin-4(3H)-one, 2-(4-methoxybenzylthio)-3-methyl-      | 2710            | -                      | -               | -               | 2.71 | -               | -  | 0.79            | 1.86 | -               | -  | -               | 2.36 | -               | -    | 0.46            | -    |
| 79. | 1H-Pyrimidine-2,4-dione, 1-benzylloxymethyl-5-bromo-6-methyl | 2373            | -                      | -               | -               | 2.23 | -               | -  | -               | 2.26 | -               | -  | 1.73            | -    | -               | -    | -               | -    |
| 80. | Docosyl ethyl carbonate                                      | 2749            | -                      | -               | -               | 0.45 | -               | -  | 1.35            | -    | -               | -  | -               | -    | -               | -    | 2.59            | -    |
| 81. | 2-Butanone, 4-phenyl-                                        | 1228            | -                      | -               | -               | 0.84 | -               | -  | -               | 1.49 | -               | -  | 0.50            | 0.50 | -               | -    | -               | -    |
| 82. | 2-Butanone, 4-(4-methoxyphenyl)-                             | 1417            | -                      | -               | -               | 0.47 | -               | -  | -               | 0.32 | -               | -  | 0.19            | 0.22 | -               | -    | -               | -    |
| 83. | Agarospinol                                                  | 1598            | -                      | -               | -               | 0.34 | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 84. | Humulene                                                     | 1579            | -                      | -               | -               | 0.41 | -               | -  | -               | 0.68 | -               | -  | -               | -    | -               | -    | -               | -    |
| 85. | Ethyl iso-allocholate                                        | 3094            | -                      | -               | -               | 0.80 | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 86. | Longipinocarveol, trans                                      | 1599            | -                      | -               | -               | 0.41 | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |

**Table 4.7** continued

| No.  | Compounds Name                                                                                                                           | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |    |                 |    |                 |      |                 |    |                 |      |                 |      |                 |      |
|------|------------------------------------------------------------------------------------------------------------------------------------------|-----------------|------------------------|-----------------|-----------------|----|-----------------|----|-----------------|------|-----------------|----|-----------------|------|-----------------|------|-----------------|------|
|      |                                                                                                                                          |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |    | F2 <sup>b</sup> |    | F2 <sup>c</sup> |      | F3 <sup>b</sup> |    | F3 <sup>c</sup> |      | NC <sup>b</sup> |      | NC <sup>c</sup> |      |
|      |                                                                                                                                          |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M | 3M              | 6M | 3M              | 6M   | 3M              | 6M | 3M              | 6M   | 3M              | 6M   | 3M              | 6M   |
| 87.  | 7-Hexadecenal, (Z)-                                                                                                                      | 1808            | -                      | -               | -               | -  | -               | -  | 2.76            | -    | -               | -  | 0.43            | -    | -               | -    | -               | -    |
| 88.  | Oxalic acid, hexyl 2-isopropylphenyl ester                                                                                               | 2072            | -                      | -               | -               | -  | -               | -  | 3.86            | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 89.  | 5-Benzyloxy pyrimidine-2-carboxylic acid                                                                                                 | 2000            | -                      | -               | -               | -  | -               | -  | 2.02            | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 90.  | Cholesta-22,24-dien-5-ol, 4,4-dimethyl-                                                                                                  | 2778            | -                      | -               | -               | -  | -               | -  | 1.05            | -    | -               | -  | -               | -    | -               | -    | -               | 0.82 |
| 91.  | Benzene, 1-chloro-4-(4-methoxybenzyloxy)-3-nitro-                                                                                        | 2307            | -                      | -               | -               | -  | -               | -  | -               | 1.55 | -               | -  | 0.52            | 0.59 | -               | -    | -               | -    |
| 92.  | Eicosane                                                                                                                                 | 2009            | -                      | -               | -               | -  | -               | -  | -               | 0.68 | -               | -  | -               | -    | -               | -    | -               | 0.96 |
| 93.  | Ledol                                                                                                                                    | 1530            | -                      | -               | -               | -  | -               | -  | -               | 0.34 | -               | -  | -               | -    | -               | -    | -               | -    |
| 94.  | 1,2,4-Methenoazulene, decahydro-1,5,5,8a-tetramethyl-, [1S-(1 $\alpha$ ,2 $\alpha$ ,3 $\alpha\beta$ ,4 $\alpha$ ,8 $\alpha\beta$ ,9R*)]- | 1184            | -                      | -               | -               | -  | -               | -  | -               | 2.44 | -               | -  | 1.73            | 1.19 | -               | -    | -               | -    |
| 95.  | Alloaromadendrene oxide-(2)                                                                                                              | 1462            | -                      | -               | -               | -  | -               | -  | -               | 0.83 | -               | -  | -               | 0.96 | -               | -    | -               | -    |
| 96.  | Epiglobulol                                                                                                                              | 1530            | -                      | -               | -               | -  | -               | -  | -               | -    | -               | -  | 0.43            | -    | -               | -    | -               | -    |
| 97.  | Farnesene epoxide, E-                                                                                                                    | 1540            | -                      | -               | -               | -  | -               | -  | -               | -    | -               | -  | 0.22            | -    | -               | -    | -               | -    |
| 98.  | Benzene, 1-trifluoromethyl-4-(2-methoxybenzyloxy)-3-nitro-                                                                               | 2075            | -                      | -               | -               | -  | -               | -  | -               | -    | -               | -  | 1.36            | 2.43 | -               | -    | -               | -    |
| 99.  | Dotriacontane                                                                                                                            | 3202            | -                      | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | 1.29            | 0.84 |
| 100. | 2-Methylhexacosane                                                                                                                       | 0               | -                      | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | 8.86            | 1.52 |
| 101. | Octadecanal                                                                                                                              | 1999            | -                      | -               | -               | -  | -               | -  | -               | -    | 0.73            | -  | -               | -    | -               | -    | -               | -    |
| 102. | Linoleic acid, ethyl ester                                                                                                               | 2193            | 1.36                   | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 103. | Behenic alcohol                                                                                                                          | 2451            | 0.56                   | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | 0.29 | -               | -    |
| 104. | Cholest-4-ene-3,6-dione                                                                                                                  | 2751            | 9.42                   | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | 11.7            | -    | -               | -    |
| 105. | Lupeol                                                                                                                                   | 2848            | 0.79                   | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |
| 106. | $\beta$ -Amyrin                                                                                                                          | 2886            | 1.52                   | -               | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    | -               | -    | -               | -    |

**Table 4.7** continued

| No.  | Compounds Name                               | RI <sup>a</sup> | Relative Peak Area (%) |                 |                 |    |                 |      |                 |    |                 |    |                 |      |                 |    |                 |      |
|------|----------------------------------------------|-----------------|------------------------|-----------------|-----------------|----|-----------------|------|-----------------|----|-----------------|----|-----------------|------|-----------------|----|-----------------|------|
|      |                                              |                 | F1 <sup>b</sup>        |                 | F1 <sup>c</sup> |    | F2 <sup>b</sup> |      | F2 <sup>c</sup> |    | F3 <sup>b</sup> |    | F3 <sup>c</sup> |      | NC <sup>b</sup> |    | NC <sup>c</sup> |      |
|      |                                              |                 | 3M <sup>d</sup>        | 6M <sup>e</sup> | 3M              | 6M | 3M              | 6M   | 3M              | 6M | 3M              | 6M | 3M              | 6M   | 3M              | 6M | 3M              | 6M   |
| 107. | Tetradecanoic acid, 12-methyl-, methyl ester | 1715            | -                      | -               | -               | -  | -               | 0.25 | -               | -  | -               | -  | -               | -    | -               | -  | -               | -    |
| 108. | Hydrastine                                   | 3176            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | -    | -               | -  | 0.66            | -    |
| 109. | 17-Pentatriacontene                          | 3508            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | -    | -               | -  | 1.05            | -    |
| 110. | Valtrate                                     | 2686            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | -    | -               | -  | -               | 0.46 |
| 111. | Cryptomeridiol                               | 0               | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | 0.93 | -               | -  | -               | -    |
| 112. | Guaia-1(10),11-diene                         | 1490            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | 0.45 | -               | -  | -               | -    |
| 113. | 3',5'-Dimethoxyacetophenone                  | 1407            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | 0.25 | -               | -  | -               | -    |
| 114. | Octacosanol                                  | 3047            | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | 0.57 | -               | -  | -               | -    |
| 115. | Heptadecanolide                              | 0               | -                      | -               | -               | -  | -               | -    | -               | -  | -               | -  | -               | 0.24 | -               | -  | -               | -    |

RI<sup>a</sup> indicates the retention indices using a BPX 5 column, F1<sup>b</sup> indicates the fungal inoculant treatment during the wet season, F1<sup>c</sup> indicate the fungal inoculant treatment during the dry season, 3M<sup>d</sup> indicates the third month inoculation samples, 6M<sup>e</sup> indicates the sixth month inoculation samples, ‘-’ indicates not detected.

## CHAPTER 5

### DISCUSSION

#### 5.1 Formulated microbial consortium

In this study, endophytic fungi used was isolated from *Aquilaria* trees in the previous study by Sulang (2017). Thus, it was believed that fungus that had originally infected the agarwood tree might integrate (involve or participate) in the resin formation in the inoculated *Aquilaria* tree. Hence, these fungi may reduce the disease symptoms and lead to a defence mechanism formed which consequently produces the resinous agarwood. Nimnoi and Pongsilp (2009) stated that endophytes known as any microorganism that isolated from inner tissues of the plants or the surface sterilized plants part where the plants themselves were unharmed by endophytes. Besides, the microorganism aids in improving and promoting plant growth as an endosymbiotic relationship was created between the microorganism with the host plants that eventually aids in reducing the symptoms of the disease by a microbial attack or environmental stress (Giordano et al., 2009).

Several studies have discovered a varied group of fungi generally found within the injured wood of *A. malaccensis* (Tamuli et al., 2000; Mohamed et al., 2010). In addition, Cui et al. (2011) showed that *Fusarium* sp. was a dominant genus that had been isolated from *A. sinensis*. However, this is not in agreement with this study as *A. malaccensis* were used in this study instead of *A. sinensis* which may harbour different effects on agarwood formation in *A. malaccensis*.

According to Mohamed et al. (2010), fungi can be manipulated in cultivated trees of *Aquilaria* to induce agarwood production. The endophytic fungi that were used in this

research include *Trichoderma* sp., *Curvularia* sp., *Fusarium* sp., *Aspergillus* sp., *Lasiodiplodia* sp., and *Penicillium* sp. which have been previously isolated from agarwood, identified and characterized by a previous study (Sulang, 2017).

It was discovered that several types of fungi may cause the formation of agarwood in the *Aquilaria* tree (Blanchette & Hauveling, 2002). These fungi include *Phialophora parasitica*, *Torula* sp., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp., *Epicoccum granulatum*, *Cylindrocladium*, *Sphaeropsis* sp., *Botryodiplodia theobromae*, *Trichoderma* sp., *Phomopsis* sp., and *Cunninghamella echinulate*. Including these fungi, only *Trichoderma* sp., *Fusarium* sp., *Aspergillus* sp., and *Penicillium* sp. used in this study were found to be proven as endophytes fungus in agarwood based on the previous findings by Blanchette and Hauveling (2002). However, it can be suggested that all the fungi used in this study were generally found in the agarwood tree as these fungi were naturally affecting *Aquilaria* trees and subsequently allowing the inducement of resinous agarwood.

The responsibility of rot fungi in agarwood formation has been studied by a few researchers (Tamuli et al., 2000; Mitra & Gogol, 2001). However, species of fungi that are important in inducing resin production in agarwood trees remain obscure. There is some imitation on the natural process by testing the effects of isolates in agarwood formation. For example, *Epicoccum granulatum* originally from infected *Aquilaria* has been tested on the standing stems of *Aquilaria* (Bhattacharyya et al., 1952; Sadgopal, 1960; Verma, 1977). Besides, it has been shown that fungal species such as *Aspergillus* sp., *Lasiodiplodia* sp., *Fusarium* sp., *Trichoderma* sp., *Penicillium* sp., *Pythium* sp., and *Diplodia* sp., are common fungi that infect *Aquilaria* sp. (Wiriadinata, 1995; Soehartono &

Mardiastuti, 1997). However, there is no clear study on specific fungus species that crucial in inducing resin for agarwood formation.

There were several limitations and not much knowledge on the formulation of the microbial consortium using endophytic fungi. Hence, in this study, three microbial consortia have been formulated. These microbial consortiums were formulated by using different types of fungus species that originated from the agarwood tree, these isolates have been identified, characterized and cultured in the previous study (Sulang, 2017). These criteria of fungus were concurrence with literature that showed the possibility of fungi infection that can induce the resin formation in agarwood (Soehartono & Mardiastuti, 1997; Blanchette & Heuveling, 2002; Santoso et al., 2011).

Even though the formulated microbial consortium consisting random combination of fungus species, the antagonistic and inhibitory effect between the different types of fungus species were observed after an incubation period to determine their symbiotic relationship. The symbiotic relationships between fungus species within the formulated microbial consortium were observed in having equal ratio of growth among the fungus species within the media. This shows that the selected microbial consortium has no antagonistic and inhibitory effect against each other by symbiotically growth in equal ratio in the microbial consortium.

Then, the best 3 types of the formulated microbial consortium were chosen to be further served as a fungal inoculant based on their symbiotic growth in a similar environment, temperature and amount of nutrient supply in PDA medium. PDA medium was used for culturing of fungus due to its convenience in observing the colour of fungal colonies and aerial hyphae (Akhsan et al., 2015). Besides, these fungi were cultured on

Potato Dextrose Agar (PDA) as PDA is common media used for the cultivation of fungi due to its ability to promotes the growth of mycelia in a variety of fungi species and it is a simple formulation of media (Stevens, 1981).

The 3 microbial consortia were formulated by randomly culture 2 types of fungus species, and 3 types of fungus species simultaneously (Appendix A). In this study, there were 2 microbial consortia made up of 2 species of fungus each of which were *T. spirale* (G4-B2) and *A. flavus* (G4-B3), *F. solani* (GKS 2) and *P. citrinum* (GB 4). These fungi in microbial consortium type 1 and 2 were observed to have similar properties of growth as they were able to symbiotically survive simultaneously in a 1:2 ratio of medium area. Blanchette et al. (2001) stated that *T. spirale* and *A. flavus* were common fungi species originated or isolated from agarwood. Meanwhile, Soehartono and Mardiasuti (1997) reported that *Aspergillus* sp. and *Penicillium* sp. are mutual fungal species that often infect *Aquilaria* spp.

Next, another microbial consortium formulated by 3 types of fungus species includes *T. harzianum* (G2-A2), *L. theobromae* (GKS 3) and *C. affinis* (G3-B2). Based on the results, it was observed that *T. harzianum*, *Lasiodiplodia*, and *Curvularia* were able to grow symbiotically in the culture medium. However, *Trichoderma harzianum* has dominated more growth areas compared to *Lasiodiplodia* and *Curvularia*, but *T. harzianum* was not completely dominant over *Lasiodiplodia* and *Curvularia* as both fungi were able to grow simultaneously in a limited medium area. Thus, these formulated microbial consortia were accepted to be further cultured as a fungal inoculant. There were very limited studies reported on the relationship between *T. harzianum*, *Lasiodiplodia* and *Curvularia*. However, these fungi species were previously found in the agarwood of *A.*

*malaccensis* (Chen et al., 2017), in which concurrence with this study that *A. malaccensis* were tested with these inoculants. Hence, positive result was expected by inducing *Aquilaria* tree with F3 fungal inoculant.

The first objective of this study has been achieved by the successful formulation of the microbial consortium to be developed as an agarwood inducer. However, the effects of these selected microbial consortia toward agarwood formation have not yet determined. Therefore, these microbial consortia were further served as fungal inoculants. These were conducted to further determine the effects of these microbial consortia on agarwood formation to identify and select the highest potential microbial consortium as agarwood inducer.

## **5.2 Fungal Inoculation Technique**

Agarwood, a resinous compound produced by *Aquilaria* spp. was formed as a response toward abiotic and biotic factors (Azren et al., 2019). The abiotic factor was defined as non-living physical and chemical elements in the ecosystem includes drought, heat, cold, flooding and salinity whereas microbial infections were an example of biotic factor as it involves living organisms in ecosystems.

In this study, the physical injury was done by drilling an artificial wound to make a hole into the xylem of *Aquilaria* trees in Serian Agarwood Farm. The formulated microbial consortium then acts as the biotic factor to serve as a fungal inoculant that applied to the wound by using a bottle drip method to increase the effectiveness of agarwood formation in short term compare to natural agarwood formation in wild environment.

Bottle drip method as described by Tang (2012) were used in this study to prevent the closure of wound as the presence of fungi aid in keeping the wound open and eventually disrupt the living cell of agarwood trees which lead to agarwood formation. This technique induces agarwood by the combination of physical injury and microbial infection by fungal inoculants in which corresponds to statement by Deodhar et al. (2002), 'physical injury or pathological wound was done to allow fungi entering the stem through holes and it represents the site of initial infection'. Hence, a substantial volume of wood was infected as the spreading of infections constantly increased with the development of pathogens on all sides of the tree.

Chemical and physical methods have constraints in terms of spreading of wound compared to biological agents that able to spread into the entire plant and efficiently yield agarwood formation. Thus, it has been stated that by using biological agents, a fulfilled quality and quantity of agarwood were produced (Soehartono & Newton 2000; Santoso et al. 2011). Hence, fungi inoculation are fundamental factors in agarwood formation as the wounded *Aquilaria* trees were stimulated by the fungal inoculant and eventually produce resin from the wounded woody tissue. Duryatmo (2009) state that the success of inoculation process is significant by determining the suitable pathogen microbes within *Aquilaria* trees. However, there were uncertainties on the cultivation agarwood trees similarity in terms of agarwood production quality compared to harvest from natural agarwood trees due to the uses of artificial agarwood inducement techniques (Nor Azah et al., 2013).

The inoculation method used in this study was slightly modified from previous artificial agarwood inducement methods by Tang (2012). Briefly, the inducement of

agarwood was done by drilling of the tree trunk and inoculate the tree stem with the fungi inoculants by using the bottle drip method.

### **5.3 Formation of agarwood in inoculated trees**

Formation of oleoresin has been related to the aging of early infection and the colour of wood turned to darker scented wood called “Agar or Gaharu” after the accumulation of oleoresin. The sweet smelling formed of oleoresins concentrates within the phloem part of the wood. The amount of deposited oleoresin within the wood affects the colour intensity of the infected wood (Rao & Dayal, 1992). Accumulation of resin in the wood over time makes it darker (Liu et al., 2013).

In this research, it was observed that the healthy *Aquilaria* stem changed to whitish yellow by negative control (sterile distilled water). López-Sampson & Page (2018) state that the resin production accompanied with the changes of colour in internal tissues of injured trees can occur in a year. It shows that the abiotic factors in this study where the *Aquilaria* tree was injured and inoculate with negative control instead of fungal inoculant, may induce agarwood but it's a time-consuming process for a goal achieved. Hence, a combination of biotic and abiotic factors has been done in this study in agreement with the statement by Santoso et al. (2011) that biotic and abiotic factors can induce agarwood formation. However, further confirmation by GC-MS analysis was needed in terms of the agarwood quality formed by fungal inoculants and negative controls.

Besides, the mechanically wounded of a tree stem can only allow the formation of agarwood around the edge of an infected area. Thus, the drilled wound need to further introduce by chemicals or microbes that can kill the tree cells for the larger formation of resin (Blanchette & Heuveling, 2002). This statement can be approved based on the current

study as the mean value of the infected zone length in negative control was less compared to fungal inoculants as the mean values were higher when fungi were used as bioinoculant to induce resin in sample trees. In addition, the pathogenic fungus was the main organism that weakens the tree which leads to the formation of resin inside the stem of the tree (Mohamed et al., 2010). It had been suggested that the colonization and propagation of endophytes might benefit their host plants by promoting the growth of the plant and reducing symptoms of the disease that trigger by the microbial attack or environmental stress (Strobel et al., 2004).

Based on the quantitative analysis of the infected zone length, it can deduce that the fungal inoculant and negative control were significantly different with each other in terms of formation of agarwood within three months and six months inoculation period in both seasons (wet and dry). The mean value of the infected zone length increased in the sixth month after inoculation showed that the length of the infected zone was significantly affected by the fungi inoculants.

In this present study, environmental factors do affect the infected zone length of fungal inoculants and negative control as inoculation periods take place within different seasons which in wet and dry seasons. Ng et al. (1997) have mentioned that factors such as tree age, genetic background, seasonal and environmental variation may also be important in agarwood formation. The rainfall pattern in Malaysia was determined by the pattern of seasonal wind with properties of local topographic (Malaysian Meteorological Department, n.d.). Based on the Malaysian Meteorological Department, northeast monsoon (wet season) in Sarawak were usually from December to March whereas for southeast monsoon (dry season) began from Jun to August. Moreover, November to January was described as most

wet months meanwhile rainfall start to decrease in March as April to September were dry months in Sarawak (Responsible Travel, n.d.).

The inoculation process in this study was done twice. The first inoculation process has been done in November which was near the beginning of the wet season and secondly in March which was the end of the wet season and near begins of the dry season. Thus, the difference in the seasonal inoculation period may have affected the agarwood production. Based on the statistical analysis on different seasons (wet and dry seasons), it can be determined that there were significant differences between wet and dry seasons as the mean value of infected zone in dry seasons were higher compare to wet seasons even among fungal inoculants and negative control (sterile distilled water). Based on T-test results, dry seasons were chosen as suitable seasons for inoculations to be done. Besides, the current study of wet season results concurred with the previous study by Santoso et al. (2011) which shows that the agarwood production may decelerate due to the humidity of location even by inoculating with *Fusarium* spp.. Based on observation, it can be deduced agarwood inducement by fungal inoculants during the wet season was not efficient compared to the dry season. Although the equal volume of 1000 mL fungi inoculant has been injected into an *Aquilaria* tree in both wet and dry seasons, the mechanism of fungal inoculants may not be distributed well within the cells of the tree due to low transpiration rate during the inoculation period in the wet season.

All the fungal inoculants used in this study were found effective in inducing the *Aquilaria* trees by the existence of an infected zone in the stem of the trees. After three months of inoculation periods, early symptoms of agarwood formation can be observed based on the infected zone. Moreover, each of the fungi inoculants gave different results

towards the formation of agarwood due to the different levels of virulence and compatibility with the host tree. Hence, it was crucial to consider the compatibility of fungal strain with the host tree in the agarwood induction process (Santoso et al., 2011).

F3 inoculants that have been formulated in this study was selected as the best fungal inoculant compared to others due to its positive result toward the resin formation in *Aquilaria* tree as this inoculant able to produce the dark colour of infected zone which specifies induction of resin occurred in both wet and dry seasons. This showed that the combination of *T. harzianum*, *L. theobromae*, and *C. affinis* was matched with the host tree. Besides, endophytic fungi include *Curvularia* and *L. theobromae* were found in the resinous plant part of *A. malaccensis* (Mohamed et al., 2010; Premalatha & Kalra, 2013). In addition, all the fungi that have been used in this study originated isolated from agarwood (Sulang, 2017). The incompatibility of fungal strain with the host tree may affects the agarwood formation. Semangun (2001) stated that the infection take place when there was compatibility between pathogens with host tree. Thus, it was believed that these fungi have potential in resin formation.

Based on the result in wet and dry seasons, the F2 inoculants (a combination of *F. solani* and *P. citrinum*) showed that the colour level of infected zoned starts to decreased from three to six months inoculation periods. This result may be affected by the type of fungal in F2 inoculant. Even though *Fusarium* sp. and *Lasiodiplodia* sp. were found abundantly in resinous wood as stated by Mohamed et al. (2010), in this research, *Fusarium* was combined with *Penicillium* which has antimicrobial properties against host tree. Hence, the result may differ due to the antimicrobial property of the fungi inoculants (Louw, 2014). Citrinin was mainly produced by *P. citrinum* and known to have effective

antibacterial properties (Stoev et al., 2010; Louw, 2014) that can disrupt the development of endophytes in *Aquilaria* tree. Eventually, the metabolic reaction between fungal inoculants with the host tree was affected. It has been stated that *P. citrinum* have antifungal activities against plant pathogens which affect endophytes activities within *Aquilaria* trees (Wakana et al., 2006; Fatma et al., 2010). In this study, F2 inoculant might secrete its antifungal properties of *P. citrinum* in the *Aquilaria* tree. Thus, the results show a decreased colour level of the infected zone for three months and six months inoculation period.

Local tree environment and wound microclimate factors may affect the succession pattern of the fungal population (Mohamed et al., 2014). Similarly, previous studies have been stated on artificial infection done by *Aspergillus* sp., *Lasiodiplodia* sp., *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp. (Yunita, 2009; Akter et al., 2013). Hence, fungi virulence, inoculation technique, and ecological conditions particularly climate conditions are significant factors that can impact the process of agarwood formation artificially.

Thus, the objective of determining the effect of the formulated fungi inoculants on the formation of resin in the *Aquilaria* tree by bottle drip method was achieved. Then, the colour of infection zone between different seasons of inoculation periods was observed and significant difference in terms of the statistical analysis for third and sixth months between different seasons has been analysed by using SPSS software and concluded.

#### 5.4 Chemical compound found in inoculated trees

From the GC-MS results, it best to state that dry season inoculations have better agarwood formation due to better results of metabolic reaction between fungal with plant defence system as dry season produced slightly more number of chemical compounds compared to wet season inoculations (82>79 chemical compounds). The chemical compounds listed in Table 4.7 were chosen by comparing with chemical compounds that were previously detected in induced agarwood and wild agarwood. Five group of agarwood compounds were identified in this study which includes sesquiterpenes, fatty acid, fatty acid methyl esters, sterols groups, and aromatic compounds. Sesquiterpenes are common agarwood compound that can be found in *A. sinensis*, *A. malaccensis*, *A. subintegra*, and *A. crassna* (Gao et al., 2019).

From the GC-MS results in both wet and dry inoculation season, the chemical compounds in this study contained major compounds presence in agarwood such as benzaldehyde, 4-hydroxy-3,5-dimethoxy, 4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl (Palustrol), and guaia-1(10),11-diene which has been reported by Jong et al. (2014). It was revealed that these major chemical compounds were found mostly in inoculated trees compared to negative control (sterile distilled water). Agarwood formation could be further influenced when fungal diversity was directly affected by volatile compounds of agarwood (Liu et al., 2019). Hence, this finding shows the fungal inoculation method used are more success in inducing agarwood in *Aquilaria* trees due to greater amount of major compound of agarwood in the fungal inoculated trees.

It was found that guaia-1(10),11-diene only presence in F3 inoculated tree after six months inoculation in the dry season. Current study was concurrence with previous study

by Ahmaed and Kulkarni (2017) that chemical compound guaia-1(10),11-diene was presented in the analysis of chemical constituents in Malaysian high qualities of agarwood oil. Hence, F3 inoculated tree produce good results of agarwood formation due to presence of high quality agarwood compound, guaia-1(10),11-diene. In addition, there were no furanone compounds detected in inoculated trees in this study. Therefore, the absence of furanone compounds in the current study concurred with the previous study of Jong et al. (2014) which stated that furanone compounds can only be detected in a healthy *Aquilaria* tree.

In this study, cryptomeridiol were not found in previous studies relates to chemical constituents of agarwood. A study by Junwei et al. (2018), stated that cryptomeridiol were natural products found in rosewood: *Pterocarpus cambodianus* as results from GC-MS analysis. Hence, it can be deduced that cryptomeridiol was a new compound found in inoculated agarwood and can only be detected in F3 inoculated trees after six months inoculation in the dry season. Besides, cryptomeridiol was a eudesmane sesquiterpenoid that known to have a significant medicinal value as anti-Alzheimer disease and antispasmodic characteristics (Tebbaa et al., 2011).

In this study, n-hexadecanoic acid was the only chemical compound present for each inoculant and negative control in a wet season whereas total absence in the dry season. n-hexadecanoic acid was the most common saturated long chain fatty acid that functions as 5-alpha reductase inhibitor, flavouring agents, an antioxidant, lubricant, hemolytic, antiandrogenic, nematocidal, pesticidal and hypocholesterolemic effect (Rajeswari et al., 2013).

The fungal inoculants used in this study especially F3 inoculants were strongly approved as agarwood inducer due to the production of chemical constituents that similar in super A grade agarwood chip (Jong et al., 2014). Those chemical compounds alloaromadendrene oxide-(2) and aromadendrene oxide-(1) that were detected in F3 inoculated tree in dry season inoculations.

Besides that, some identified aromatic compounds in the current study were known as constituents in agarwood essential oil that has been commercially used in the fragrance industry due to their aromatic characteristics. The finding of the aromatic compound includes cubenol and cis-z- $\alpha$ -bisabolene epoxide in F3 inoculated trees agree with Chen et al. (2011) although different artificial methods were used to induce the formation of agarwood. Based on the previous study by Chen et al. (2011), the chemical method was used to induce the agarwood formation and cubenol was found with a great amount in the essential oils which contributes to its smell and state. Hence, cubenol was used for reviewing the quality of agarwood as a reference (Chen et al., 2011).

In this study, there was a single naphthalene compound 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1 $\alpha$ ,7 $\beta$ ,8a. $\alpha$ )]-. Based on previous study by Bhuiyan (2009), Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1 $\alpha$ ,7 $\beta$ ,8a. $\alpha$ )]- was identified in healthy and artificially screw *Aquilaria agallocha* which is also present in this current studies of inoculated trees and negative control (sterile distilled water) except for F1 trees in both wet and dry inoculation periods.

The tree extractives of the resin contained aromatic terpenes. In agarwood, sesquiterpenes, and 2-(2-phenylethyl) chromone derivatives are the main active compound

(Chen et al., 2011; Naef, 2011). Hoque et al. (2019) mentioned that sesquiterpenes are less volatile compared to other terpenes as it contributes to fragrance (essence) of plants which also has greater potential for stronger odours too.

Based on the result in wet and dry season inoculation periods, it was determined that sesquiterpenes include 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-one, 2-Phenethyl-4H-chromen-4-one, 6-Methoxy-2-phenethyl-4H-chromen-4-one, and 2-(4-Methoxyphenethyl)-4H-chromen-4-one were mainly presented in all inoculated trees and some also presented in the negative control. Hence, this finding showed positive evidence that these inoculants have induced the agarwood formation in *the Aquilaria* tree.

After sixth month inoculation in the dry season, results of GC-MS analysis detect chemical compounds that possess fragrance properties such as crytomeridiol, 3',5'-dimethoxyacetophenone, guaia-1(10), 11-diene, octacosanol and heptadecanolide only present in F3 inoculated trees. Based on the previous study, guaia-1(10), 11-diene were detected in the agarwood chipwood which contribute to the release of aromatic smell during burning (Ismail et al., 2016). These aromatic compounds were revealed absent in other inoculants and negative control (sterile distilled water) in the both wet and dry season of inoculation periods. Hence, the presence of these chemical compounds might contribute to the distinct smell of agarwood inoculated by F3 compare to other inoculants. High quality of agarwood would have a strong and sweet aroma emitted during burning (Ismail et al., 2016).

Agarospinol, sesquiterpene alcohol was only found in the F1 inoculated tree after six months inoculation period in the dry season. Presence of agarospinol in this study shown that F1 inoculant able to induce agarwood as the previous study by Varma et al. (1965)

reported that agarospirol has been found from infected agarwood essential oil. However, steroid compounds in this study include stigmasterol, and  $\gamma$ -sitosterol were detected in inoculated trees and negative control (sterile distilled water).

In this study, there were some similarities in terms of chemical compounds between extracts of the negative control (sterile distilled water) with extracts of other inoculants. The physically wounding of the *Aquilaria* tree in this study may trigger agarwood formation which eventually produces agarwood chemical constituents even without inoculated with fungal inoculant. However, this method requires a longer time of agarwood formation with uncertain qualities in which concurrence to this study that negative control trees have lesser quality and quantity of major agarwood compound (Rasool & Mohamed, 2016; Wu et al., 2017).

From the inoculated plant, a wide variety of chemical constituents including monoterpene, sesquiterpenes, and phenylpropanoids have been found. Most identified chemical compounds in this study were known to have medicinal properties. For instance, Stigmasterol and  $\gamma$ -Sitosterol were known as plant steroid that has antioxidant, anti-inflammatory and antibacterial properties (Gabay et al., 2010).

A wide variety of bioactivities was exhibited by the extracts of *Aquilaria malaccensis* in recent studies which include the antimelanogenesis activities, antimicrobial, antibiofilm, anti-inflammatory, antivector, insect repellent, antidiabetic, and antifertility (Wang et al., 2018). Hence, agarwood not only function as an essential material for the fragrance production but also valuable in terms of agriculture and medicinal sector.

Based on the finding in this study, it can be observed that the long inoculation period by fungal inoculation method shown to have positive effects in inducing agarwood

formation over time and season. It can be detected after six months inoculation periods, more chemical compound includes sesquiterpenes and aromatic compounds were produced compared to the third month inoculation period in both wet and dry season. This result was concurrence with Liao et al. (2018) that longer inoculation time results in better production of sesquiterpenes and aromatic compounds in agarwood.

In agarwood, benzylacetone was stated as a major compound (Naef, 2011). However, benzylacetone was absent in all samples in this study. Even though the extraction method used were methanol extracts according to Jong et al. (2014) research but benzylacetone were still absent in both wet and dry inoculation season. Therefore, it can be deduced that the genetic factor or natural variant and environment factor may cause the presence and absence of chemical constituents (Saiful & Mashitah, 2010). The samples in this study were from Sarawak and involve two different seasons of inoculation which was wet and dry. Meanwhile, the previous samples were from Peninsular Malaysia (Jong et al., 2014). Hence, this indicated that the agarwood chemical compounds from this study might be slightly different from the previous study of agarwood.

Adams et al. (2016) discovered that the agarwood chemical compounds are the products formed by the fungi that infected the cell wall of the host, including lignin and starch grains. Agar formation has been studied by the researcher on the relations of mould and decay fungi within the agar zones (Bhattacharyya et al., 1952; Tamuli et al., 2000).

In this research, fungi act as the elicitors that stimulate and activate the signal that allows the production of secondary metabolites to be expressed by genes involved (Zhao et al., 2005). The formations of agarwood eventually lead to the production of secondary metabolite. Secondary metabolites function as recreational drugs, flavouring, pigments and

medicines (Craney et al., 2013). Agarwood resin was considered as plant secondary metabolites as a response toward the infection or stress on the *Aquilaria* trees (Tan et al., 2019).

Based on Bhuiyan (2009), the existence of a specific host-pathogen relationship may relate to artificial inoculation results. Thus, the specific host might produce a positive result based on the artificial supplement of inoculum in inducing plant disease. Hence, this statement may relate to the current study that certain chemical compounds can only be detected in a specific inoculated tree. For instance, compound  $\alpha$ -amyrin and  $\beta$ -amyrin were only found in F1 inoculants after three months inoculation in the wet season. Triterpene alcohol,  $\beta$ -amyrin were previously isolated from *Aspergillus nidulans* (Gealt, 1983). Besides that, *Aspergillus* sp. were isolated from various host plant tissues as endophytic fungi (Al-Fakih, 2019). Thus, this factor might contribute to the existence of  $\beta$ -amyrin in the F1 inoculated tree as F1 inoculant consist of *Aspergillus* species.

Furthermore, Ibrahim et al. (2015) stated that stigmast-4-ene-3-one, a steroid compound was also derived from *Aspergillus* species. It can be observed in this study that after three months inoculation in the wet season, stigmast-4-ene-3-one was detected in F1 inoculated tree whereby F1 inoculant consist of *Aspergillus* species. Stigmast-4-ene-3 one was known as a steroid that has antimicrobial, anti-inflammatory, anticancer and hepatoprotective properties. Hence, the fungal inoculants in this study were able to induce agarwood in the *Aquilaria* tree due to the existence of chemical constituents that has a similar chemical profile of induced agarwood and wild agarwood.

The artificial fungal inoculants used in this study were all able to artificially induce agarwood. This study concurred with the previous study by Naef (2011). Agarwood in F3

inoculated trees contained more major constituents of agarwood such as sesquiterpenes, chromones, fatty acid, and aromatic compounds compared to other inoculants after every three months and six months inoculation in both wet and dry seasons.

The presence of these major compounds causes agarwood oil to be distinctive from other oils. Hence, this suggested that the agarwood in F3 might be more superior than others. Although major compounds were also present in other inoculants, the distinct fragrance and colour of the agarwood formed were considered. In this study, F3 inoculated tree has the most distinct smell and darker colour of agarwood formation compare to other inoculated trees. Therefore, it can be suggested that F3 inoculant was recommended as the highest potential inoculants to be used for artificial agarwood inducer due to the greater presence of major compounds with previous chemical compounds found in natural agarwood and induced agarwood.

In this chapter, the presence of bioactive secondary metabolites which also known as the chemical compounds in each inoculated agarwood that induced by a different type of fungal inoculant has proven the interaction of endophytic fungi associated with agarwood formation. A study by Akhsan et al. (2015) stated that factors such as season, genera, species, trees within species, part (trunk, branch, root) and external factors (biotic and abiotic) might influence the secondary metabolites in the plant. In this study, the inoculation season (wet and dry) and external factor (inoculant type) may be related to the presence of chemical constituents in inoculated trees.

## CHAPTER 6

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

As a conclusion, all objectives of this study were achieved. The objectives on develop a new formulation of microbial consortia as agarwood inducer was achieved when a total of three microbial consortia have been successfully formulated which includes Formulation 1 (F1) consists of the combination of *Trichoderma* sp. and *Aspergillus* sp., Formulation 2 (F2) consists of the combination of *Fusarium* sp. and *Penicillium* sp., whereas Formulation 3 (F3) consists of the combination of *Trichoderma* sp., *Lasiodiplodia* sp. and *Curvularia* sp.. Then, the selected formulated microbial consortium was served as fungi inoculants for inoculation of the *Aquilaria* tree stem. The negative control used in this study were sterile distilled water to compare results with fungal inoculants.

After three months and six months inoculation periods in each wet and dry season, the quantitative and qualitative analysis of inoculated trees was done by observation and measurement on the colour and length of the infected zone. Based on the morphological observation on the colour of the inoculated zone, it can be found that initial colour of inoculated *Aquilaria* tree gradually changed from white to darker colour within three months and the infected zone becomes more developed after six months inoculation. Thus, the objectives to determine the effects of these selected microbial consortia toward agarwood formation were achieved.

In the meantime, different seasons of inoculations were also considered in this study. In the dry season, the transpiration rate of plants is higher compared to the wet season. This factor might cause the fungal inoculant distributed well throughout the inoculated tree

during the dry season. Thus, the agarwood formation in the dry season was more effective compared to the wet season based on the colour and length of the inoculated zone after three months and six months inoculation period in each season. Besides, dry season has slightly more agarwood compound compare to wet seasons. The objective to evaluate the outcomes of analysis between different seasons of inoculation periods were achieved as dry seasons were more suitable for inoculations to be done.

In this study, fungal inoculants have shown their capability in production agarwood chemical constituents includes sesquiterpenes, chromone derivatives, steroid compounds, fatty acid methyl ester, and aromatic compounds. For instance, 2-Phenethyl-4H-chromen-4-one, guaia-1(10),11-diene, aromadendrene oxide-(1), alloaromadendrene oxide-(2) and n-hexadecanoic, methyl ester was identified in GC-MS analysis of inoculated *Aquilaria* trees. All these chemical compounds were responsible for good grade quality agarwood oil as mentioned in previous literature. Thus, the objective to identify the chemical constituents in the inoculated agarwood were achieved. A new sesquiterpene compound known as cryptomeridiol was found in F3 inoculated *Aquilaria* trees after six months inoculation in the dry season. The findings suggested that F3 inoculant that contains *Trichoderma* sp., *Lasiodiplodia* sp., and *Curvularia* sp. as the most potential fungal inoculant toward agarwood formation in *Aquilaria* tree. In every three months and six months inoculation periods, F3 inoculant has produced the darkest colour and largest mean length of the infected zone in *Aquilaria* trees for both wet and dry seasons.

## 6.2 Recommendations

It is recommended to do more research on the formulation of microbial consortia for better inoculants to have a better yield of agarwood by artificial infection. More studies should focus on the roles of endophytes in the mechanism of agarwood formation. A longer inoculation period and a large replication of agarwood inoculants are required for better knowledge on fungus effect with the production of secondary metabolites in *Aquilaria* trees for agarwood formation.

The bottle drip method used in this study gave a successful outcome. In each third and sixth months of inoculation, the process of fungal inoculants was seen to be spread well throughout the tree. Thus, the bottle drip method is highly recommended as it involved physical and biological methods to induce agarwood artificially. Bottle drip method allows the fungal inoculant to fully spread and diffused inside the tree. Furthermore, further study on environmental factors such as inoculation seasons in agarwood formation was recommended for better knowledge.

Lastly, F3 inoculant is recommended to further commercialized as agarwood inducer due to the good quality and quantity of agarwood formation. However, further examination of fungal inoculant was required to have more efficient development of resinous agarwood.

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## APPENDICES

**Appendix A:** The matrix method of the fungal isolates growth ratio after 7 days in formulated microbial consortium are recorded accordingly.

Table A.1 Formulation for 2 different species (Ratio 1:1)

| Fungal species | G3-B2 | G4-B3 | G4-B2 | G2-A2 | GKS 2 | GKS 3 | GB 4 |
|----------------|-------|-------|-------|-------|-------|-------|------|
| G3-B2          | X     | -     | -     | -     | -     | -     | -    |
| G4-B3          | -     | X     | 1:1   | -     | -     | -     | -    |
| G4-B2          | -     | -     | X     | -     | -     | -     | -    |
| G2-A2          | -     | -     | -     | X     | -     | -     | -    |
| GKS 2          | -     | -     | -     | -     | X     | -     | -    |
| GKS 3          | -     | -     | -     | -     | -     | X     | -    |
| GB 4           | -     | -     | -     | -     | 1.1   | -     | X    |

“-“ indicates unequal growth ratio, “X” indicates same fungal species

Table A.2 Formulation for 3 different species (Ratio 1:1:1 or 2:1)

| Fungal species   | G3-B2 | G4-B3 | G4-B2 | G2-A2 | GKS 2 | GKS 3 | GB 4 |
|------------------|-------|-------|-------|-------|-------|-------|------|
| G3-B2 +<br>G4-B3 | X     | X     | -     | -     | -     | -     | -    |
| G3-B2 +<br>G4-B2 | X     | -     | X     | -     | -     | -     | -    |
| G3-B2 +<br>G2-A2 | X     | -     | -     | X     | -     | 1:1:1 | -    |
| G3-B2 +<br>GKS 2 | X     | -     | -     | -     | X     | -     | -    |
| G3-B2 +<br>GKS 3 | X     | -     | -     | -     | -     | X     | -    |
| G3-B2 +<br>GB 4  | X     | -     | -     | -     | -     | -     | X    |

“-“ indicates unequal growth ratio, “X” indicates same fungal species

**Appendix B:** Raw Data of Growth Rate of Fungal Microbial Consortium Within 7 Days

Table B The diameter (cm) of mycelia in plate of each fungal species in microbial consortium are measured accordingly.

| Microbial consortium | Fungal Isolate | Day 1                | Day 2               | Day 3                       | Day 4  | Day 5  | Day 6  | Day 7  |
|----------------------|----------------|----------------------|---------------------|-----------------------------|--------|--------|--------|--------|
| F1                   | G4-B2          | 1.3 cm               | 2.1 cm              | 3.0 cm                      | 3.5 cm | -      | -      | -      |
|                      | G4-B3          | 2.5 cm               | 3.5 cm              | 4.0 cm                      | -      | -      | -      | -      |
| F2                   | GKS 2          | Mycelia              | 1.2 cm              | 2.2 cm                      | 3.1 cm | 3.5 cm | -      | -      |
|                      | GB 4           | Only a colony formed | A few colonies grow | Colony fully grow in 4.0 cm | -      | -      | -      | -      |
| F3                   | G2-A2          | 1.0 cm               | 3.5 cm              | 4.0 cm                      | -      | -      | -      | -      |
|                      | GKS 3          | 2.0 cm               | 3.0 cm              | 3.5 cm                      | -      | -      | -      | -      |
|                      | G3-B2          | 1.5 cm               | 2.2 cm              | 3.0 cm                      | 3.0 cm | 3.2 cm | 3.2 cm | 3.5 cm |

‘-’ indicates no more growth detected.

### Appendix C: Perimeter of *Aquilaria* Stem Trees

Table C Mean value for perimeter of *Aquilaria* stem trees in Serian Agarwood Farm.

| Fungi inoculant tree | Replication, R | Perimeter (cm) | Mean  | Standard deviation | Standard error ( $\pm$ ) |
|----------------------|----------------|----------------|-------|--------------------|--------------------------|
| F1                   | R1             | 61             | 52.33 | 9.02               | 5.21                     |
|                      | R2             | 43             |       |                    |                          |
|                      | R3             | 53             |       |                    |                          |
| F2                   | R1             | 56             | 60    | 3.61               | 2.08                     |
|                      | R2             | 61             |       |                    |                          |
|                      | R3             | 63             |       |                    |                          |
| F3                   | R1             | 46             | 48.33 | 2.52               | 1.45                     |
|                      | R2             | 51             |       |                    |                          |
|                      | R3             | 48             |       |                    |                          |
| Negative control     | R1             | 54             | 53    | 2.65               | 1.53                     |
|                      | R2             | 50             |       |                    |                          |
|                      | R3             | 55             |       |                    |                          |

### Appendix D: Raw Data for Infected Longitudinal Measurement

Table D.1 Longitudinal measurement of infected zone on January 2018

| Fungi inoculant tree | Replication, R | Length (L)/ cm | Mean | Standard deviation |
|----------------------|----------------|----------------|------|--------------------|
| F1                   | R1             | 6.0            | 6.2  | 0.25               |
|                      | R2             | 6.5            |      |                    |
|                      | R3             | 6.2            |      |                    |
| F2                   | R1             | 6.0            | 5.8  | 0.44               |
|                      | R2             | 6.1            |      |                    |
|                      | R3             | 5.3            |      |                    |
| F3                   | R1             | 5.0            | 6.3  | 1.14               |
|                      | R2             | 7.1            |      |                    |
|                      | R3             | 6.8            |      |                    |
| Negative control     | R1             | 1.0            | 1.3  | 0.29               |
|                      | R2             | 1.5            |      |                    |
|                      | R3             | 1.5            |      |                    |

Table D.2 Longitudinal measurement of infected zone on April 2018

| Fungi inoculant tree | Replication, R | Length (L)/ cm | Mean | Standard deviation |
|----------------------|----------------|----------------|------|--------------------|
| F1                   | R1             | 7.5            | 7.6  | 0.40               |
|                      | R2             | 8.0            |      |                    |
|                      | R3             | 7.2            |      |                    |
| F2                   | R1             | 7.0            | 7.1  | 0.15               |
|                      | R2             | 7.3            |      |                    |
|                      | R3             | 7.1            |      |                    |
| F3                   | R1             | 7.5            | 7.8  | 0.25               |
|                      | R2             | 8.0            |      |                    |
|                      | R3             | 7.8            |      |                    |
| Negative control     | R1             | 2.5            | 2.3  | 0.29               |
|                      | R2             | 2.5            |      |                    |
|                      | R3             | 2.0            |      |                    |

Table D.3 Longitudinal measurement of infected zone on June 2018

| Fungi inoculant tree | Replication, R | Length (L)/ cm | Mean | Standard deviation |
|----------------------|----------------|----------------|------|--------------------|
| F1                   | R1             | 11.0           | 10.9 | 0.36               |
|                      | R2             | 10.5           |      |                    |
|                      | R3             | 11.2           |      |                    |
| F2                   | R1             | 10.0           | 9.5  | 0.47               |
|                      | R2             | 9.1            |      |                    |
|                      | R3             | 9.3            |      |                    |
| F3                   | R1             | 12.0           | 12.1 | 0.36               |
|                      | R2             | 12.5           |      |                    |
|                      | R3             | 11.8           |      |                    |
| Negative control     | R1             | 2.0            | 1.7  | 0.25               |
|                      | R2             | 1.5            |      |                    |
|                      | R3             | 1.7            |      |                    |

Table D.4 Longitudinal measurement of infected zone on September 2018

| Fungi inoculant tree | Replication, R | Length (L)/ cm | Mean | Standard deviation |
|----------------------|----------------|----------------|------|--------------------|
| F1                   | R1             | 13.5           | 13.9 | 0.36               |
|                      | R2             | 14.2           |      |                    |
|                      | R3             | 14.0           |      |                    |
| F2                   | R1             | 11.0           | 10.5 | 0.47               |
|                      | R2             | 10.3           |      |                    |
|                      | R3             | 10.1           |      |                    |
| F3                   | R1             | 15.0           | 14.4 | 0.60               |
|                      | R2             | 14.5           |      |                    |
|                      | R3             | 13.8           |      |                    |
| Negative control     | R1             | 3.0            | 2.7  | 0.25               |
|                      | R2             | 2.7            |      |                    |
|                      | R3             | 2.5            |      |                    |

## Appendix E: Data Analysis

### E.1 Data Analysis for January Sampling (3 months inoculation period in wet season)

#### ANOVA

Length

|                           | Sum of Squares | df | Mean Square | F      | Sig. |
|---------------------------|----------------|----|-------------|--------|------|
| Between Groups (Combined) | 51.803         | 3  | 17.268      | 42.462 | .000 |
| Linear Term Contrast      | 30.246         | 1  | 30.246      | 74.375 | .000 |
| Deviation                 | 21.557         | 2  | 10.779      | 26.505 | .000 |
| Within Groups             | 3.253          | 8  | .407        |        |      |
| Total                     | 55.057         | 11 |             |        |      |

### Multiple Comparisons

Dependent Variable: Length

Tukey HSD

| (I) Formulation | (J) Formulation | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|-----------------|-----------------|-----------------------|------------|------|-------------------------|-------------|
|                 |                 |                       |            |      | Lower Bound             | Upper Bound |
| 1               | 2               | .43333                | .52068     | .838 | -1.2341                 | 2.1007      |
|                 | 3               | -.06667               | .52068     | .999 | -1.7341                 | 1.6007      |
|                 | 4               | 4.90000*              | .52068     | .000 | 3.2326                  | 6.5674      |
| 2               | 1               | -.43333               | .52068     | .838 | -2.1007                 | 1.2341      |
|                 | 3               | -.50000               | .52068     | .775 | -2.1674                 | 1.1674      |
|                 | 4               | 4.46667*              | .52068     | .000 | 2.7993                  | 6.1341      |
| 3               | 1               | .06667                | .52068     | .999 | -1.6007                 | 1.7341      |
|                 | 2               | .50000                | .52068     | .775 | -1.1674                 | 2.1674      |
|                 | 4               | 4.96667*              | .52068     | .000 | 3.2993                  | 6.6341      |
| 4               | 1               | -4.90000*             | .52068     | .000 | -6.5674                 | -3.2326     |
|                 | 2               | -4.46667*             | .52068     | .000 | -6.1341                 | -2.7993     |
|                 | 3               | -4.96667*             | .52068     | .000 | -6.6341                 | -3.2993     |

### E.2 Data Analysis for April Sampling (6 months inoculation period in wet season)

### ANOVA

Length

|                           | Sum of Squares | df | Mean Square | F       | Sig. |
|---------------------------|----------------|----|-------------|---------|------|
| Between Groups (Combined) | 60.433         | 3  | 20.144      | 241.733 | .000 |
| Linear Term Contrast      | 34.051         | 1  | 34.051      | 408.608 | .000 |
| Deviation                 | 26.383         | 2  | 13.191      | 158.296 | .000 |
| Within Groups             | .667           | 8  | .083        |         |      |
| Total                     | 61.100         | 11 |             |         |      |

### Multiple Comparisons

Dependent Variable: Length

Tukey HSD

| (I) Formulation | (J) Formulation | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|-----------------|-----------------|-----------------------|------------|------|-------------------------|-------------|
|                 |                 |                       |            |      | Lower Bound             | Upper Bound |
| 1               | 2               | .4333                 | .2357      | .324 | -.321                   | 1.188       |
|                 | 3               | -.2000                | .2357      | .830 | -.955                   | .555        |
|                 | 4               | 5.2333*               | .2357      | .000 | 4.479                   | 5.988       |
| 2               | 1               | -.4333                | .2357      | .324 | -1.188                  | .321        |
|                 | 3               | -.6333                | .2357      | .103 | -1.388                  | .121        |
|                 | 4               | 4.8000*               | .2357      | .000 | 4.045                   | 5.555       |
| 3               | 1               | .2000                 | .2357      | .830 | -.555                   | .955        |
|                 | 2               | .6333                 | .2357      | .103 | -.121                   | 1.388       |
|                 | 4               | 5.4333*               | .2357      | .000 | 4.679                   | 6.188       |
| 4               | 1               | -5.2333*              | .2357      | .000 | -5.988                  | -4.479      |
|                 | 2               | -4.8000*              | .2357      | .000 | -5.555                  | -4.045      |
|                 | 3               | -5.4333*              | .2357      | .000 | -6.188                  | -4.679      |

### E.3 Data Analysis for Mei Sampling (3 months inoculation period in dry season)

#### ANOVA

Length

|                           | Sum of Squares | df | Mean Square | F       | Sig. |
|---------------------------|----------------|----|-------------|---------|------|
| Between Groups (Combined) | 196.297        | 3  | 65.432      | 478.772 | .000 |
| Linear Term Contrast      | 92.753         | 1  | 92.753      | 678.678 | .000 |
| Deviation                 | 103.544        | 2  | 51.772      | 378.820 | .000 |
| Within Groups             | 1.093          | 8  | .137        |         |      |
| Total                     | 197.390        | 11 |             |         |      |

### Multiple Comparisons

Dependent Variable: Length

Tukey HSD

| (I) Formulation | (J) Formulation | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|-----------------|-----------------|-----------------------|------------|------|-------------------------|-------------|
|                 |                 |                       |            |      | Lower Bound             | Upper Bound |
| 1               | 2               | 1.4333 <sup>*</sup>   | .3018      | .006 | .467                    | 2.400       |
|                 | 3               | -1.2000 <sup>*</sup>  | .3018      | .017 | -2.167                  | -.233       |
|                 | 4               | 9.1667 <sup>*</sup>   | .3018      | .000 | 8.200                   | 10.133      |
| 2               | 1               | -1.4333 <sup>*</sup>  | .3018      | .006 | -2.400                  | -.467       |
|                 | 3               | -2.6333 <sup>*</sup>  | .3018      | .000 | -3.600                  | -1.667      |
|                 | 4               | 7.7333 <sup>*</sup>   | .3018      | .000 | 6.767                   | 8.700       |
| 3               | 1               | 1.2000 <sup>*</sup>   | .3018      | .017 | .233                    | 2.167       |
|                 | 2               | 2.6333 <sup>*</sup>   | .3018      | .000 | 1.667                   | 3.600       |
|                 | 4               | 10.3667 <sup>*</sup>  | .3018      | .000 | 9.400                   | 11.333      |
| 4               | 1               | -9.1667 <sup>*</sup>  | .3018      | .000 | -10.133                 | -8.200      |
|                 | 2               | -7.7333 <sup>*</sup>  | .3018      | .000 | -8.700                  | -6.767      |
|                 | 3               | -10.3667 <sup>*</sup> | .3018      | .000 | -11.333                 | -9.400      |

### E.4 Data Analysis for August Sampling (6 months inoculation period in dry season)

#### ANOVA

Length

|                           | Sum of Squares | df | Mean Square | F       | Sig. |
|---------------------------|----------------|----|-------------|---------|------|
| Between Groups (Combined) | 261.897        | 3  | 87.299      | 447.687 | .000 |
| Linear Term Contrast      | 130.833        | 1  | 130.833     | 670.937 | .000 |
| Deviation                 | 131.064        | 2  | 65.532      | 336.062 | .000 |
| Within Groups             | 1.560          | 8  | .195        |         |      |
| Total                     | 263.457        | 11 |             |         |      |

### Multiple Comparisons

Dependent Variable: Length

Tukey HSD

| (I) Formulation | (J) Formulation | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|-----------------|-----------------|-----------------------|------------|------|-------------------------|-------------|
|                 |                 |                       |            |      | Lower Bound             | Upper Bound |
| 1               | 2               | 3.4333*               | .3606      | .000 | 2.279                   | 4.588       |
|                 | 3               | -.5333                | .3606      | .491 | -1.688                  | .621        |
|                 | 4               | 11.1667*              | .3606      | .000 | 10.012                  | 12.321      |
| 2               | 1               | -3.4333*              | .3606      | .000 | -4.588                  | -2.279      |
|                 | 3               | -3.9667*              | .3606      | .000 | -5.121                  | -2.812      |
|                 | 4               | 7.7333*               | .3606      | .000 | 6.579                   | 8.888       |
| 3               | 1               | .5333                 | .3606      | .491 | -.621                   | 1.688       |
|                 | 2               | 3.9667*               | .3606      | .000 | 2.812                   | 5.121       |
|                 | 4               | 11.7000*              | .3606      | .000 | 10.545                  | 12.855      |
| 4               | 1               | -11.1667*             | .3606      | .000 | -12.321                 | -10.012     |
|                 | 2               | -7.7333*              | .3606      | .000 | -8.888                  | -6.579      |
|                 | 3               | -11.7000*             | .3606      | .000 | -12.855                 | -10.545     |

### E.5 Data Analysis for F1 (3 months inoculation period in wet and dry season)

#### Paired Samples Statistics

|            | Mean   | N | Std. Deviation | Std. Error Mean |
|------------|--------|---|----------------|-----------------|
| Pair 1 wet | 6.233  | 3 | .2517          | .1453           |
| dry        | 10.900 | 3 | .3606          | .2082           |

#### Paired Samples Test

| Paired Samples Test |           |                    |                |                 |                                           |         |         |    |                 |
|---------------------|-----------|--------------------|----------------|-----------------|-------------------------------------------|---------|---------|----|-----------------|
|                     |           | Paired Differences |                |                 |                                           |         | t       | df | Sig. (2-tailed) |
|                     |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |         |         |    |                 |
|                     |           |                    |                |                 | Lower                                     | Upper   |         |    |                 |
| Pair 1              | wet - dry | -4.6667            | .5774          | .3333           | -6.1009                                   | -3.2324 | -14.000 | 2  | .005            |

### E.6 Data Analysis for F1 (6 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean   | N | Std. Deviation | Std. Error Mean |
|--------|-----|--------|---|----------------|-----------------|
| Pair 1 | wet | 7.567  | 3 | .4041          | .2333           |
|        | dry | 13.900 | 3 | .3606          | .2082           |

**Paired Samples Test**

|        |           | Paired Differences |                |                 |                                           |         | t       | df | Sig. (2-tailed) |
|--------|-----------|--------------------|----------------|-----------------|-------------------------------------------|---------|---------|----|-----------------|
|        |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |         |         |    |                 |
|        |           |                    |                |                 | Lower                                     | Upper   |         |    |                 |
| Pair 1 | wet – dry | -6.3333            | .4163          | .2404           | -7.3676                                   | -5.2991 | -26.348 | 2  | .001            |

### E.7 Data Analysis for F2 (3 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean  | N | Std. Deviation | Std. Error Mean |
|--------|-----|-------|---|----------------|-----------------|
| Pair 1 | wet | 5.800 | 3 | .4359          | .2517           |
|        | dry | 9.467 | 3 | .4726          | .2728           |

**Paired Samples Test**

|        |           | Paired Differences |                |                 |                                           |         | t       | df | Sig. (2-tailed) |
|--------|-----------|--------------------|----------------|-----------------|-------------------------------------------|---------|---------|----|-----------------|
|        |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |         |         |    |                 |
|        |           |                    |                |                 | Lower                                     | Upper   |         |    |                 |
|        |           |                    |                |                 |                                           |         |         |    |                 |
| Pair 1 | wet - dry | -3.6667            | .5774          | .3333           | -5.1009                                   | -2.2324 | -11.000 | 2  | .008            |

### E.8 Data Analysis for F2 (6 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean   | N | Std. Deviation | Std. Error Mean |
|--------|-----|--------|---|----------------|-----------------|
| Pair 1 | wet | 7.133  | 3 | .1528          | .0882           |
|        | dry | 10.467 | 3 | .4726          | .2728           |

**Paired Samples Test**

|        |           | Paired Differences |                |                 |                                           | t       | df      | Sig. (2-tailed) |       |
|--------|-----------|--------------------|----------------|-----------------|-------------------------------------------|---------|---------|-----------------|-------|
|        |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |         |         |                 |       |
|        |           |                    |                |                 | Lower                                     |         |         |                 | Upper |
|        |           |                    |                |                 |                                           |         |         |                 |       |
| Pair 1 | wet - dry | -3.3333            | .5774          | .3333           | -4.7676                                   | -1.8991 | -10.000 | 2               | .010  |

### E.9 Data Analysis for F3 (3 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean   | N | Std. Deviation | Std. Error Mean |
|--------|-----|--------|---|----------------|-----------------|
| Pair 1 | wet | 6.300  | 3 | 1.1358         | .6557           |
|        | dry | 12.100 | 3 | .3606          | .2082           |

**Paired Samples Test**

|        |           | Paired Differences |                |                 |                                           | t       | df     | Sig. (2-tailed) |       |
|--------|-----------|--------------------|----------------|-----------------|-------------------------------------------|---------|--------|-----------------|-------|
|        |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |         |        |                 |       |
|        |           |                    |                |                 | Lower                                     |         |        |                 | Upper |
| Pair 1 | wet - dry | -5.8000            | 1.0583         | .6110           | -8.4290                                   | -3.1710 | -9.492 | 2               | .011  |

E.10 Data Analysis for F3 (6 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean   | N | Std. Deviation | Std. Error Mean |
|--------|-----|--------|---|----------------|-----------------|
| Pair 1 | wet | 7.767  | 3 | .2517          | .1453           |
|        | dry | 14.433 | 3 | .6028          | .3480           |

**Paired Samples Test**

|        |           | Paired Differences |                   |                    |                                              | t       | df      | Sig.<br>(2-<br>tailed) |       |
|--------|-----------|--------------------|-------------------|--------------------|----------------------------------------------|---------|---------|------------------------|-------|
|        |           | Mean               | Std.<br>Deviation | Std. Error<br>Mean | 95% Confidence Interval<br>of the Difference |         |         |                        |       |
|        |           |                    |                   |                    | Lower                                        |         |         |                        | Upper |
| Pair 1 | wet - dry | -6.6667            | .7638             | .4410              | -8.5640                                      | -4.7694 | -15.119 | 2                      | .004  |

E.11 Data Analysis for Negative control (3 months inoculation period in wet and dry season)

**Paired Samples Statistics**

|        |     | Mean  | N | Std. Deviation | Std. Error Mean |
|--------|-----|-------|---|----------------|-----------------|
| Pair 1 | wet | 1.333 | 3 | .2887          | .1667           |
|        | dry | 1.733 | 3 | .2517          | .1453           |

**Paired Samples Test**

|        |           | Paired Differences |                |                 |                                           |       | t      | df | Sig. (2-tailed) |
|--------|-----------|--------------------|----------------|-----------------|-------------------------------------------|-------|--------|----|-----------------|
|        |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |       |        |    |                 |
|        |           |                    |                |                 | Lower                                     | Upper |        |    |                 |
|        |           |                    |                |                 |                                           |       |        |    |                 |
| Pair 1 | wet - dry | -.4000             | .5292          | .3055           | -1.7145                                   | .9145 | -1.309 | 2  | .321            |

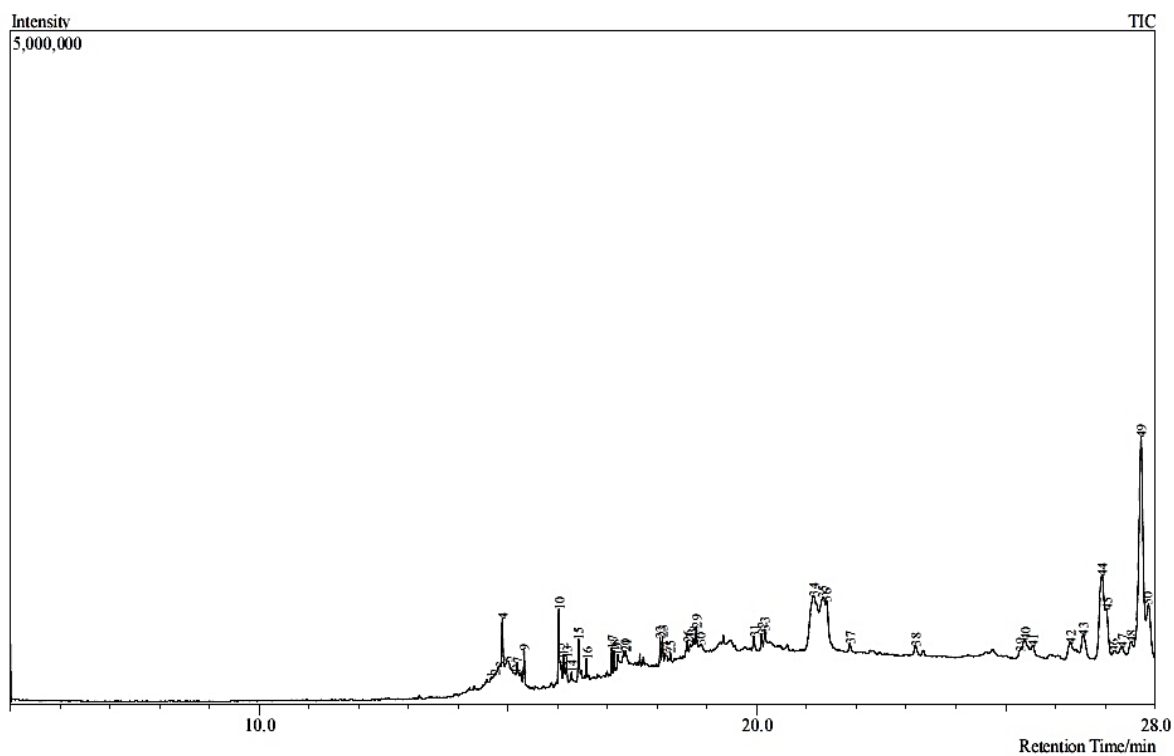
E.12 Data Analysis for Negative control (6 months inoculation period in wet and dry season)

| Paired Samples Statistics |     |       |   |                |                 |
|---------------------------|-----|-------|---|----------------|-----------------|
|                           |     | Mean  | N | Std. Deviation | Std. Error Mean |
| Pair 1                    | wet | 2.333 | 3 | .2887          | .1667           |
|                           | dry | 2.733 | 3 | .2517          | .1453           |

| Paired Samples Test |           |                    |                |                 |                                           |       |        |    |                 |
|---------------------|-----------|--------------------|----------------|-----------------|-------------------------------------------|-------|--------|----|-----------------|
|                     |           | Paired Differences |                |                 |                                           |       | t      | df | Sig. (2-tailed) |
|                     |           | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |       |        |    |                 |
|                     |           |                    |                |                 | Lower                                     | Upper |        |    |                 |
|                     |           |                    |                |                 |                                           |       |        |    |                 |
| Pair 1              | wet - dry | -.4000             | .1732          | .1000           | -.8303                                    | .0303 | -4.000 | 2  | .057            |

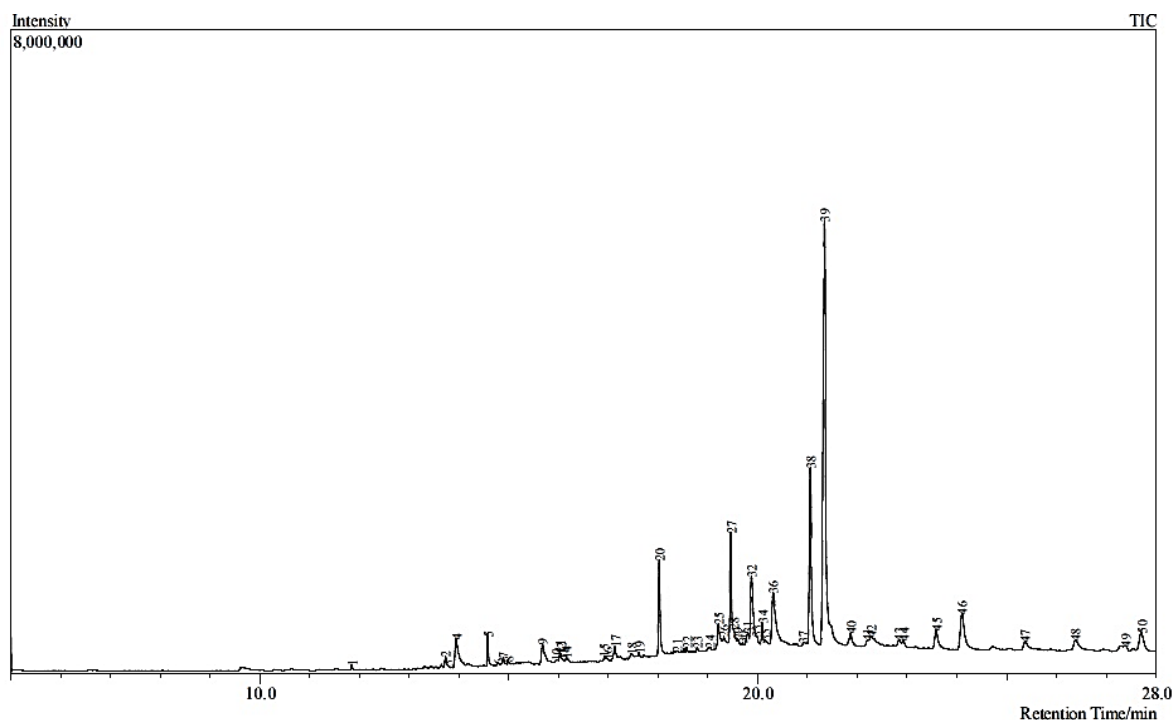
**Appendix F:** Total ion chromatography (TIC) for all sample inoculants and negative control for third month inoculation in wet seasons

F1. Results for third month inoculation of F1 inoculant



| Peak Report TIC |        |          |        |          |         |                                                |
|-----------------|--------|----------|--------|----------|---------|------------------------------------------------|
| Peak#           | R.Time | Area     | Area%  | Height   | Height% | Name                                           |
| 1               | 14.665 | 340901   | 0.76   | 75265    | 0.71    | Hexacosane                                     |
| 2               | 14.725 | 268927   | 0.60   | 96064    | 0.90    | Stigmast-4-en-3-one                            |
| 3               | 14.825 | 873671   | 1.95   | 157524   | 1.48    | Stigmast-4-en-3-one                            |
| 4               | 14.885 | 1343354  | 3.00   | 509716   | 4.78    | Hexadecanoic acid, methyl ester                |
| 5               | 14.990 | 1264178  | 2.82   | 192638   | 1.81    | Stigmast-4-en-3-one                            |
| 6               | 15.085 | 373951   | 0.84   | 122315   | 1.15    | Stigmast-4-en-3-one                            |
| 7               | 15.184 | 782767   | 1.75   | 178727   | 1.68    | n-Hexadecanoic acid                            |
| 8               | 15.255 | 210792   | 0.47   | 94047    | 0.88    | Oxacycloheptadecan-2-one                       |
| 9               | 15.321 | 497620   | 1.11   | 280554   | 2.63    | Hexadecanoic acid, ethyl ester                 |
| 10              | 16.019 | 1042384  | 2.33   | 567036   | 5.32    | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester |
| 11              | 16.070 | 186962   | 0.42   | 148462   | 1.39    | 9,12,15-Octadecatrienoic acid, methyl ester, ( |
| 12              | 16.115 | 453688   | 1.01   | 219067   | 2.05    | Phytol                                         |
| 13              | 16.179 | 342047   | 0.76   | 195262   | 1.83    | Methyl stearate                                |
| 14              | 16.275 | 125663   | 0.28   | 67419    | 0.63    | Tricyclo[4.2.2.0(2,5)]dec-7-ene, 7-(5-hexynyl  |
| 15              | 16.417 | 606778   | 1.36   | 315787   | 2.96    | Linoleic acid ethyl ester                      |
| 16              | 16.575 | 234559   | 0.52   | 156170   | 1.46    | Octadecanoic acid, ethyl ester                 |
| 17              | 17.083 | 257461   | 0.58   | 197457   | 1.85    | Hexadecanoic acid, hexyl ester                 |
| 18              | 17.130 | 203875   | 0.46   | 163295   | 1.53    | Hexadecanoic acid, hexyl ester                 |
| 19              | 17.202 | 331477   | 0.74   | 118189   | 1.11    | Erucic acid                                    |
| 20              | 17.329 | 434167   | 0.97   | 116116   | 1.09    | Stigmast-3,5-dien-7-one                        |
| 21              | 17.369 | 271938   | 0.61   | 108509   | 1.02    | Methyl 18-methylnonadecanoate                  |
| 22              | 18.058 | 305880   | 0.68   | 204736   | 1.92    | Isopropyl linoleate                            |
| 23              | 18.106 | 346539   | 0.77   | 206538   | 1.94    | Isopropyl linoleate                            |
| 24              | 18.160 | 142201   | 0.32   | 79304    | 0.74    | i-Propyl 9,12,15-octadecatrienoate             |
| 25              | 18.262 | 155421   | 0.35   | 73130    | 0.69    | Tetracosane                                    |
| 26              | 18.604 | 297061   | 0.66   | 116733   | 1.09    | n-Propyl 9,12-octadecadienoate                 |
| 27              | 18.665 | 235582   | 0.53   | 90244    | 0.85    | 2H-Pyran, 2-(2-heptadecynyl)tetrahydro-        |
| 28              | 18.724 | 384045   | 0.86   | 128889   | 1.21    | Octadecanoic acid, hexyl ester                 |
| 29              | 18.781 | 508486   | 1.14   | 205995   | 1.93    | Tetrapentacontane, 1,54-dibromo-               |
| 30              | 18.872 | 349126   | 0.78   | 68062    | 0.64    | Stigmastane-3,6-dione, (5.alpha.)-             |
| 31              | 19.939 | 250946   | 0.56   | 104478   | 0.98    | Behenic alcohol                                |
| 32              | 20.092 | 205319   | 0.46   | 112126   | 1.05    | Squalene                                       |
| 33              | 20.156 | 271380   | 0.61   | 122557   | 1.15    | Estra-1,3,5(10)-trien-17-one, 3,4-bis[(trimeth |
| 34              | 21.138 | 4218990  | 9.42   | 400948   | 3.76    | Cholest-4-ene-3,6-dione                        |
| 35              | 21.323 | 2682801  | 5.99   | 380588   | 3.57    | Stigmastane-3,6-dione, (5.alpha.)-             |
| Peak#           | R.Time | Area     | Area%  | Height   | Height% | Name                                           |
| 36              | 21.402 | 1344656  | 3.00   | 355561   | 3.33    | Heptacosane, 1-chloro-                         |
| 37              | 21.867 | 170923   | 0.38   | 63832    | 0.60    | 9(11)-Dehydroergosteryl benzoate               |
| 38              | 23.188 | 268402   | 0.60   | 73085    | 0.69    | Vitamin E                                      |
| 39              | 25.285 | 274592   | 0.61   | 57997    | 0.54    | 6,6-Dimethyl-1,4-dioxaspiro[4.5]dec-7-ene      |
| 40              | 25.378 | 924959   | 2.07   | 137156   | 1.29    | Stigmasterol                                   |
| 41              | 25.543 | 374584   | 0.84   | 80840    | 0.76    | 22-Desoxycarpesterol                           |
| 42              | 26.297 | 471806   | 1.05   | 89808    | 0.84    | 22-Stigmasten-3-one                            |
| 43              | 26.561 | 837565   | 1.87   | 154372   | 1.45    | Lup-20(29)-en-3-one                            |
| 44              | 26.938 | 4129061  | 9.22   | 616072   | 5.78    | 7,22-Ergostadienone                            |
| 45              | 27.025 | 1338969  | 2.99   | 362410   | 3.40    | Olean-18-ene                                   |
| 46              | 27.170 | 353032   | 0.79   | 52605    | 0.49    | Lupeol                                         |
| 47              | 27.333 | 512283   | 1.14   | 86120    | 0.81    | Cholest-4-en-3-one, 26-(acetyloxy)-            |
| 48              | 27.519 | 678283   | 1.52   | 121022   | 1.14    | .beta.-Amyrin                                  |
| 49              | 27.720 | 10104002 | 22.57  | 1643608  | 15.42   | 4,22-Cholestadien-3-one                        |
| 50              | 27.871 | 2182726  | 4.88   | 393353   | 3.69    | .alpha.-Amyrin                                 |
|                 |        | 44766780 | 100.00 | 10661788 | 100.00  |                                                |

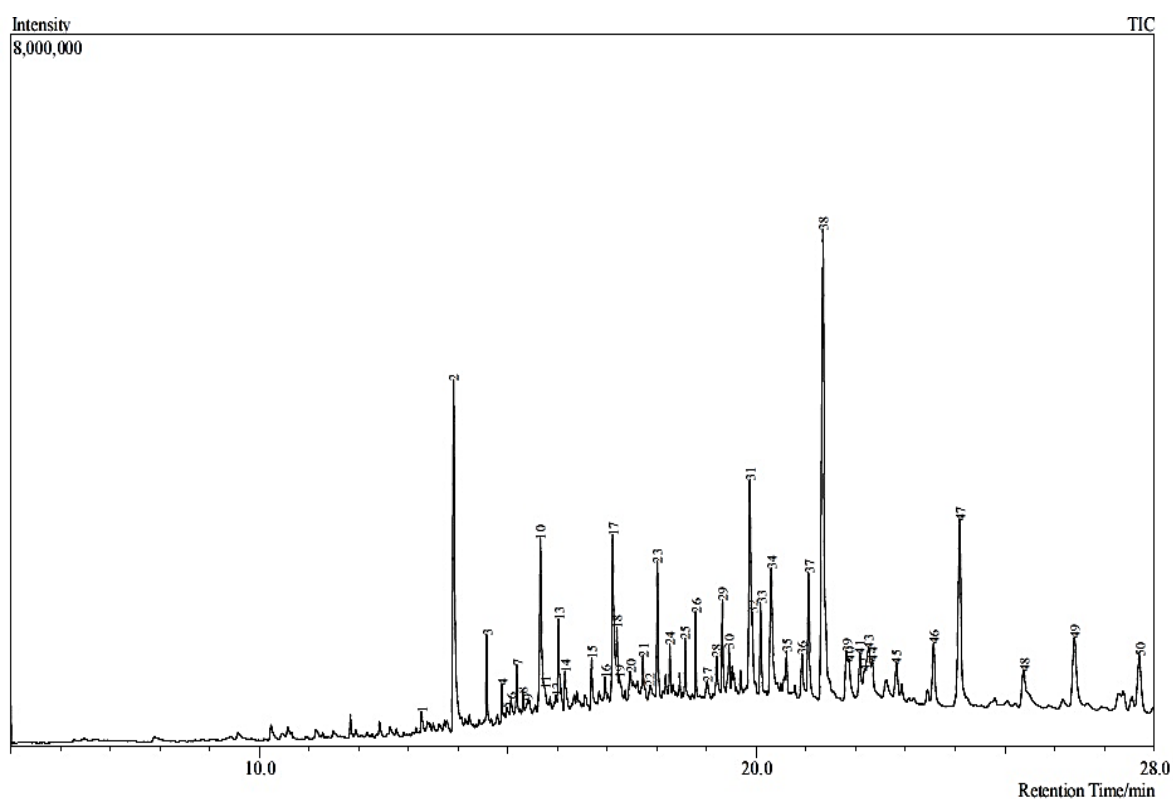
## F.2 Results for third month inoculation of F2 inoculant



| Peak Report TIC |        |         |       |         |         |                                                |
|-----------------|--------|---------|-------|---------|---------|------------------------------------------------|
| Peak#           | R.Time | Area    | Area% | Height  | Height% | Name                                           |
| 1               | 11.843 | 110056  | 0.19  | 68307   | 0.40    | Phenol, 3,5-bis(1,1-dimethylethyl)-            |
| 2               | 13.731 | 344928  | 0.60  | 142708  | 0.84    | cis-5,8,11,14,17-Eicosapentaenoic acid         |
| 3               | 13.775 | 90527   | 0.16  | 43034   | 0.25    | 1,1':3',1''-Tercyclopentane, 2'-dodecyl-       |
| 4               | 13.940 | 1876720 | 3.28  | 356087  | 2.10    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl      |
| 5               | 14.576 | 618811  | 1.08  | 372808  | 2.20    | 1,2-Benzenedicarboxylic acid, bis(2-methylp    |
| 6               | 14.780 | 85174   | 0.15  | 25542   | 0.15    | cis-Z-.alpha.-Bisabolene epoxide               |
| 7               | 14.888 | 186458  | 0.33  | 94661   | 0.56    | Hexadecanoic acid, methyl ester                |
| 8               | 14.985 | 96557   | 0.17  | 44750   | 0.26    | Caryophyllene oxide                            |
| 9               | 15.679 | 1006583 | 1.76  | 238270  | 1.41    | trans-Sinapyl alcohol                          |
| 10              | 15.965 | 118657  | 0.21  | 33065   | 0.19    | 1,3-Bis(cinnamoyloxymethyl)adamantane          |
| 11              | 16.023 | 197039  | 0.34  | 129781  | 0.77    | Methyl 10-trans,12-cis-octadecadienolate       |
| 12              | 16.045 | 142529  | 0.25  | 104582  | 0.62    | 15-Octadecenoic acid, methyl ester             |
| 13              | 16.080 | 92229   | 0.16  | 48916   | 0.29    | 9,12,15-Octadecatrienoic acid, methyl ester, ( |
| 14              | 16.157 | 118692  | 0.21  | 61135   | 0.36    | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a   |
| 15              | 16.924 | 140782  | 0.25  | 66508   | 0.39    | 1-Penten-3-one, 1,5-diphenyl-                  |
| 16              | 16.980 | 80661   | 0.14  | 26780   | 0.16    | Kauren-18-ol, acetate, (4.beta.)-              |
| 17              | 17.131 | 433279  | 0.76  | 150645  | 0.89    | Andrographolide                                |
| 18              | 17.468 | 118625  | 0.21  | 40491   | 0.24    | Andrographolide                                |
| 19              | 17.613 | 94270   | 0.16  | 48435   | 0.29    | 4,8,12,16-Tetramethylheptadecan-4-olide        |
| 20              | 18.020 | 2692919 | 4.70  | 1187216 | 7.00    | 2-Phenethyl-4H-chromen-4-one                   |
| 21              | 18.373 | 133908  | 0.23  | 34811   | 0.21    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 22              | 18.572 | 89858   | 0.16  | 55475   | 0.33    | Di-n-octyl phthalate                           |
| 23              | 18.785 | 90871   | 0.16  | 50537   | 0.30    | Benzeneacetic acid, 3-pentadecyl ester         |
| 24              | 19.040 | 164787  | 0.29  | 41944   | 0.25    | 2-Cyclohexen-1-one, 3-methoxy-2-(2,4,5-tri     |
| 25              | 19.209 | 1259917 | 2.20  | 318526  | 1.88    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 26              | 19.323 | 464741  | 0.81  | 159109  | 0.94    | 1-Tributylsilyloxymethyl-4-methoxybenzene      |
| 27              | 19.456 | 3603797 | 6.29  | 1439731 | 8.49    | 6-Methoxy-2-phenethyl-4H-chromen-4-one         |
| 28              | 19.520 | 719202  | 1.26  | 237176  | 1.40    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one        |
| 29              | 19.602 | 325951  | 0.57  | 106454  | 0.63    | 8-Benzyloxy-1,2,3,4-tetrahydro-dibenzofuran    |
| 30              | 19.690 | 140868  | 0.25  | 51889   | 0.31    | 4-Methoxy-4',5'-methylendioxybiphenyl-2-ol     |
| 31              | 19.804 | 595538  | 1.04  | 168597  | 0.99    | Imidazolidin-4-one, 5-benzyl-2-thioxo-3-p-tol  |
| 32              | 19.875 | 3286268 | 5.74  | 864671  | 5.10    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 33              | 19.985 | 276281  | 0.48  | 113135  | 0.67    | Decanedioic acid, bis(2-ethylhexyl) ester      |
| 34              | 20.090 | 534137  | 0.93  | 281097  | 1.66    | Squalene                                       |
| 35              | 20.154 | 145478  | 0.25  | 46903   | 0.28    | Ethanone, 1-(2-hydroxy-6-methoxyphenyl)-       |

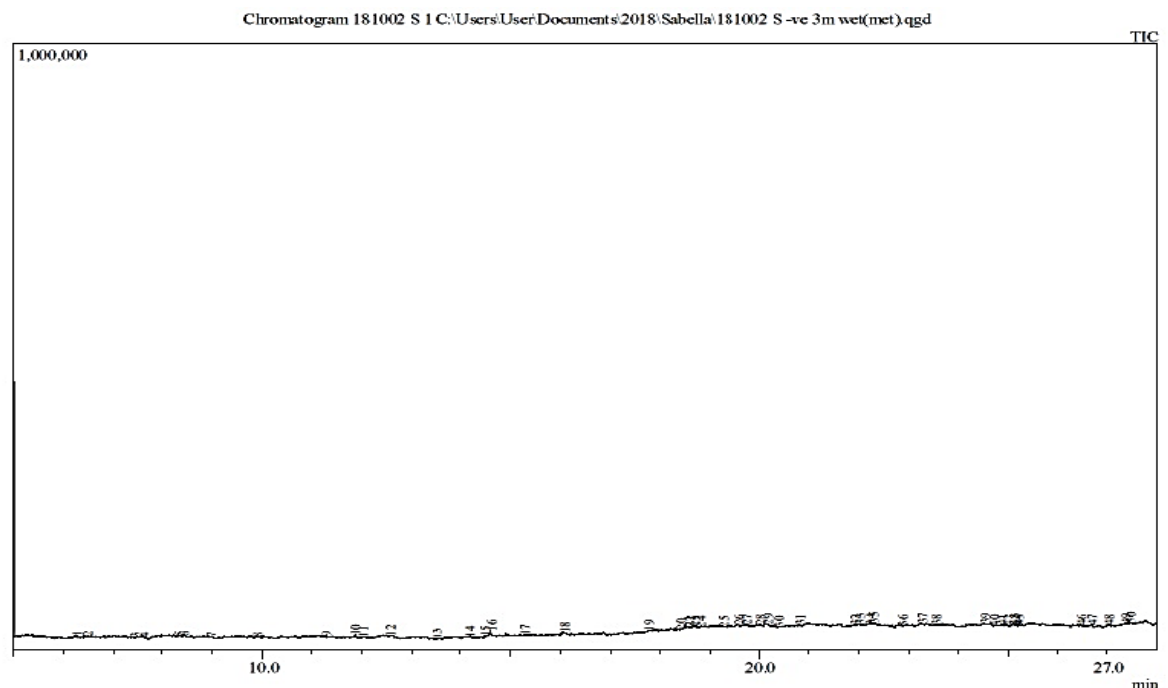
| Peak# | R.Time | Area     | Area%  | Height   | Height% | Name                                          |
|-------|--------|----------|--------|----------|---------|-----------------------------------------------|
| 36    | 20.313 | 3236631  | 5.65   | 630523   | 3.72    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-     |
| 37    | 20.920 | 113245   | 0.20   | 26736    | 0.16    | Methyl 20-methyl-heneicosanoate               |
| 38    | 21.058 | 6155813  | 10.75  | 2198083  | 12.96   | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin       |
| 39    | 21.343 | 19270888 | 33.65  | 5266674  | 31.06   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c      |
| 40    | 21.864 | 641409   | 1.12   | 158098   | 0.93    | Anthraergostatetraenol benzoate               |
| 41    | 22.210 | 162786   | 0.28   | 40714    | 0.24    | Trifluoroacetic acid, 4-benzyloxyphenyl ester |
| 42    | 22.283 | 175092   | 0.31   | 51224    | 0.30    | 3.beta.-Acetoxystigmasta-4,6,22-triene        |
| 43    | 22.838 | 299178   | 0.52   | 81387    | 0.48    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim      |
| 44    | 22.926 | 296940   | 0.52   | 88797    | 0.52    | Cholesta-4,6-dien-3-ol, (3.beta.)-            |
| 45    | 23.584 | 992107   | 1.73   | 228800   | 1.35    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 46    | 24.104 | 2450831  | 4.28   | 442257   | 2.61    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 47    | 25.373 | 455680   | 0.80   | 91505    | 0.54    | Stigmasterol                                  |
| 48    | 26.384 | 705911   | 1.23   | 114956   | 0.68    | .gamma.-Sitosterol                            |
| 49    | 27.389 | 504723   | 0.88   | 58389    | 0.34    | Cholestan-3-one, (5.alpha.)-                  |
| 50    | 27.703 | 1325012  | 2.31   | 225290   | 1.33    | 4,22-Stigmastadiene-3-one                     |
|       |        | 57263344 | 100.00 | 16957209 | 100.00  |                                               |

### F.3 Results for third month inoculation of F3 inoculant



| Peak Report TIC |        |           |        |          |                                                     |
|-----------------|--------|-----------|--------|----------|-----------------------------------------------------|
| Peak#           | R.Time | Area      | Area%  | Height   | Height% Name                                        |
| 1               | 13.261 | 644056    | 0.54   | 235868   | 0.56 Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpl   |
| 2               | 13.907 | 11145165  | 9.30   | 3925037  | 9.36 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl      |
| 3               | 14.571 | 1476844   | 1.23   | 971901   | 2.32 1,2-Benzenedicarboxylic acid, bis(2-methylp    |
| 4               | 14.879 | 655276    | 0.55   | 428022   | 1.02 Hexadecanoic acid, methyl ester                |
| 5               | 14.975 | 693293    | 0.58   | 148045   | 0.35 7-Dehydrocholesteryl isocaproate               |
| 6               | 15.062 | 727659    | 0.61   | 239750   | 0.57 Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpl   |
| 7               | 15.178 | 1309664   | 1.09   | 597261   | 1.42 n-Hexadecanoic acid                            |
| 8               | 15.302 | 765337    | 0.64   | 275183   | 0.66 5,8,11-Eicosatrienoic acid, (Z)-, TMS derivat  |
| 9               | 15.402 | 919456    | 0.77   | 179447   | 0.43 Diepicedrene-1-oxide                           |
| 10              | 15.655 | 5953566   | 4.97   | 1952500  | 4.65 trans-Sinapyl alcohol                          |
| 11              | 15.745 | 614808    | 0.51   | 263189   | 0.63 11,14-Octadecadiynoic acid, methyl ester       |
| 12              | 15.968 | 558199    | 0.47   | 177031   | 0.42 Pentadecafluorooctanoic acid, heptadecyl est   |
| 13              | 16.015 | 1999342   | 1.67   | 1027801  | 2.45 9,12-Octadecadienoic acid (Z,Z)-, methyl est   |
| 14              | 16.149 | 1152495   | 0.96   | 428422   | 1.02 Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dim    |
| 15              | 16.686 | 966077    | 0.81   | 525327   | 1.25 Cycloprop[elindene-1a,2(1H)-dicarboxaldeh      |
| 16              | 16.953 | 730632    | 0.61   | 291268   | 0.69 1H-Cycloprop[e]azulene, decahydro-1,1,7-tr     |
| 17              | 17.110 | 5272020   | 4.40   | 1880615  | 4.48 1,2,4-Methenoazulene, decahydro-1,5,5,8a-te    |
| 18              | 17.194 | 1556604   | 1.30   | 834838   | 1.99 Eucic acid                                     |
| 19              | 17.245 | 996997    | 0.83   | 265674   | 0.63 Isocaromadendrene epoxide                      |
| 20              | 17.462 | 561850    | 0.47   | 226250   | 0.54 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trime  |
| 21              | 17.720 | 698963    | 0.58   | 454368   | 1.08 Heneicosane                                    |
| 22              | 17.860 | 691581    | 0.58   | 145815   | 0.35 4-Hydroxy-beta.-ionone                         |
| 23              | 18.008 | 3016201   | 2.52   | 1533236  | 3.66 2-Phenethyl-4H-chromen-4-one                   |
| 24              | 18.258 | 871839    | 0.73   | 556863   | 1.33 Heneicosane                                    |
| 25              | 18.567 | 1020265   | 0.85   | 640724   | 1.53 Di-n-octyl phthalate                           |
| 26              | 18.777 | 1399880   | 1.17   | 964818   | 2.30 Nonacosane                                     |
| 27              | 19.013 | 650017    | 0.54   | 195857   | 0.47 4-Trifluoroacetoxy-diphenylmethane             |
| 28              | 19.201 | 1373290   | 1.15   | 453869   | 1.08 Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 29              | 19.318 | 1986014   | 1.66   | 1062716  | 2.53 Nonacosane                                     |
| 30              | 19.447 | 1002018   | 0.84   | 511252   | 1.22 6-Methoxy-2-phenethyl-4H-chromen-4-one         |
| 31              | 19.868 | 6131369   | 5.12   | 2354072  | 5.61 Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 32              | 19.920 | 1592838   | 1.33   | 883234   | 2.11 Heneicosane                                    |
| 33              | 20.085 | 1935437   | 1.61   | 1013639  | 2.42 Squalene                                       |
| 34              | 20.299 | 4718922   | 3.94   | 1372933  | 3.27 Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 35              | 20.603 | 844121    | 0.70   | 457106   | 1.09 Tetratetracontane                              |
| Peak#           | R.Time | Area      | Area%  | Height   | Height% Name                                        |
| 36              | 20.920 | 1513169   | 1.26   | 491585   | 1.17 .alpha.-d-Glucose, 2-O-benzoyl-1-methylthio    |
| 37              | 21.051 | 3790696   | 3.16   | 1398407  | 3.33 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin        |
| 38              | 21.340 | 18107595  | 15.11  | 5186549  | 12.36 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c      |
| 39              | 21.813 | 1682671   | 1.40   | 544765   | 1.30 Propanedinitrile, [3-(4-methoxyphenyl)-1-me    |
| 40              | 21.865 | 1540638   | 1.29   | 438137   | 1.04 Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22 |
| 41              | 22.083 | 1595617   | 1.33   | 492096   | 1.17 4H-1-Benzopyran-4-one, 5-hydroxy-7-methc       |
| 42              | 22.170 | 1149326   | 0.96   | 282030   | 0.67 Trifluoroacetic acid, 4-benzyloxyphenyl ester  |
| 43              | 22.260 | 2473256   | 2.06   | 527767   | 1.26 Benzene, 1-methoxy-3-(3-methyl-3-butenyl)-     |
| 44              | 22.335 | 1194202   | 1.00   | 376676   | 0.90 Heneicosane                                    |
| 45              | 22.816 | 1176460   | 0.98   | 362773   | 0.86 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim       |
| 46              | 23.564 | 2481255   | 2.07   | 672204   | 1.60 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 47              | 24.092 | 8261888   | 6.89   | 2024124  | 4.83 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 48              | 25.377 | 1339404   | 1.12   | 316958   | 0.76 Stigmasterol                                   |
| 49              | 26.400 | 3942419   | 3.29   | 763522   | 1.82 .gamma.-Sitosterol                             |
| 50              | 27.704 | 2988818   | 2.49   | 595325   | 1.42 4,22-Stigmastadiene-3-one                      |
|                 |        | 119869509 | 100.00 | 41946849 | 100.00                                              |

#### F.4 Results for third month inoculation of negative control

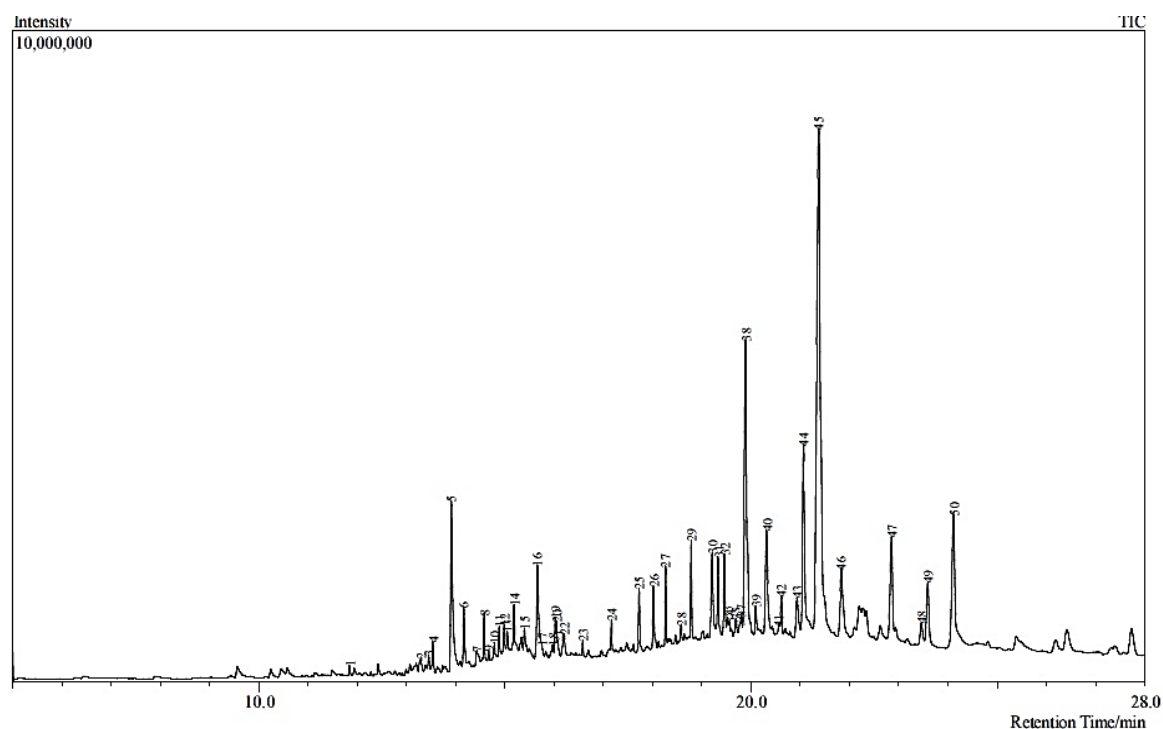


| Peak Report TIC |        |         |       |         |         |                                               |
|-----------------|--------|---------|-------|---------|---------|-----------------------------------------------|
| Peak#           | R.Time | Area    | Area% | Height  | Height% | Name                                          |
| 1               | 11.839 | 514725  | 1.24  | 331213  | 3.27    | Phenol, 3,5-bis(1,1-dimethylethyl)-           |
| 2               | 13.298 | 229049  | 0.55  | 63840   | 0.63    | 1,2-Dihexyleyclopropene-3-carboxylic acid     |
| 3               | 13.447 | 190679  | 0.46  | 77942   | 0.77    | Tetradecanal                                  |
| 4               | 13.922 | 2598819 | 6.27  | 628146  | 6.20    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp      |
| 5               | 14.575 | 1651793 | 3.99  | 1076675 | 10.63   | 1,2-Benzenedicarboxylic acid, bis(2-methylp   |
| 6               | 14.815 | 1474205 | 3.56  | 176141  | 1.74    | gamma-Sitosterone                             |
| 7               | 14.884 | 1059723 | 2.56  | 420494  | 4.15    | Hexadecanoic acid, methyl ester               |
| 8               | 14.941 | 450223  | 1.09  | 203525  | 2.01    | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dic |
| 9               | 15.013 | 1252523 | 3.02  | 207395  | 2.05    | Stigmast-4-en-3-one                           |
| 10              | 15.095 | 281275  | 0.68  | 164198  | 1.62    | Stigmast-4-en-3-one                           |
| 11              | 15.173 | 1028249 | 2.48  | 183367  | 1.81    | L-(+)-Ascorbic acid 2,6-dihexadecanoate       |
| 12              | 15.244 | 361447  | 0.87  | 119432  | 1.18    | Dibutyl phthalate                             |
| 13              | 15.317 | 272904  | 0.66  | 106535  | 1.05    | Hexadecanoic acid, ethyl ester                |
| 14              | 15.674 | 1123151 | 2.71  | 268504  | 2.65    | trans-Sinapyl alcohol                         |
| 15              | 16.020 | 779237  | 1.88  | 306847  | 3.03    | Methyl 10-trans,12-cis-octadecadienoate       |
| 16              | 17.250 | 288952  | 0.70  | 40798   | 0.40    | (E)-13-Docosenoic acid                        |
| 17              | 17.613 | 306592  | 0.74  | 163172  | 1.61    | 4,8,12,16-Tetramethylheptadecan-4-olide       |
| 18              | 18.395 | 487135  | 1.18  | 116352  | 1.15    | Ethanedioic acid, bis(1-methylpropyl) ester   |
| 19              | 18.460 | 328775  | 0.79  | 165621  | 1.63    | Docosanoic acid, methyl ester                 |
| 20              | 18.522 | 389506  | 0.94  | 197518  | 1.95    | Heicosanal                                    |
| 21              | 18.572 | 334130  | 0.81  | 177060  | 1.75    | Di-n-octyl phthalate                          |
| 22              | 18.665 | 771824  | 1.86  | 85482   | 0.84    | Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7( |
| 23              | 18.781 | 455506  | 1.10  | 173782  | 1.72    | Sulfurous acid, octadecyl 2-propyl ester      |
| 24              | 18.866 | 495371  | 1.20  | 91924   | 0.91    | Stigmastane-3,6-dione, (5.alpha.)-            |
| 25              | 18.985 | 251729  | 0.61  | 56977   | 0.56    | Tricosanoic acid, methyl ester                |
| 26              | 19.060 | 300632  | 0.73  | 115806  | 1.14    | Octadecanal                                   |
| 27              | 19.135 | 195107  | 0.47  | 50199   | 0.50    | Cyclopropane, 1,1-dichloro-2,2,3,3-tetrametl  |
| 28              | 19.210 | 323720  | 0.78  | 126958  | 1.25    | Docosanoic acid, 2-hydroxy-, methyl ester     |
| 29              | 19.254 | 467948  | 1.13  | 149266  | 1.47    | Diazprogesterone                              |
| 30              | 19.328 | 537839  | 1.30  | 147030  | 1.45    | Octadecane, 1-chloro-                         |
| 31              | 19.442 | 1139486 | 2.75  | 184750  | 1.82    | 15-Tetracosenoic acid, methyl ester, (Z)-     |
| 32              | 19.554 | 406325  | 0.98  | 166410  | 1.64    | Tetracosanoic acid, methyl ester              |
| 33              | 19.699 | 479187  | 1.16  | 115518  | 1.14    | 7-(3,4-Methylenedioxy)-tetrahydrobenzofura    |
| 34              | 19.814 | 214501  | 0.52  | 80552   | 0.80    | Methyl 2-hydroxy-heptadecanoate               |
| 35              | 19.933 | 446881  | 1.08  | 111843  | 1.10    | Benzeneacetic acid, 4-pentadecyl ester        |

| Peak# | R.Time | Area     | Area%  | Height   | Height% | Name                                           |
|-------|--------|----------|--------|----------|---------|------------------------------------------------|
| 36    | 19.987 | 213339   | 0.51   | 94864    | 0.94    | Decanedioic acid, bis(2-ethylhexyl) ester      |
| 37    | 20.086 | 265405   | 0.64   | 88611    | 0.87    | .beta.-d-Mannofuranoside, 2,3:5,6-di-ethylbc   |
| 38    | 20.785 | 267261   | 0.64   | 103022   | 1.02    | 15-Tetracosenoic acid, methyl ester, (Z)-      |
| 39    | 20.923 | 356458   | 0.86   | 134285   | 1.33    | Hexacosanoic acid, methyl ester                |
| 40    | 21.087 | 4849999  | 11.70  | 464675   | 4.59    | Cholest-4-ene-3,6-dione                        |
| 41    | 21.331 | 3780907  | 9.12   | 499662   | 4.93    | 9,12-Octadecadienoic acid (Z,Z)-, phenylmet    |
| 42    | 21.863 | 342690   | 0.83   | 123363   | 1.22    | Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22 |
| 43    | 22.924 | 541694   | 1.31   | 158912   | 1.57    | Cholesta-4,6-dien-3-ol, (3.beta.)-             |
| 44    | 23.705 | 201660   | 0.49   | 58691    | 0.58    | 6-Methoxypurine, TBDMS derivative              |
| 45    | 25.365 | 1295410  | 3.13   | 223798   | 2.21    | Stigmasterol                                   |
| 46    | 26.386 | 2780585  | 6.71   | 468464   | 4.62    | .gamma.-Sitosterol                             |
| 47    | 27.293 | 922259   | 2.23   | 169416   | 1.67    | 4-Campestene-3-one                             |
| 48    | 27.378 | 830246   | 2.00   | 173251   | 1.71    | Cholestan-3-one                                |
| 49    | 27.546 | 234800   | 0.57   | 54398    | 0.54    | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimet    |
| 50    | 27.700 | 2670021  | 6.44   | 464833   | 4.59    | 4,22-Cholestadien-3-one                        |
|       |        | 41441882 | 100.00 | 10131517 | 100.00  |                                                |

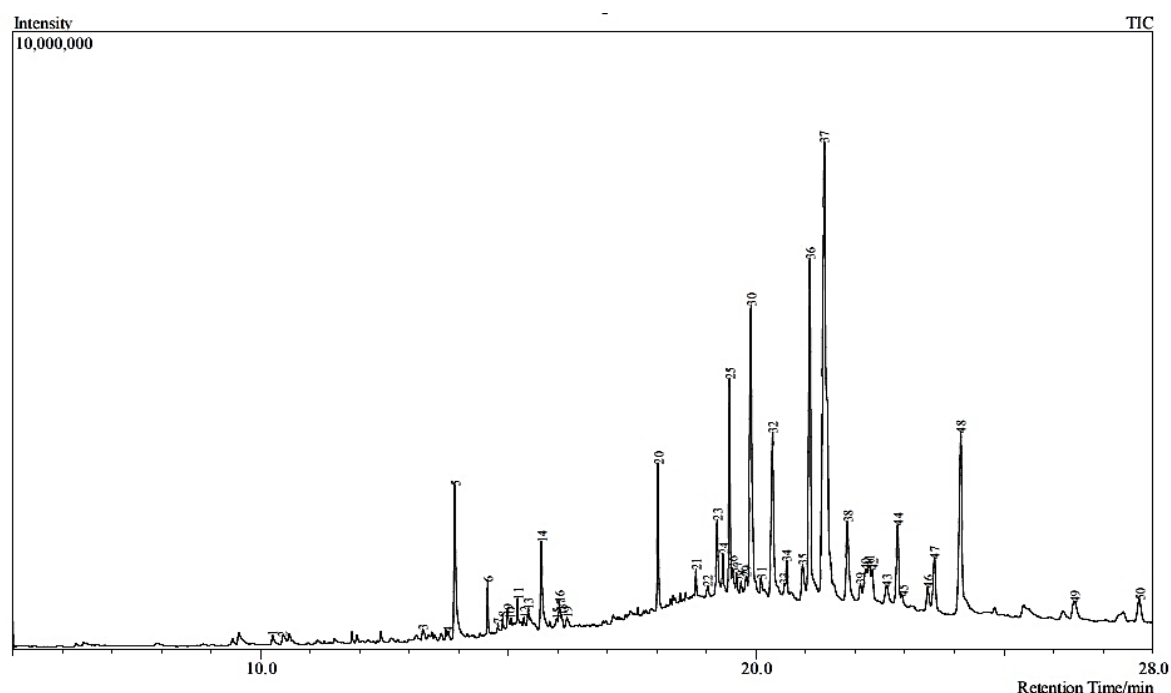
**Appendix G:** Total ion chromatography (TIC) for all sample inoculants and negative controls for sixth month inoculation in wet seasons

#### G.1 Results for sixth month inoculation of F1 inoculant



| Peak Report TIC |        |           |        |          |         |                                              |
|-----------------|--------|-----------|--------|----------|---------|----------------------------------------------|
| Peak#           | R.Time | Area      | Area%  | Height   | Height% | Name                                         |
| 1               | 11.841 | 215165    | 0.16   | 149923   | 0.33    | Phenol, 3,5-bis(1,1-dimethylethyl)-          |
| 2               | 13.296 | 631428    | 0.47   | 210828   | 0.47    | 2-((2R,4aR,8aR)-4a,8-Dimethyl-1,2,3,4,4a,5   |
| 3               | 13.452 | 335286    | 0.25   | 212976   | 0.47    | Cubenol                                      |
| 4               | 13.537 | 589762    | 0.44   | 427274   | 0.94    | 2-((2R,4aR,8aR)-4a,8-Dimethyl-1,2,3,4,4a,5   |
| 5               | 13.911 | 7658025   | 5.73   | 2590322  | 5.72    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl    |
| 6               | 14.165 | 1674743   | 1.25   | 890172   | 1.97    | 2-((2R,4aR,8aR)-4a,8-Dimethyl-1,2,3,4,4a,5   |
| 7               | 14.425 | 1094983   | 0.82   | 224934   | 0.50    | Methyl 6-O-[1-methylpropyl]-.beta.-d-galact  |
| 8               | 14.576 | 1067195   | 0.80   | 729726   | 1.61    | 1,2-Benzenedicarboxylic acid, bis(2-methylp  |
| 9               | 14.669 | 276585    | 0.21   | 166589   | 0.37    | Kessanyl acetate                             |
| 10              | 14.783 | 507658    | 0.38   | 254334   | 0.56    | Undec-10-ynoic acid, tridec-2-yn-1-yl ester  |
| 11              | 14.884 | 699734    | 0.52   | 457421   | 1.01    | Hexadecanoic acid, methyl ester              |
| 12              | 14.985 | 829741    | 0.62   | 426964   | 0.94    | 4,5-di-epi-aristolochene                     |
| 13              | 15.046 | 571174    | 0.43   | 307838   | 0.68    | 7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl- |
| 14              | 15.185 | 943645    | 0.71   | 621284   | 1.37    | n-Hexadecanoic acid                          |
| 15              | 15.396 | 474539    | 0.35   | 307510   | 0.68    | 4aH-Cycloprop[elazulen-4a-ol, decahydro-1,   |
| 16              | 15.660 | 4482436   | 3.35   | 1428103  | 3.15    | trans-Sinapyl alcohol                        |
| 17              | 15.750 | 243094    | 0.18   | 171908   | 0.38    | 4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyet  |
| 18              | 15.971 | 162578    | 0.12   | 122164   | 0.27    | Sulfurous acid, 2-propyl tetradecyl ester    |
| 19              | 16.021 | 767941    | 0.57   | 553130   | 1.22    | Methyl 10-trans,12-cis-octadecadienoate      |
| 20              | 16.046 | 655870    | 0.49   | 505228   | 1.12    | 9-Octadecenoic acid (Z)-, methyl ester       |
| 21              | 16.080 | 338437    | 0.25   | 205542   | 0.45    | 8,11,14-Docosatrienoic acid, methyl ester    |
| 22              | 16.200 | 656277    | 0.49   | 263343   | 0.58    | 6,9-Octadecadiynoic acid, methyl ester       |
| 23              | 16.581 | 297039    | 0.22   | 235506   | 0.52    | Heneicosane                                  |
| 24              | 17.166 | 556357    | 0.42   | 469423   | 1.04    | Heneicosane                                  |
| 25              | 17.726 | 1179237   | 0.88   | 910629   | 2.01    | Heneicosane                                  |
| 26              | 18.015 | 1773474   | 1.33   | 957810   | 2.12    | 2-Phenethyl-4H-chromen-4-one                 |
| 27              | 18.264 | 1585679   | 1.19   | 1206920  | 2.67    | Heneicosane                                  |
| 28              | 18.580 | 454037    | 0.34   | 257021   | 0.57    | Bis(tridecyl) phthalate                      |
| 29              | 18.784 | 2054730   | 1.54   | 1493712  | 3.30    | Nonacosane                                   |
| 30              | 19.209 | 3494515   | 2.61   | 1303985  | 2.88    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 31              | 19.327 | 2333146   | 1.74   | 1213696  | 2.68    | Nonacosane                                   |
| 32              | 19.458 | 2450037   | 1.83   | 1222651  | 2.70    | 6-Methoxy-2-phenethyl-4H-chromen-4-one       |
| 33              | 19.524 | 432118    | 0.32   | 225860   | 0.50    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one      |
| 34              | 19.565 | 218730    | 0.16   | 145033   | 0.32    | Tetracosanoic acid, methyl ester             |
| 35              | 19.692 | 363801    | 0.27   | 218833   | 0.48    | SPIROMESIFEN                                 |
| Peak#           | R.Time | Area      | Area%  | Height   | Height% | Name                                         |
| 36              | 19.765 | 310250    | 0.23   | 119655   | 0.26    | 3-Methylheptacosane                          |
| 37              | 19.805 | 431469    | 0.32   | 254345   | 0.56    | 2,4-Diamino-5,6,7,8-tetrahydro-5-phenyl[1]t  |
| 38              | 19.887 | 14676324  | 10.98  | 4504434  | 9.95    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim    |
| 39              | 20.095 | 685480    | 0.51   | 394879   | 0.87    | Squalene                                     |
| 40              | 20.316 | 4883564   | 3.65   | 1562790  | 3.45    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 41              | 20.560 | 301155    | 0.23   | 110070   | 0.24    | p-Acetamidobenzaldehyde 6-methylpyridine-    |
| 42              | 20.616 | 1033221   | 0.77   | 574995   | 1.27    | Tetracontane                                 |
| 43              | 20.935 | 1846035   | 1.38   | 570783   | 1.26    | 2-Methoxybenzyl alcohol, 2-methylpropyl et   |
| 44              | 21.068 | 6949645   | 5.20   | 2775647  | 6.13    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin      |
| 45              | 21.375 | 40051088  | 29.95  | 7652963  | 16.90   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c     |
| 46              | 21.833 | 3892998   | 2.91   | 1011011  | 2.23    | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-       |
| 47              | 22.853 | 4862636   | 3.64   | 1493544  | 3.30    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim     |
| 48              | 23.464 | 973666    | 0.73   | 311119   | 0.69    | Octadecane, 1-bromo-                         |
| 49              | 23.590 | 3342252   | 2.50   | 954892   | 2.11    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
| 50              | 24.113 | 7400244   | 5.53   | 1905365  | 4.21    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
|                 |        | 133709223 | 100.00 | 45285084 | 100.00  |                                              |

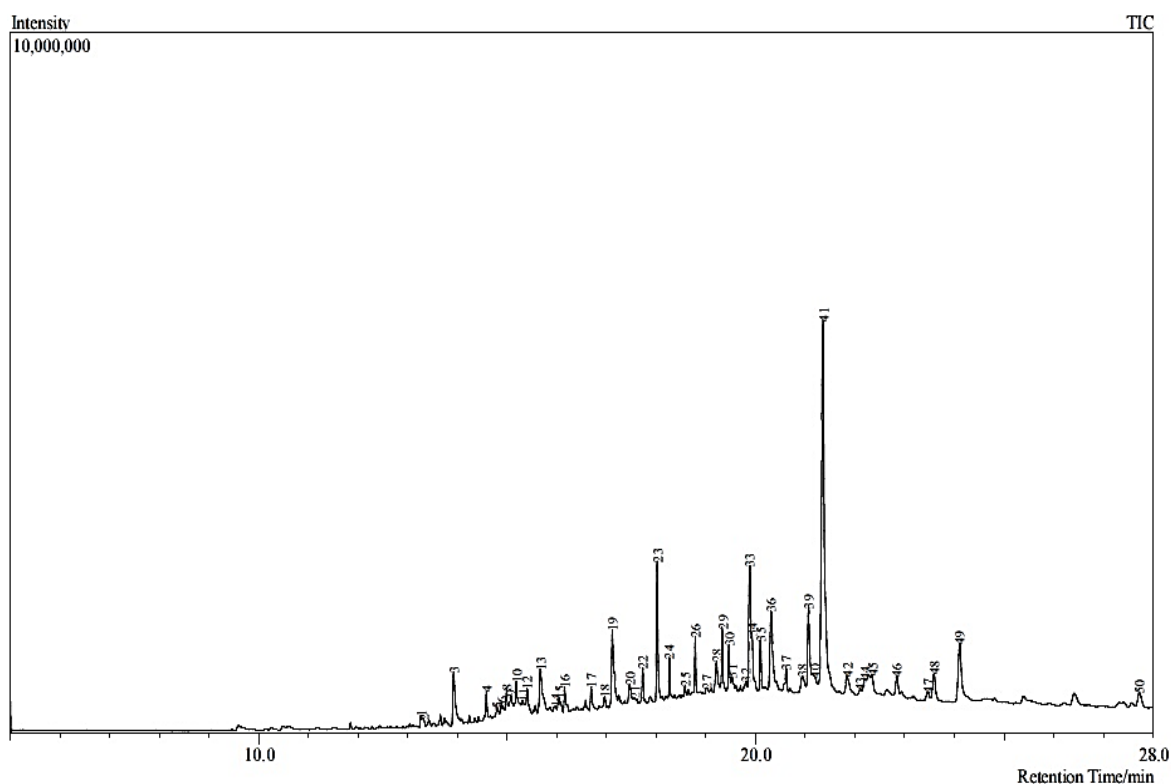
## G.2 Results for sixth month inoculation of F2 inoculant



| Peak# | R.Time | Area     | Area% | Height  | Height% | Name                                         |
|-------|--------|----------|-------|---------|---------|----------------------------------------------|
| 1     | 10.238 | 449113   | 0.27  | 149917  | 0.30    | 2-Methoxy-4-vinylphenol                      |
| 2     | 10.445 | 491631   | 0.30  | 122549  | 0.25    | Hydrocinnamic acid                           |
| 3     | 13.274 | 543404   | 0.33  | 169208  | 0.34    | Benzaldehyde, 4-hydroxy-3,5-dimethoxy-       |
| 4     | 13.783 | 355773   | 0.21  | 130258  | 0.26    | Tetrahydroedulan                             |
| 5     | 13.915 | 7181362  | 4.31  | 2480123 | 5.03    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl    |
| 6     | 14.580 | 1157156  | 0.69  | 845600  | 1.72    | 1,2-Benzenedicarboxylic acid, bis(2-methylp  |
| 7     | 14.787 | 333193   | 0.20  | 150856  | 0.31    | 10-Methyl-8-tetradecen-1-ol acetate          |
| 8     | 14.887 | 407576   | 0.24  | 257574  | 0.52    | Hexadecanoic acid, methyl ester              |
| 9     | 14.989 | 907146   | 0.54  | 358899  | 0.73    | Doconexent                                   |
| 10    | 15.052 | 354602   | 0.21  | 177657  | 0.36    | (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-di    |
| 11    | 15.183 | 1330812  | 0.80  | 475129  | 0.96    | n-Hexadecanoic acid                          |
| 12    | 15.311 | 452375   | 0.27  | 147133  | 0.30    | 3.beta.-Hydroxyguaia-4(15),10(14),11(13)-tr  |
| 13    | 15.397 | 567867   | 0.34  | 273143  | 0.55    | 4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,   |
| 14    | 15.663 | 4415127  | 2.65  | 1427194 | 2.90    | trans-Sinapyl alcohol                        |
| 15    | 15.977 | 526752   | 0.32  | 160426  | 0.33    | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione    |
| 16    | 16.023 | 695710   | 0.42  | 431804  | 0.88    | Methyl 10-trans,12-cis-octadecadienoate      |
| 17    | 16.050 | 351422   | 0.21  | 292832  | 0.59    | 9-Octadecenoic acid (Z)-, methyl ester       |
| 18    | 16.077 | 379445   | 0.23  | 214286  | 0.43    | Methyl 6,8-octadecadiynoate                  |
| 19    | 16.185 | 420280   | 0.25  | 158919  | 0.32    | Tetradecanoic acid, 12-methyl-, methyl ester |
| 20    | 18.015 | 4398413  | 2.64  | 2381756 | 4.83    | 2-Phenethyl-4H-chromen-4-one                 |
| 21    | 18.785 | 636724   | 0.38  | 434170  | 0.88    | 2-Methylhexacosane                           |
| 22    | 19.024 | 551782   | 0.33  | 176986  | 0.36    | Octadecane, 3-ethyl-5-(2-ethylbutyl)-        |
| 23    | 19.211 | 3624259  | 2.18  | 1234835 | 2.51    | Acetamide, N-(4-benzoyloxyphenyl)-2-cyano-   |
| 24    | 19.327 | 1674685  | 1.01  | 676917  | 1.37    | Nonacosane                                   |
| 25    | 19.461 | 6952552  | 4.17  | 3497749 | 7.10    | 6-Methoxy-2-phenethyl-4H-chromen-4-one       |
| 26    | 19.524 | 1214734  | 0.73  | 431827  | 0.88    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one      |
| 27    | 19.605 | 649480   | 0.39  | 331520  | 0.67    | 8-Benzoyloxy-1,2,3,4-tetrahydro-dibenzofura  |
| 28    | 19.694 | 397542   | 0.24  | 184087  | 0.37    | 1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl-   |
| 29    | 19.804 | 790018   | 0.47  | 262628  | 0.53    | Imidazolidin-4-one, 5-benzyl-2-thioxo-3-p-tc |
| 30    | 19.891 | 14794755 | 8.88  | 4649249 | 9.44    | Coumarin, 6-benzoyloxy-3,4-dihydro-4,4-dim   |
| 31    | 20.097 | 430956   | 0.26  | 220926  | 0.45    | Squalene                                     |
| 32    | 20.333 | 8317471  | 4.99  | 2571922 | 5.22    | Acetamide, N-(4-benzoyloxyphenyl)-2-cyano-   |
| 33    | 20.570 | 373100   | 0.22  | 177585  | 0.36    | p-Acetamidobenzaldehyde 6-methylpyridine-    |
| 34    | 20.616 | 1024884  | 0.62  | 546198  | 1.11    | Tetracontane                                 |
| 35    | 20.937 | 2273245  | 1.36  | 562195  | 1.14    | Benzene, 1-trifluoromethyl-4-(2-methoxyben   |

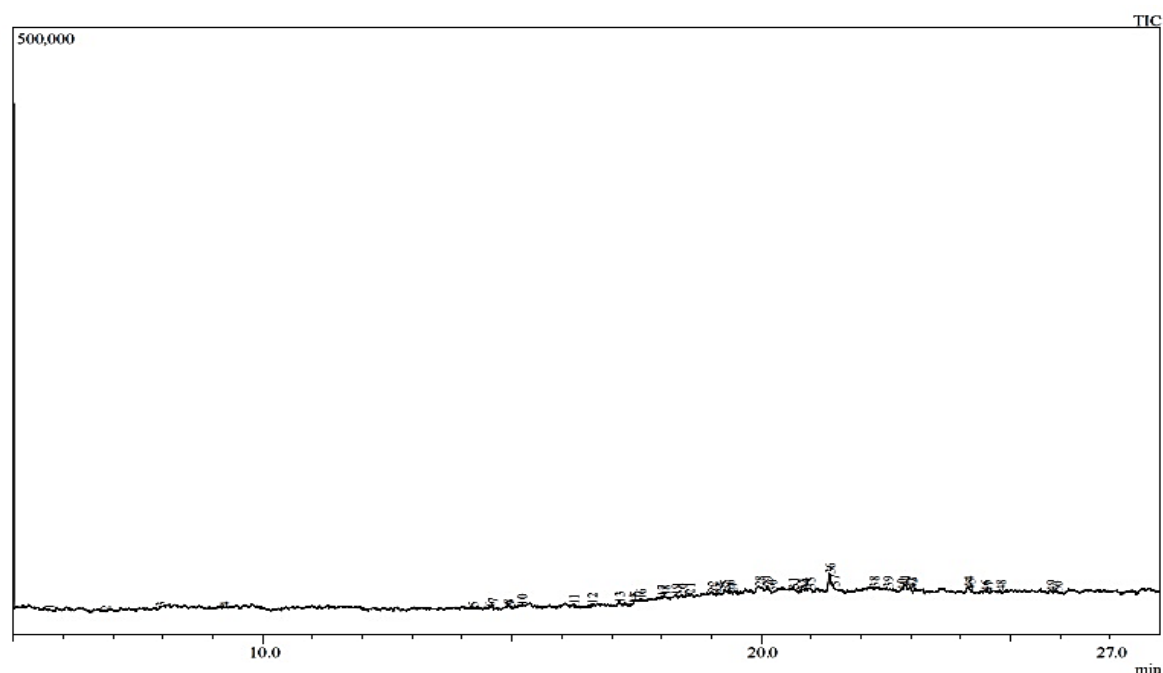
| Peak# | R.Time | Area      | Area%  | Height   | Height% | Name                                          |
|-------|--------|-----------|--------|----------|---------|-----------------------------------------------|
| 36    | 21.083 | 16959605  | 10.18  | 5513959  | 11.19   | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin       |
| 37    | 21.377 | 41864238  | 25.13  | 7387246  | 15.00   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c      |
| 38    | 21.837 | 5051865   | 3.03   | 1269557  | 2.58    | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-        |
| 39    | 22.101 | 870144    | 0.52   | 269397   | 0.55    | 4H-1-Benzopyran-4-one, 5-hydroxy-7-methc      |
| 40    | 22.211 | 2225627   | 1.34   | 514505   | 1.04    | Trifluoroacetic acid, 4-benzyloxyphenyl ester |
| 41    | 22.278 | 2193489   | 1.32   | 557569   | 1.13    | Benzene, 1-methoxy-4-pentyl-                  |
| 42    | 22.341 | 1442628   | 0.87   | 504659   | 1.02    | Tetracontane                                  |
| 43    | 22.629 | 1376932   | 0.83   | 287882   | 0.58    | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-        |
| 44    | 22.854 | 4531708   | 2.72   | 1293265  | 2.63    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim      |
| 45    | 22.950 | 444853    | 0.27   | 141692   | 0.29    | Cholesta-4,6-dien-3-ol, (3.beta.)-            |
| 46    | 23.464 | 1337690   | 0.80   | 379817   | 0.77    | Triacontane, 1-bromo-                         |
| 47    | 23.598 | 3281259   | 1.97   | 855604   | 1.74    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 48    | 24.130 | 12240592  | 7.35   | 2907347  | 5.90    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 49    | 26.417 | 1539923   | 0.92   | 265047   | 0.54    | .gamma.-Sitosterol                            |
| 50    | 27.730 | 1793465   | 1.08   | 352300   | 0.72    | 4,22-Stigmastadiene-3-one                     |
|       |        | 166579364 | 100.00 | 49263903 | 100.00  |                                               |

### G.3 Results for sixth month inoculation of F3 inoculant



| Peak# | R.Time | Area     | Area%  | Peak Report TIC |         | Name                                           |
|-------|--------|----------|--------|-----------------|---------|------------------------------------------------|
|       |        |          |        | Height          | Height% |                                                |
| 1     | 13.268 | 350164   | 0.40   | 169715          | 0.62    | 2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H     |
| 2     | 13.320 | 317977   | 0.36   | 110778          | 0.40    | (E)-Labda-8(17),12-diene-15,16-dial            |
| 3     | 13.922 | 2321308  | 2.65   | 730516          | 2.66    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl      |
| 4     | 14.580 | 607749   | 0.69   | 380445          | 1.38    | 1,2-Benzenedicarboxylic acid, bis(2-methylp    |
| 5     | 14.790 | 387734   | 0.44   | 132397          | 0.48    | Caryophyllene oxide                            |
| 6     | 14.888 | 409781   | 0.47   | 201054          | 0.73    | Hexadecanoic acid, methyl ester                |
| 7     | 14.925 | 288555   | 0.33   | 127561          | 0.46    | Androst-1-en-3-one, (5.alpha.)-                |
| 8     | 14.990 | 974772   | 1.11   | 367138          | 1.34    | cis-Z-.alpha.-Bisabolene epoxide               |
| 9     | 15.072 | 914152   | 1.04   | 291117          | 1.06    | Cyclohexanemethanol, 4-ethenyl-.alpha.,.alp    |
| 10    | 15.184 | 1866772  | 2.13   | 473638          | 1.72    | n-Hexadecanoic acid                            |
| 11    | 15.307 | 965819   | 1.10   | 203290          | 0.74    | 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydr     |
| 12    | 15.402 | 1346753  | 1.54   | 362077          | 1.32    | 1H-Cycloprop[c]azulen-4-ol, decahydro-1,1,     |
| 13    | 15.668 | 2872130  | 3.28   | 629351          | 2.29    | trans-Sinapyl alcohol                          |
| 14    | 15.973 | 360360   | 0.41   | 92650           | 0.34    | 2-Methyltetraacosane                           |
| 15    | 16.049 | 292389   | 0.33   | 207853          | 0.76    | 11-Octadecenoic acid, methyl ester, (Z)-       |
| 16    | 16.159 | 729210   | 0.83   | 344830          | 1.25    | .beta.-Longipinene                             |
| 17    | 16.694 | 576515   | 0.66   | 313024          | 1.14    | Cycloprop[c]indene-1a,2(1H)-dicarboxaldeh      |
| 18    | 16.962 | 314060   | 0.36   | 156654          | 0.57    | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,   |
| 19    | 17.117 | 3694984  | 4.22   | 1080090         | 3.93    | Aromadendrene oxide-(1)                        |
| 20    | 17.464 | 732783   | 0.84   | 260908          | 0.95    | Andrographolide                                |
| 21    | 17.580 | 338374   | 0.39   | 70072           | 0.25    | trans-Z-.alpha.-Bisabolene epoxide             |
| 22    | 17.729 | 675740   | 0.77   | 468481          | 1.70    | Heneicosane                                    |
| 23    | 18.019 | 3957971  | 4.52   | 1995800         | 7.26    | 2-Phenethyl-4H-chromen-4-one                   |
| 24    | 18.267 | 926960   | 1.06   | 596330          | 2.17    | Heneicosane                                    |
| 25    | 18.583 | 290050   | 0.33   | 154777          | 0.56    | 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-di    |
| 26    | 18.786 | 1112054  | 1.27   | 790561          | 2.88    | Nonacosane                                     |
| 27    | 19.012 | 330441   | 0.38   | 87353           | 0.32    | Butylaldehyde, 4-benzoyloxy-4-[2,2,-dimethy    |
| 28    | 19.212 | 1568334  | 1.79   | 455656          | 1.66    | Acetamide, N-(4-benzoyloxyphenyl)-2-cyano-     |
| 29    | 19.329 | 1930410  | 2.20   | 930659          | 3.38    | Nonacosane                                     |
| 30    | 19.462 | 1548679  | 1.77   | 679806          | 2.47    | 6-Methoxy-2-phenethyl-4H-chromen-4-one         |
| 31    | 19.527 | 686036   | 0.78   | 222504          | 0.81    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one        |
| 32    | 19.809 | 510742   | 0.58   | 151359          | 0.55    | Imidazolidin-4-one, 5-benzyl-2-thioxo-3-p-tc   |
| 33    | 19.883 | 4851455  | 5.54   | 1797579         | 6.54    | Coumarin, 6-benzoyloxy-3,4-dihydro-4,4-dim     |
| 34    | 19.930 | 1665799  | 1.90   | 802517          | 2.92    | Heneicosane                                    |
| 35    | 20.098 | 1238436  | 1.41   | 705206          | 2.56    | Squalene                                       |
| Peak# | R.Time | Area     | Area%  | Height          | Height% | Name                                           |
| 36    | 20.314 | 4238283  | 4.84   | 1119978         | 4.07    | Acetamide, N-(4-benzoyloxyphenyl)-2-cyano-     |
| 37    | 20.616 | 608817   | 0.69   | 317752          | 1.16    | Tetratetracontane                              |
| 38    | 20.941 | 1180494  | 1.35   | 230184          | 0.84    | 1H-1,2,3,4-Tetrazole-1,5-diamine, N(1)-[(2-r   |
| 39    | 21.068 | 3684658  | 4.21   | 1208102         | 4.39    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin        |
| 40    | 21.200 | 1445105  | 1.65   | 213181          | 0.78    | Octatriacontane, 1,38-dibromo-                 |
| 41    | 21.356 | 22697383 | 25.91  | 5303687         | 19.29   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c       |
| 42    | 21.835 | 1061879  | 1.21   | 243344          | 0.89    | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-         |
| 43    | 22.095 | 314297   | 0.36   | 68264           | 0.25    | 4H-1-Benzopyran-4-one, 5-hydroxy-7-methc       |
| 44    | 22.190 | 2178501  | 2.49   | 212747          | 0.77    | Trifluoroacetic acid, 4-benzoyloxyphenyl ester |
| 45    | 22.343 | 783624   | 0.89   | 270479          | 0.98    | Heneicosane                                    |
| 46    | 22.841 | 1027888  | 1.17   | 282998          | 1.03    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim       |
| 47    | 23.465 | 368978   | 0.42   | 109597          | 0.40    | Dotriacontane, 1-iodo-                         |
| 48    | 23.590 | 1471321  | 1.68   | 375999          | 1.37    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 49    | 24.110 | 3588111  | 4.10   | 800424          | 2.91    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 50    | 27.722 | 1007843  | 1.15   | 193454          | 0.70    | 4,22-Stigmastadiene-3-one                      |
|       |        | 87612630 | 100.00 | 27493936        | 100.00  |                                                |

#### G.4 Results for sixth month inoculation of negative control

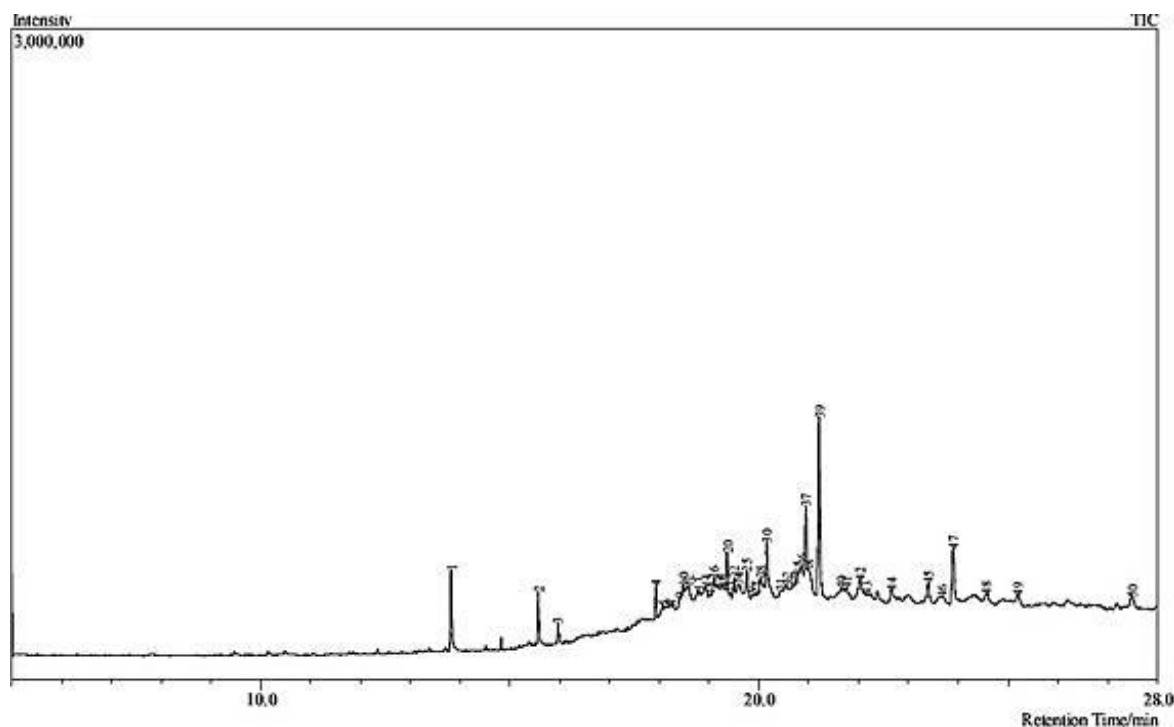


| Peak# | R.Time | Area    | Area% | Peak Report TIC |         | Name                                         |
|-------|--------|---------|-------|-----------------|---------|----------------------------------------------|
|       |        |         |       | Height          | Height% |                                              |
| 1     | 11.840 | 249590  | 0.34  | 168078          | 0.73    | Phenol, 3,5-bis(1,1-dimethylethyl)-          |
| 2     | 12.135 | 316018  | 0.43  | 109533          | 0.48    | Silane, [(1,1-dimethyl-2-propenyl)oxy]dimet  |
| 3     | 13.295 | 726520  | 0.99  | 214506          | 0.94    | 1,2-Dihexyleyclopropene-3-carboxylic acid    |
| 4     | 13.385 | 282602  | 0.39  | 80308           | 0.35    | Butan-2-one, 4-(3-hydroxy-2-methoxypheny     |
| 5     | 13.911 | 6499086 | 8.87  | 2036848         | 8.90    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxy      |
| 6     | 14.576 | 1326428 | 1.81  | 934976          | 4.09    | 1,2-Benzenedicarboxylic acid, bis(2-methylp  |
| 7     | 14.780 | 422193  | 0.58  | 93313           | 0.41    | cis-Z.-alpha.-Bisabolene epoxide             |
| 8     | 14.884 | 644153  | 0.88  | 410362          | 1.79    | Hexadecanoic acid, methyl ester              |
| 9     | 14.985 | 279362  | 0.38  | 117789          | 0.51    | cis-Z.-alpha.-Bisabolene epoxide             |
| 10    | 15.055 | 554711  | 0.76  | 106580          | 0.47    | .gamma.-Gurjunenepoxide-(2)                  |
| 11    | 15.183 | 1054709 | 1.44  | 598791          | 2.62    | n-Hexadecanoic acid                          |
| 12    | 15.315 | 778204  | 1.06  | 153800          | 0.67    | cis-Z.-alpha.-Bisabolene epoxide             |
| 13    | 15.399 | 310938  | 0.42  | 124274          | 0.54    | 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,   |
| 14    | 15.445 | 200465  | 0.27  | 102066          | 0.45    | 3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-d  |
| 15    | 15.660 | 4459375 | 6.09  | 1341515         | 5.86    | trans-Sinapyl alcohol                        |
| 16    | 16.020 | 482132  | 0.66  | 327298          | 1.43    | Methyl 9-cis,11-trans-octadecadienoate       |
| 17    | 16.045 | 380665  | 0.52  | 309527          | 1.35    | 9-Octadecenoic acid (Z)-, methyl ester       |
| 18    | 16.154 | 501277  | 0.68  | 205622          | 0.90    | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8- |
| 19    | 17.135 | 478323  | 0.65  | 181091          | 0.79    | Reynosin                                     |
| 20    | 17.163 | 245375  | 0.34  | 201078          | 0.88    | Heneicosane                                  |
| 21    | 17.456 | 286415  | 0.39  | 110409          | 0.48    | Reynosin                                     |
| 22    | 17.725 | 328077  | 0.45  | 265220          | 1.16    | Heneicosane                                  |
| 23    | 18.016 | 567344  | 0.77  | 304260          | 1.33    | 2-Phenethyl-4H-chromen-4-one                 |
| 24    | 18.262 | 416337  | 0.57  | 294200          | 1.29    | Heneicosane                                  |
| 25    | 18.324 | 215778  | 0.29  | 141900          | 0.62    | Behenic alcohol                              |
| 26    | 18.575 | 237350  | 0.32  | 125305          | 0.55    | Di-n-octyl phthalate                         |
| 27    | 18.782 | 713062  | 0.97  | 489318          | 2.14    | 2-Methylhexacosane                           |
| 28    | 19.204 | 1407769 | 1.92  | 453458          | 1.98    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 29    | 19.324 | 978972  | 1.34  | 426201          | 1.86    | Nonacosane                                   |
| 30    | 19.454 | 508221  | 0.69  | 199632          | 0.87    | 6-Methoxy-2-phenethyl-4H-chromen-4-one       |
| 31    | 19.523 | 279246  | 0.38  | 136608          | 0.60    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one      |
| 32    | 19.871 | 7172945 | 9.79  | 2184031         | 9.55    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim    |
| 33    | 20.092 | 991442  | 1.35  | 560363          | 2.45    | Squalene                                     |
| 34    | 20.295 | 1957057 | 2.67  | 572936          | 2.50    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 35    | 20.565 | 710438  | 0.97  | 304167          | 1.33    | 2,5-Cyclohexadien-1-one, 2-methyl-4-[[[(2-)  |

| Peak# | R.Time | Area     | Area%  | Height   | Height% | Name                                        |
|-------|--------|----------|--------|----------|---------|---------------------------------------------|
| 36    | 20.607 | 355679   | 0.49   | 197833   | 0.86    | Heneicosane                                 |
| 37    | 20.926 | 701362   | 0.96   | 227669   | 1.00    | .alpha.-d-Glucose, 2-O-benzoyl-1-methylthio |
| 38    | 21.058 | 1567879  | 2.14   | 601426   | 2.63    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin     |
| 39    | 21.330 | 13476593 | 18.40  | 2702163  | 11.81   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c    |
| 40    | 21.817 | 2020820  | 2.76   | 417129   | 1.82    | 2-Pentenitrile, 5-(p-anisyl)-3-methyl-      |
| 41    | 22.091 | 1228539  | 1.68   | 430651   | 1.88    | 4H-1-Benzopyran-4-one, 5-hydroxy-7-metho    |
| 42    | 22.829 | 3685738  | 5.03   | 1022150  | 4.47    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim    |
| 43    | 22.933 | 853308   | 1.17   | 239639   | 1.05    | Cholesta-4,6-dien-3-ol, (3.beta.)-          |
| 44    | 23.452 | 610828   | 0.83   | 159832   | 0.70    | Dodecane, 1-bromo-                          |
| 45    | 23.572 | 1410985  | 1.93   | 364533   | 1.59    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
| 46    | 24.091 | 3796366  | 5.18   | 924481   | 4.04    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
| 47    | 25.381 | 746978   | 1.02   | 180277   | 0.79    | Stigmasterol                                |
| 48    | 26.176 | 1423988  | 1.94   | 236615   | 1.03    | Noscapine                                   |
| 49    | 26.400 | 2672007  | 3.65   | 476365   | 2.08    | .gamma.-Sitosterol                          |
| 50    | 27.710 | 1719860  | 2.35   | 315160   | 1.38    | 4,22-Stigmastadiene-3-one                   |
|       |        | 73233509 | 100.00 | 22881286 | 100.00  |                                             |

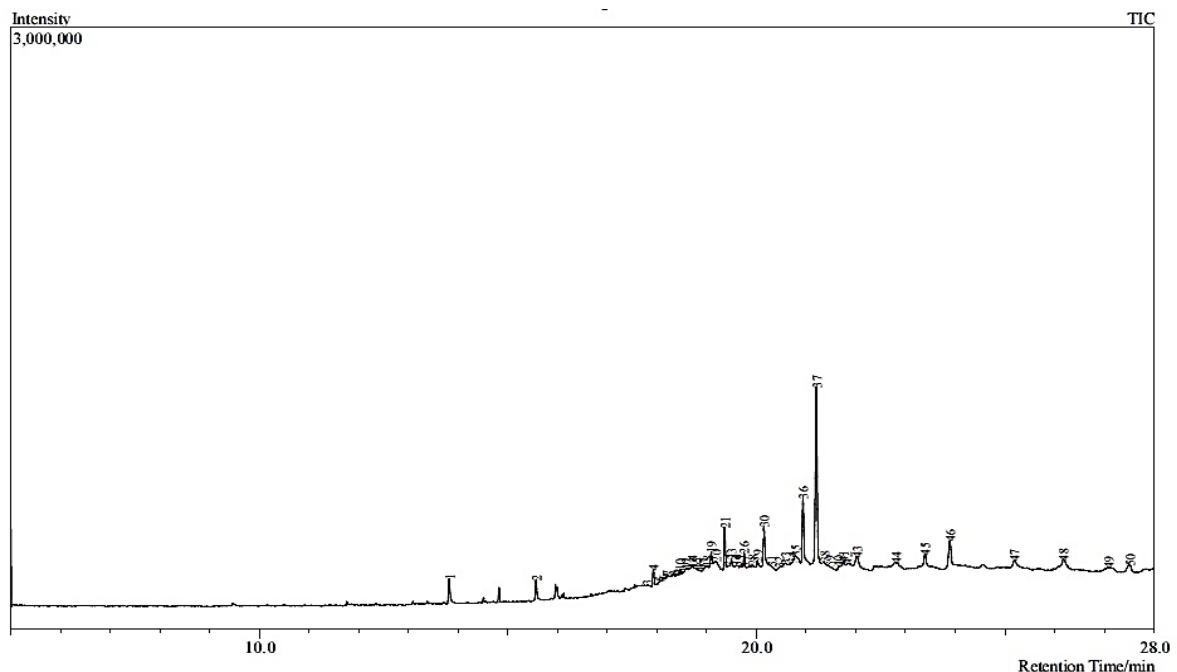
**Appendix H:** Total ion chromatography (TIC) for all sample inoculants and negative control for third month inoculation in dry seasons

#### H.1 Results for third month inoculation of F1 inoculant



| Peak Report TIC |        |          |        |         |         |                                              |
|-----------------|--------|----------|--------|---------|---------|----------------------------------------------|
| Peak#           | R.Time | Area     | Area%  | Height  | Height% | Name                                         |
| 1               | 13.817 | 811950   | 3.15   | 388321  | 5.52    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl    |
| 2               | 15.569 | 463832   | 1.80   | 254296  | 3.62    | trans-Sinapyl alcohol                        |
| 3               | 15.961 | 207200   | 0.80   | 105448  | 1.50    | 9,12-Octadecadienoic acid (Z,Z)-, methyl est |
| 4               | 17.929 | 259900   | 1.01   | 167760  | 2.39    | 2-Phenethyl-4H-chromen-4-one                 |
| 5               | 18.067 | 308226   | 1.20   | 63767   | 0.91    | Hexadecanal, 2-methyl-                       |
| 6               | 18.120 | 263883   | 1.02   | 54065   | 0.77    | Oxalic acid, 3,5-difluorophenyl nonyl ester  |
| 7               | 18.207 | 181294   | 0.70   | 66435   | 0.94    | 7-Methyl-Z-tetradecen-1-ol acetate           |
| 8               | 18.263 | 243338   | 0.94   | 62561   | 0.89    | 1-Heptacosanol                               |
| 9               | 18.413 | 350416   | 1.36   | 93237   | 1.33    | Docosanoic acid, methyl ester                |
| 10              | 18.468 | 504558   | 1.96   | 151132  | 2.15    | Hexacosanal                                  |
| 11              | 18.520 | 331878   | 1.29   | 128498  | 1.83    | Diisooctyl phthalate                         |
| 12              | 18.578 | 786877   | 3.05   | 139619  | 1.98    | Hexacontane                                  |
| 13              | 18.785 | 879172   | 3.41   | 118753  | 1.69    | Hexacontane                                  |
| 14              | 18.931 | 974856   | 3.78   | 129023  | 1.83    | Hexacontane                                  |
| 15              | 19.000 | 223533   | 0.87   | 89086   | 1.27    | Eicosen-1-ol, cis-9-                         |
| 16              | 19.097 | 930080   | 3.61   | 166255  | 2.36    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 17              | 19.180 | 348602   | 1.35   | 116806  | 1.66    | 1,2-15,16-Diepoxyhexadecane                  |
| 18              | 19.235 | 342389   | 1.33   | 117694  | 1.67    | Acetamide, N-(4-cyanomethylphenyl)-2-(4-n    |
| 19              | 19.265 | 498885   | 1.94   | 118749  | 1.69    | 2-Methylhexacosane                           |
| 20              | 19.363 | 773014   | 3.00   | 280711  | 3.99    | 6-Methoxy-2-phenethyl-4H-chromen-4-one       |
| 21              | 19.424 | 198960   | 0.77   | 83845   | 1.19    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one      |
| 22              | 19.502 | 525357   | 2.04   | 154725  | 2.20    | Octanoic acid, 8-(phenylmethoxy)-, methyl e  |
| 23              | 19.550 | 166328   | 0.65   | 93497   | 1.33    | 2-Nitro-1,3-bis-octyloxy-benzene             |
| 24              | 19.591 | 589026   | 2.29   | 121577  | 1.73    | Hexacontane                                  |
| 25              | 19.755 | 936404   | 3.64   | 180588  | 2.57    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim    |
| 26              | 19.865 | 159605   | 0.62   | 60768   | 0.86    | 2-Methylhexacosane                           |
| 27              | 19.919 | 227272   | 0.88   | 67544   | 0.96    | Decanedioic acid, bis(2-ethylhexyl) ester    |
| 28              | 20.029 | 585093   | 2.27   | 138545  | 1.97    | Squalene                                     |
| 29              | 20.080 | 332790   | 1.29   | 109239  | 1.55    | Hexacontane                                  |
| 30              | 20.157 | 1511701  | 5.87   | 308053  | 4.38    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-    |
| 31              | 20.435 | 298319   | 1.16   | 67247   | 0.96    | 1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentam   |
| 32              | 20.540 | 608954   | 2.36   | 91040   | 1.29    | Tetrapentacontane                            |
| 33              | 20.635 | 375243   | 1.46   | 72006   | 1.02    | Hexacontane                                  |
| 34              | 20.705 | 337139   | 1.31   | 90383   | 1.28    | 2-Chloropropionic acid, hexadecyl ester      |
| 35              | 20.790 | 441009   | 1.71   | 138134  | 1.96    | Benzene, 1-trifluoromethyl-4-(2-methoxyben   |
| Peak#           | R.Time | Area     | Area%  | Height  | Height% | Name                                         |
| 36              | 20.847 | 815898   | 3.17   | 174112  | 2.48    | Hexacosanoic acid, methyl ester              |
| 37              | 20.940 | 1491690  | 5.79   | 456238  | 6.49    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin      |
| 38              | 21.010 | 661512   | 2.57   | 141113  | 2.01    | Stigmastane-3,6-dione, (5.alpha.)-           |
| 39              | 21.210 | 2352655  | 9.13   | 871379  | 12.39   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c     |
| 40              | 21.653 | 267906   | 1.04   | 52839   | 0.75    | Quinazolin-4(3H)-one, 2-(4-methoxybenzyltl   |
| 41              | 21.751 | 220027   | 0.85   | 55896   | 0.79    | 9(11)-Dehydroergosteryl benzoate             |
| 42              | 22.026 | 383796   | 1.49   | 84157   | 1.20    | 5-Benzyloxypyrimidine-2-carboxylic acid      |
| 43              | 22.154 | 160051   | 0.62   | 22095   | 0.31    | Tetrapentacontane, 1,54-dibromo-             |
| 44              | 22.663 | 268318   | 1.04   | 61449   | 0.87    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim     |
| 45              | 23.399 | 356338   | 1.38   | 96340   | 1.37    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
| 46              | 23.664 | 350307   | 1.36   | 34800   | 0.49    | Tetrapentacontane, 1,54-dibromo-             |
| 47              | 23.898 | 848088   | 3.29   | 258885  | 3.68    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
| 48              | 24.567 | 169110   | 0.66   | 38347   | 0.55    | Ergosterol                                   |
| 49              | 25.199 | 154553   | 0.60   | 41262   | 0.59    | 5.alpha.-Pregn-9(11)-en-12-one               |
| 50              | 27.493 | 281883   | 1.09   | 55422   | 0.79    | 4,22-Cholestadien-3-one                      |
|                 |        | 25759215 | 100.00 | 7033741 | 100.00  |                                              |

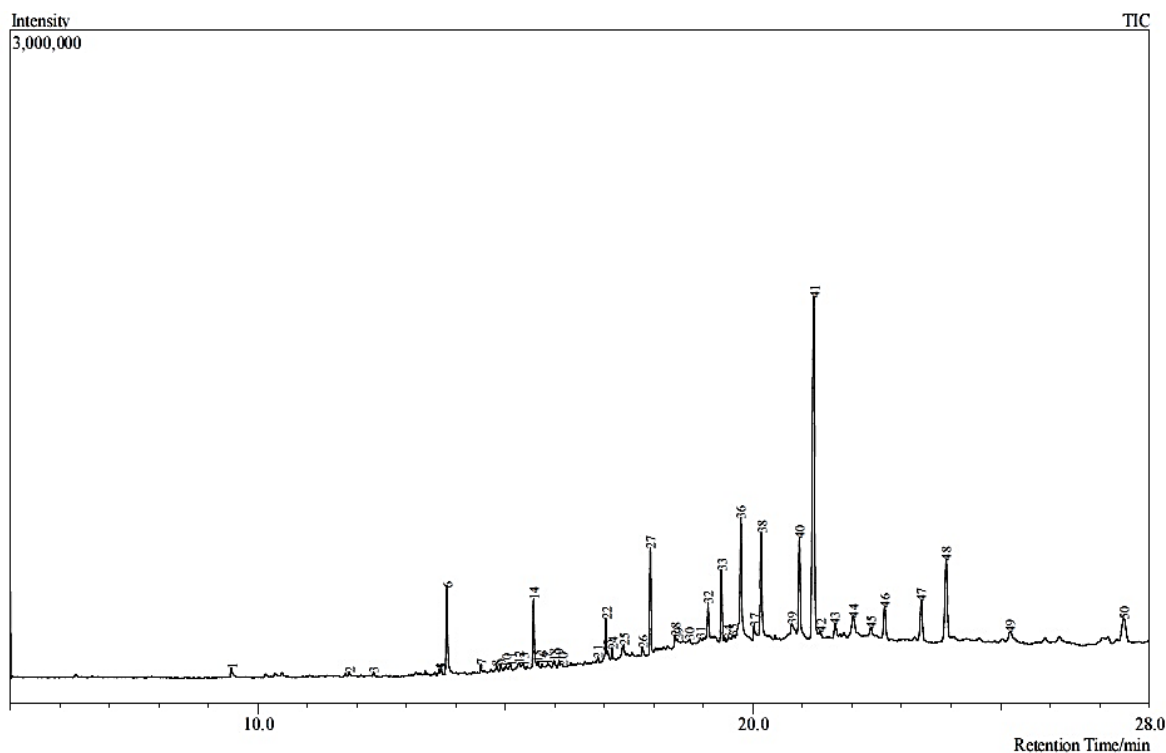
## H.2 Results for third month inoculation of F2 inoculant



| Peak Report TIC |         |         |       |        |         |                                               |
|-----------------|---------|---------|-------|--------|---------|-----------------------------------------------|
| Peak#           | R. Time | Area    | Area% | Height | Height% | Name                                          |
| 1               | 13.816  | 288703  | 1.35  | 129329 | 2.41    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl     |
| 2               | 15.566  | 222420  | 1.04  | 108637 | 2.02    | trans-Sinapyl alcohol                         |
| 3               | 17.810  | 284275  | 1.33  | 21469  | 0.40    | 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl- |
| 4               | 17.928  | 231339  | 1.08  | 99294  | 1.85    | 2-Phenethyl-4H-chromen-4-one                  |
| 5               | 18.060  | 219787  | 1.03  | 38070  | 0.71    | 3-Cyclopentylpropionic acid, 2-dimethylamir   |
| 6               | 18.130  | 208680  | 0.97  | 49387  | 0.92    | Oxalic acid, 3,5-difluorophenyl nonyl ester   |
| 7               | 18.212  | 265453  | 1.24  | 66364  | 1.24    | 7-Hexadecenal, (Z)-                           |
| 8               | 18.329  | 437111  | 2.04  | 59414  | 1.11    | 2,6,10,14-Tetramethyl-7-(3-methylpent-4-en    |
| 9               | 18.408  | 190823  | 0.89  | 64013  | 1.19    | Docosanoic acid, methyl ester                 |
| 10              | 18.465  | 252560  | 1.18  | 85766  | 1.60    | Eicosanal-                                    |
| 11              | 18.518  | 182654  | 0.85  | 71140  | 1.33    | Di-n-octyl phthalate                          |
| 12              | 18.590  | 364191  | 1.70  | 86964  | 1.62    | Tetrapentacontane, 1,54-dibromo-              |
| 13              | 18.628  | 182562  | 0.85  | 89989  | 1.68    | E-8-Methyl-9-tetradecen-1-ol acetate          |
| 14              | 18.713  | 590933  | 2.76  | 94746  | 1.77    | 7-Hexadecenal, (Z)-                           |
| 15              | 18.780  | 272308  | 1.27  | 85752  | 1.60    | Tetrapentacontane, 1,54-dibromo-              |
| 16              | 18.875  | 192538  | 0.90  | 70378  | 1.31    | Nonadecyl pentafluoropropionate               |
| 17              | 18.940  | 334579  | 1.56  | 80996  | 1.51    | 2-Propenoic acid, 3-(1-acetyl-2,2-dimethyle   |
| 18              | 18.999  | 445526  | 2.08  | 93537  | 1.74    | Tetrapentacontane, 1,54-dibromo-              |
| 19              | 19.093  | 605086  | 2.82  | 161352 | 3.01    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-     |
| 20              | 19.175  | 956376  | 4.46  | 114022 | 2.12    | Tetrapentacontane, 1,54-dibromo-              |
| 21              | 19.362  | 672864  | 3.14  | 282246 | 5.26    | 6-Methoxy-2-phenethyl-4H-chromen-4-one        |
| 22              | 19.420  | 272431  | 1.27  | 85193  | 1.59    | 3-Allyloxy-6-(4-methoxy-benzyloxy)-cycloh     |
| 23              | 19.497  | 587377  | 2.74  | 115041 | 2.14    | Octanoic acid, 8-(phenylmethoxy)-, methyl e   |
| 24              | 19.601  | 393970  | 1.84  | 81242  | 1.51    | Tetrapentacontane, 1,54-dibromo-              |
| 25              | 19.671  | 177035  | 0.83  | 76651  | 1.43    | 6-Methoxy-2-phenethyl-4H-chromen-4-one        |
| 26              | 19.753  | 422630  | 1.97  | 144779 | 2.70    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim     |
| 27              | 19.825  | 447059  | 2.09  | 67844  | 1.26    | erythro-7,8-Bromochlorodisparlure             |
| 28              | 19.924  | 215079  | 1.00  | 77926  | 1.45    | Decanedioic acid, bis(2-ethylhexyl) ester     |
| 29              | 20.025  | 562400  | 2.63  | 95744  | 1.78    | Squalene                                      |
| 30              | 20.152  | 1199913 | 5.60  | 271099 | 5.05    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-     |
| 31              | 20.305  | 203357  | 0.95  | 62863  | 1.17    | erythro-7,8-Bromochlorodisparlure             |
| 32              | 20.465  | 248241  | 1.16  | 53219  | 0.99    | 2,6,10,14-Tetramethyl-7-(3-methylpent-4-en    |
| 33              | 20.590  | 306674  | 1.43  | 69516  | 1.30    | Tetrapentacontane, 1,54-dibromo-              |
| 34              | 20.715  | 205802  | 0.96  | 71036  | 1.32    | 9-Octadecenoic acid, methyl ester, (E)-       |
| 35              | 20.782  | 827429  | 3.86  | 104011 | 1.94    | Oxalic acid, hexyl 2-isopropylphenyl ester    |

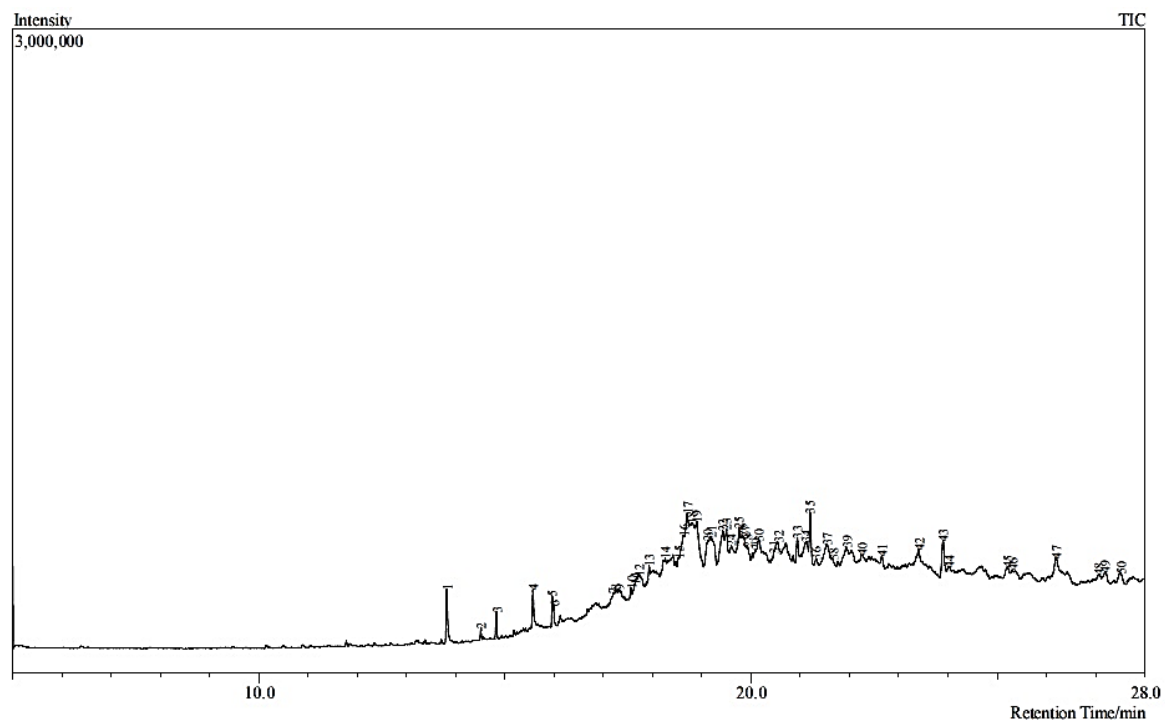
| Peak# | R. Time | Area     | Area%  | Height  | Height% | Name                                        |
|-------|---------|----------|--------|---------|---------|---------------------------------------------|
| 36    | 20.937  | 1506242  | 7.03   | 399347  | 7.44    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin     |
| 37    | 21.207  | 2925736  | 13.66  | 965523  | 17.99   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c    |
| 38    | 21.359  | 349764   | 1.63   | 59580   | 1.11    | 1,5-Di(4-formyl-phenoxy)-3-methyl-pentane   |
| 39    | 21.465  | 189084   | 0.88   | 36443   | 0.68    | Tetrapentacontane, 1,54-dibromo-            |
| 40    | 21.655  | 169858   | 0.79   | 28583   | 0.53    | Quinazolin-4(3H)-one, 2-(4-methoxybenzyl)   |
| 41    | 21.753  | 182380   | 0.85   | 51890   | 0.97    | Ergosta-5,7,9(11),22-tetraen-3-ol, (3.β.,22 |
| 42    | 21.832  | 289960   | 1.35   | 41271   | 0.77    | Docosyl ethyl carbonate                     |
| 43    | 22.019  | 431679   | 2.02   | 70819   | 1.32    | 5-Benzoyloxypyrimidine-2-carboxylic acid    |
| 44    | 22.796  | 169491   | 0.79   | 32089   | 0.60    | Tetrapentacontane, 1,54-dibromo-            |
| 45    | 23.393  | 221132   | 1.03   | 66790   | 1.24    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
| 46    | 23.892  | 454066   | 2.12   | 131169  | 2.44    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
| 47    | 25.195  | 224842   | 1.05   | 40856   | 0.76    | Cholesta-22,24-dien-5-ol, 4,4-dimethyl-     |
| 48    | 26.180  | 452423   | 2.11   | 54132   | 1.01    | .gamma.-Sitosterol                          |
| 49    | 27.089  | 185962   | 0.87   | 20476   | 0.38    | 7-Hexadecenal, (Z)-                         |
| 50    | 27.503  | 199873   | 0.93   | 38027   | 0.71    | Ergosta-4,6,8(14),22-tetraen-3-one          |
|       |         | 21422657 | 100.00 | 5366024 | 100.00  |                                             |

### H.3 Results for third month inoculation of F3 inoculant



| Peak Report TIC |        |          |        |         |                                                     |
|-----------------|--------|----------|--------|---------|-----------------------------------------------------|
| Peak#           | R.Time | Area     | Area%  | Height  | Height% Name                                        |
| 1               | 9.460  | 90444    | 0.50   | 41658   | 0.60 2-Butanone, 4-phenyl-                          |
| 2               | 11.846 | 35069    | 0.19   | 19648   | 0.28 2-Butanone, 4-(4-methoxyphenyl)-               |
| 3               | 12.338 | 35398    | 0.20   | 19928   | 0.29 3',5'-Dimethoxyacetophenone                    |
| 4               | 13.659 | 39469    | 0.22   | 23448   | 0.34 Farnesene epoxide, E-                          |
| 5               | 13.700 | 48139    | 0.27   | 30793   | 0.45 Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-  |
| 6               | 13.814 | 791426   | 4.36   | 402396  | 5.82 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl      |
| 7               | 14.507 | 61567    | 0.34   | 37294   | 0.54 Dibutyl phthalate                              |
| 8               | 14.822 | 40792    | 0.22   | 22560   | 0.33 Hexadecanoic acid, methyl ester                |
| 9               | 14.909 | 64796    | 0.36   | 32297   | 0.47 cis-Z-.alpha.-Bisabolene epoxide               |
| 10              | 15.012 | 61388    | 0.34   | 20376   | 0.29 Aromadendrene oxide-(1)                        |
| 11              | 15.103 | 52983    | 0.29   | 22273   | 0.32 1-(+)-Ascorbic acid 2,6-dihexadecanoate        |
| 12              | 15.275 | 71492    | 0.39   | 25539   | 0.37 (2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-me   |
| 13              | 15.370 | 78194    | 0.43   | 19312   | 0.28 7-Hexadecenal, (Z)-                            |
| 14              | 15.567 | 595523   | 3.28   | 314458  | 4.55 trans-Sinapyl alcohol                          |
| 15              | 15.659 | 57900    | 0.32   | 29612   | 0.43 Dihydro-isosteviol methyl ester                |
| 16              | 15.740 | 37824    | 0.21   | 20096   | 0.29 Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-     |
| 17              | 15.850 | 75301    | 0.42   | 17271   | 0.25 10-12-Pentacosadiynoic acid                    |
| 18              | 15.985 | 45433    | 0.25   | 30559   | 0.44 9-Octadecenoic acid, methyl ester, (E)-        |
| 19              | 16.080 | 64468    | 0.36   | 30710   | 0.44 Valerenol                                      |
| 20              | 16.155 | 36346    | 0.20   | 10326   | 0.15 .gamma.-Gurjunenepoxide-(2)                    |
| 21              | 16.868 | 40487    | 0.22   | 24663   | 0.36 Cycloheptane, 4-methylene-1-methyl-2-(2-m      |
| 22              | 17.027 | 314204   | 1.73   | 186892  | 2.70 1,2,4-Methenoazulene, decahydro-1,5,5,8a-t     |
| 23              | 17.069 | 60817    | 0.34   | 36646   | 0.53 5,8,11-Eicosatriynoic acid, tert-butylidimethy |
| 24              | 17.150 | 77733    | 0.43   | 52574   | 0.76 Epiglobulol                                    |
| 25              | 17.390 | 135334   | 0.75   | 46489   | 0.67 (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,8   |
| 26              | 17.765 | 84947    | 0.47   | 38999   | 0.56 Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a     |
| 27              | 17.927 | 749521   | 4.13   | 486263  | 7.03 2-Phenethyl-4H-chromen-4-one                   |
| 28              | 18.429 | 100988   | 0.56   | 51459   | 0.74 2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,      |
| 29              | 18.480 | 39692    | 0.22   | 17440   | 0.25 Tricyclo[7.2.2.0(3,8)]trideca-5,12-dien-2-one  |
| 30              | 18.712 | 35135    | 0.19   | 15478   | 0.22 Tetrapentacotane, 1,54-dibromo-                |
| 31              | 18.930 | 69591    | 0.38   | 15588   | 0.23 Silane, dimethyl(2-methylphenoxy)heptyloxy     |
| 32              | 19.094 | 334752   | 1.84   | 164968  | 2.39 Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 33              | 19.361 | 579925   | 3.20   | 333373  | 4.82 6-Methoxy-2-phenethyl-4H-chromen-4-one         |
| 34              | 19.495 | 54770    | 0.30   | 15833   | 0.23 Tetracosanoic acid, methyl ester               |
| 35              | 19.590 | 49791    | 0.27   | 17283   | 0.25 1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl-     |
| Peak#           | R.Time | Area     | Area%  | Height  | Height% Name                                        |
| 36              | 19.755 | 1425428  | 7.86   | 546775  | 7.91 Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 37              | 20.024 | 140552   | 0.77   | 64944   | 0.94 Squalene                                       |
| 38              | 20.164 | 1232031  | 6.79   | 484377  | 7.00 Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 39              | 20.785 | 247611   | 1.36   | 53402   | 0.77 Benzene, 1-trifluoromethyl-4-(2-methoxyben     |
| 40              | 20.937 | 1181822  | 6.51   | 458906  | 6.63 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin        |
| 41              | 21.228 | 5052706  | 27.85  | 1573542 | 22.75 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c      |
| 42              | 21.356 | 98584    | 0.54   | 32499   | 0.47 benzenesulfonyl fluoride, 3-[(2-hydroxybenz    |
| 43              | 21.658 | 202194   | 1.11   | 66090   | 0.96 Benzene, (1-methoxy-4-methyl-3-pentenyl)-      |
| 44              | 22.027 | 313008   | 1.73   | 80467   | 1.16 1H-Pyrimidine-2,4-dione, 1-benzyloxymethy      |
| 45              | 22.376 | 95249    | 0.52   | 34961   | 0.51 Benzene, 1-chloro-4-(4-methoxybenzyloxy)-      |
| 46              | 22.659 | 451899   | 2.49   | 149855  | 2.17 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim       |
| 47              | 23.398 | 587213   | 3.24   | 185726  | 2.69 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 48              | 23.902 | 1270562  | 7.00   | 368598  | 5.33 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 49              | 25.194 | 145290   | 0.80   | 36869   | 0.53 5.alpha.-Pregn-9(11)-en-12-one                 |
| 50              | 27.490 | 588152   | 3.24   | 105236  | 1.52 4,22-Stigmastadiene-3-one                      |
|                 |        | 18143940 | 100.00 | 6916749 | 100.00                                              |

#### H.4 Results for third month inoculation of negative control

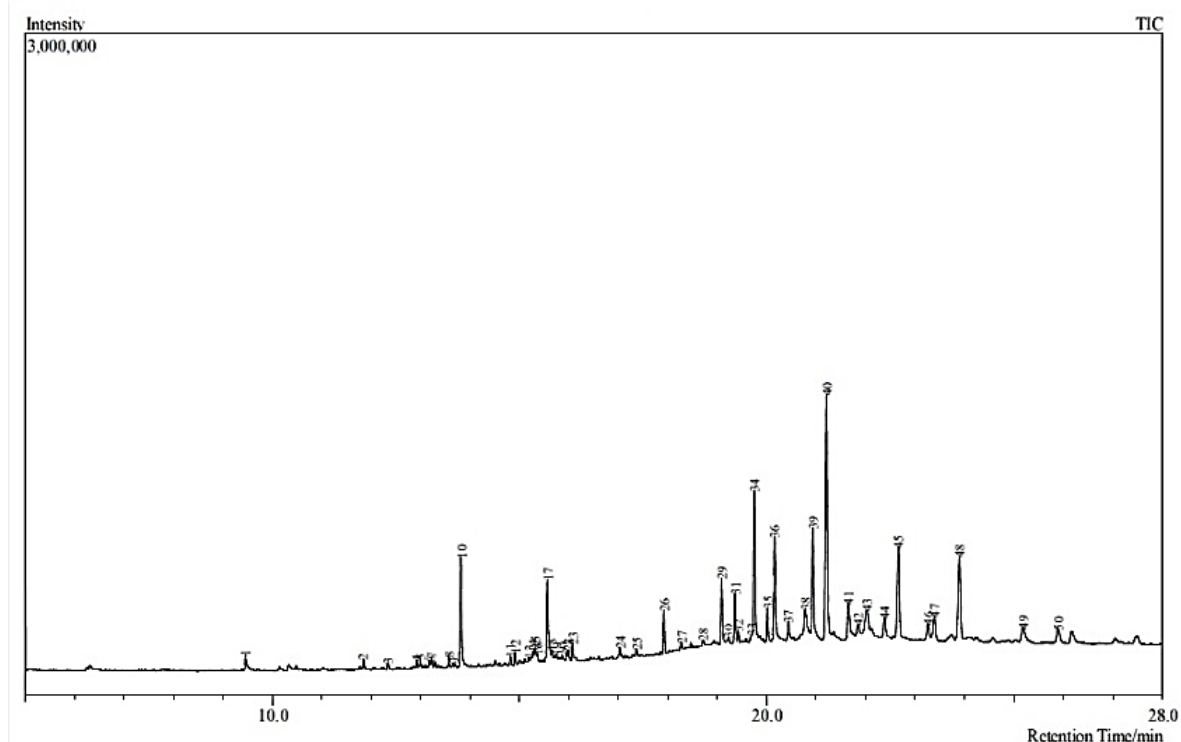


| Peak Report TIC |        |         |       |        |         |                                                |
|-----------------|--------|---------|-------|--------|---------|------------------------------------------------|
| Peak#           | R.Time | Area    | Area% | Height | Height% | Name                                           |
| 1               | 13.817 | 536269  | 2.83  | 259545 | 5.27    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl      |
| 2               | 14.514 | 79816   | 0.42  | 59144  | 1.20    | 1,2-Benzenedicarboxylic acid, bis(2-methylp    |
| 3               | 14.824 | 182984  | 0.96  | 130476 | 2.65    | Hexadecanoic acid, methyl ester                |
| 4               | 15.568 | 345153  | 1.82  | 178543 | 3.62    | trans-Sinapyl alcohol                          |
| 5               | 15.962 | 200646  | 1.06  | 148278 | 3.01    | Methyl 10-trans,12-cis-octadecadienoate        |
| 6               | 15.990 | 129122  | 0.68  | 101274 | 2.06    | 9-Octadecenoic acid (Z)-, methyl ester         |
| 7               | 17.200 | 161809  | 0.85  | 54073  | 1.10    | 2-Hexyldecyl acetate                           |
| 8               | 17.245 | 249086  | 1.31  | 68866  | 1.40    | Tetrapentacontane, 1,54-dibromo-               |
| 9               | 17.317 | 274291  | 1.45  | 66187  | 1.34    | Methyl 8,10-dimethyl-hexadecanoate or 8,10     |
| 10              | 17.558 | 90086   | 0.47  | 51669  | 1.05    | 4,8,12,16-Tetramethylheptadecan-4-olide        |
| 11              | 17.640 | 155170  | 0.82  | 52944  | 1.07    | Tetracontane-1,40-diol                         |
| 12              | 17.716 | 552418  | 2.91  | 76537  | 1.55    | Tetrapentacontane, 1,54-dibromo-               |
| 13              | 17.930 | 85077   | 0.45  | 56333  | 1.14    | Benzeneacetic acid, 10-undecenyl ester         |
| 14              | 18.256 | 206713  | 1.09  | 39014  | 0.79    | Tetrapentacontane, 1,54-dibromo-               |
| 15              | 18.520 | 84186   | 0.44  | 45498  | 0.92    | Di-n-octyl phthalate                           |
| 16              | 18.625 | 719460  | 3.79  | 150389 | 3.05    | Tetrapentacontane                              |
| 17              | 18.699 | 1201297 | 6.33  | 259033 | 5.26    | Tetrapentacontane                              |
| 18              | 18.795 | 1680087 | 8.86  | 210598 | 4.28    | 2-Methylhexacosane                             |
| 19              | 18.898 | 775279  | 4.09  | 215666 | 4.38    | Triphenylphosphine oxide                       |
| 20              | 19.120 | 491318  | 2.59  | 122772 | 2.49    | Docosyl ethyl carbonate                        |
| 21              | 19.175 | 942542  | 4.97  | 141197 | 2.87    | Tetrapentacontane, 1,54-dibromo-               |
| 22              | 19.426 | 849050  | 4.48  | 161283 | 3.27    | Tetrapentacontane, 1,54-dibromo-               |
| 23              | 19.499 | 584660  | 3.08  | 166689 | 3.38    | Tetracosanoic acid, methyl ester               |
| 24              | 19.602 | 413005  | 2.18  | 83803  | 1.70    | Hexacontane                                    |
| 25              | 19.756 | 714637  | 3.77  | 164490 | 3.34    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 26              | 19.830 | 464227  | 2.45  | 127233 | 2.58    | Tetracontane-1,40-diol                         |
| 27              | 19.860 | 244888  | 1.29  | 116744 | 2.37    | Dotriacontane                                  |
| 28              | 19.930 | 263755  | 1.39  | 72405  | 1.47    | Carbonic acid, 2-ethylhexyl heptadecyl ester   |
| 29              | 20.085 | 93184   | 0.49  | 35334  | 0.72    | 1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentam     |
| 30              | 20.155 | 264689  | 1.40  | 64178  | 1.30    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 31              | 20.465 | 91077   | 0.48  | 37429  | 0.76    | erythro-7,8-Bromochlorodisparlure              |
| 32              | 20.541 | 334383  | 1.76  | 71311  | 1.45    | Tetrapentacontane                              |
| 33              | 20.941 | 334379  | 1.76  | 123450 | 2.51    | 1-Phenyl-2-[5-thia-6,9-diazatriecyclo[7.3.0.0( |
| 34              | 21.107 | 747687  | 3.94  | 106726 | 2.17    | Tetrapentacontane, 1,54-dibromo-               |
| 35              | 21.201 | 637958  | 3.36  | 251125 | 5.10    | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c       |

| Peak# | R.Time | Area     | Area%  | Height  | Height% | Name                                         |
|-------|--------|----------|--------|---------|---------|----------------------------------------------|
| 36    | 21.324 | 87396    | 0.46   | 31223   | 0.63    | Tetrapentacontane                            |
| 37    | 21.535 | 725187   | 3.82   | 103144  | 2.09    | Tetrapentacontane, 1,54-dibromo-             |
| 38    | 21.660 | 87612    | 0.46   | 33049   | 0.67    | Quinazolin-4(3H)-one, 2-(4-methoxybenzyl)    |
| 39    | 21.933 | 282553   | 1.49   | 51630   | 1.05    | Oxalic acid, isohexyl octadecyl ester        |
| 40    | 22.240 | 96137    | 0.51   | 26284   | 0.53    | Hexacontane                                  |
| 41    | 22.660 | 125193   | 0.66   | 47707   | 0.97    | Hydrastine                                   |
| 42    | 23.407 | 198117   | 1.04   | 67412   | 1.37    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
| 43    | 23.901 | 620719   | 3.27   | 170327  | 3.46    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-     |
| 44    | 24.017 | 115979   | 0.61   | 30687   | 0.62    | erythro-7,8-Bromochlorodisparlure            |
| 45    | 25.209 | 193133   | 1.02   | 46676   | 0.95    | 1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2 |
| 46    | 25.304 | 293560   | 1.55   | 37721   | 0.77    | Tetrapentacontane, 1,54-dibromo-             |
| 47    | 26.192 | 333831   | 1.76   | 79079   | 1.61    | .gamma.-Sitosterol                           |
| 48    | 27.055 | 198676   | 1.05   | 30402   | 0.62    | 17-Pentatriacontene                          |
| 49    | 27.186 | 186192   | 0.98   | 51259   | 1.04    | Cholestan-3-one                              |
| 50    | 27.509 | 243049   | 1.28   | 48708   | 0.99    | Spinasterone                                 |
|       |        | 18967722 | 100.00 | 4925544 | 100.00  |                                              |

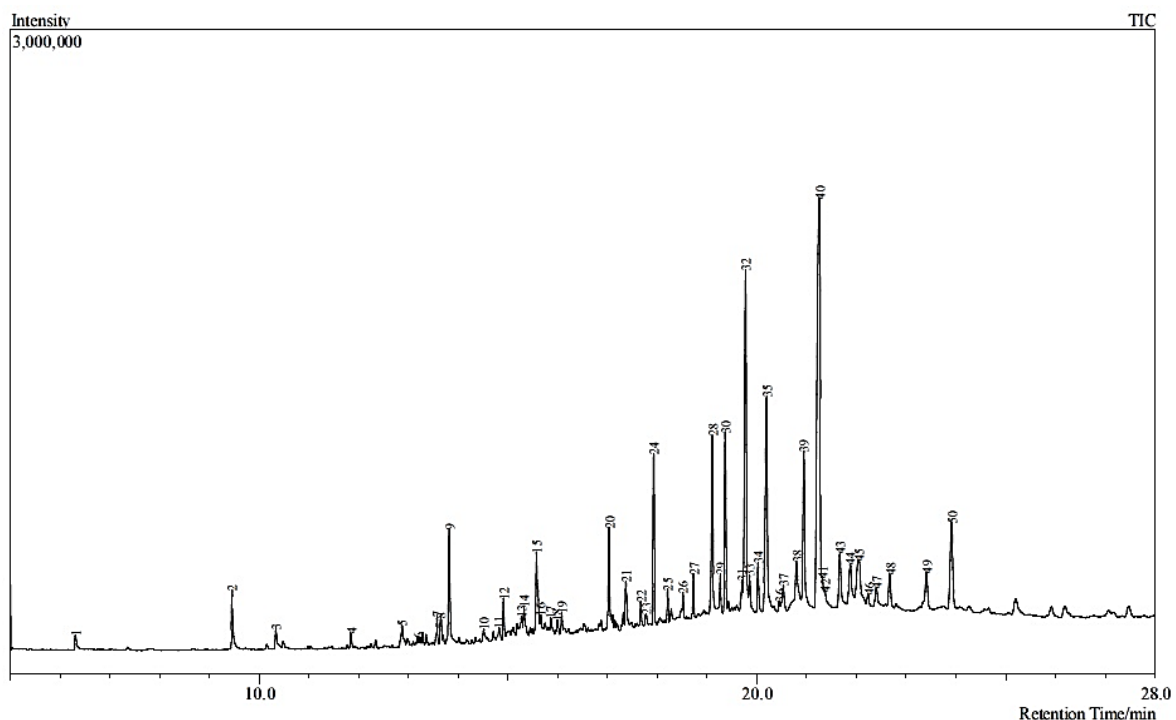
**Appendix I:** Total ion chromatography (TIC) for all sample inoculants and negative control for sixth month inoculation in dry seasons

#### I.1 Results for sixth month inoculation of F1 inoculant



| Peak Report TIC |        |         |       |         |         |                                                                   |
|-----------------|--------|---------|-------|---------|---------|-------------------------------------------------------------------|
| Peak#           | R.Time | Area    | Area% | Height  | Height% | Name                                                              |
| 1               | 9.455  | 155342  | 0.84  | 68397   | 0.91    | 2-Butanone, 4-phenyl-                                             |
| 2               | 11.839 | 86564   | 0.47  | 50125   | 0.67    | 2-Butanone, 4-(4-methoxyphenyl)-                                  |
| 3               | 12.334 | 66822   | 0.36  | 34311   | 0.46    | 3-tert-Butyl-4-hydroxyanisole                                     |
| 4               | 12.921 | 48874   | 0.27  | 35232   | 0.47    | 2-((2S,4aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,7-c                      |
| 5               | 12.994 | 61912   | 0.34  | 40578   | 0.54    | Agarospirol                                                       |
| 6               | 13.180 | 43675   | 0.24  | 22815   | 0.31    | Benzaldehyde, 4-hydroxy-3,5-dimethoxy-                            |
| 7               | 13.223 | 59379   | 0.32  | 43597   | 0.58    | 3-Hydroxy- $\alpha$ -ionene                                       |
| 8               | 13.573 | 76091   | 0.41  | 48720   | 0.65    | Humulene                                                          |
| 9               | 13.660 | 26101   | 0.14  | 15646   | 0.21    | Cyclobutene, 4,4-dimethyl-1-(2,7-octadienyl)-                     |
| 10              | 13.810 | 952128  | 5.17  | 510290  | 6.82    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyph                         |
| 11              | 14.818 | 64469   | 0.35  | 43911   | 0.59    | Hexadecanoic acid, methyl ester                                   |
| 12              | 14.907 | 92341   | 0.50  | 57327   | 0.77    | 10,12-Tricosadiynoic acid, methyl ester                           |
| 13              | 15.180 | 111358  | 0.60  | 24176   | 0.32    | 1,3-Cyclohexadiene, 1,3,5,5,6,6-hexamethyl-                       |
| 14              | 15.275 | 98272   | 0.53  | 49553   | 0.66    | (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-di                         |
| 15              | 15.313 | 147088  | 0.80  | 67837   | 0.91    | Ethyl iso-allocholate                                             |
| 16              | 15.365 | 67175   | 0.36  | 39160   | 0.52    | 3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-d                       |
| 17              | 15.564 | 887502  | 4.82  | 384214  | 5.14    | trans-Sinapyl alcohol                                             |
| 18              | 15.658 | 108187  | 0.59  | 52518   | 0.70    | 3,7-Cyclodecadiene-1-methanol, $\alpha$ , $\alpha$ , $\alpha$ -tr |
| 19              | 15.744 | 115904  | 0.63  | 35557   | 0.48    | 10-12-Pentacosadiynoic acid                                       |
| 20              | 15.858 | 112457  | 0.61  | 30707   | 0.41    | 4,7,10,13,16,19-Docosahexaenoic acid, meth                        |
| 21              | 15.955 | 71876   | 0.39  | 40351   | 0.54    | Methyl 10-trans,12-cis-octadecadienoate                           |
| 22              | 15.985 | 89214   | 0.48  | 51513   | 0.69    | 9-Octadecenoic acid (Z)-, methyl ester                            |
| 23              | 16.075 | 105366  | 0.57  | 74341   | 0.99    | 1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-oct                       |
| 24              | 17.023 | 57180   | 0.31  | 40699   | 0.54    | 1,2,4-Methenoazulene, decahydro-1,5,5,8a-t                        |
| 25              | 17.363 | 75958   | 0.41  | 35905   | 0.48    | Longipinocarveol, trans-                                          |
| 26              | 17.923 | 302077  | 1.64  | 201283  | 2.69    | 2-Phenethyl-4H-chromen-4-one                                      |
| 27              | 18.268 | 82349   | 0.45  | 32639   | 0.44    | Docosyl ethyl carbonate                                           |
| 28              | 18.715 | 66049   | 0.36  | 26369   | 0.35    | Enic acid                                                         |
| 29              | 19.092 | 618398  | 3.36  | 308857  | 4.13    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-                         |
| 30              | 19.223 | 98666   | 0.54  | 30838   | 0.41    | Acetamide, N-(4-cyanomethylphenyl)-2-(4-n                         |
| 31              | 19.358 | 396597  | 2.15  | 231256  | 3.09    | 6-Methoxy-2-phenethyl-4H-chromen-4-one                            |
| 32              | 19.422 | 89182   | 0.48  | 51844   | 0.69    | 2-(4-Methoxyphenethyl)-4H-chromen-4-one                           |
| 33              | 19.695 | 87328   | 0.47  | 28891   | 0.39    | Benzothioephene-3-carbonitrile, 4,5,6,7-tetra                     |
| 34              | 19.753 | 1493245 | 8.10  | 696279  | 9.31    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim                         |
| 35              | 20.019 | 270145  | 1.47  | 153479  | 2.05    | Squalene                                                          |
| Peak#           | R.Time | Area    | Area% | Height  | Height% | Name                                                              |
| 36              | 20.160 | 1109008 | 6.02  | 475754  | 6.36    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-                         |
| 37              | 20.440 | 197894  | 1.07  | 84280   | 1.13    | p-Acetamidobenzaldehyde 6-methylpyridine,                         |
| 38              | 20.780 | 438118  | 2.38  | 116916  | 1.56    | 2-Methoxybenzyl alcohol, 1-methylpropyl et                        |
| 39              | 20.935 | 1289380 | 7.00  | 509376  | 6.81    | 1-Phenyl-2-[5-thia-6,9-diazatricyclo[7.3.0.0(                     |
| 40              | 21.213 | 3186085 | 17.29 | 1139791 | 15.24   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c                          |
| 41              | 21.655 | 499446  | 2.71  | 157144  | 2.10    | Quinazolin-4(3H)-one, 2-(4-methoxybenzyl)                         |
| 42              | 21.847 | 162289  | 0.88  | 48991   | 0.65    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim                         |
| 43              | 22.022 | 411448  | 2.23  | 96903   | 1.30    | 1H-Pyrimidine-2,4-dione, 1-benzyloxymethy                         |
| 44              | 22.385 | 261719  | 1.42  | 88388   | 1.18    | Benzene, 1-chloro-4-(4-methoxybenzyloxy)-                         |
| 45              | 22.666 | 1276650 | 6.93  | 421765  | 5.64    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim                          |
| 46              | 23.264 | 197020  | 1.07  | 67887   | 0.91    | 4H-1,2,4-Triazole-3-thiol, 5-(3-dimethylamir                      |
| 47              | 23.392 | 351702  | 1.91  | 108098  | 1.45    | 6,7-Dimethoxy-2-(4-methoxyphen                                    |

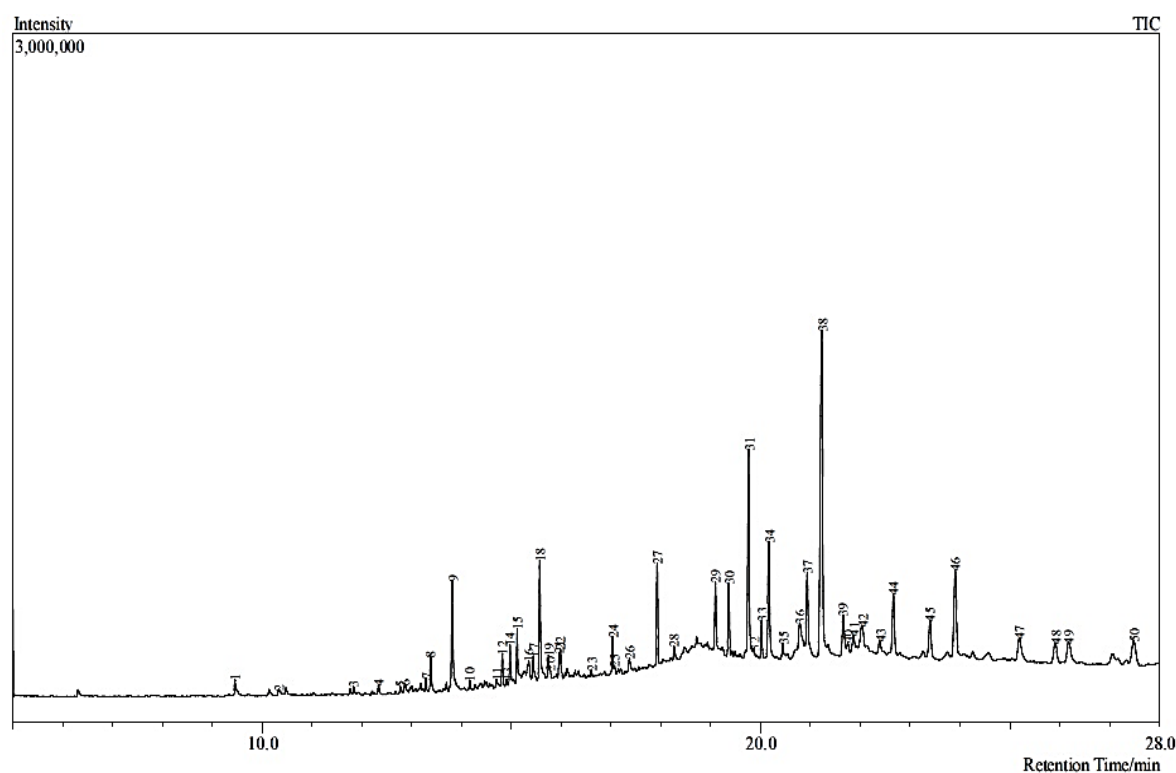
## I.2 Results for sixth month inoculation of F2 inoculant



| Peak# | R.Time | Area    | Area% | Height  | Height% | Name                                          |
|-------|--------|---------|-------|---------|---------|-----------------------------------------------|
| 1     | 6.309  | 118782  | 0.31  | 65111   | 0.44    | Benzaldehyde                                  |
| 2     | 9.455  | 566231  | 1.49  | 282057  | 1.93    | 2-Butanone, 4-phenyl-                         |
| 3     | 10.337 | 195194  | 0.51  | 93922   | 0.64    | Hydrocinnamic acid                            |
| 4     | 11.841 | 122744  | 0.32  | 72365   | 0.49    | 2-Butanone, 4-(4-methoxyphenyl)-              |
| 5     | 12.869 | 196757  | 0.52  | 80385   | 0.55    | Ethyl alpha.-d-glucopyranoside                |
| 6     | 13.180 | 107933  | 0.28  | 29994   | 0.20    | 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,    |
| 7     | 13.575 | 259933  | 0.68  | 139052  | 0.95    | Humulene                                      |
| 8     | 13.653 | 315775  | 0.83  | 130779  | 0.89    | Alloaromadendrene oxide-(2)                   |
| 9     | 13.815 | 1055602 | 2.77  | 541833  | 3.70    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxypl     |
| 10    | 14.514 | 121039  | 0.32  | 54393   | 0.37    | Butyl 6,9,12,15-octadecatetraenoate           |
| 11    | 14.821 | 156288  | 0.41  | 58849   | 0.40    | Hexadecanoic acid, methyl ester               |
| 12    | 14.910 | 310920  | 0.82  | 191983  | 1.31    | cis-Z.-alpha.-Bisabolene epoxide              |
| 13    | 15.273 | 297871  | 0.78  | 90096   | 0.62    | Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl |
| 14    | 15.318 | 315718  | 0.83  | 139982  | 0.96    | 4aH-cycloprop[e]azulen-4a-ol, decahydro-1,    |
| 15    | 15.571 | 1032410 | 2.71  | 383632  | 2.62    | trans-Sinapyl alcohol                         |
| 16    | 15.658 | 128080  | 0.34  | 71157   | 0.49    | Ledol                                         |
| 17    | 15.860 | 111850  | 0.29  | 59702   | 0.41    | 4,7,10,13,16,19-Docosahexanoic acid, meth     |
| 18    | 15.989 | 135845  | 0.36  | 64924   | 0.44    | 9,12-Octadecadienoyl chloride, (Z,Z)-         |
| 19    | 16.078 | 140665  | 0.37  | 91435   | 0.62    | .beta.-Longipinene                            |
| 20    | 17.029 | 928020  | 2.44  | 489678  | 3.35    | 1,2,4-Methenoazulene, decahydro-1,5,5,8a-t    |
| 21    | 17.368 | 484938  | 1.27  | 211148  | 1.44    | Andrographolide                               |
| 22    | 17.668 | 141885  | 0.37  | 114732  | 0.78    | Eicosane                                      |
| 23    | 17.769 | 151314  | 0.40  | 56583   | 0.39    | 4-Hydroxy-.beta.-ionone                       |
| 24    | 17.927 | 1315799 | 3.46  | 824409  | 5.63    | 2-Phenethyl-4H-chromen-4-one                  |
| 25    | 18.207 | 221480  | 0.58  | 156892  | 1.07    | Eicosane                                      |
| 26    | 18.515 | 114761  | 0.30  | 99936   | 0.68    | Di-n-octyl phthalate                          |
| 27    | 18.726 | 258990  | 0.68  | 201522  | 1.38    | Eicosane                                      |
| 28    | 19.104 | 1810185 | 4.76  | 858430  | 5.87    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-     |
| 29    | 19.265 | 402465  | 1.06  | 187730  | 1.28    | Tetracontane                                  |
| 30    | 19.365 | 1482390 | 3.90  | 869631  | 5.94    | 6-Methoxy-2-phenethyl-4H-chromen-4-one        |
| 31    | 19.702 | 339303  | 0.89  | 146786  | 1.00    | Imidazolidin-4-one, 5-benzyl-2-thioxo-3-p-t   |
| 32    | 19.774 | 4193222 | 11.02 | 1634968 | 11.17   | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim     |
| 33    | 19.858 | 313142  | 0.82  | 163045  | 1.11    | Tetracontane                                  |
| 34    | 20.024 | 467556  | 1.23  | 237869  | 1.63    | Squalene                                      |
| 35    | 20.194 | 3051664 | 8.02  | 1023397 | 6.99    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-     |

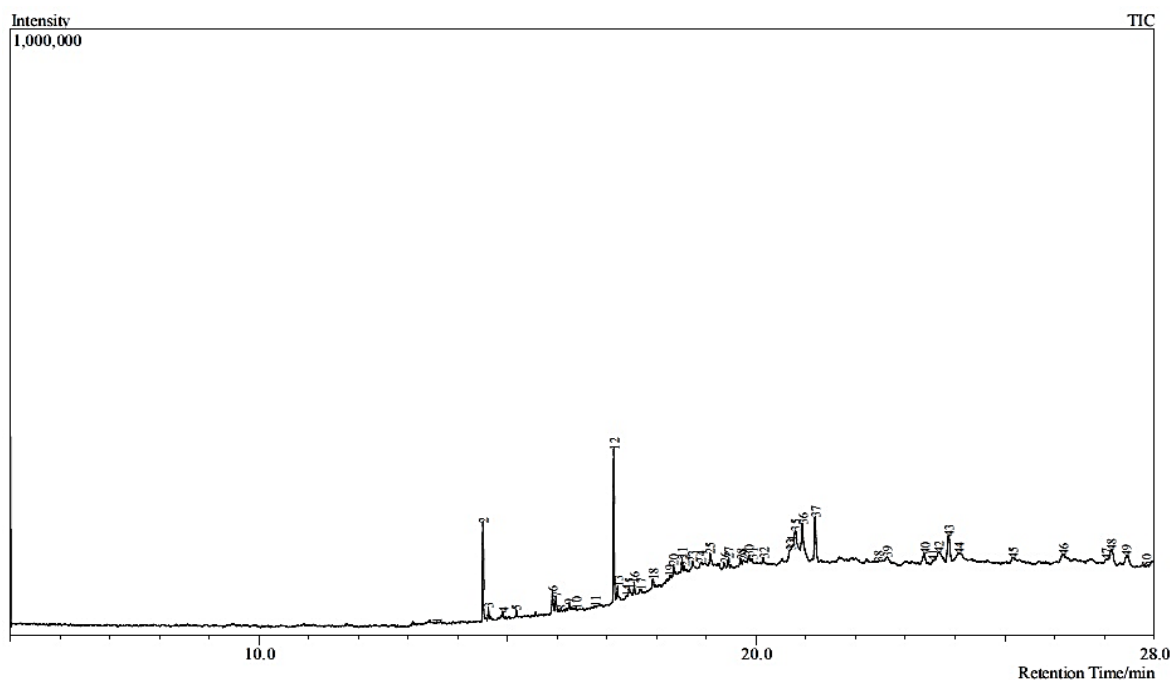
| Peak# | R.Time | Area     | Area%  | Height   | Height% | Name                                        |
|-------|--------|----------|--------|----------|---------|---------------------------------------------|
| 36    | 20.450 | 109686   | 0.29   | 50478    | 0.34    | p-Acetamidobenzaldehyde 6-methylpyridine-   |
| 37    | 20.532 | 379543   | 1.00   | 122503   | 0.84    | Hexatriacontane                             |
| 38    | 20.796 | 591016   | 1.55   | 190413   | 1.30    | Benzene, 1-chloro-4-(4-methoxybenzyloxy)-   |
| 39    | 20.944 | 1781931  | 4.68   | 730917   | 4.99    | 2-[p-Methoxybenzyloxy]-6-methoxy-8-amin     |
| 40    | 21.255 | 8432145  | 22.16  | 1961276  | 13.40   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c    |
| 41    | 21.315 | 265946   | 0.70   | 128779   | 0.88    | Tetracontane                                |
| 42    | 21.370 | 140093   | 0.37   | 59481    | 0.41    | benzenesulfonyl fluoride, 3-[(2-hydroxybenz |
| 43    | 21.667 | 706714   | 1.86   | 250858   | 1.71    | Quinazolin-4(3H)-one, 2-(4-methoxybenzyltl  |
| 44    | 21.876 | 660423   | 1.74   | 182697   | 1.25    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim   |
| 45    | 22.037 | 861423   | 2.26   | 182550   | 1.25    | 1H-Pyrimidine-2,4-dione, 1-benzyloxymethy   |
| 46    | 22.235 | 120022   | 0.32   | 50733    | 0.35    | Tetracontane                                |
| 47    | 22.400 | 251075   | 0.66   | 82935    | 0.57    | Benzene, 1-chloro-4-(4-methoxybenzyloxy)-   |
| 48    | 22.669 | 438762   | 1.15   | 154802   | 1.06    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim    |
| 49    | 23.409 | 453467   | 1.19   | 154770   | 1.06    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
| 50    | 23.913 | 1493564  | 3.93   | 412787   | 2.82    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-    |
|       |        | 38052561 | 100.00 | 14634386 | 100.00  |                                             |

### I.3 Results for sixth month inoculation of F3 inoculant



| Peak Report TIC |        |          |        |         |         |                                                |
|-----------------|--------|----------|--------|---------|---------|------------------------------------------------|
| Peak#           | R.Time | Area     | Area%  | Height  | Height% | Name                                           |
| 1               | 9.456  | 116633   | 0.50   | 63427   | 0.68    | 2-Butanone, 4-phenyl-                          |
| 2               | 10.325 | 61807    | 0.26   | 21354   | 0.23    | Hydrocinnamic acid                             |
| 3               | 11.840 | 52422    | 0.22   | 32850   | 0.35    | 2-Butanone, 4-(4-methoxyphenyl)-               |
| 4               | 12.335 | 57212    | 0.25   | 37851   | 0.40    | 3',5'-Dimethoxyacetophenone                    |
| 5               | 12.770 | 66754    | 0.29   | 30457   | 0.32    | 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-oct    |
| 6               | 12.865 | 168787   | 0.72   | 33960   | 0.36    | Ethyl alpha.-d-glucopyranoside                 |
| 7               | 13.287 | 105254   | 0.45   | 59492   | 0.63    | Guaiac-1(10),11-diene                          |
| 8               | 13.381 | 299498   | 1.28   | 156380  | 1.67    | Cubenol                                        |
| 9               | 13.812 | 1008511  | 4.32   | 488228  | 5.21    | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp       |
| 10              | 14.165 | 68377    | 0.29   | 42853   | 0.46    | Cryptomenidol                                  |
| 11              | 14.706 | 62616    | 0.27   | 34479   | 0.37    | 4aH-Cycloprop[c]azulen-4a-ol, decahydro-1,     |
| 12              | 14.818 | 236347   | 1.01   | 148962  | 1.59    | Hexadecanoic acid, methyl ester                |
| 13              | 14.910 | 62267    | 0.27   | 29881   | 0.32    | 10,12-Tricosadiynoic acid, methyl ester        |
| 14              | 14.973 | 271818   | 1.16   | 176419  | 1.88    | (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-di      |
| 15              | 15.116 | 305273   | 1.31   | 222022  | 2.37    | n-Hexadecanoic acid                            |
| 16              | 15.349 | 218305   | 0.93   | 74038   | 0.79    | Cryptomenidol                                  |
| 17              | 15.440 | 224274   | 0.96   | 105279  | 1.12    | Allotomadenrene oxide-(2)                      |
| 18              | 15.569 | 1097905  | 4.70   | 539192  | 5.75    | trans-Sinapyl alcohol                          |
| 19              | 15.746 | 222647   | 0.95   | 107746  | 1.15    | (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-di      |
| 20              | 15.780 | 61499    | 0.26   | 41852   | 0.45    | (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-di      |
| 21              | 15.957 | 153086   | 0.66   | 112689  | 1.20    | Methyl 10-trans,12-cis-octadecadienoate        |
| 22              | 15.983 | 200112   | 0.86   | 132580  | 1.41    | 9-Octadecenoic acid (Z)-, methyl ester         |
| 23              | 16.606 | 52624    | 0.23   | 31844   | 0.34    | 1H-Cycloprop[c]azulene, 1a,2,3,5,6,7,7a,7b-    |
| 24              | 17.028 | 277635   | 1.19   | 168310  | 1.79    | 1,2,4-Methenoazulene, decahydro-1,5,5,8a-te    |
| 25              | 17.075 | 56858    | 0.24   | 34662   | 0.37    | 6-(Dimethylamino)-4-oxohexanoic acid, TBI      |
| 26              | 17.368 | 131253   | 0.56   | 55610   | 0.59    | Andrographolide                                |
| 27              | 17.926 | 686546   | 2.94   | 456489  | 4.87    | 2-Phenethyl-4H-chromen-4-one                   |
| 28              | 18.263 | 134232   | 0.57   | 60568   | 0.65    | Octacosanol                                    |
| 29              | 19.095 | 569721   | 2.44   | 301148  | 3.21    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 30              | 19.360 | 560601   | 2.40   | 323920  | 3.45    | 6-Methoxy-2-phenethyl-4H-chromen-4-one         |
| 31              | 19.758 | 1972914  | 8.45   | 916696  | 9.78    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 32              | 19.870 | 54889    | 0.24   | 26587   | 0.28    | Heptadecanolide                                |
| 33              | 20.019 | 288359   | 1.24   | 169209  | 1.80    | Squalene                                       |
| 34              | 20.166 | 1247484  | 5.34   | 519303  | 5.54    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-      |
| 35              | 20.443 | 111191   | 0.48   | 58665   | 0.63    | p-Acetamidobenzaldehyde 6-methylpyridine       |
| Peak#           | R.Time | Area     | Area%  | Height  | Height% | Name                                           |
| 36              | 20.787 | 566772   | 2.43   | 125753  | 1.34    | Benzene, 1-trifluoromethyl-4-(2-methoxyben     |
| 37              | 20.935 | 969919   | 4.15   | 364024  | 3.88    | 1-Phenyl-2-[5-thia-6,9-diazatricyclo[7.3.0.0(  |
| 38              | 21.228 | 4747218  | 20.33  | 1447438 | 15.44   | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c       |
| 39              | 21.656 | 552171   | 2.36   | 178564  | 1.90    | Quinazolin-4(3H)-one, 2-(4-methoxybenzylt      |
| 40              | 21.750 | 120681   | 0.52   | 48396   | 0.52    | Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22 |
| 41              | 21.860 | 231588   | 0.99   | 73110   | 0.78    | Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dim      |
| 42              | 22.037 | 465503   | 1.99   | 100596  | 1.07    | Benzene, (methoxymethyl)-                      |
| 43              | 22.389 | 138455   | 0.59   | 50538   | 0.54    | Benzene, 1-chloro-4-(4-methoxybenzyloxy)-      |
| 44              | 22.664 | 788378   | 3.38   | 269026  | 2.87    | 4H-1-Benzopyran-4-one, 7-hydroxy-2,5-dim       |
| 45              | 23.399 | 506882   | 2.17   | 163192  | 1.74    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 46              | 23.905 | 1329171  | 5.69   | 384339  | 4.10    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-       |
| 47              | 25.189 | 416318   | 1.78   | 93174   | 0.99    | 3-Buten-2-one, 4-(2,2,3-trimethyl-6-methyl     |
| 48              | 25.909 | 330329   | 1.41   | 83215   | 0.89    | Noscapine                                      |
| 49              | 26.176 | 409987   | 1.76   | 83801   | 0.89    | gamma.-Sitosterol                              |
| 50              | 27.482 | 509748   | 2.18   | 96741   | 1.03    | 4,22-Stigmastadiene-3-one                      |
|                 |        | 23348831 | 100.00 | 9376909 | 100.00  |                                                |

#### I.4 Results for sixth month inoculation of negative control



| Peak Report TIC |        |        |       |        |         |                                             |
|-----------------|--------|--------|-------|--------|---------|---------------------------------------------|
| Peak#           | R.Time | Area   | Area% | Height | Height% | Name                                        |
| 1               | 13.570 | 17497  | 0.56  | 2667   | 0.21    | Silane, trichlorooctadecyl-                 |
| 2               | 14.506 | 225104 | 7.24  | 164821 | 13.24   | 1,2-Benzenedicarboxylic acid, bis(2-methylp |
| 3               | 14.612 | 29675  | 0.96  | 21317  | 1.71    | Eicosane                                    |
| 4               | 14.906 | 18903  | 0.61  | 12535  | 1.01    | Eicosane                                    |
| 5               | 15.175 | 16610  | 0.53  | 11965  | 0.96    | Dibutyl phthalate                           |
| 6               | 15.903 | 79141  | 2.55  | 38566  | 3.10    | n-Nonadecanol-1                             |
| 7               | 15.973 | 47323  | 1.52  | 29036  | 2.33    | 2-Methylhexacosane                          |
| 8               | 16.070 | 27357  | 0.88  | 5062   | 0.41    | Cyclopropane, 1-chloro-2,2-dimethyl-3-(3,3- |
| 9               | 16.235 | 25996  | 0.84  | 14312  | 1.15    | Dotriacontane                               |
| 10              | 16.380 | 16915  | 0.54  | 5034   | 0.40    | Undec-10-ynoic acid, octadecyl ester        |
| 11              | 16.773 | 15991  | 0.51  | 3262   | 0.26    | 5-Methyl-Z-5-docosene                       |
| 12              | 17.137 | 338915 | 10.91 | 252974 | 20.32   | Hexadecen-1-ol, trans-9-                    |
| 13              | 17.209 | 39172  | 1.26  | 23774  | 1.91    | Tetrapentacontane                           |
| 14              | 17.390 | 14304  | 0.46  | 5288   | 0.42    | Valtrate                                    |
| 15              | 17.447 | 31190  | 1.00  | 15673  | 1.26    | Tetracosane                                 |
| 16              | 17.548 | 35972  | 1.16  | 22760  | 1.83    | 4,8,12,16-Tetramethylheptadecan-4-olide     |
| 17              | 17.665 | 21265  | 0.68  | 8352   | 0.67    | Dodecane, 2,6,10-trimethyl-                 |
| 18              | 17.925 | 29630  | 0.95  | 16080  | 1.29    | 2-Phenethyl-4H-chromen-4-one                |
| 19              | 18.261 | 19049  | 0.61  | 7799   | 0.63    | n-Nonadecanol-1                             |
| 20              | 18.337 | 32651  | 1.05  | 20976  | 1.68    | Tetrapentacontane                           |
| 21              | 18.510 | 33601  | 1.08  | 23235  | 1.87    | Di-n-octyl phthalate                        |
| 22              | 18.555 | 15185  | 0.49  | 10526  | 0.85    | Tetrapentacontane                           |
| 23              | 18.719 | 29158  | 0.94  | 13265  | 1.07    | 2-Methylhexacosane                          |
| 24              | 18.895 | 14441  | 0.46  | 6450   | 0.52    | Triphenylphosphine oxide                    |
| 25              | 19.075 | 49544  | 1.59  | 20053  | 1.61    | Propanoic acid, 3-mercapto-, dodecyl ester  |
| 26              | 19.357 | 21348  | 0.69  | 10157  | 0.82    | Benzaldehyde, 3-benzyloxy-2-fluoro-4-meth   |
| 27              | 19.440 | 31772  | 1.02  | 16952  | 1.36    | Tetrapentacontane                           |
| 28              | 19.690 | 24714  | 0.80  | 11466  | 0.92    | Tetrapentacontane                           |
| 29              | 19.752 | 27028  | 0.87  | 9643   | 0.77    | 2,4-Difluorobenzene, 1-benzyloxy-           |
| 30              | 19.855 | 56677  | 1.82  | 15414  | 1.24    | Tetrapentacontane, 1,54-dibromo-            |
| 31              | 19.915 | 22240  | 0.72  | 10325  | 0.83    | Decanedioic acid, bis(2-ethylhexyl) ester   |
| 32              | 20.139 | 19738  | 0.64  | 9767   | 0.78    | Acetamide, N-(4-benzyloxyphenyl)-2-cyano-   |
| 33              | 20.680 | 57485  | 1.85  | 18917  | 1.52    | Eicosyl methyl ether                        |
| 34              | 20.710 | 39935  | 1.29  | 22056  | 1.77    | Fumaric acid, 2-chloropropyl tridecyl ester |
| 35              | 20.790 | 275399 | 8.86  | 51314  | 4.12    | 4-Benzyloxy-3-nitromethyl-pentanoic acid, n |

| Peak# | R. Time | Area    | Area%  | Height  | Height% | Name                                          |
|-------|---------|---------|--------|---------|---------|-----------------------------------------------|
| 36    | 20.924  | 275584  | 8.87   | 62396   | 5.01    | 1-Phenyl-2-[5-thia-6,9-diazatricyclo[7.3.0.0( |
| 37    | 21.183  | 181678  | 5.85   | 73751   | 5.92    | 6,7-Dimethoxy-2-phenethyl-4H-chromen-4-c      |
| 38    | 22.450  | 24940   | 0.80   | 1881    | 0.15    | 9-Bromo-10-[2-pyrimidylamino]-deoxoarten      |
| 39    | 22.634  | 51802   | 1.67   | 11251   | 0.90    | 4-Oxo-2-(4-fluorophenyl)-3,4-dihydropyrimi    |
| 40    | 23.379  | 66328   | 2.13   | 17551   | 1.41    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 41    | 23.545  | 30233   | 0.97   | 5904    | 0.47    | 11-Octadecadienoic acid, pyrrolidide          |
| 42    | 23.667  | 118120  | 3.80   | 18112   | 1.45    | trans-Sinapyl alcohol                         |
| 43    | 23.870  | 154659  | 4.98   | 44284   | 3.56    | 6,7-Dimethoxy-2-(4-methoxyphenethyl)-4H-      |
| 44    | 24.083  | 91679   | 2.95   | 11433   | 0.92    | 7H-6,9a-Methanocycloocta[b]furan-7,10-dio     |
| 45    | 25.173  | 25483   | 0.82   | 6938    | 0.56    | Cholesta-22,24-dien-5-ol, 4,4-dimethyl-       |
| 46    | 26.159  | 40895   | 1.32   | 9969    | 0.80    | .gamma.-Sitosterol                            |
| 47    | 27.035  | 36644   | 1.18   | 6569    | 0.53    | 4-Campestene-3-one                            |
| 48    | 27.149  | 107657  | 3.46   | 24343   | 1.96    | Cholestan-3-one                               |
| 49    | 27.444  | 85850   | 2.76   | 16271   | 1.31    | 4,22-Cholestadien-3-one                       |
| 50    | 27.877  | 14818   | 0.48   | 2594    | 0.21    | Pregn-5-en-20-one, 3,8,11,12,14-pentahydro:   |
|       |         | 3107295 | 100.00 | 1245040 | 100.00  |                                               |