Potential of Gelam (*Melaleuca cajuputi)* Stem as Natural Insecticide

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DECLARATION

I declare that this thesis entitled "Potential of Gelam (*Melaleuca cajuputi*) Stem as Natural Insecticide"is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _____

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Date :

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Potential of Gelam (*Melaleuca cajuputi*) Stem as Natural Insecticide

ABSTRACT

Carpenter ant, *Camponotus* spp. is one of the most common and destructive insect pest in residential area. Synthetic insecticide is used to control the population of carpenter ants. Synthetic insecticides create various problems to the environment and human health causes research to look into alternative strategies. Present study was conducted to determine the potential of *Melaleuca cajuputi* stem as natural insecticide by evaluating the toxicity and repellency rate. Repellency and toxicity of the plant to carpenter ants were tested by using crude extract extracted using *n*-hexane, dichloromethane and methanol. Repellency test was conducted using modified WHO by using 20% concentration of crude extract diluted with acetone. Repellency rate was calculated based on the number of ants entered the treated area in the experimental setup. For toxicity test, 10% of each crude extract was prepared by mixing with honey. The toxicity rate was calculated based on the number of ants died after eating the honey mixed with extract. The results for repellency test showed a significantly higher repellency rate in hexane extract with the percentage of 97.30%, followed by dichloromethane extract with percentage of 83.40% and lastly 42.80% repellency for methanol extract. Hexane extract has the highest repellency rate because it might contain essential oil which is a non-polar compound that the smell of the oil repels the ants. The extract smell of dichloromethane and methanol, which was mild to almost no smell, was not strong enough to repel the ants. The result for toxicity test showed a significantly higher toxicity rate in methanol extracts to ants with the percentage of 84.30%, followed by dichloromethane extract with the percentage of 77.70% and hexane with 54.30%. Methanol extract showed higher percentage due to higher content of active ingredients that are toxic to the carpenter ants. In conclusion, *Melaleuca cajuputi* stem has higher repellency effect compared to toxicity effect.

Potensi Batang Gelam (*Melaleuca cajuputi*) sebagai Racun Serangga Semula Jadi

ABSTRAK

Semut carpenter, *Camponotus* spp. merupakan salah satu serangga perosak yang paling biasa ditemui di kawasan perumahan. Racun serangga sintetik biasanya digunakan untuk mengawal populasi semut ini. Racun serangga sintetik menyebabkan kesan negatif kepada kesihatan manusia dan juga alam sekitar. Oleh itu, banyak penyelidikan dijalankan untuk meneliti strategi alternatif untuk menggantikan penggunaan racun serangga sintetik. Kajian ini telah dijalankan untuk menganalisasi potensi batang *Melaleuca cajuputi* sebagai racun serangga semula jadi dengan menilai kadar ketoksikan dan keupayaan menghalau semut dari sesuatu kawasan. Kajian ini dijalankan menggunakan ekstrak mentah yang diekstrak menggunakan *n*-heksana, diklorometana dan metanol. Keupayaan menghalau dianalisis menggunakan cara WHO yang diubahsuai dengan menggunakan 20% kepekatan ekstrak mentah yang dicairkan dengan aseton. Kadar menghalau dikira berdasarkan bilangan semut yang memasuki kawasan yang telah dirawat. Untuk ujian ketoksikan, 10% kepekatan ekstrak mentah telah disediakan dengan mencampurkan ekstrak dengan madu. Kadar ketoksikan dikira berdasarkan kepada bilangan semut mati selepas makan madu yang dicampur dengan ekstrak. Keputusan bagi ujian menghalau menunjukkan kadar yang lebih tinggi menghalau bagi ekstrak heksana dengan peratusan 97.30%, diikuti dengan ekstrak diklorometana dengan peratusan 83.40% dan akhir sekali 42.80% untuk ekstrak metanol. Ekstrak heksana mempunyai kadar menghalau tertinggi kerana ia mungkin mengandungi minyak pati yang merupakan sebatian bukan kutub yang berbau kuat sehinnga menghalau semut. Bau ekstrak diklorometana dan metanol tidak cukup kuat untuk menghalau semut. Keputusan untuk ujian ketoksikan menunjukkan kadar yang lebih tinggi ketoksikan dalam ekstrak metanol dengan peratusan 84.30%, diikuti dengan ekstrak diklorometana dengan peratusan 77.70% dan heksana dengan 54,30%. Ekstrak metanol menunjukkan peratusan yang lebih tinggi kerana kandungan tinggi bahan-bahan aktif yang toksik kepada semut. Kesimpulannya, batang *Melaleuca cajuputi* mempunyai kesan menghalau lebih tinggi berbanding dengan kesan ketoksikan.

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LIST OF ABBREVIATIONS

BHC	Benzene Hexa Chloride
WP	Wettable Powder
spp	Species (Plural)
m	Metres
mm	Millimetre
USA	United States of America
RH	Relative Humidity
rpm	Revolutions per minute
no.	Number
ml	Millilitre
cm	Centimetre
ANOVA	Analysis of Variance

LIST OF SYMBOLS

%	Percentage
º C	Degree Celcius

POTENTIAL OF GELAM (*Melaleuca Cajuputi*) STEM AS NATURAL INSECTICIDE

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Synthetic insecticides are manufactured from synthetic chemicals created from other chemicals (David, 2010). Most popular synthetic insecticides that are commonly used by people to control insects is known as DDT (dichlorodiphenyltrichloroethane). Synthetic insecticides are used by many people which includes householders and industrialist (Zibaee & Khorram, 2015). However, there are many environmental issues and harmful human health effects related to the high usage of synthetic insecticides which have been the matter of concern to many people in current years, including both scientists and public (Koul et al., 2008). Besides that, development of insecticide resistance in the insects against synthetic insecticide causes the problem to reduce and control the population of insects.

Issues related to the high usage of synthetic insecticide can be solved by developing and using natural insecticides which have been identified as an alternative source to synthetic insecticides in order control the population of insects (Isman, 2006). The vital need for a sustainable and ecological friendly approach for numerous practices and applications are rising greatly, especially in the developing nations in recent years (Baskar & Ignacimuthu, 2012). Therefore, there is a growing interest in the study of natural insecticide as it has reduced ecological effects and minimal cost of expenses (Zibaee & Khorram, 2015).

Natural insecticides are defined as a vital group of plants that can be found naturally in the wild, often slow growing protectant crops (Mhazo et al., 2011). Protectants which can considered as an insecticide are considered safe and also beneficial to environment and humans when compared to synthetic insecticides which are also known as chemical insecticides, and has minimal residual effects (Dadang et al., 2009). Natural insecticides are mainly made of active components of the selected plant extracts and usually are very safe to be used in daily life in comparison to the use of synthetic insecticides (Khater, 2012).

Natural insecticides are used to control widely spreading population of insects for many centuries in certain countries. To date, natural insecticide produced are mainly to control the population of mosquitoes, cockroaches, termites and also pest that causes reduction in the agricultural crops production (Bashir et al., 2013). Studies show that most of the compound of natural insecticide are secondary plant substances which include quinones, alkaloids, essential oils, glycosides and flavonoids (Appel et al., 2001). Each natural insecticide which have derived from plants has its own mode of action to kill and repel insects. The mode of actions includes poisonous, irritation and respiration problem (Devanand & Usha, 2008). The difference in the mode of action of insecticides from different plant is due to the type and concentration of active ingredient contain in the selected plant to be used as natural insecticide.

In many countries, including Malaysia and Thailand, natural insecticides which are derived from plants have been used to kill and repel domestic insects such as cockroaches and mosquitoes that causes harm to human health (Busse and Mitchell, 2007) or human properties (Chopa et al., 2006). There are two approved natural

insecticides which are being used commercially in many countries; Pyrethrins from *Chrysanthemum* species and Rotenone from *Derris* species (Isman, 2006). Another common example, Azadirachtin from the Neem tree (*Azadirachta indica*), is under commercial development in several countries and being used commercially in a few countries for many years such as in India (Isman, 2006). There are many more plants that has the potential to be used as natural insecticide which have not been studied in details such as *Melaleuca cajuputi* tree (Azlinda et al., 2009). Different part of plants has different concentration and type of active ingredient present. For example, leaves of a plant has different active ingredient compared to the bark of the plant.

In developing countries, pests become the most concern issues as they can pose terrifying risks and rendered many problem and diseases. Most common pest that causes threat to human being are cockroaches, mosquitoes and ants. Carpenter ants (*Camponotus* spp.) is one of the largest and most abundant species of ants that can be found in residential areas, which are usually in baits, gardens, cupboards, and kitchens (Appel et al., 2001). *Camponotus* spp. has the ability to contaminate food and transmit many bacterial and viral diseases such as dysentery, leprosy, typhoid fever, cholera and poliomyelitis, through contact or food contamination, by virtue of their cohabitation with human (Thavara et al., 2007). *Camponotus* spp. are one of the most significant structural pest in the world. They cause serious damage by burrowing or nesting into the wood (Chen et al., 2002). It is very important to control the population of *Camponotus* spp. to reduce property damage and contamination of food.

The efficient and effective control method of *Camponotus* spp. depends highly on the usage of synthetic insecticides (Rejitha et al., 2014). Based on a few studies it has

been identified that the high usage of synthetic insecticide causes damage to the ozone layer and human health (Rejitha et al., 2014). The misuse or overuse of synthetic insecticide has also led to the development of insecticide resistance in *Camponotus* spp. and destruction of non – target organisms causing imbalance ecological system (Chopa et al., 2006). These problems have made the use of synthetic insecticides undesirable. The high cost of these insecticides are also a factor contributing for rural folks revert to the use of plant materials to control insect pests. These problems have led to the search for cheaper, safer and more biodegradable alternatives to synthetic pesticides (Chopa et al., 2006). Thus, the interest on studies regarding development of natural insecticides from plants has increased.

Melaleuca cajuputi is one of the well-known plants that can be found abundantly in Peninsular Malaysia and Thailand (Widiana et al., 2015). The *Melaleuca cajuputi* plant has high medicinal value. Studies proved that the leaves of *Melaleuca cajuputi* retain antibacterial, anti-inflammatory and anaesthetic properties and have the potential to repel and kill insects (Ujjan et al., 2014). *Melaleuca cajuputi* has been used for many purposes in human daily life, such as flavouring in cooking, the good smell of the leaves is used for fragrance and refreshing agent in the cosmetics, perfumes, detergents and soap.

Different part of the tree plays different function to the ecosystem and people. Leaves of *Melaleuca cajuputi* is used to produce cajuput oil that can be used to cure skin problems while stem is used as fuel wood. There are studies proved the effectiveness of *Melaleuca cajuputi* as natural insecticide against dengue vectors (Azlinda et al., 2009). Therefore, it is an assumption that *Melaleuca cajuputi* is capable to be used as natural insecticide to kill and repel *Camponotus* spp. (Ko et al., 2009).

Thus, this research is conducted to determine the potential and effectiveness of *Melaleuca cajuputi* stem as natural insecticide to reduce the major insect population that can be found abundantly such as *Camponotus* spp. This experiment is carried out by utilising the stem part of *Melaleuca cajuputi.* Sequential extraction is conducted using three different extraction solvent; *n*-hexane, dichloromethane and methanol. Three different type of crude extracts with different content of active ingredient are produced to react with insect to be controlled (Pavela, 2009). As till date, there are lack of study conducted on insecticidal activities of *Melaleuca cajuputi* stem against Carpenter Ants.

1.2 Problem Statement

Recently, there are more focus on *Melaleuca* species in the Myrtaceae family. Although the potential and importance of this family species have been studied in detail, there is still lack of research on the *Melaleuca cajuputi,* which is one of the abundance *Melaleuca* species that can be found in Peninsular Malaysia. There are many studies proved the potential of the leaves of this plant to act as natural insecticide where the leaves are distilled to get the essential oil called cajuput oil that also acts as the source of medical and antiseptic.

However, there has been less studies published on the potential of *Melaleuca cajuputi* stem to act as natural insecticide. Based on observation, it is found that the stem of this tree has less fungus infection or any other threat caused by insects. It is assumed that the stem might have compounds that has the ability to repel and kill the insect. Thus, this experiment is conducted to identify and evaluate the potential of

Melaleuca cajuputi as natural insecticide against *Camponotus* spp. using sequential extraction and WHO method.

1.3 Research Question

Do *Melaleuca cajuputi* stem's crude extract has the potential to be used as natural insecticide against *Camponotus* spp.?

1.4 Objectives

The objectives of this study were:

1.4.1 To evaluate the repellency and toxicity rate of *Melaleuca cajuputi* extract against *Camponotus* spp.

1.4.2 To identify the potential of *Melaleuca cajuputi* stem as new natural insecticide to replace the usage of synthetic insecticides in the market.

1.5 Scope of the Study

The focus of this study is to determine the potential of *Melaleuca cajuputi* stem as natural insecticide to repel *Camponotus* spp. The plant material was extracted using three different extraction solvent: *n*-hexane, dichloromethane and methanol to produce crude extract. The repellency and toxicity test were conducted to determine the effectiveness and potential of *Melaleuca cajuputi* stem as natural insecticide. The WHO method was adapted and modified for the repellency and toxicity test. Repellency and toxicity test were conducted to identify the rate of the crude extract to kill and also repel the *Camponotus* spp. for a fixed time of period with specified intervals.

1.6 Significance of the Study

This study was conducted to determine the effectiveness of *Melaleuca cajuputi* stem as natural insecticide. The extraction of this plant material was conducted to obtain three different crude extract from hexane, dichloromethane and methanol respectively. This study is very significant to reduce the usage of synthetic insecticides which causes harm to the environment and human health and to control the population of insects that causes diseases. Even though people are aware of insect-related diseases, but action and methods used to control the population of *Camponotus* spp. using natural plants are very less, mostly depending on the use of synthetic insecticide.

In this study, the aim is to identify the potential of *Melaleuca cajuputi* stem as natural insecticides. The result of this study are beneficial information and significance to public where the outcome of this research will help to minimize the spread of insect related diseases. Other than that, this study will be a useful reference and will provide guidance to other related researches.

CHAPTER 2

LITERARUTE REVIEW

2.1 Insecticides

Insecticides are a category of pesticides that functions to kill, harm, repel, and disrupt hormones of insect pests such as cockroaches and mosquitoes that causes harm to the environment and human health (Khater, 2012). Insecticides substantively play a greater role especially in the food production in these days besides used in household areas to expel the insects that damage the properties and pose risks to the human health (Miller et al., 2010).

Insecticides can be divided into two, which are synthetic insecticide and natural insecticide (Bommarco et al., 2011). Synthetic insecticides are mostly made up of chemicals, whereas natural insecticide are made up of naturally occurring materials such as plants (David, 2010).

2.1.2 Synthetic Insecticide

The application of synthetic insecticide (Figure 2.1) is usually to control the population of insects from continuous growing and spreading (Bommarco et al., 2011). Today, synthetic insecticides have been purchased by most peoples around the world as the fastest way to eradicate pests instantly without considering its effects mainly to environment and human health. Synthetic insecticide that are commonly used such as dichlorodiphenyltrichloroethane (DDT), was the first generation insecticide that has been used especially in urban aerial spray in United States to control mosquito, moth,

beetle and others in 1940's before it was banned due to the effect it causes to the environment and others (Isman, 2006).

The areas that are highly infested by insect population such as buildings or warehouse will be cleaned thoroughly and sprayed with BHC (Benzene Hexa Chloride) or Malathion. If there are any parts of the building which cannot be reached or controlled with sprays mentioned above, fumigation will be carried out (Johnson & Townsend, n.d.). Methyl bromide, or carbon tetrachloride and an ethylene dichloride mixture are few examples of effective fumigants which can be used for fumigation of infested grain or area. However, due to the toxicity hazards of the fumigants, fumigation can only be conducted by the authorized operators (Dennis, 2003). Synthetic insecticide can be divided into many classes based on the area the insecticide effects (Cordova et al., 2006). The main classes of synthetic insecticide are carbamates, organochlorines, pyrethroids and organophosphates (Table 2.1).

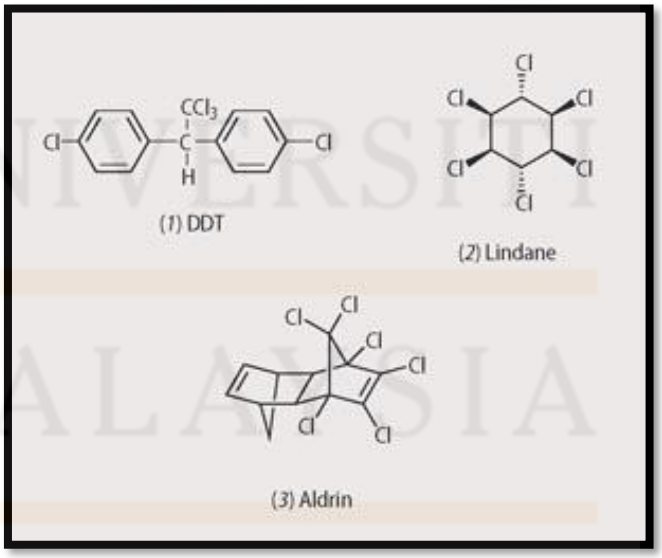

Figure 2.1: Chemical structure of some synthetic insecticides.
(Source: Royal Society of Chemistry, 2007)

Table 2.1: Main classes of synthetic insecticides. (Source: Cordova et al., 2006)

Class	Examples	Area of Effect
Carbamates	Carbofuran, aldicarb, carbaryl	Central nervous system
Organochlorines	DDT, toxaphene, dieldrin, Aldrin	Reproductive, nervous, endocrine, and immune system
Pyrethroids	Fenpropanthrin, deltamethrin, cypermethrin	Skin and endocrine system
Organophosphates	Diazinon, glyphosate, malathion	Central nervous system

According to The World Health Organization (2014), there are about three million cases of pesticide poisoning are reported each year. Young children are more easily exposed to the harmful effects of synthetic insecticides (Lah, 2011). This proves that synthetic insecticides are very dangerous to environment and human health.

2.1.3 Natural Insecticide

There are three main categories of natural insecticides (Figure 2.2), which are chemical, mineral, and biological. The purpose of all three categories of natural insecticide is to repel, kill, or damage the behaviour of insect pests (Azlinda et al., 2009). In order to reduce negative impacts to human health and environment, natural insecticides have been identified has an excellent alternative to replace the usage of synthetic insecticide. Natural insecticides are usually derived from plants (David, 2010). Natural insecticide is less toxic to human, easily biodegradable, appropriate to be used by small scale farmers as it is cost effective and able to protect crops from being attacked by a wide range of insect pests (Logita, 2015).

As there are many undesirable and unavoidable side effects or negative impacts of synthetic insecticides, awareness of human being towards toxicological and environmental problems in the use of synthetic insecticide is growing (Ko et al., 2009). This growing awareness has led to a clearly increasing effort to the usage of a more environmentally efficient insecticide which reduces the usage of toxic synthetic insecticides and other chemicals related to agriculture in an attempt to save the human health and the environment (Ko et al., 2009).

Natural insecticides contain rich sources of bioactive ingredient that can act as an alternative method to control insect populations (Asmanizar et al., 2012). There are many ways to extract the plant material to obtain the bioactive ingredient. There are plant materials that can be extracted and applied directly on the insect infected area while there is some bioactive ingredient of plants that can only be extracted using Hydrodistillation, infusion and other extraction methods (Silva et al., 2013). Meanwhile, phytochemicals which derived from plant sources can act as repellent and these activities have been observed by many researchers (Silva et al., 2013; Suthisut et al., 2011).

The effectiveness of plant extracts to repel insects have been observed by many researchers (Silva et al., 2013; Dadang et al., 2009; Norashiqin et al., 2011). Rotenone from *Derris* sp., nicotine from tobacco, neem from *Azadiracta indica* and pyrethrins from *Chrysanthemum* sp. have been widely used especially in small-scale industry as well as in commercial agriculture (David, 2010). Rotenone and pyrethrins are the most popular traditional botanical insecticides among other natural insecticides that have been used since sixtieth century. Rotenone is a terpene that was applied as a spray on fruits or crop and become potentially toxins to several insects such as aphids,

cockroaches and houseflies (Chen et al., 2002). While, pyrethrins can paralyzes the insects in contact within a few seconds only but in mammals, the toxicity is low since the pyrethrins ester can be converted into nontoxic compound in the stomach (Chen et al., 2002).

Other than pyrethrins, rotenone, nicotine, ryania, sabadilla and neem oil, which are the existing natural products, there are several other products of plant origin that have been identified to be influenced by the properties of having toxic, repellent, antifeedant, and interfere with the growth and development behavior of arthropod pests (Coats, 2004).

As people are getting more aware about the consequences of high usage of synthetic insecticides, there are more studies to identify potential botanical insecticides from different plants. Appel et al. (2001) proved that mint oil is an effective control method against American cockroach. Norashiqin et al. (2011) reported the effectiveness of *Piper aduncum* (Spiked pepper) to repel and kill mosquito species. Rejitha et al. (2014) conducted a study to determine the effectiveness of six different plant powders to repel American cockroach. According to Rejitha et al. (2014), *Curcuma longa* (Tumeric), *Lantana camara* (Lantana), *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Basil), *Adhatoda vasica* (Adusa) and *Vitex negundo* (Chaste tree) are very effective as a natural insecticide to repel American cockroaches.

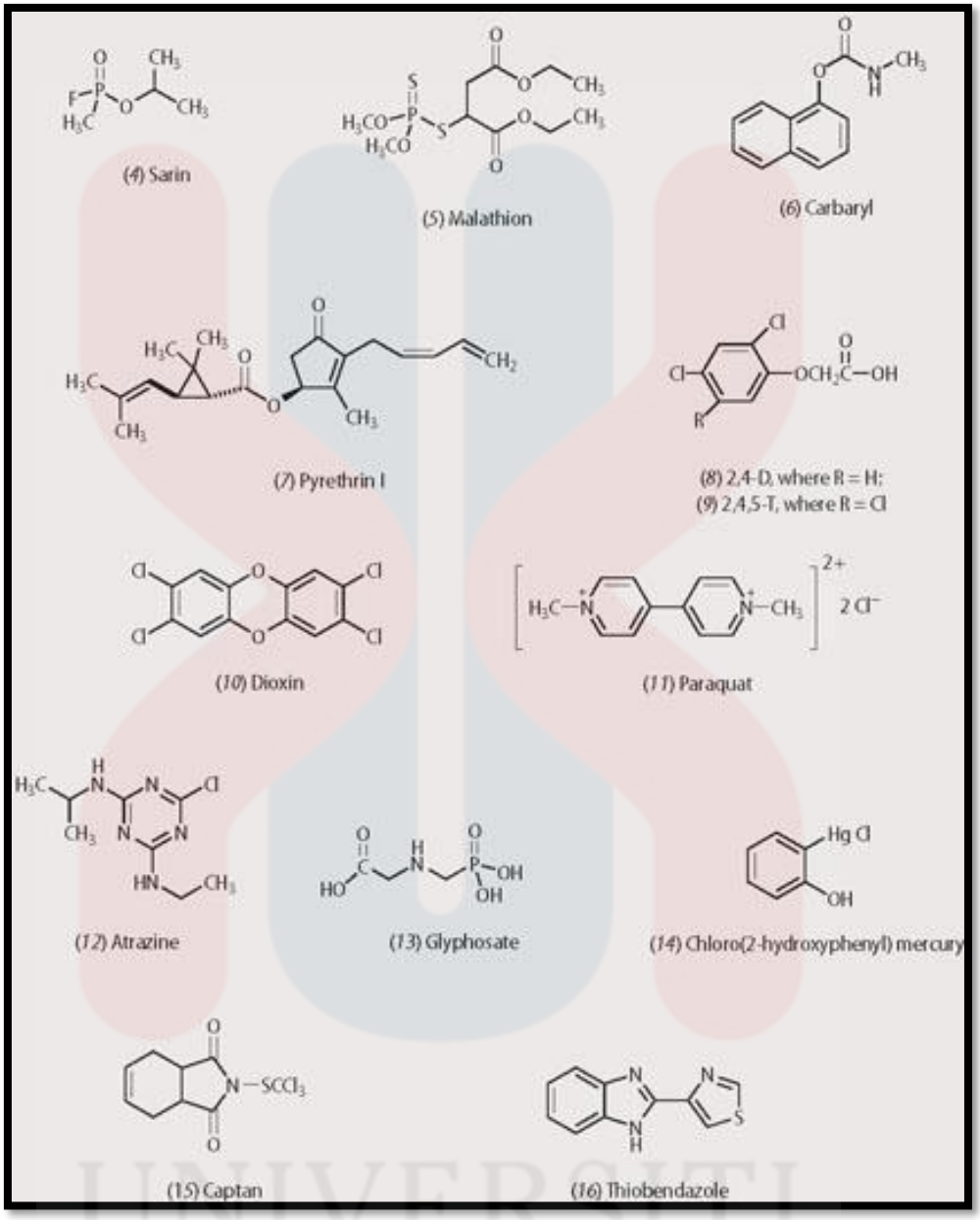

Figure 2.2: Chemical structure of some natural insecticides
(Source: Royal Society of Chemistry, 2007)

2.2 Gelam (*Melaleuca cajuputi)*

Melaleuca cajuputi can be described as a large perennial tree which belongs to the family of Myrtaceae. It has the ability to grow to the height up to 40 meters, where mature trees will be having trunks up to 1.2 m in diameter (Figure 2.3) (Ko et al., 2009). This tree grows in a condition of swampy soils, drain lines and in seasonally flooded soils, but they can also occur in dry, rocky and infertile soils.

Melaleuca cajuputi is the main source of cajuput oil, which as multiple uses and widely used in the folk medicine of Southeastern Asia (Barbosa et al., 2013). Other members of the Myrtaceae family that are also well known include *Syzygium* spp., *Rhodamnia* spp., *Leptospermum* spp., and *Trifitaniopis* spp. (Lim & Midon, 2001).

The species of *Melaleuca* can be found growing in natural conditions in the swamp forests between the old raised sea beaches. In Malacca, *Melaleuca cajuputi* are also used as shade on roadside in low lying areas where they cross rice-swamps, but the crown size is not sufficient to cover wide road (Lim & Midon, 2001). The identification of the tree is very easy because it has distinctive thick papery flaky bark that can be peeled off easily.

As these trees is only available in certain locations, the use of timber of this tree is very less. The physical appearance of the trees is often said to be twisted and small in size, the timber is considered as unattractive to be used as sawn except for firewood. The cajuput oil yielded from the leaves of this tree has been used as an external application for ear – ache, headache, rheumatism cramp, tooth-ache and fresh wounds (Lim & Midon, 2001).

There are a few researches related to *Melaleuca cajuputi* that has been carried out to identify the potential as natural insecticide. Azlinda et al. (2009) conducted a study on the evaluation of *Melaleuca cajuputi* Powell extract in aerosol can against dengue vectors in the laboratory. The study has proved that the essential oil extract from the leaves of this tree has potential to reduce dengue vectors. According to Ko et al. (2009), *Melaleuca cajuputi* leaf essential oil possesses the characteristics that are

able to act as fumigant, repellency and contact toxicities against *Sitophilus zeamais* and *Tribolium castaneum.*

(a)

Figure2.3 (a): Full tree of Gelam *Melaleuca cajuputi* (Source: Kwan, 2007).

(b)

Figure 2.3 (b): Gelam *Melaleuca Cajuputi* tree at Bachok taken on 11th April 2016.

Melaleuca cajuputi belongs to the family Myrtaceae which consists of at least 133 genera and 3, 800 woody shrubs tall tree (Asgar, 2013). For a fast track view, *Melaleuca cajuputi* classification is as follow (Table 2.2):

Table 2.2: Classification of *Melaleuca cajuputi* (Source: Nuyim, 2001)

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	*Melaleuca*
Species	*cajuputi* Powell

2.2.1 Stem of *Melaleuca cajuputi*

The stem of *Melaleuca cajuputi* (Figure 2.4) has been used for many purposes. The ability of the stem to be peeled and rolled into sheets has led it to be used as roofing material and a sealant material in boat construction. The stem not only used in construction but it is also used in seed propagation, fabrication of water and heat resistant nets for drying materials and other heat resistant materials (Widiana et al., 2015).

Utilization of *Melaleuca cajuputi* timber is of many uses for local people in the communities surrounding *Melaleuca cajuputi* forests. In particular, use of stem as fuel wood, and is particularly suitable for and produces high quality charcoal. Although relatively soft, *Melaleuca cajuputi* stem is adapted to anaerobic conditions and able to withstand water damage more strongly compared to other woods. Based on the density

of timber, it is proved by Lim and Midon (2001) that *Melaleuca cajuputi* tend to be stronger than light red meranti (*Shorea* sp.), rubberwood (*Hevea brasiliensis*), and mersawa (*Anisoptera*) but weaker than kempas (*Koompassia malaccensis*) and keruing (*Dipterocarpus* sp.). Therefore, these unique characteristics of this stem allow it to be used for many purposes.

On the smallest scale it is used widely for construction of fences and border markers to delimit land administration, while it is also used in the construction of houses, particularly as supporting columns or piles and for floorboards and frames. Smaller stems of *Melaleuca cajuputi* are also used for a many other purposes such as for fishing rods, fish trap stakes and supports and stakes for agricultural purposes (Somchai et al, 1999).

Figure 2.4 (a): Stem of *Melaleuca cajuputi* collected from Pattani, Thailand.

Figure 2.4 (b): Stem of *Melaleuca cajuputi* covered with bark layers.

2.2.2 Cajuput oil

Cajuput oil is produced from the essential oil extraction process from *Melaleuca cajuputi* (Azlinda et al., 2009) (Figure 2.5). Cajuput oil is very effective to cure digestive and skin problems and also helps to keep human mind balance by appealing mind, clearing thoughts (Sabir et al., 2014) and allaying the feeling of lethargy (Markham,1999). The main chemical components of Cajuput oil include a-pinene, myrcene, b-pinene, limonene, a-terpinene, y-terpinene, p-cymene, 1, 8-cineole, terpinolene, linalool, terpinen-4-ol and a-terpineol (Basta & Spooner – Hart, 2004) (Table 2.2).

Figure 2.5: Example of ready extract Cajuput essential oil.
(Source: 101 Herbs, 2002).

Table 2.3: Chemical composition of cajuput oil.
(Source: Basta & Spooner – Hart, 2004).

Peak No	Compound	Composition (%)
1	a-pinene	Tr- 0.8
2	1,8-cineole	Tr – 0.7
3	B-caryophyllene	0.6 – 3.2
4	Humulene	0.6 – 1.3
5	Spathulenol	4.0 – 9.0
6	Caryophyllene oxide	Tr – 3.6
7	Platyphyllol	64 – 71
8	MW 234	4.3

2.3 Carpenter Ants

Carpenter ants belongs to the family Formicidae (Figure 2.6) (Table 2.3). The scientific name for carpenter ants is *Camponotus* spp. This species is a pest that can threat human health. ants commonly known as Carpenter ants. Carpenter ants is the largest of the house-infesting ants (Chen et al., 2002). It has been spread throughout the world by commerce. It is able to spread bacterial diseases by contaminating food to be consumed by human being, which leads to food poisoning, dysentery, diarrhoea and childhood asthma (Maketon et al., 2010).

Figure 2.6: Carpenter ants.
(Source: Steven, 2008)

Table 2.4: Classification of *Camponotus* spp. (Source: Myers et al., 2016)

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Family	Formicidae
Genus	*Camponotus*

2.3.1 Morphology of Carpenter ants

Carpenter ants (*Camponotus* spp.) are large (0.3 to 1.0 in or 0.76 to 2.54 cm) ants indigenous to many forested parts of the world. Overall body size of Carpenter ants is not a significant characteristic for identification because body size of carpenter ants varies significantly across and within species (Pararas-Carayannis, 2015). Carpenter ants have dark bodies, narrow waists and elbowed antennae (Steve, 2008). The front and hind wings of carpenter are not alike, where their hind wings are shorter, often different in shape and few vein patterns on it (Figure 2.7). Other than that, when it's wings detach, there are no wing stubs that can be observed. Carpenter ants can be observed flying about in the open, during daylight (Chen et al., 2002).

Carpenter ants build nests inside wood consisting of galleries chewed out with their mandibles, preferably in dead, damp wood. They do not consume the wood, however, unlike termites. Sometimes, carpenter ants hollow out sections of trees. They also commonly infest wooden buildings and structures, and are a widespread nuisance and major cause of structural damage. The genus includes over 1,000 species (Ryan, 2006).

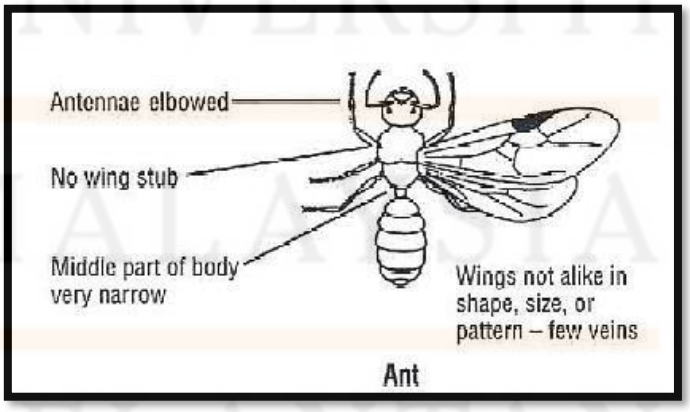

Figure 2.7: Morphology of carpenter ants.
(Source: Pararas-Carayannis, 2015)

2.3.2 Life Cycle of Carpenter Ants

The life cycle of carpenter ants begins with the nuptial flight, which usually occurs in the late spring or early summer, depending on environmental factors. During this mating flight, male winged carpenter ants, or swarmer's, mate with winged females. Soon after mating, the females shed their wings and the males die. The female ants then search for a new site to build their colonies (Ryan, 2006). The queen typically seeks a small crack in a wooden structure. She then closes herself inside that chamber, and lays the first batch of eggs. She remains inside the chamber until her first batch of eggs becomes adult workers. During this time, the queen uses her stored fat reserves and wing muscles for nourishment (Chen et al., 2002).

The queen provides food for the young by means of her salivary glands until they become workers capable of foraging (Johnson and Townsend, n.d.). The queen looks after her first brood, and, once grown, that first brood of adult workers takes care of subsequent broods. It takes three to six years to establish a large and stable colony. The life cycle of a carpenter ant is estimated to be 6 to 12 weeks from egg to adult (Figure 2.8). Cold weather can stretch the development time of carpenter ants up to 10 months. The only role of the carpenter ant queen is to lay eggs, but as soon as worker carpenter ants mature into adults, they take on the responsibilities of the colony. They forage for food, tend to the eggs, larvae and pupae, and excavate galleries to broaden and propagate their nest. Functions are divided into two castes: major workers who act as soldiers to guard the nest, and minor workers who forage for food and take care of the young. After two or more years, the queen begins to produce winged males and females who will leave to begin other colonies of carpenter ants. A typical carpenter ant colony contains one queen (Dennis, 2003).

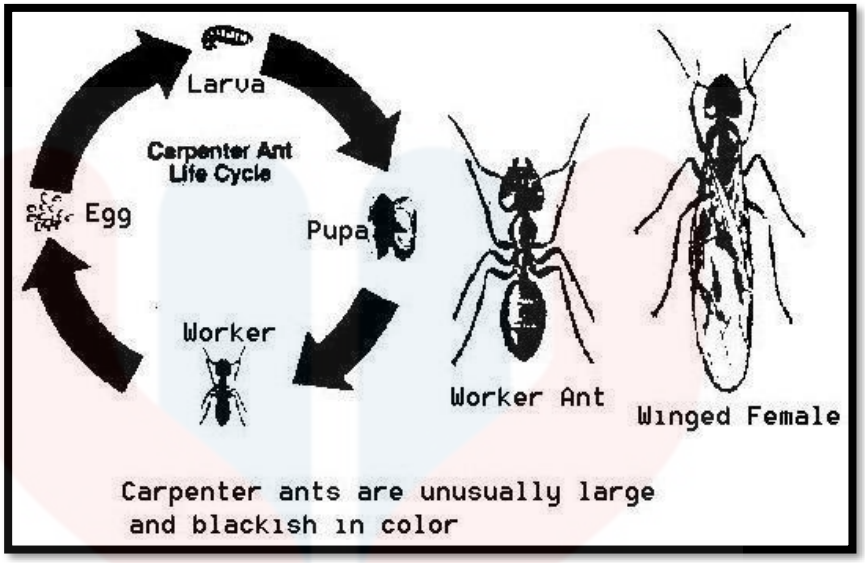

Figure 2.8: Life cycle of Carpenter ants
(Source: Vectors and Diseases P.4, n.d.)

2.3.3 Distribution and Ecology of Carpenter ants

There are two types of carpenter ant nets: parent colonies and satellite colonies. Parent colonies are typically established outdoors in moist wood including rotting trees, tree roots, tree stumps, and logs or boards lying on or buried in the ground. They may also nest in moist or decayed wood inside buildings. Wood decay may be caused by exposure to water leaks, condensation, or poor air circulation. Nests have been found behind bathroom tiles; around tubs, sinks, showers, and dishwashers; under roofing, in attic beams, and under subfloor insulation; and in hollow spaces such as doors, curtain rods, and wall voids. Areas around windows and where wood parts touch the foundation may be prone to infestation. Carpenter ants may also nest in foam insulation (Department of Entomology, 2016).

Parent carpenter ant colonies sometimes establish one or more satellite nests in nearby indoor or outdoor sites. Satellite nests are typically composed of workers, pupae, and mature larvae. A satellite nest with less moisture may only support workers

(the eggs would dry out in lower humidity). For this reason, satellite nests can be found in relatively dry locations, such as insulation, hollow doors, sound wood, and wall voids. The workers of satellite colonies move readily between their nest and the parent colony. In late summer, winged reproductives may emerge from pupae transported into satellite colonies. They may appear in structures in late winter and early spring as they swarm from a satellite nest. Carpenter ants may move eggs into satellite nests inside a house or other structure if there is enough moisture (Devanand and Usha, 2008).

2.3.4 Economic Importance of Carpenter ants

Ants foraging within a structure are a physical nuisance and may be psychologically stressful for some individuals. Infested goods are often disposed of resulting in monetary loss to the consumer. Ants often trigger strong negative emotions in homeowners who perceive these infestations of their dwelling as caused by unsanitary conditions. Subterranean termites, *Reticulitermes* spp., and carpenter ants, *Camponotus* spp., are recognized as among the most serious structural pests in Aseans. Carpenter ants are recognized as a structurally damaging pest in areas where termites have reached their geographical limits. In a survey published by the Environmental Protection Agency, ants were considered to be a more serious pest than 24 cockroaches and have displaced subterranean termites in public concern (Whitmore et al., 1992). In a recent survey of pest management professionals, nuisance ants continued.

Carpenter ants are thought to play a role in the transmission of the fungal pathogen *Cryphonectria* (Endothia*) parasitica* (Murrill), the causal agent of Chestnut blight. The pathogen has been isolated from the digestive tract of carpenter ants, though

their ability to mechanically transmit the pathogen is unknown. This is in striking contrast to actual mechanical disease transmitting capabilities and number of disease related organisms potentially carried by cockroaches (Roth and Willis, 1997). Unlike most species of pest ants, carpenter ant infestations represent a dual problem. As a nuisance pest, carpenter ants forage within structures in search of food and moisture. Carpenter ants are also a potential structural pest causing monetary loss to consumers from structural repairs to wooden timbers damaged during gallery formation by adult workers.

2.4 Crude Extracts of *Melaleuca cajuputi*

Crude extraction method will be used for plant materials that are not able to withstand heat or heated forms of extraction such as hot water distillation. High pressure or high temperature action on this type of plant will damage the plant, once damaged, the efficiency of their essential oil will be affected and cannot be extracted (Odey et al., 2012). Therefore, to overcome this damage to the plant material, solvents such as ether, ethanol, methanol, hexane, alcohol and petroleum are used.

Before starting the crude extraction process, plant material will have washed thoroughly using hydrocarbon solvents such as hexane. This step is very important to ensure waxy matter, pigment, aromatic molecules and other necessary plant materials are completely dissolved with the solvent (Ferdinand, 2007). Under low pressure, the solvent mixture is then filtered and distilled using low pressure. A resin or a concentrated concrete remain after the distillation process. Additional processing using alcohol enhance the quality of extracting the essential oils (Sasidharan et al., 2011).

2.5 Bioassay in General

Bioassay or biological assay is a type of scientific practice which engaging the use of live animal or plant (in vivo) or tissue or cell (in vitro) to investigating the biological activity of a substance such as hormone or drug (Miller et al., 2010). Usually, bioassay will be operated to identify the effects and causes of target substance on living organism in creating new products or drugs especially in controlling contamination and pollution. Besides, a bioassay also can be used to identify the experiment of a certain structure of a mixture that can pose disastrous effects whether on environment or on organisms (Miller et al., 2010).

Bioassay can be both qualitative and quantitative where qualitative are applied to reach the physical effects of a materials that maybe cannot be quantified. While, quantitative involve consideration of the dose response curve and how the response changes to the increasing dose level (Miller et al., 2010).

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

There were several instruments and apparatus that were used to carry out this study. The main instruments that were used to complete this study are electric grinder and rotary evaporator attached to a vacuum and water cooler to control the pressure and temperature of the solution to be evaporated.

Chemicals that were used for the extraction process of crude extracts are *n* – hexane, dichloromethane and ethanol. Other chemical that were used throughout the experiment was anhydrous sodium sulphate. A square container, filter paper, filter funnel, filtration flask, 20cm diameter ring, honey and aluminium foil were used to conduct the repellency and toxicity test. For the control of this experiment, honey was used as the control of the toxicity test, whereas acetone was used as the control for repellency test.

3.2 Method

There were five main processes involved in this study. The steps were collection of plant material, collection of insect, processing plant material, bioassay technique and statistical analysis. The method used for this study is adopted and modified from the steps used by WHO (World Health Organisation) to control the disease causing vector, *Aedes* mosquito (World Health Organisation, 2013). The method used by WHO is modified because the life cycle and characteristics of carpenter ants and mosquito is different.

3.2.1 Collection of *Melaleuca cajuputi* stem

Plant material studied in this research was the stem of *Melaleuca cajuputi* that were collected from the wild of Pattani, Thailand at the coordinate of 6°49'24.04" N, 101°9'44.4" E. The dust and sand from stem sample was removed to prevent contamination. The stem sample were collected using knife, axe and chisel. The collected stem sample were air dried under shade at room temperature (27 ºC) for five days until the stem were completely dried and in crispy form (Ogunsina et al., 2011). The moisture content of the sample before drying and after dying were calculated every 24 hours (Equation 3.1) to ensure the stem sample was completely dry.

$$Moisture\ content = \frac{Ww - Wd}{Ww} X\ 100\% \quad \text{..... (Equation 3.1)}$$

Where 'Ww' stands for wet weight, 'Wd' stands for dry weight.

The dried stem sample were cut into small pieces using axe (Figure 3.1). The small pieces of stem were ground into fine powder by using electric grinder (Figure 3.2). The finely ground bark sample were sealed in polyethylene bags (Figure 3.3) and stored in refrigerator at four degrees Celsius before being used for the process of solid crude extraction (Bussaman et al., 2012).

Figure 3.1: Stem sample that were cut into small pieces using axe on 21st July 2016.

Figure 3.2: The small pieces of stem were ground into fine powder using electric grinder on 26th July 2016.

Figure 3.3: The ground stem sample taken on 26th July 2016.

3.2.2 Collection of Carpenter ants (*Camponotus* spp.)

The insect that were used for this experiment was Carpenter ants, the common household insect. Carpenter ants were collected by using honey as a trap to find the colony of the carpenter ants (Figure 3.4). Honey were mixed with three drops of water in an aluminium foil that was shaped into the size of a 500 ml mineral water bottle cap and kept in a garden around Prince of Songkla University which was suspected to have carpenter ants. The trail of the carpenter ants had led to the colony of those ants. The

colony of ants was brushed into a plastic bag smoothly using a small and soft painting brush to prevent injuries to the ants. The plastic was poked with tiny holes for aeration and supplied with honey as a source of food and water before being used for bioassay test. The collected live carpenter ants sample were kept in a transparent plastic bag at room temperature of 26 to 30°C and 70% RH (Relative Humidity).

Figure 3.4: Honey used as a trap to find the colony of carpenter ants on 5th August 2016.

3.2.3 Crude Extract Preparation Using Stem Powder

This study used the crude extract of *Melaleuca cajuputi*. In order to obtain the crude extract, the plant material was extracted using three different extraction solvents: *n*-hexane, dichloromethane and methanol (Nostro et al., 2005). Crude extract of *Melaleuca cajuputi* stem was prepared by modifying the method described by Shankar (2015).

The crude extract of *Melaleuca cajuputi* stem were extracted by using sequential extraction technique. Extraction were started with the most non-polar extraction solvent, n-hexane, followed by dichloromethane and methanol. The ground stem sample were tied using a sieve cloth and placed into a glass jar (Figure 3.5). Extraction solvent was measured and poured into the glass jar till the sieve cloth was totally soaked and covered with the solvent (Figure 3.6). The sample was allowed to soak for 24 hours. After 24 hours, the sieve cloth was removed from the glass jar and the sieve cloth was squeezed completely to ensure all the solution to drop into the glass jar (Figure 3.7). The volume of the solution obtained was measured.

The solution obtained were filtered using filter funnel and Whatman filter paper No. 1. One spoon of anhydrous sodium sulphate was added into the solution to remove excess water molecules in the solution before being filtered. Finally, the filtered solution was evaporated using a rotary evaporator that was connected to a vacuum and water cooler (Figure 3.8). The concentrated extracts (Figure 3.9) were allowed to dry in hot air oven and stored in refrigerator four degree Celsius until required for bioassays test. The time taken for each extract to evaporate was recorded. Methanol extract were oven dried as extract solution cannot be evaporated completely although have been evaporating for more than six hours. Methanol extract was oven dried at temperature 50ºC for 24 hours. The moisture content of the oven dried methanol extract (Figure 3.10) was calculated.

Figure 3.5: Stem sample were tied using a sieve cloth and placed into a glass jar on 28th July 2016.

Figure 3.6: Stem sample were soaked with extraction solvent and left to soak for 24 hours on 28th July 2016.

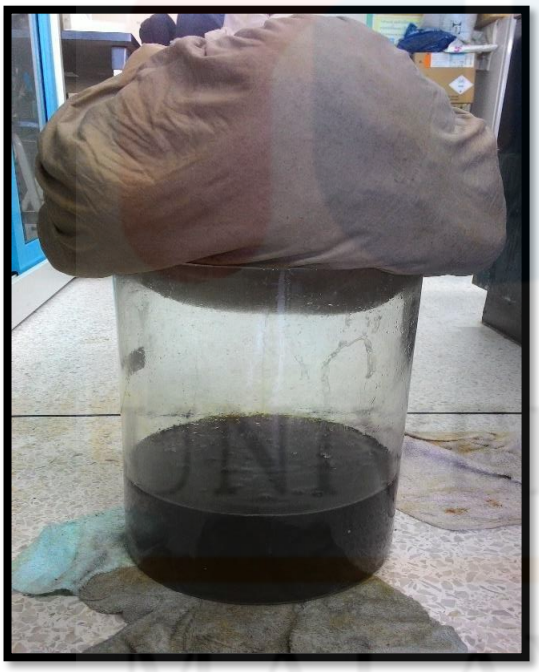

Figure 3.7: The sieve cloth was squeezed completely on 29th July 2016.

Figure 3.8: The solution (hexane mixed active ingredient of the plant) was evaporated using rotary evaporator on 29th July 2016.

Figure 3.9: The concentrated extract after evaporation of extract solution taken on 1st August 2016.

Figure 3.10: Oven dried methanol crude extract taken on 5th August 2016

3.2.4 Bioassay Techniques of Extractives

The bioassay technique for this study involves two types of test, which is the repellency test and toxicity test. Repellency test was conducted using a method which was modified from the WHO method that was used to determine suitable natural insecticide to control disease causing mosquito population. WHO method for mosquito repellency was conducted using volunteers who were used to apply the prepared insecticide against mosquitoes on their hand and exposed to mosquitoes, in order to identify whether the mosquitoes are repelled from biting the volunteers hand (WHO, 2014). This WHO method was modified based on (Mensah et al., 2014) to the suitability of carpenter ants. Mensah et al. (2014) conducted research on evaluation of

the insecticidal and repellent properties of the volatile oils of lime, sweet orange and lemon on Carpenter ants.

Toxicity test was conducted to identify the toxicity rate of the prepared crude extract using force feed method. Toxicity test for this research was conducted by modifying method described by Miguelena and Baker (2014). The study conducted by Miguelena and Baker (2014) was on evaluation of liquid and bait insecticides against Dark Rover ants. The bait insecticide study method was modified and used for this study. Force feed method can be described as a method that causes the insect to be forced to eat the bait prepared. In this study, the ants were starved and exposed to the bait, so that the ants will eat the bait. Both repellency and toxicity test was conducted using *n*-hexane, dichloromethane and methanol crude extract.

a) Repellency of *Melaleuca cajuputi* against *Camponotus* spp.

WHO's method for mosquito repellent testing was modified and used for this research (WHO, 2014) and followed the steps and experimental setup as described by Mensah et al. (2014). Repellent activities of the extracts were evaluated by preparing 20% w/v of the extract in acetone. A volume of one millilitre of 20% w/v solution of the diluted crude extract was used to soak the edges of a nine centimetre round Whatman filter paper (Figure 3.11) and was introduced into the center of a ring. A bait of two gram of honey was placed in the center of the filter paper as a food source to attract the ants (Figure 3.12). The control of this experiment was one millilitre of acetone.

A total of 30 live ants were used for this experiment. The ants were starved for 24 hours before repellency test were conducted. A total of 30 live ants were introduced onto each ring (150 mm x 25 mm) but outside the 9 cm Whatman filter paper. A three setup of ring were prepared to triplicate the experiment. The experiment was monitored for three hours with intervals of every 15 minutes to calculate the repellency rate of each extract. The final repellency rate for each extract was calculated after 180 minutes of exposure time using modified WHO's landing inhibition formula as used by Thavara et al., 2007 (Equation 3.2). Number of ants found on the treated zone (Figure 3.13) and control zone was recorded.

$$Repellecny\ rate\ (\%) = 100 - (\mathrm{T} \times 100)/N \quad \ldots\ldots\ldots\ldots \text{(Equation 3.2)}$$

Where, 'T' stands for the number of ants found inside the treated zone containing the bait and 'N' represents the total number of ants per set-up, which in this experiment it was 30 ants per each set-up.

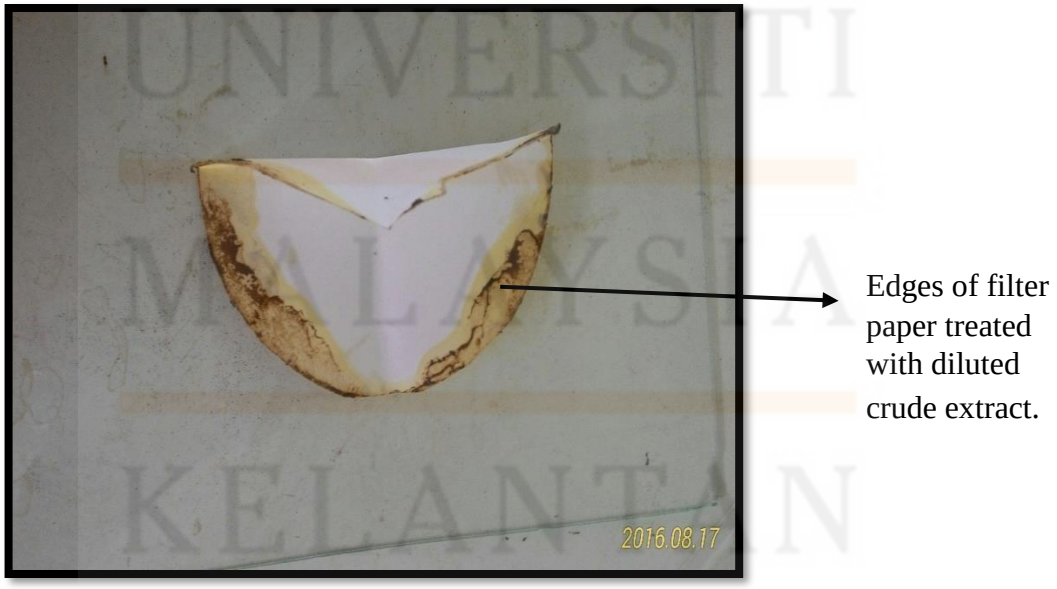

Figure 3.11: Filter paper soaked with crude extract om 17th August 2016.

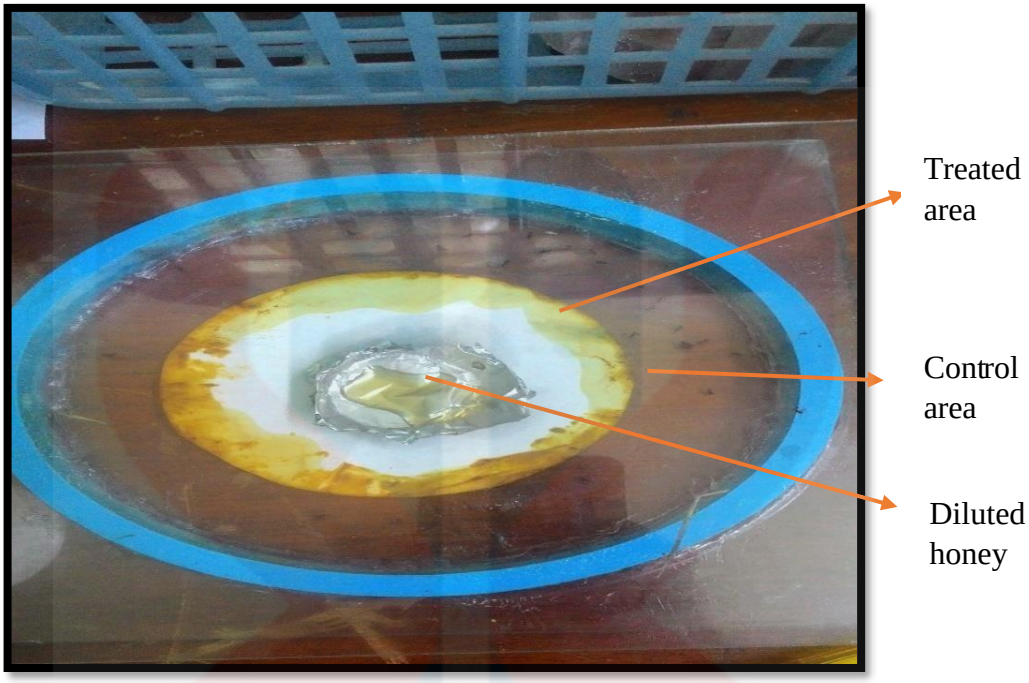

Figure 3.12: Experimental setup for repellency test taken on 17th August 2016.

Figure 3.13: Number of ants found in the treated area taken on 17th August 2016.

b) Toxicity of *Melaleuca cajuputi* against Carpenter ants

The toxicity rate of prepared crude extract to Carpenter ants were identified by conducting toxicity test using force feed method. The ants sample were force feed with the prepared crude extract. A model set up of box which consists of foraging area and habitat were prepared (Figure 3.14). In the habitat area, the box was filled with damp soil and dry leaves, a condition where Carpenter ants prefer (Figure 3.15). In the foraging area, the box was used to hold water and honey mixed with 10% extract (Hexane, Dichloromethane and Methanol) (Figure 3.16). The foraging area were connected to the habitat by using a pipe for the ants to travel from the habitat box to foraging area.

The collected ants were transferred into the habitat box, 30 live ants per box and provided with honey and water to allow the ants to adapt to the environment. The ants were allowed to adapt in the box for 24 hours. After 24 hours, the ants were starved for 24 hours to make the ants feel hungry. After starving for 24 hours, the ants were provided with water and food of honey mixed with the extract. The experiment was replicated 3 times. The same method was repeated using different crude extract. The number of dead ants were recorded for 24 hours with the interval of three hours. Toxicity against the cockroach were calculated using the formula (Equation 3.3).

$$\text{Mortality (\%)} = \frac{D}{N} x\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(Equation 3.3)}$$

Where D stands for the number of cockroaches found dead in the container and N stands for the number of cockroaches used in the for container of each set.

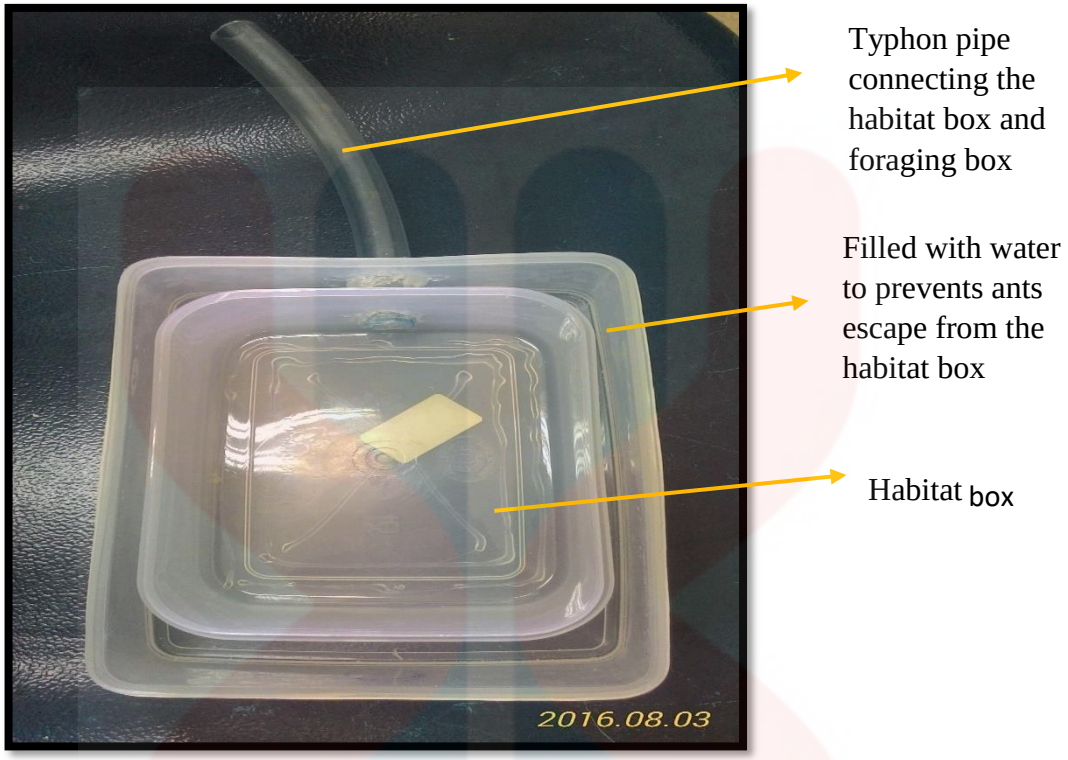

Figure 3.14: Experimental habitat setup for toxicity test taken on 3rd August 2016.

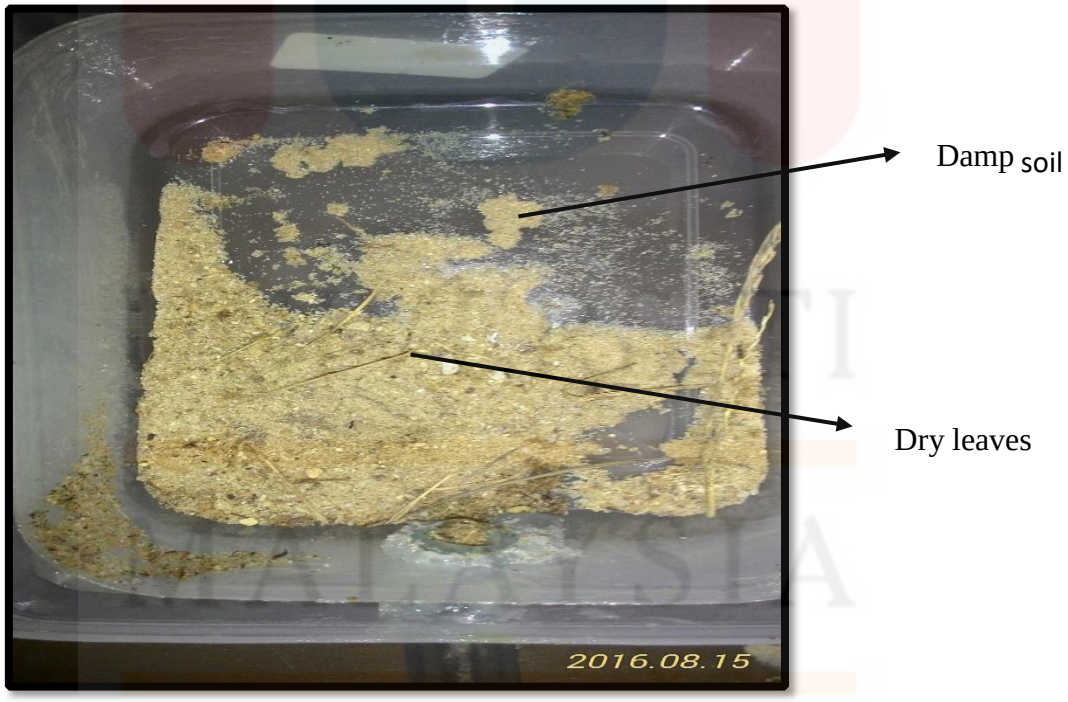

Figure 3.15: Habitat box of ants filled with damp soil and dry leaves taken on 15th August 2016.

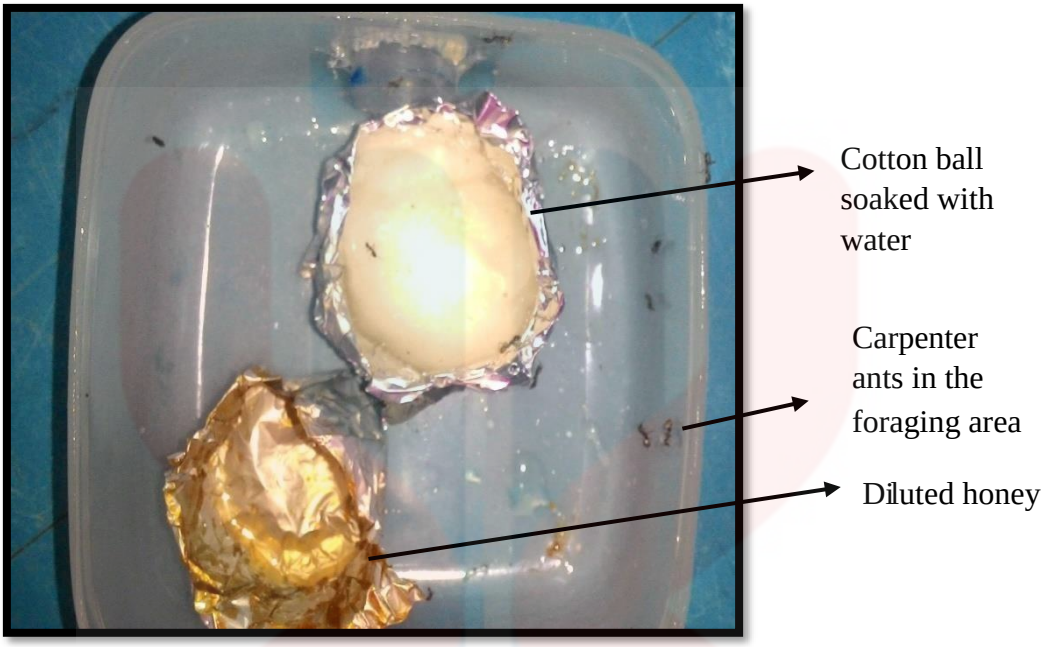

Figure 3.16: Foraging area with water and honey mixed with extract taken on 11th August 2016.

3.1.5 Statistical Analysis

The statistical analysis for toxicity test were carried out using Probit's analysis to identify the lethal time taken for 50% and 90% of the population of carpenter ants to die whereas ANOVA followed by LSD were conducted for repellency test to determine the most effective extract to repel carpenter ants (Thavara et al., 2007).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Stem Extraction of *Melaleuca cajuputi*

Melaleuca cajuputi stem collected from the wild was air dried first before being used for extraction instead of using the freshly collected stem because dried stem will not undergo any further metabolic reaction and enzymatic alteration (Zygmunt & Namiesnik, 2003). Furthermore, at dried condition all active ingredient and compounds in the stem can be recovered in a natural and unmodified form, while fresh stem has the possibility to form new or intermediates compounds. Drying process of the stem helps to remove excess enzyme action and also water (Dadang, 2009). Therefore, drying the stem is very important to reduce errors throughout the experiment.

There are many drying methods that can be used to dry plant materials, such as air drying and oven drying. Air drying method is used for this experiment because it does not use heat that has the possibilities to degrade the active ingredients contain in the stem of *Melaleuca cajuputi* (Yi & Wetzstein, 2011). The stem sample were cut into smaller size to increase the efficiency and easiness to grind the stem sample. The smaller the surface area, the higher the efficiency. After grinding, the total weight of the stem sample in the powder form were weighed and recorded as 2487.1g.

The ground stem sample were sealed in a polyethylene bags and refrigerated before being used for extraction to prevent degradation of active ingredient that present in the stem sample. The active ingredient plays an important role to identify the potential and effectiveness of *Melaleuca cajuputi* stem as natural insecticide.

The total of 2487.1 g of the ground stem sample were used for sequential extraction. This method was referred to Oparaeke (2007) where the study used 2500 g of powdered plant material for sequential extraction to determine the insecticidal potential of extracts of *Gmelina arborea* (Beechwood). In sequential extraction, the extraction is carried out on the same plant material successively in the order of polarity of the solvent. It is also known as successive extraction. In sequential extraction a particular solvent is used for the extraction and once the extraction is over, fresh plant material is used for further extraction with other solvents (Shankar, 2015). Hexane is a nonpolar extraction solvent, Dichloromethane is moderately polar extraction solvent and methanol is strongly polar solvent. Therefore, this study was conducted by starting with hexane (non-polar), dichloromethane (moderately polar) and lastly methanol (strongly polar) (Johnson et al., 2012). Sequential extraction was conducted for this study because the active metabolites present in the stem of *Melaleuca cajuputi* stem is unknown (Shankar, 2015). If the active metabolites present in the stem sample were identified before, different extraction method suitable for the active ingredient can be used, such as ethanol extraction mainly for hydrophilic compounds or hexane extraction for mainly non – polar hydrophobic compounds (Ying, 2015).

Before extraction solvent was poured, the ground stem sample was tied in a sieve cloth to allow the active ingredient to dissolve out from the cloth into the extraction solvent in the out. After 24 hours of soaking, the solution that has the extraction solution and dissolved active compound was measured and filtered using Whatman No.1 filter paper to remove dissolved stem particles from the solution (Zygmunt & Namiesnik, 2003). Small sized stem particles were not able to be filtered by the sieve cloth as the pore size in the sieve cloth is larger compared to the size of

the dissolved stem particle (Sinclair, 1998), therefore filtration was conducted. Followed by evaporation of the solution using rotary evaporator to evaporate the extraction solvent and remain with the active compound in the form of sticky solid crude extract. Evaporation was conducted at the temperature of 45 ºC and 48 rpm. During evaporation, the rotary evaporator was connected to the water cooler to prevent the solvent from freezing during the evaporation process and vacuum to reduce the boiling point to be significantly lower than an ambient pressure (Odey et al., 2012), so that solution can be evaporated at a faster rate. The volume of solution remaining in the glass jar after 24 hours and the time taken for evaporation was recorded as shown in Table 4.1.

Table 4.1: The volume of remaining solution and time taken for the extraction solvent to be evaporated using rotary evaporator.

Extraction Solvent	**Volume of solution after 24 hours (mL)**	**Evaporation time (Minutes)**
Hexane	3000	150
Dichloromethane	2300	65
Methanol	2550	545

According the Table 4.1**,** it can be seen clearly that dichloromethane has the lowest time taken (65 min) to be evaporated, followed by hexane (150 min) and lastly methanol (545 min). Factors influencing the evaporation time is the boiling point of the extraction solvent used. The boiling point for hexane is 68 ºC (Jay, 2001), dichloromethane is 39.6 ºC (Jay, 2004) and methanol 64.7 ºC (Hugg & George, 2007). Methanol is an alcohol with the lowest boiling point. The higher the boiling point of the extraction solvent the longer the time taken to evaporate the extraction solvent to form crude extract. However, methanol extract with lower boiling point (64.7 ºC)

compared to hexane extract (68 ºC), took longer time to evaporate because it is hypothesised that there is presence of stem particles in the solution, presence of water absorbed by methanol from the stem or presence of supernatant particles that are not able to be filtered using normal filtration method (Pakhathirathien, 2016). Therefore, double filtration was conducted using Buchner Funnel filtration method with Whatman No. 1 filter paper 50 mm. This filtration method able to remove supernatant particles of stem that was not removed in the normal filtration. The supernatant particles that was removed using Buchner Funnel filtration is shown in Figure 4.1.

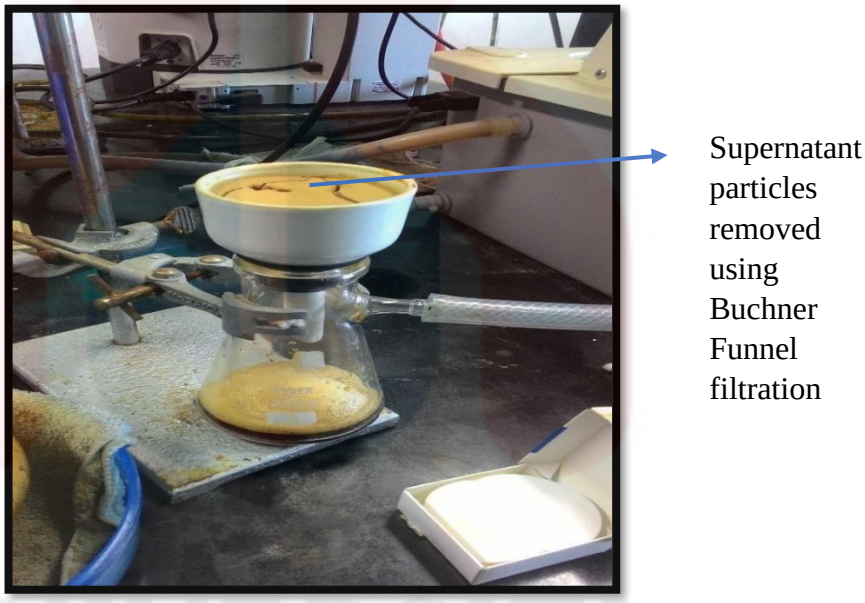

Figure 4.1: Double filtration method using Buchner Funnel filtration method on 1st August 2016.

Although the supernatant particles were removed from the methanol extract using Buchner Funnel filtration method, still the solution were not able to be evaporated completely, as there is still presence of liquid solution can be observed in the evaporation flask, instead of a sticky solid crude extract. Methanol is a polar solvent that is completely miscible in water, therefore when it exposed to the air methanol will absorb water, which reduces the potential for methanol to be evaporated completely

(Martin, 2003). It is believed that the evaporation was not complete due to the presence of water absorbed by methanol which cannot be evaporated at the temperature of 45 ºC and 48rpm. Therefore, the water is removed from the solution by oven drying for 24 hours to get methanol crude extract.

The volume of solution remaining after 24 hours is also influenced by the boiling point (Jay, 2004). Dichloromethane has the lowest volume of solution remaining after 24 hours because it has a lowest boiling point of 39.6 ºC, where it is able to evaporate at room temperature, whereas less solvent of hexane and methanol has been evaporated at room temperature due to their higher boiling point. On the other hand, the volume of extraction solvent used for each extract is not the same because the way the stem sample is tied and placed again is not the same as previous one, so the volume of extraction solvent used to cover the sieve cloth is different is also one of the reason of different volume of solution remaining after 24 hours. For example, the volume of extraction solvent used for flatly tied sieve cloth will be less compared to the one which was not that flat.

The percentage yield of each extract was calculated to know which polarity compound is the highest in the stem sample (Appendix A). The percentage yield was calculated by dividing the total weight of the solid crude extract with total weight of stem sample used for this experiment, which was 2487.1 g (Figure 4.2). The yield obtained was refrigerated at four degrees Celsius before used for bioassay test to prevent microbial growth and degradation of the active compound (Rejitha et al., 2014). It is important to prevent microbial growth and degradation of the extract prepared in order to prevent error of toxicity and repellency against the ants caused by

other factors such as bacteria or degradation of the extract which reduces the efficiency of study.

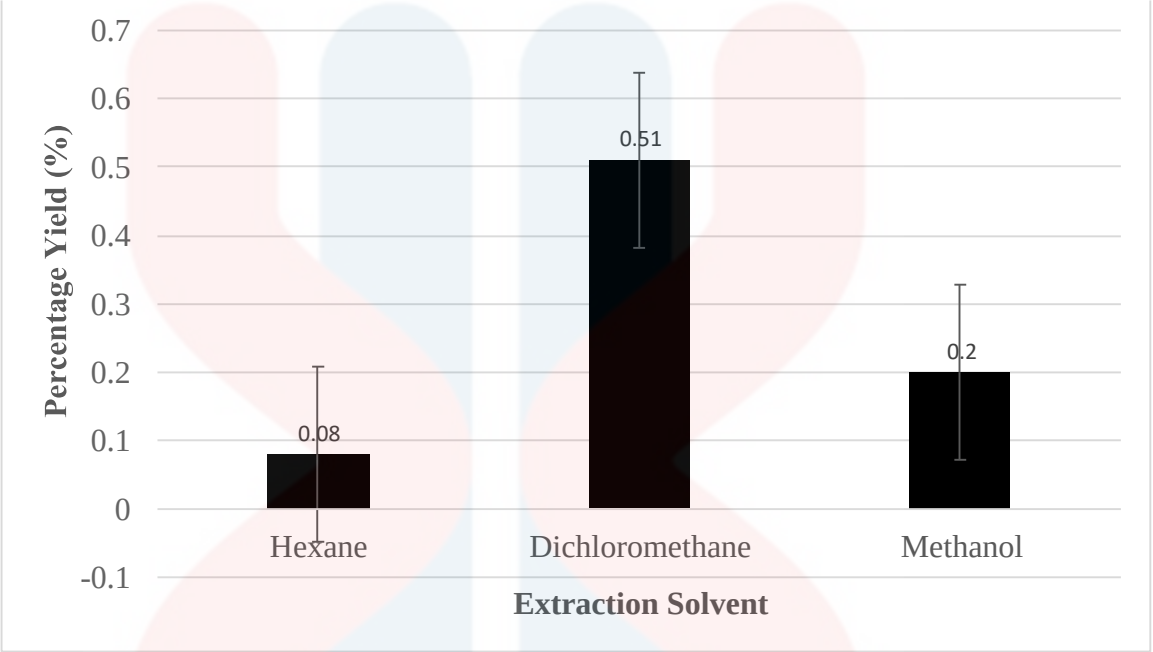

Figure 4.2: Percentage Yield of Crude Extract using Different Extraction Solvent.

Based on the bar chart above, it can be concluded that the active compound that has been extracted from stem sample of *Melaleuca cajuputi* were mostly moderately polar substance. This is because dichloromethane extract has the highest percentage yield of crude extract which was 0.51%. Followed by, methanol extract with the percentage of 0.2% and lastly hexane extract with the lowest percentage of 0.08%. This result is supported by the study conducted by Pattarawadee et al. (2014). The study was conducted using *Melaleuca cajuputi* leaf and branch extracts using hexane, methanol and dichloromethane as solvent of extraction, and the result of the branch extract percentage yield showed similar result to this study, where dichloromethane extract of the branch has highest percentage yield while hexane extract has the lowest percentage yield. However, the percentage yield of stem extract is higher compared to branch extract because quantity of active compound resulting from secondary

metabolism in the stem is higher compared to the branch (Ghasemzadeh et al., 2016). Hexane is a non-polar solvent that will extract non-polar compounds from the stem sample. The percentage yield of non-polar compounds was very low because non-polar compounds in plants are usually in the form of essential oil that are mostly found in the leaves of the plant not stem of the plant (Koul et al., 2008). Koul et al. (2008) proved that essential oil is main non-polar compound in *Melaleuca cajuputi*.

Moreover, essential oil is usually extracted via hot water distillation method or steam distillation method (Paranagama et al., 2008). Paranagame et al. (2008) conducted research on the efficiency of hot water distillation and steam distillation method, and result showed that steam distillation is more effective to yield essential oil from plant compared to hot water distillation method for normal conditions, while hot water distillation is better to extract essential oil for plants whose essential oil are difficult to be extracted at high temperature. Essential oil extracted via crude extraction method has very low percentage yield although the plant material (Melaleuca cajuputi) has high concentration of essential oil (Charles & Simon, 2009).

According to the bar chart, it can be concluded that *Melaleuca cajuputi* stem has very low percentage of active compound present. The percentage yield for each crude extract is less than 1%. This shows that high quantity of stem sample will be required to prepare a natural insecticide product from this plant material to be used in the market, which is not economical. It is better to produce a product that is effective and produce in higher percentage yield although small quantity of plant material is used.

4.2 Repellency rate of *Melaleuca cajuputi* stem against Carpenter Ants

Repellency test was conducted using 30 live ants and placed into a ring with treated filter paper. The concentration of crude extract used for this experiment is 20%. The control for repellency test was acetone. Acetone was used as control because it was the solvent used to dilute the crude extract before being applied on the filter paper. Acetone was used to dilute because it has a very low boiling point and can evaporate easily in room temperature. Control test was conducted using acetone to ensure there is no any repellency effect of acetone to the ants. The control test using acetone resulted in 0% repellency, which means that acetone does not affect the repellency rate of the extract against carpenter ants. The repellency rate was observed for three hours with the interval of 15 minutes. The number of ants repelled from entering the treated area to eat the honey was recorded (Appendix B). The ants were starved for 24 hours before the experiment to test whether hunger causes the ant to cross the treated border to eat the honey.

Based on the bar chart shown below (Figure 4.3), hexane extract has the highest repellency effect against carpenter ants at the percentage of 97.3%, dichloromethane at the percentage of 83.4% and lastly methanol with the percentage of 42.8%. The nonpolar compound which is believed to have essential oil has the highest repellency effect because the smell of the essential oil repels the ants from going to the treated area (Bakkali et al., 2008). On the other hand, methanol and dichloromethane extract has no strong smell that distract the ants from going to the treated area.

The repellency rate decreases as the polarity increases in the order of hexane > dichloromethane > methanol. This result is supported by the study on activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the ants (Hymenoptera: Formicidae).

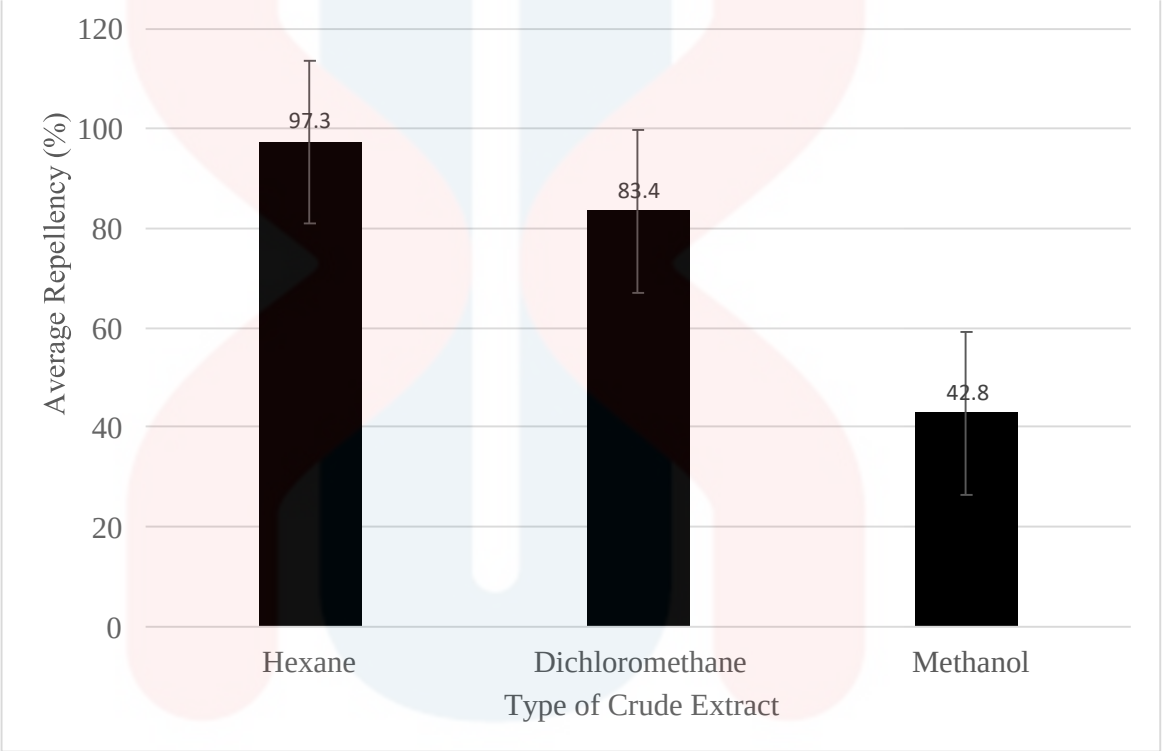

Figure 4.3: Average Repellency Rate of Different Crude Extract against Carpenter Ants.

In order to determine the most effective extract, one-way ANOVA analysis was conducted. Analysis of the data showed that the repellency rate differed among types of crude extract used. Further analysis using one-way ANOVA technique revealed that the difference was significant at least at the 0.05 level, but the analysis did not mention which extract differed from other extract groups. To detect this post hoc test was conducted (Appendix C). All the extract showed difference with at least 0.05

significant. This shows that there is significant difference between the repellency rate and type of crude extract used.

4.3 Toxicity rate of *Melaleuca cajuputi* stem against Carpenter Ants

Toxicity test conducted by using 30 live ants and exposing them to diluted honey solution mixed with crude extract. Honey was diluted because ants doesn't prefer concentrated pure honey. Concentrated pure honey is very thick and ants that try to eat it will stick to it and unable to move, this might cause injuries and even death to ants. In order to prevent these errors in the experiment, the honey must be diluted with few drops of water. The ants to be tested was starved 24 hours before experiment conducted so that they will be hungry during the period of experiment. Ants will not die due to starvation of 24 hours because carpenter ants are able to live without food for 2 months.

Honey is used as the food source to attract the carpenter ants. It is proved that Carpenter ants will eat sweet food such as honey and sucrose solutions. The ants were allowed to adapt in the habitat and foraging area one day before the experiment, so that the ant colony will not feel disturbed. The toxicity test was conducted for 24 hours with the interval of three hours to calculate the number of dead ants and live ants in the box. The number of dead ants were recorded for all the extract (Appendix D).

The experiment was started with hexane extract toxicity test. The ants were exposed to 10% concentration of hexane extract mixed in the honey and placed into the foraging area. The number of ants dead after eating the bait were recorded and the Probit's of Mortality was calculated (Appendix E). Probit's of Mortality was tabulated

to determine the time taken for 50% and 90% of the ants' population to die. The experiment was repeated by using different crude extracts; dichloromethane and methanol.

Based on the bar chart below (Figure 4.4), it can be concluded that methanol extract has the highest toxicity effect to carpenter ants at the percentage of 84.3%, followed by dichloromethane extract at the percentage of 77.7% and lastly hexane extract with the percentage of 84.3%. The control used for this experiment was honey, which shows 0% of toxicity effect to carpenter ants, which means that the ants will not die due to eating honey but due to the crude extract mixed in the honey.

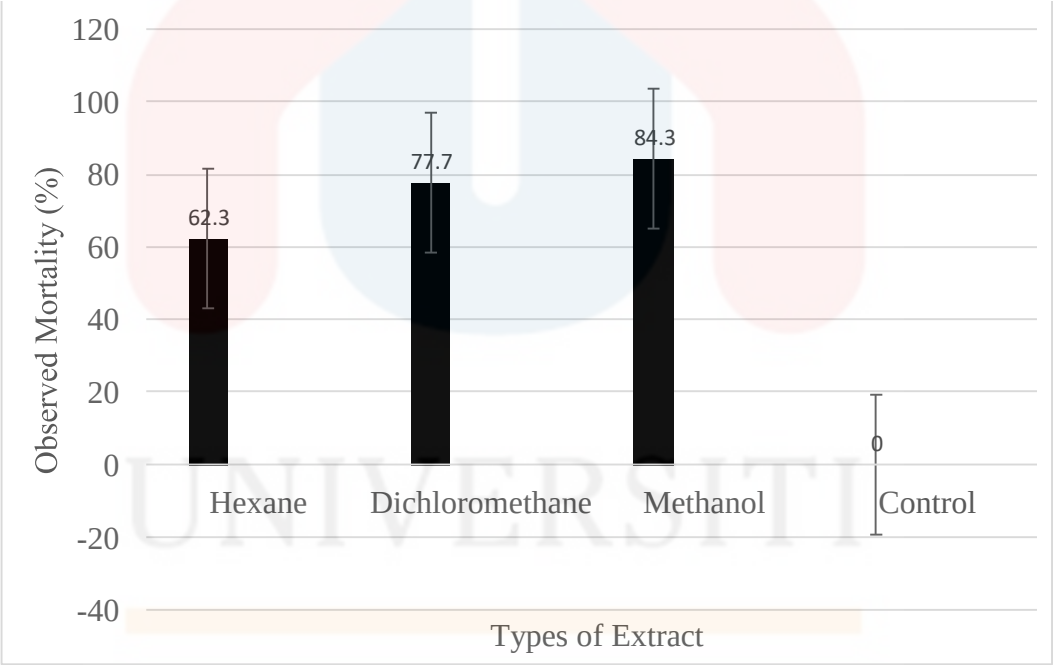

Figure 4.4: Total observed Mortality of Carpenter Ants using Different Crude Extracts.

Polar compound has higher toxicity effect compared to non-polar compound because, the smell that present in the hexane extract prevent the ants from eating the honey bait which results in the less toxicity effect. On the other hand, dichloromethane

and methanol extract has no any strong smell that prevent the ants from eating the honey bait, which results in higher toxicity effect against carpenter ants.

Figure 4.5 shows the Probit's mortality of hexane extract against carpenter ants where the LT_{50} and LT_{90} were calculated using Probit's value. LT_{50} stands for lethal time taken for the extract to kill 50 % of the ant population, whereas LT_{90} stands for lethal time taken for the extract to kill 90% of the ant population. The Probit value of 5 in the y-axis is observed to determine the time taken for 50% of the population to die and 6.28 on the y-axis to determine time taken to kill 90% of the population. Based on the graph below, it has been estimated that to the time taken for hexane extract to kill 50% of the population was 19.11 hours and 58.42 hours to kill 90% of the population. The time taken to kill ants with hexane extract was very long because number of ants ate the honey mixed with hexane extract was very few. Ants were disturbed by the smell of the hexane extract that causes it to be afraid to consume it. Therefore, hexane extract shows very low toxicity effect.

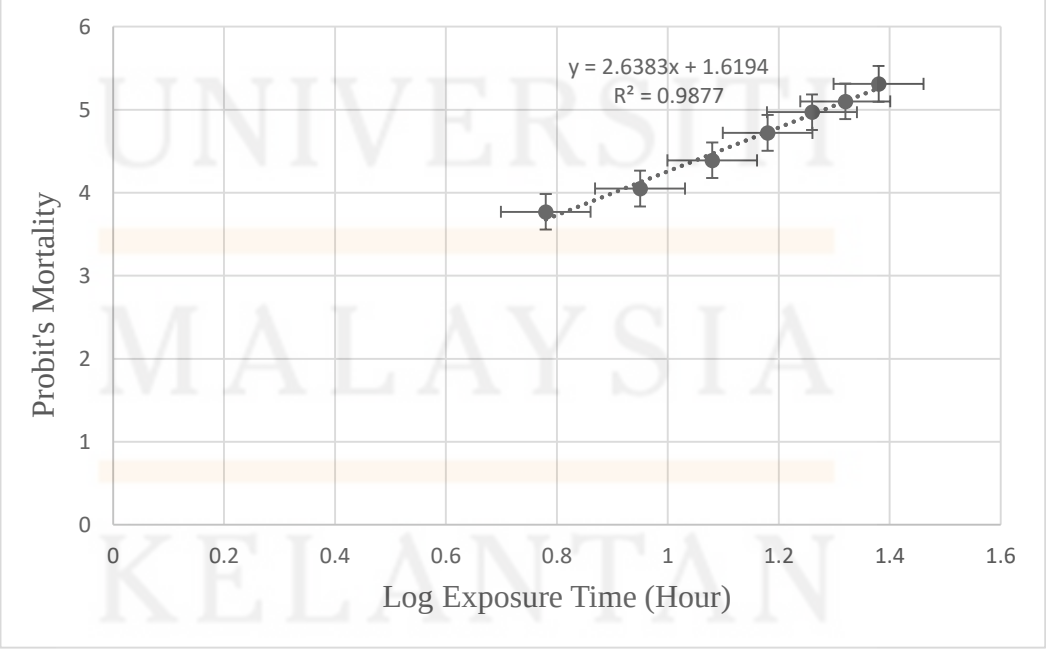

Figure 4.5: Probit's Mortality of Hexane Extract against Carpenter ants

Figure 4.6 shows the Probit's mortality for dichloromethane extract against carpenter ants, which was used to estimate the LT_{50} and LT_{90}. Dichloromethane extract shows that to kill 50 % of the carpenter ant population the time taken is 11.89 hours and 43.53 hours to kill 90% of the population. The time taken for dichloromethane extract to kill carpenter ants is less compared to hexane extract. This proves that mortality rate increases as the polarity of the compound increases. Besides that, the smell of dichloromethane is very mild compared to the smell of hexane extract, which might not trigger the eating habit of the ants. It can be said that the smell of the compound does plays role in influencing the toxicity rate of the compound.

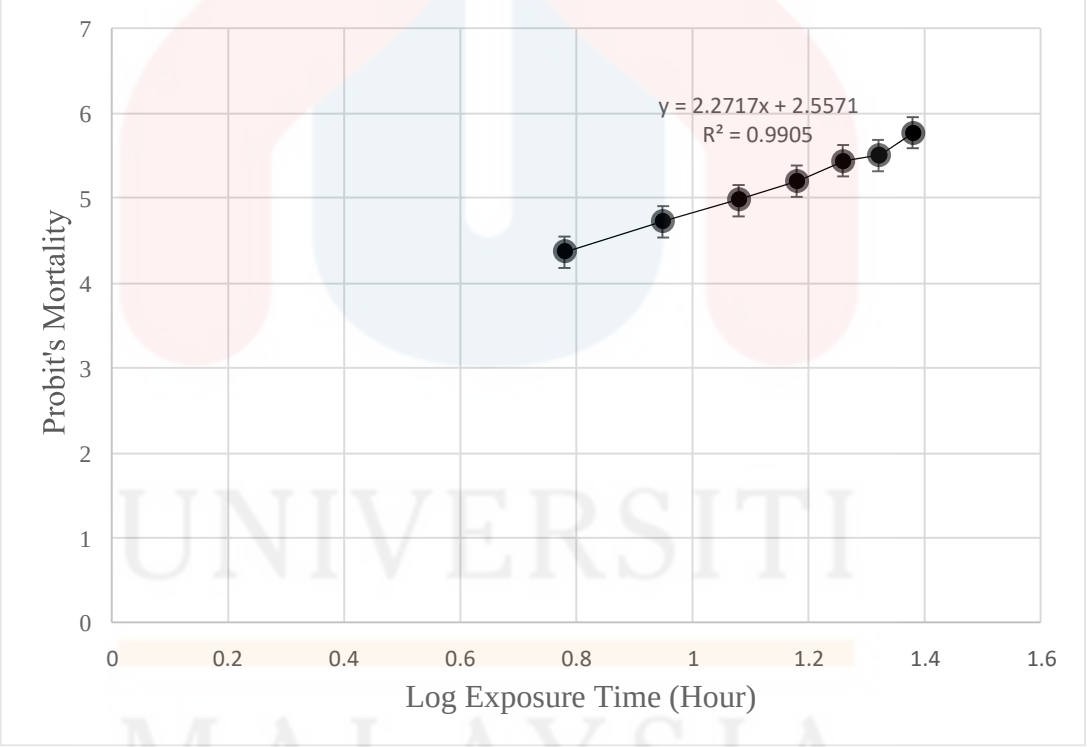

Figure 4.6: Probit's Mortality of Dichloromethane Extract against Carpenter Ants.

Figure 4.7 shows the Probit's mortality for methanol extract against carpenter ants, which was used to estimate LT_{50} and LT_{90}. The time taken for methanol extract to kill 50% of the carpenter ant population is 9.43 hours and 32.93 hours to kill 90%

of the population. The time take for methanol extract to kill the carpenter ants was the fastest compared to hexane and dichloromethane extract. This strongly proves that the toxicity rate of carpenter ants increases with the increasing polarity in the order of methanol > dichloromethane > hexane.

At the same time, methanol extract has almost no any smell that can be detected. This influences the ants to eat the honey mixed with extract as no smell disturbs them. This result of increasing toxicity rate with increasing polarity was proved by Terezan et al. (2010) on the study of activities of extracts and compounds from *Spiranthera odoratissima* St. Hil. (Rutaceae) in ants. This study proves that methanol extract is more toxic to ants compared to dichloromethane and hexane extract.

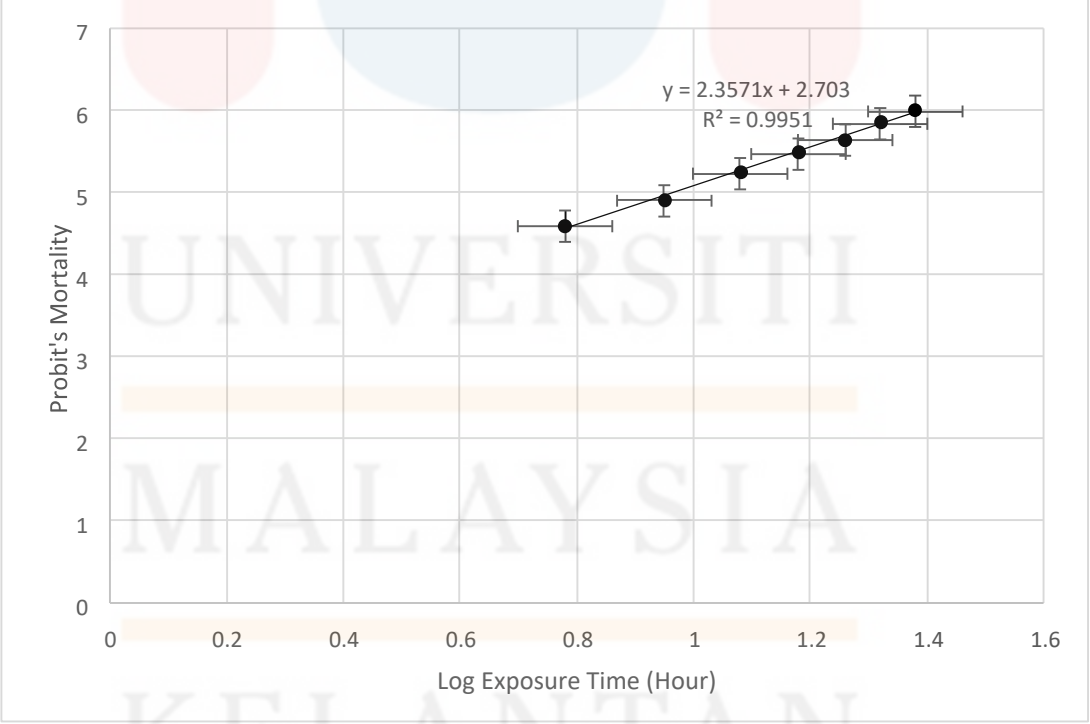

Figure 4.7: Probit's Mortality of Methanol Extract against Carpenter Ants.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In a nutshell, *Melaleuca cajuputi* stem has a moderate potential to be used as natural insecticide to repel and kill carpenter ants. It is not 100% effective to be used as natural insecticide to control the population of carpenter ants. The repellency rate and toxicity rate were determined. The maximum repellency rate of *Melaleuca cajuputi* stem to carpenter ants was shown by using hexane extract which showed 97.3% repellency. The repellency rate tends to decrease over time as the smell of the extract that repels the ants' decreases.

The maximum toxicity rate of *Melaleuca cajuputi* stem to carpenter ants was shown by methanol extract with the percentage of 84.3%. Probit's analysis showed that the minimum time needed to kill 50% of the carpenter ants' population using methanol extract will require 9.43 hours and 32.93 hours to kill 90% of the population. *Melaleuca cajuputi* stem is more effective as repellent to carpenter ants compared to toxic effect. Non polar compounds of *Melaleuca cajuputi* stem is significantly effective as repellent, whereas polar compound is effective for causing toxic to the ants.

5.2 Recommendations

There are few recommendations to improve and continue further research on the potential of *Melaleuca cajuputi* stem as natural insecticide. Current study

conducted is only the basic to evaluate whether there is any potential for the stem of *Melaleuca cajuputi* to be used as natural insecticide.

5.2.1 Identification of Chemical Compound Present in the Stem Sample

Further studies can be conducted to identify the chemical compound or active ingredient present in the stem of *Melaleuca cajuputi*. Identification of the compound present will help to determine the compounds that are responsible for the repellent and toxicity effect to carpenter ants. Chemical compound present can be identified by using gas chromatography-mass spectrometry (GC-MS). GC-MS is an analytical technique that merge the separation characteristics of gas-liquid chromatography with disclosure feature of mass spectrometry to identify different compounds that is present in a sample (Hossain et al., 2013). Therefore, GC-MS can be used to identify chemical compound present in the stem sample. Other than GC-MS, there are other several effective methods that can be used to identify chemical compound present in the stem sample such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The high compound composition identified is chemically and biologically important in a research.

5.2.2 Evaluating the Repellency and Toxicity Rate of Extract at Different Concentration

Present study was conducted using only one concentration to evaluate the repellency and toxicity effect of *Melaleuca cajuputi* stem to carpenter ants. The concentration used for repellency test was 20% whereas 10% concentration for toxicity test. The result was moderately effective as natural insecticide. Therefore, it is recommended to evaluate using different concentration which might have higher

toxicity and repellency rate. It is not economical to use more quantity of stem to produce natural insecticide but can be used for research propose to identify the minimum concentration needed to kill and repel 100% of the carpenter ants. When a test is conducted with different concentration Probit analysis can be conducted to evaluate the minimum concentration need to kill 50%, 70%, 90% or even 100% of the population.

5.2.3 Determine the Effect of the Mixed Polar and Non-Polar Compound

Present study was conducted by evaluating hexane extract, dichloromethane extract and methanol extract separately, and the result showed that hexane extract (non-polar compound) showed high repellency rate whereas methanol extract (polar compound) showed high toxicity rate to carpenter ants (Khummueng, 2016). Therefore, it will be better to further the studies by mixing the polar and non-polar compound to determine whether the mixture will result in both high toxicity and repellency rate. The mixed compound will have both the active ingredient that repelled and killed the insect. If the mixture resulted as a good repellent and toxic compound it can be used as an effective natural insecticide.

5.2.4 Mixing *Melaleuca cajuputi* Stem Extract with Other Plant Extract

Melaleuca cajuputi stem extract showed moderately effective result as natural insecticide. As to date, lemongrass natural insecticide has been used commercially in the market as a natural product (Pakhathirathiren, 2016). In order to increase the effectiveness, lemongrass or other effective plants extract can be mixed with *Melaleuca cajuputi* stem extract to evaluate whether the effectiveness increases or

decreases. If the effectiveness increases, it can be used as new effective natural insecticide in the market.

5.2.5 Evaluate the Efficiency by Spraying Directly on Carpenter Ants

Present study conducted was evaluated by laboratory testing, where ants were collected from their habitat and tested in laboratory condition. The effectiveness of the extract must be evaluated by conducting field test, where the prepared extract should be modified and used as spray and spray it directly on the carpenter ants as other insecticide that is available in the market. The efficiency of the extract as natural insecticide product can only be evaluated by applying it as used in daily life.

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APPENDIX A

Table A1: Percentage yield of crude extract from different extraction solvent.

Extraction Solvent	Percentage Yield (%)
Hexane	0.08
Dichloromethane	0.51
Methanol	0.20

APPENDIX B

Table B1: Repellency rate of Different Crude extract against Carpenter Ants.

TIME (MIN)	HEXANE EXTRACT OBSERVED REPELLENCY (%) ± SE	DICHLOROMETHANE EXTRACT OBSERVED REPELLENCY (%) ± SE	METHANOL EXTRACT OBSERVED REPELLENCY (%) ± SE
15	100.0 ± 0.0	96.7 ± 0.6	65.7 ± 0.3
30	96.7 ± 0.6	95.7 ± 0.7	60.0 ± 0.6
45	99.0 ± 0.3	93.3 ± 0.6	63.3 ± 0.6
60	94.3 ± 0.3	89.0 ± 0.3	53.3 ± 0.6
75	95.7 ± 0.7	81.0 ± 0.7	41.0 ± 0.3
90	94.3 ± 0.3	80.0 ± 0.6	36.7 ± 0.6
105	99.0 ± 0.0	82.3 ± 0.3	36.7 ± 0.6
120	99.0 ± 0.0	82.3 ± 0.3	34.3 ± 0.3
135	97.7 ± 0.3	84.3 ± 0.7	33.3 ± 0.6
150	97.7 ± 0.3	76.7 ± 0.6	35.7 ± 0.7
165	96.7 ± 0.6	71.0 ± 0.3	31.0 ± 0.9
180	97.7 ± 0.3	69.0 ± 0.3	22.3 ± 0.3

Table B2: The Summary of Average Repellency of Different Crude Extract against Carpenter Ants.

CRUDE EXTRACT	AVERAGE REPELLENCY (%) ± SE
Hexane Extract	97.3 ± 0.3
Dichloromethane Extract	83.4 ± 0.5
Methanol Extract	42.8 ± 0.5

APPENDIX C

Table C1: One-way ANOVA descriptive result.

Descriptives

Repellency

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Hexane	12	97.3167	1.85170	.53454	96.1402	98.4932	94.30	100.00
Dichloromethane	12	83.4417	8.96503	2.58798	77.7456	89.1378	69.00	96.70
Methanol	12	42.7750	14.14471	4.08323	33.7879	51.7621	22.30	65.70
Total	36	74.5111	25.30215	4.21702	65.9501	83.0721	22.30	100.00

Table C2: ANOVA result

ANOVA

Repellency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19284.347	2	9642.174	101.899	.000
Within Groups	3122.608	33	94.624		
Total	22406.956	35			

Table C3: Multiple comparisons of Extracts

Multiple Comparisons

Dependent Variable: Repellency

LSD

(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Hexane	Dichloromethane	13.87500*	3.97124	.001	5.7955	21.9545
	Methanol	54.54167*	3.97124	.000	46.4621	62.6212
Dichloromethane	Hexane	-13.87500*	3.97124	.001	-21.9545	-5.7955
	Methanol	40.66667*	3.97124	.000	32.5871	48.7462
Methanol	Hexane	-54.54167*	3.97124	.000	-62.6212	-46.4621
	Dichloromethane	-40.66667*	3.97124	.000	-48.7462	-32.5871

*. The mean difference is significant at the 0.05 level.

APPENDIX D

Table D1: The summary of toxicity rate of different crude extract against carpenter ants.

Crude Extracts	No. of Exposed ants	Toxicity rate (%)							
		3	6	9	12	15	18	21	24
Hexane	30	5.6	10.0	13.3	15.7	22.3	34.3	42.3	62.3
Dichloromethane	30	15.7	27.7	40.0	50.0	60.0	69.0	77.7	77.7
Methanol	30	20.0	34.3	45.7	59.0	67.8	74.4	80.0	84.3
Control	30	0	0	0	0	0	3.3	3.3	3.3

APPENDIX E

Table E1: Toxicity rate and Probit's Mortality of Hexane extract against carpenter ants.

EXPOSURE TIME (HOUR)	LOG TIME	OBSERVED MORTALITY (%)	ROUND OFF VALUE OF OBSERVED MORTALITY (%)	PROBIT'S MORTALITY
3	0.48	7.80	8	3.59
6	0.78	11.0	11	3.77
9	0.95	16.7	17	4.05
12	1.08	26.7	27	4.39
15	1.18	39.0	39	4.72
18	1.26	48.7	49	4.97
21	1.32	54.3	54	5.10
24	1.38	62.3	62	5.31

Table E2: Toxicity rate and Probit's Mortality of Dichloromethane extract against carpenter ants.

EXPOSURE TIME (HOUR)	LOG TIME	OBSERVED MORTALITY (%)	ROUND OFF VALUE OF OBSERVED MORTALITY (%)	PROBIT'S MORTALITY
3	0.48	15.7	16	4.01
6	0.78	25.5	26	4.36
9	0.95	38.9	39	4.72
12	1.08	49.0	49	4.97
15	1.18	57.7	58	5.20
18	1.26	66.7	67	5.44
21	1.32	69.0	69	5.50
24	1.38	77.7	78	5.77

Table E3: Toxicity rate and Probit's Mortality of Methanol extract against carpenter ants.

EXPOSURE TIME	LOG TIME	OBSERVED MORTALITY (%)	ROUND OFF VALUE OF OBSERVED MORTALITY (%)	PROBIT'S MORTALITY
3	0.48	20.0	20	4.16
6	0.78	34.3	34	4.59
9	0.95	45.7	46	4.90
12	1.08	59.0	59	5.23
15	1.18	67.8	68	5.47
18	1.26	74.4	74	5.64
21	1.32	80.0	80	5.84
24	1.38	84.3	84	5.99