



EFFECTIVENESS OF SILVERY WORMWOOD  
(*ARTEMISIA ARGYI*) IN MOSQUITO CONTROL

NUR ASMA ATIKAH BINTI AZHAR

F18B0306

A thesis submitted in fulfilment of the requirement for the degree  
of Bachelor of Applied Sciences (Agrotechnology) With  
Honours

UNIVERSITY MALAYSIA KELANTAN

FACULTY OF AGRO-BASED INDUSTRY

UNIVERSITY MALAYSIA KELANTAN

2022



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**DECLARATION**

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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Signature

Student's Name :

Matric No : Date :

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Supervisor Signature

Supervisor's Name :

Stamp :

Date :

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## ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful.

All praise and thanks to Allah and His blessings for the accomplishment of this study. I thank God for all of the opportunities, experiences, and strength that have been bestowed upon me to complete the thesis. My heartfelt thanks go to the Holy Prophet Muhammad (Peace be upon him), whose view of life has been a source of encouragement for me.

First and foremost, I would want to express my gratitude to my supervisor, Prof Madya Dr.Kumara, for his advice, understanding, patience, and, especially, for giving positive support and a warm heart for me to complete my thesis. All your advice and guidance will be kept safely in mind for the plan. Special credit to the Faculty of Agro-Based Industry for allowing me to conduct the Final Year Project to complete my studies here at Universiti Malaysia Kelantan. I would also want to thank all of the lecturers who participated in providing brief information on the proposal writing format along with management, and indeed all laboratory assistants for their support and cooperation.

I'd also want to thank all of my dear friends who were there for me and supported me constantly, providing motivation, prayers, and genuine assistance while I finished my studies. Finally, I want to express my heartfelt appreciation to all of my family members. It would be impossible to accomplish this thesis without their aid. May God grant the aforementioned individuals prosperity and honor in their lives.

## Keberkesanan *Artemisia Argyi* dalam Kawalan Nyamuk

### ABSTRAK

Nyamuk menghantar pelbagai agen berjangkit yang menjejaskan kesihatan manusia. Malaria, demam denggi, demam kuning dan penyakit bawaan nyamuk lain menjangkiti individu yang tidak terkira banyaknya dan menyumbang beribu-ribu kematian setiap tahun, menyebabkan beban yang besar kepada kesihatan awam dan proses pembangunan ekonomi negara. Kadar kematian rakyat Malaysia meningkat sebanyak 143 kematian. Kadar pembiakan yang tinggi dan kekurangan vaksin yang berkesan terhadap beberapa patogen bawaan nyamuk menjadikan kita sangat ketagih dengan penggunaan racun serangga untuk menguruskan populasi nyamuk untuk mengelakkan penyakit akibat jangkitan. Kebanyakan rakyat Malaysia masih menggunakan semburan ridsect dan ubat nyamuk lingkaran untuk menghalau nyamuk. Penghalau ini bertujuan untuk membunuh nyamuk dan biasanya mengandungi racun serangga yang mempunyai kesan sisa, seperti deltamethrin. Untuk menangani isu ini, satu kajian eksperimen telah dijalankan menggunakan artemisia argyi sebagai komponen utama penghalau nyamuk. Tujuan penyelidikan ini adalah untuk membuktikan keberkesanan ubat nyamuk menggunakan bahan organik. Kajian ini direka untuk melindungi dan menyumbang kepada masyarakat yang lebih sihat dengan cara mengawal jika tidak memerangi populasi nyamuk yang semakin meningkat dan mengurangkan kebarangkalian orang ramai dijangkiti penyakit bawaan nyamuk. Kajian ini terdiri daripada tiga rawatan: (i) Rawatan organik (ii) Rawatan kimia (iii) Tiada rawatan. Data dikumpul dengan mengira bilangan minit nyamuk mati diantara tiga (3) rawatan. Berdasarkan keputusan tersebut, ujian sangkar membuktikan bahawa ubat nyamuk yang dibangunkan adalah sangat cekap terhadap nyamuk dan dengan itu boleh digunakan dengan lebih berkesan untuk mengawal nyamuk.

Kata kunci: Nyamuk, penyakit bawaan nyamuk *Artemisia Argyi*, Gegelung nyamuk kimia, Ubat nyamuk organik

## Effectiveness of *Artemisia Argyi* in Mosquito Control

### ABSTRACT

Mosquitoes transmit a range of infectious agents that affects human health. Malaria, dengue fever, yellow fever and other mosquito-borne diseases infect countless individuals and account for thousands of deaths annually, causing a large burden to public health and on the economic process of developing countries. The fatality rate of Malaysians increased by 143 deaths. High rate of reproduction and lack of effective vaccines against several mosquito-borne pathogens makes us extremely addicted to the utilization of insecticides to manage the mosquito populations so as to prevent illness due to transmission. Most Malaysians still use ridsect spray and spiral mosquito repellent to repel mosquitoes. These repellents are intended to kill mosquitoes and usually contain insecticides that have a residual effect, such as deltamethrin. To address this issue, an experimental study was conducted using *artemisia argyi* as a primary component of a mosquito repellent. The purpose of this research is to substantiate the effectiveness of a mosquito repellent using organic materials. This study is designed to protect and contribute to a healthier society by way of controlling if not combatting the growing population of mosquitoes and lowering the probability of people getting infected with mosquito-borne diseases. This study consists of three treatments: (i) Organic treatment (ii) Chemical treatment (iii) No treatment. The data were gathered by tallying the number of minutes the mosquitoes died between three (3) treatment. Based on the result, cage test proves that the developed mosquito repellent is highly efficient against mosquitoes and hence can be more effectively used for control of mosquitoes.

Keywords: Mosquitoes, mosquito-borne diseases, *Artemisia Argyi*, Chemical mosquito coils, Organic mosquito repellent.

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## LIST OF ABBREVIATIONS/ SYMBOLS

*A. albopictus*

*Aedes albopictus*

*A. argyi*

*Artemisia argyi*

DDT

Dichlorodiphenyltrichloroethane

-OH

an alcohol group

-COOH

carboxylic acid group

DEET

N, N-diethyl-meta-toluamide

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Research Background

Mosquitoes are found throughout the world and recognised as a dangerous vector in the world (Cheng et al., 2009; Wang et al., 2011). Apart from being a global issue, the majority of mosquito-borne diseases result in human mortality and economic loss. Worldwide, the genus *Aedes* contains significant vectors of mosquito-borne diseases. Dengue, Zika, chikungunya, and yellow fever are all diseases spread by *Aedes*. The primary carrier of dengue fever is *Aedes aegypti* (Linnaeus), while the secondary carrier is *Aedes albopictus* (Skuse). Dengue fever is one of the mosquito-borne diseases that often infect humans (Adanan et al., 2005). Increased cases of dengue fever and dengue hemorrhagic fever from year to year, resulting in a high death rate in Malaysia (Lee and Zairi, 2005).

Mosquitoes breed successfully in both artificial and natural habitats. Mosquitoes were abundant in discarded tyres, ant traps, jars, coconut shells, flower pots and water tanks, according to Dom et al. (2013). In around housing zones and building sites, *Aedes* mosquitos have been seen reproducing in man-made and natural containers. Significant infrastructural changes cause human industrial and economic growth, indirectly created a man -made environment conducive to the breeding of *Aedes* mosquitoes. (Chua et al., 2005). *Aedes albopictus* and *Aedes aegypti* are the main vectors of dengue fever transmission in Malaysia. Dengue fever has emerged as a major public health issue in Malaysia. As of November 22, Malaysia's total number of recorded Dengue cases was 93402, with 178 confirmed fatalities. (World Health Organization, 2014).

Controlling the disease's transmission, particularly control of the vectors, has taken a great deal of effort. Insecticides are the primary method of mosquito control. According to Gratz (1999), If ultra-low-volume (ULV) is applied may be helpful in controlling dengue vectors during an outbreak. Deltamethrin, s-bioallethrin, piperonyl butoxide, and cyfluthrin are also utilised in high-rise apartment complexes in Malaysia to combat dengue vectors, according to Sulaiman et al. (2000). Although pesticides can efficiently eliminate mosquito-borne illnesses, long-term usage can cause insects to evolve resistance to these insecticides. Since the 1940s, when DDT was introduced, pesticide treatment directed at the adult or larval stage has been the major approach for controlling mosquitoes, despite the fact that there are many other options.

In mosquito control programmes, chemical control measures such as larviciding and adulticiding are successful. Adulticide is used to kill mosquitos that are flying or resting on surfaces, whereas larvicide is used to kill mosquitos in their larval stage. Because mosquito larvae have a smaller range of movement than free-flying adult mosquitoes, controlling larvae is more effective than controlling adult mosquitoes (Lee and Zairi, 2005; Amer and Mehlhorn, 2006a; Rajkumar and Jebanesan, 2009; Waliwitiya et al., 2009). For millennia, chemical and synthetic insecticides have been employed in vector control programmes to limit the spread of mosquito-borne illnesses. However, its application produced in a plethora of issues, including pesticide resistance, pollution, and negative impacts on people and other non-target creatures. (Georghiou and Taylor, 1977; Hemingway and Ranson, 2000; Nauen, 2007; Ghosh et al., 2012). For example, dichlorodiphenyltrichloroethane (DDT) was initially employed in 1946, but within a year, many obstacles occur. (Hemingway and Ranson, 2000).

Sublethal effects research is still very limited and poorly studied. Synthetic pesticides have been shown to have sublethal effects on *Aedes albopictus*. Varied pesticides in the same insecticide family, however, have different sub-lethal effects. *Aedes albopictus* egg production was suppressed by three pyrethroids: d-phenothrin, d-allethrin, and tetramethrin. Only d-phenothrin and d-allethrin, on the other hand, showed a decrease in blood engorgement. (Liu et al., 1986). Dieldrin has an influence on *Aedes albopictus*, eating and egg-laying ability, according to another study, the effect is not passed down to the progeny. (Duncan, 1963).

To address these issues, over the last few years, research on plant-based products has been conducted (Karmegam et al., 1997; Isman, 2006; Jbilou et al., 3 2008). A good mosquito repellent should keep humans safe from bug bites for a long time without generating negative health consequences or responses in those who are exposed to it. Botanical products like *artemisia argyi* leaves, which are used as a mosquito repellent and pest management they are widely accessible, non-toxic to non-target species and biodegradable, (Isman, 2006; Jbilou et al., 2008; Pavela, 2008; Shekari et al., 2008; Cheng et al., 2009). Shaalan et al. (2005) reported that no resistance was detected in botanical compounds as a result of their limited use in vector control programmes.

A chemical identified in this plant, *A.argyi*, is effective against the malaria parasite *plasmodium falciparum*. (Tawfiq et al., 1989; Paniego and Giulietti, 1994; Bhakuni et al., 2001). *A.argyi* might be a viable antimalarial plant that could be utilised to treat malaria in malaria-endemic areas like Africa. It can also be used as an antimicrobial, antifeedant, and anti-inflammatory medication in addition to treating cancer. (Bhakuni et al., 2001; Baldi and Dixit, 2008). Liu et al. (1992) reported that an extract from *A.argyi* cell suspension culture exhibited antimalarial activity against *Plasmodium falciparum*. While *A.argyi* is well-known for its antimalarial activities, there is little information on its antibacterial action against mosquitos. As a result, the potential for this plant to act as an insecticide against mosquitoes is extremely important to investigate



## 1.2 Problem statement

Mosquitoes are insects that may thrive in a variety of environments and on surfaces. *Aedes albopictus* (Skuse), the principal vector for dengue disease, is also a secondary vector in dengue transmission. Most Malaysians still use ridsect spray and spiral mosquito repellent to repel mosquitoes. These repellents are intended to kill mosquitoes and usually contain insecticides that have a residual effect, such as deltamethrin. The purpose is to curb the spread of malaria. We, therefore, have to devise alternative strategies to target the Aedes mosquito vector using natural-based active compounds than chemical-based compounds.

### 1.3 Objectives

The objectives of this research are:

1. To investigate the repellent activity of *Artemisia argyi* towards mosquitoes.
2. To examine the bio-efficacy and insecticidal activity of *Artemisia argyi*

### 1.4 Hypothesis

H<sub>1</sub> - A mixture of *Artemisia argyi* leaves react to mosquitoes.

H<sub>2</sub> - Tested the effectiveness of mosquito repellent cones using *Artemisia argyi* leaves mixture which has been dried

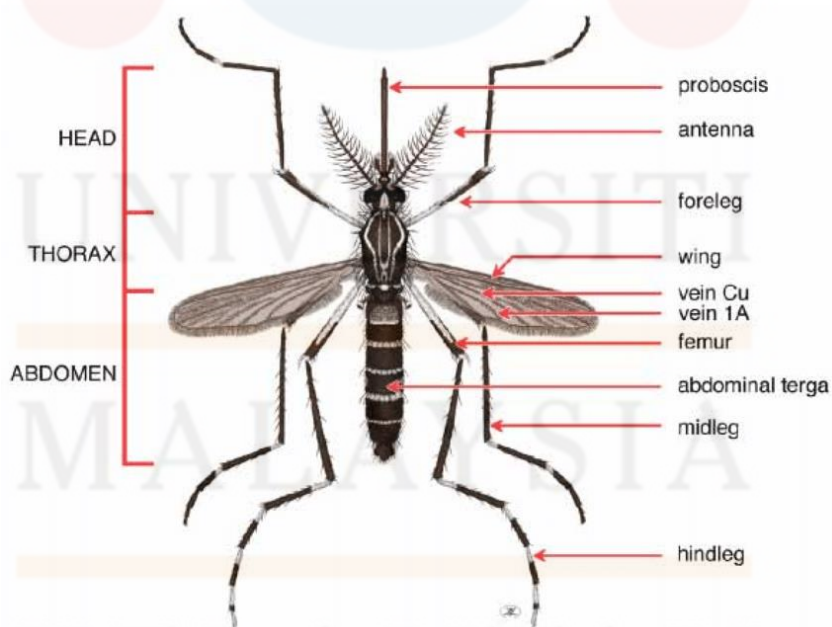
## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction of Mosquito

There are over 3000 species of mosquitos worldwide, the majority of which are found in tropical regions (Ogunmodede A,2020). Mosquitoes will always exist in the presence of humans. Certain mosquito genera, on the other hand, are restricted to specific regions. In tropical climes, mosquitoes are the most common home insect (Adanan et al., 2005), causing more problems for the public than any other group of arthropods by transmitting a variety of medically significant diseases (Ghosh et al., 2012). Although some of the species are not disease vectors, they are a serious biting nuisance to the public (Service, 1997).

Mosquitoes belong to the order Diptera and the family Culicidae. The biting Diptera is a two-winged flying insect that feeds on the blood of humans and animals. A mosquito's body is divided into three sections: the head, thorax, and abdomen, all of which are covered in scales. Adult mosquitoes have a long, slender body, long legs, and long needle-shaped mouthparts referred to collectively as proboscis (Figure 2.1). An adult mosquito measures between 2 and 19 millimeters in length (Service, 1997). The Culicidae family is further divided into three subfamilies: Anophelinae, Culicinae, and Toxorhynchitinae. Among these, the subfamilies Anophelinae and Culicinae are important vectors of disease, whereas the subfamily Toxorhynchitinae has no medical significance. The genera *Anopheles*, *Aedes*, and *Culex* contain mosquito species that are significant vectors of mosquito-borne diseases (Penget al., 1998).



**Figure 2.1** Mosquito structure

Source: researchgate et al., (2020)

## 2.2 Taxonomical Classification

Mosquitoes are divided into 38 genera and include roughly 3450 species and subspecies.

Sample Classification of *Aedes albopictus* (*Stegomyia albopicta*)



**Figure 2.2** *Aedes albopictus* (*Stegomyia albopicta*)

Source: Entomology., (2014)

Class: Insecta

Order: Diptera

Family: Culicidae

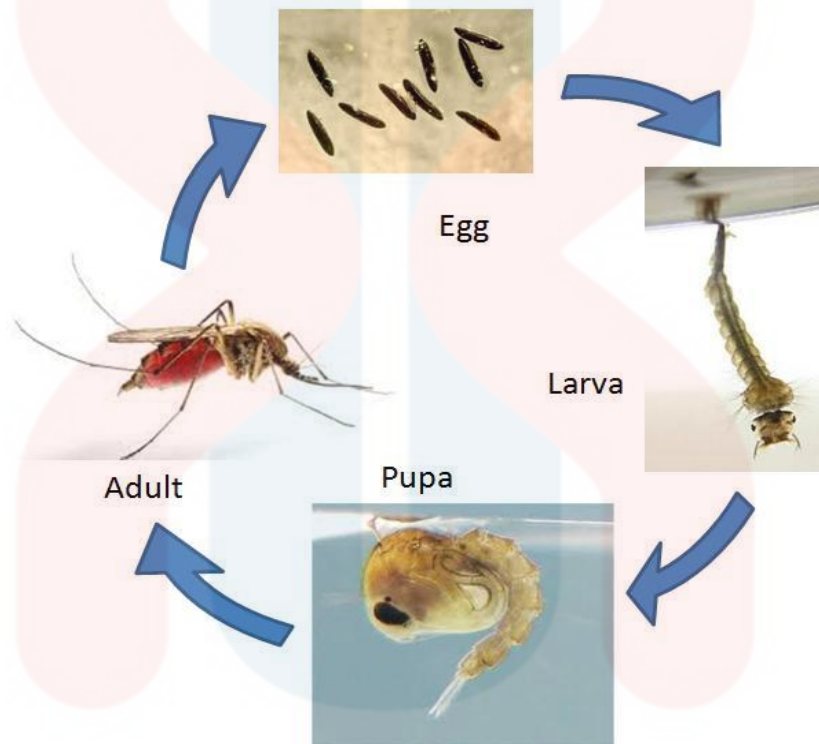
Genus: *Aedes*

Species: *A. albopictus*

Subgenus: *Stegomyia*

Kingdom: Animalia

All mosquitoes undergo a complete metamorphosis which includes egg, larval, pupal, and adult stages. (Figure 2.3) *A. albopictus*



**Figure 2.3** Life Cycle of *A. albopictus*

Source: Centers For Disease Control And Prevention., (2020)

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## **2.3 Biology of *Aedes albopictus***

### **2.3.1 Life cycle of *Aedes albopictus***

The *Aedes* mosquito has 4 life stages: egg, larva, pupae and adult. Mosquitoes can live and reproduce at home and outside. The entire life cycle from egg to adult takes about 8-10 days.

### **2.3.2 Egg Stage**

*Aedes* mosquitoes lay elongated eggs that are always placed alone. This is wrapped in a thick, one-millimeter-long shell. The egg is white on the inside. It was a bright red when it was originally laid, but it quickly faded to a smooth black. The eggs are smooth and Ovoid in shape. They have a front taper and a back taper (Clements, 1963; Dengue Virus Network, 2012; Reinert, 1972). The eggs can resist and tolerate dryness for an extended period of time, frequently more than a year. If the eggs are submerged in water, they will hatch right away. However, because the eggs do not all hatch at the same time, the process of hatching may take a long time (Dengue Virus Network, 2012). *Aedes* like to lay their eggs in tree holes on the edge of the water. Pools, damp clay pots and containers that are easily flooded after rain.

*Aedes* mosquitos do not normally lay all of their eggs in one place; instead, they disperse their eggs throughout several places (Foster and Walker 2002). In their lives, *Aedes* mosquitos may deposit roughly 5 batches of eggs. The amount of blood ingested by female *Aedes* mosquitos has a direct correlation with egg production (Maricopa, 2006)

### **2.3.3 Larval Stage**

Mosquito larvae are unlike any other aquatic insect larvae. They don't have any legs. A spherical breast that is broader than the head and abdomen is also present. There is frequently a small barrel-shaped syphon and a pair of sub-abdominal clusters in *Aedes* species 1. Section 9 also features at least three pairs of ventral brushes as well as a few bristles on the chest. Most *Aedes* larvae have comb teeth, whereas *Anopheles* larvae do not (service,1997; Abu Hassan and Yap, 1999). Mosquito larvae will feed on garbage and algae to survive after hatching. They will peel off three times over the course of four years. between 5 and 12 days ( Becker et al. 2010) If the larva acquires enough energy and achieves the lowest size when needed, it will begin to metamorphosis and turn into pupae during the fourth instar (Norris, 2004; Centers for Disease Control and Prevention, 2012). They are most commonly seen in clear water. Temperature, competition, water quality, and food are all factors that influence the growth of *Aedes* larvae. The larval stage, on the other hand, normally lasts 5 to 12 days (Becker et al. 2010).



#### 2.3.4 Pupal Stage

The pupal stage is only two to three days long. The pupae are unconscious, and their body is separated into three sections: head, chest, and abdomen. A pair of breathing horns and a pair of eyeballs are located on the head and chest (Sulaiman, 1990). They can tell what it is by looking at the abdominal parts. They don't have spines in the abdominal segments like *Anopheles* pupae, which contain sections 2 to 7. The pupal stage spends the most of its time on the water's surface, and the breathing horn is utilised to intake air, even when foraging. If they are disturbed, they will jerkily move up and down (Service, 1997).

#### 2.3.5 Adult Stage

The *Aedes* mosquito's life cycle can be relatively short, spanning about 6 to 8 days from egg to adult. Nevertheless, it generally takes 10 to 12 days; however, the life cycle of some temperate species might take several months. The thorax and legs of most mature *Aedes* mosquitos exhibit distinct black and white markings. They normally have a black and white mark on their abdomen. *Aedes* mosquitos also have scales covering their veins, which are narrow and generally black. The silver scales in the form of a lyre distinguish *A. albopictus*. *A. albopictus* is identified by wide stripes of white scales running vertically in the middle of the shield on the outer border of the breastplate. (Abu Hasan and Ye, 1999; Service, 1997; Centers for Disease Control and Prevention, 2011).

*A. albopictus* is active all day, as well as during dawn and night. They bite the most in the morning and when there is a lot of dust. Within one to three days of hatching, female mosquitos begin feeding on blood. *A.albopictus* bites people inside their homes throughout the day, most notably in the morning and at dawn. On the other hand, *A. albopictus* enjoys biting outside of buildings and resting on plants such as shrubs. Both species are capable of flying distances exceeding 400 metres (Sulaiman, 1990). *Aedes* are capable of surviving in the absence of blood consumption. Female *A.albopictus* may survive in the absence of blood meals by feeding on sugar solution, according to one study (Briegel et al., 2001). Adult *A. albopictus* fed sucrose solution has a longer life span than adults fed blood, according to Xue et al. (2010). On the other hand, blood meal is necessary for mosquito reproduction. Because egg development requires protein, a blood meal is required (Klowden, 1990).

## 2.4 *Artemisia argyi*

### 2.4.1 Biology and uses



**Figure 2.4** *Artemisia argyi*

Source: Samiksha et al., (2020)

*Artemisia argyi*, often known as Chinese mugwort or silvery wormwood, is a perennial herbaceous plant with a spreading rhizome. China, Korea, Mongolia, Japan, and the Russian Far East are all home to this species (Amur Oblast, Primorye). In Chinese, it is known as àico or ài yè, while in Japanese, it is known as gaiyou. It's used in herbal therapy to treat liver, spleen, and renal problems. In Chinese cuisine, it is a popular flavouring and colourant. Artemisias belong to the Asteraceae, a family of plants which also includes asters and daisies; however, Artemisia flowers, though typical, are usually quite small. There are over 300 species in the genus, distinguished by the silkiness and divisions of their leaves and the arrangements of their flowers.

With the exception of several species in tropical environs, the genus originated in and mostly belongs to the drier climes of the Northern Hemisphere (Watson, p. 4). *A. argyi* is a one-metre tall erect grey herbaceous perennial with small branches and a creeping rhizome. The stalked leaves are oblong, deeply split, and covered with tiny oil-producing glands. They are hairy on top and thickly white tomentose on the bottom. The lower leaves are bipinnate, with wide lanceolate lobes and small teeth along the edges, and are about six cm long. The bracteal leaves are simple, linear, and lanceolate, while the upper leaves are smaller and three-partite.

The inflorescence is a leafy panicle with a slender inflorescence. Individual blooms are pale yellow and tubular, with spherical turned-down crowns. The blooms in the centre are bisexual, while the flowers in the periphery are female. The petals are small and cylindrically folded, with a cobwebby pubescence on the bracts. The entire plant has a pungent scent. Temperate areas in Asia, such as China and Vietnam, are home to *A. argyi* (Abdin et al., 2003). It has been used in Chinese medicine to treat fever for over 2000 years and is known in China as Qinghao (green herb). Paniago and Giulietti (1994); Tawfiq et al., 1989).

*A. argyi*, a plant that contains a significant amount of artemisinin, is gaining popularity for clinical use across the world. Artesunate, artemether, and arteether are some of the derivatives that can be used to treat malaria parasites that are multidrug resistant. (Titulaer and colleagues, 1991). In 1972, artemisinin was isolated from the plant's aerial parts, including the leaves, buds, and flowers, in China. Luo and Shen (Luo and Shen, 1987). The artemisinin content of the plant, on the other hand, was found to be exceedingly low. Variable cultivars of *A. argyi* have different amounts of artemisinin depending on the plant's origin and growth conditions such as temperature and photoperiod (Christen and Veuthey, 2001). The leaves of Chinese forms of the plant, for example, were found to have the greatest concentration of artemisinin (Nair et al., 1986). According to Jain et al. (1996), more than 80% of the artemisinin was identified in the leaves and inflorescences, with just 5% to 15% found in stem tissue and no artemisinin found in the root. Artemisinin was found in the highest concentrations before to blooming or throughout the flowering phase, according to previous research (Dharam et al., 1996).

#### **2.4.2 Uses of *Artemisia Argyi***

The artemisinin found in this plant is effective against both chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium falciparum*. (Tawfiq et al., 1989; Liu et al., 1992; Paniego and Giuliatti, 1994); Tawfiq et al., 1989; Liu et al., 1992; Paniego and Giuliatti, 1994). As a result, *A. argyi* is employed in the treatment of malaria, particularly in malaria-endemic areas such as Africa. *A. argyi* can also be used to treat skin conditions and tumours including leukaemia and colon cancer (Baldi and Dixit, 2008). Furthermore, investigations have revealed that some isolated chemicals from this plant

have antibacterial, antifeedant, and anti-inflammatory properties (Bhakuni et al., 2001). The activity of this molecule is dependent on an endoperoxide bridge, which has been proven to be important (Van der Kooy and colleagues, 2008).

Lactone is a cyclic ester generated when an alcohol group  $-OH$  and a carboxylic acid group  $-COOH$  combine to form a cyclic ester. Free radicals are thought to be involved in the mechanism of action for antimalarial activity, according to Halliwell and Gutteridge (1999). Free radicals may be released when artemisinin interacts with heme or iron. The Plasmodium parasites will be damaged by these free radicals, which will eventually kill them (Meshnick, 2002). According to previous research, the medicine is particularly effective against the parasites' trophozoites stage (Geary et al., 1989). There have been no reported reports of artemisinin resistance in malaria parasites.

To begin with, the medicine has a short half-life and parasites do not come into touch with it for an extended period of time. Furthermore, artemisinin destroys malaria parasite gametocytes, which are the sexual stage of the parasites that cause infection in Anopheles mosquitoes. Parasites in patients treated with artemisinin will have a lower likelihood of infecting mosquitos and transmitting the disease to additional patients as a result of this. As a result, the likelihood of malaria parasites developing resistance to the medicine is reduced (Hommel, 2008). In the treatment of malaria, artemisinin is usually used in conjunction with other antimalarials such as chloroquine and mefloquine. This is one of the reasons why resistance to artemisinin therapy takes so long (Meshnick, 2002). Monotherapy using artemisinin is uncommon.

### 2.4.3 Potential in pest control

Although *A. Argyi* is well-known as an antimalarial herb, nothing is known about its impact on animal pests. Several studies have shown that *A. argyi* is efficient against insect pests such as mosquitoes (Sharma et al., 2006a), elm leaf beetles (Shekari et al., 2008), stored-product beetles (Tripathi et al., 2000), and lesser mulberry pyralids (Tripathi et al., 2000). (Roya et al., 2010). According to Liu et al. (1992), extract from *A. argyi* cell suspension culture has antimalarial action against the malaria parasite *P. falciparum*. Aroma *A. argyi* are highly fragrant due to the presence of cineole. The aroma of each species is distinctive some camphor-like, clean and refreshing. *Aedes* egg hatching was significantly disrupted, and the larval and pupae periods were significantly extended.

### 2.4.4 Mechanism of action of insect repellents against insects

Insect repellents work by interfering with mosquitoes' odorant receptors (ORs) and gustatory receptors (GRs) (Dicken and Bohbot, 2013). The proposed mechanism is that they bind to the "odour binding proteins" (OBP) in the insect antenna, causing them to flee (Ditzen et al., 2008). DEET's action suggests that insects dislike the odour, while another study found that DEET may confuse insects by impairing their sense of smell. Insects have a variety of different olfactory sense organs that collect chemical signals.

The majority of these neurons are equipped with an odour receptor, which is composed of two snap-together proteins. Each odour receptor is made up of two proteins called Orco. Orco can covalently link to any of sixty other proteins. Odor molecules in the air bind to these receptors, activating some nerves and inhibiting others. As a result, repellents bind to their "odour binding proteins" and confuse them (Howard Hughes Medical Institute, 2011).



## 2.5 Mosquito Control

Mosquitoes can be effectively controlled if we have a thorough understanding of their biology. The more we learn about mosquito behaviour, the simpler it will be to keep them under control (Goma, 1966). Surveillance, source reduction, larviciding, adulticide, biological control, and public education are the main components of integrated mosquito management (Rose, 2001). Mosquito control tactics are divided into four categories by Yap et al. (2003a): source reduction, physical barrier or personal protection, chemical control, and biological control. The most efficient means of control are larviciding and adulticiding, which are often focused at a single or a few of the most prominent species. Adulticide is used to control adult mosquitoes that are flying or resting in space spraying and surface residual spraying. In order for pesticides to be effective against adult mosquitos, the little droplets must reach and kill the adults. Large pesticide droplets that fall to the ground without coming into touch with mosquitos are a waste and may have unforeseen repercussions for other creatures (Rose, 2001).

Because mosquito larvae have a smaller range of movement than adult mosquitoes, larval control is more successful than adult control (Lee and Zairi, 2005; Amer and Mehlhorn, 2006a). Mosquito control requires the identification of breeding places. In order to get the greatest effect in reducing young mosquitoes, the choice of larvicide and formulation of larvicide must be carefully evaluated and used appropriately (Pates and Curtis, 2005). As a result of various reasons, including fast and unorganised urbanisation, greater movement between endemic and non-endemic areas, vector

resistance to conventional pesticides, and global warming, mosquito-borne illnesses have become a serious global concern (Yap et al., 2003).

### 2.5.1 Chemical control

Since the development of organic pesticides in the 1940s, chemical control has remained a significant component of mosquito control (Yap et al., 2003b). The most often used insecticide categories include organochlorines, organophosphates, carbamates, and pyrethroids. From 2003 to 2005, the World Health Organization (WHO) claimed that 547 tonnes of organochlorine active components, 437 tonnes of organophosphates, 25 tonnes of carbamates, and 161 tonnes of pyrethroids were used yearly for vector control on a worldwide scale. In 2005, pyrethroids were used more frequently than organophosphates in Malaysia's dengue control programme. Between 2001 and 2005, only pyrethroids were used to combat malaria (WHO, 2007). In general, the pesticides most often used to control mosquitoes are organophosphates, carbamates, and synthetic pyrethroids (Kamaraju et al., 2011). The organophosphate larvicides temephos, chlorpyrifos, and fenthion are used to manage clean water breeders like *Aedes sp.*, whereas chlorpyrifos and fenthion are used to control contaminated water breeders (Yap et al., 2003b).

However, their usage has led in a plethora of issues, including pesticide resistance, environmental residues, and disturbance of natural systems (Busvine, 1978; Georghiou and Taylor, 1977; Karmegam et al., 1997; Nauen, 2007). DDT, for example, was initially used in 1946, but within a year after its introduction, resistance emerged (Hemingway and Ranson, 2000). The capacity of a species to survive a dose of pesticide that would

ordinarily kill it is known as insecticide resistance (Goma, 1966). Insect growth regulators, which are divided into two groups: juvenile hormone analogues and inhibitors of chitin synthesis, are another sort of pesticide. Insect growth regulators work by interfering with the moulting process or reducing the synthesis of chitin in the juvenile stage of the insect. The insect growth regulator is thought to be less harmful to other beneficial insects and to be more ecologically friendly. However, compared to synthetic pesticides, they have a slower mode of action. Mosquito larvae are also controlled by *Bacillus thuringiensis* and *Bacillus sphaericus*.

### **2.5.2 Botanical insecticide**

A botanical pesticide has a repellent effect, which keeps insect pests away and protects the crops (Isman, 2006). M. B. Isman & C. M. Machial (2006). Pesticides derived from essential oils of plants: From traditional use to commercialization. Naturally occurring bioactive compounds. Rai, M., and Carpinella, M.C. (Eds.). Chemicals having therapeutic and pesticide activities have been discovered in over 2000 plant species (Ghosh et al., 2012). For the development of botanical pesticides, the plant families Asteraceae, Meliaceae, Piperaceae, Lamiaceae, and Annonaceae have all been intensively investigated (Isman, 2006; Pavela, 2008). Pyrethrum, rotenone, sabadilla, ryania, and nicotine are five groups of botanically derived chemicals that are often employed (Ware, 1983). with minimal impact on the ecosystem, as they repel insect pests from treated materials via olfactory or other receptor stimulation (Talukder, 2006; Talukder, Islam, Hossain, Rahman, & Alam, 2004). Botanical pesticides are considered safe for pest

control because they leave little or no pesticide residue on the skin, environment, or ecosystem (Talukder et al., 2004)

To far, no studies have shown resistance to botanical-based pesticides among vector pests due to the limited usage of botanical products in vector control programmes (Shaalán et al., 2005). Plant-based treatments also contain a combination of chemicals that target the biological and physiological properties of pests. As a result, plant-based pesticides are very efficient against pests while also decreasing the risk of resistance development and having no negative effects on humans or wildlife (Ghosh et al., 2012). The issues connected with synthetic pesticides, such as pollution, resistance development, and high manufacturing costs, have required the quest for a new pest control strategy. Natural products of plant origin with insecticidal qualities have been employed in recent years to control a range of insect pests and vectors, according to Kamaraj et al. (2010), while plant extracts have been utilised in mosquito control programmes since ancient times, according to Ghosh et al. (2012). Phytochemicals, however, have not been widely employed due to inadequate characterisation and the difficulties to identify active components with insecticidal characteristics.

## CHAPTER 3

### METHODOLOGY

#### 3.1 Material and Instrument

Mosquito breeding has been carried out around the agro park, Universiti Malaysia Kelantan, Jeli Campus, from eggs to adult mosquitoes. The mosquitoes were reared for (7) seven days. The study's main focus is *Aedes albopictus*. The mosquitos will then be transferred to food containers and placed in treatment boxes once they have hatched. Organic treatment, chemical treatment, and no treatment squitoes were divided into three groups. The first fifteen (15) mosquitoes were placed in a cage with organic mosquito repellent, fifteen (15) mosquitoes were placed in a cage with chemical control, and the remaining fifteen (15) mosquitoes were placed in a cage with no mosquito control. Each treatment was repeated (2) twice, requiring a total of thirty mosquitoes for one treatment. Inside the cage, the mosquitos were individually tested.

## **3.2 Preparation of Mosquito breeding**

### **3.2.1 Preparation of Material**

(10) Ten cans of empty milk, (1) one bamboo stick split into ten parts, (2) two black spray paint, (10) ten food containers, (1) one multipurpose filter, (1) one plastic spoon, and Label sticker.

### **3.2.2 Preparation of the breeding ground**

Empty cans are cleaned first. After that, the cans are sprayed using black spray paint on the inner and outer surfaces of the cans and left until they dry. As much as (1/2) of the water is put in the can, the bamboo is placed diagonally in cans. Next, the cans are placed strategically around a bamboo tree near the old Agro Park bridge. Mosquito growth monitoring is recorded daily from mosquitoes laying eggs until adult mosquitoes. Overall, *Aedes albopictus* mosquitoes take (8) eight to (10) ten days to become adult mosquitoes.

### **3.3 Preparation of Extract of *Artemisia Argyi***

#### **3.3.1 Preparation of Material**

75 grams of dried blended *Artemisia argyi* leaves, 50 grams of pulverized candle, 45 grams of ethyl alcohol, and 15 grams of corn-starch.

#### **3.3.2 Manufacturing Method**

The leaves of *Artemisia argyi* should be dried first in the sun. Then, the leaves are ground until smooth. Weigh all the ingredients that have been prepared according to the prescribed dosage, such as (i) 75 grams of *A. argyi* leaf powder that has been ground, (ii) 50 grams of pulverized candle, (iii) 45 grams of ethyl alcohol (iv) 15 grams of corn starch. All the above ingredients are put in a container and mixed well. The well-blended dough is put into a cone-shaped mold. Let the dough dry. The dried mosquito repellent mixture is removed from the mold.

### 3.4 Treatment

Three treatments were used in this experiment: organic treatments, chemical treatments, and no treatments. The investigation took place in a cardboard box with a plastic wrap wall on the outside and a net on top. A total of three cardboard boxes measuring 35cm x 17cm x 40cm were used to study *Aedes albopictus*, the study's main subject. The larvae of *A. albopictus* mosquitoes hatch into adult mosquitoes after 3-5 days. The mosquitoes are then divided into three groups once they have hatched. The first fifteen (15) mosquitoes were placed in a cage with an organic mosquito coil, a chemical mosquito coil, and a no-control mosquito in the other half. Inside the cage, the mosquitos were individually tested. When the mosquito repellent was turned on, the stopwatch was started, and observations of the fainting mosquitoes were taken. The data was collected at the rate at which the mosquito passed out.

This step is repeated once to suffice the three (3) trials needed to be undergone by each mosquito. Each mosquito fainted; data will be recorded after gathering the lapse time of the two (2) trials. A total of thirty (30) tests for the fifteen (15) mosquitoes in each cage with Organic coil, thirty (30) trials for the fifteen (15) mosquitoes in each cell with Chemical coil, and another thirty (30) trials for the last fifteen (15) mosquitoes in a different cage with the no control.

The data were gathered by tallying the number of minutes the mosquito's faint. The experiment and data were raw and legitimate as they were gathered



directly from the effect of the mosquito coil products used on the test subjects thus, making it genuine and reliable in the comparison of the organic mosquito repellent, chemical mosquito repellent and no control.

### **3.5 Statistical Analysis**

The data acquired from the experiment were compiled and analyzed using different data analyses available in Microsoft Office - Excel. ANOVA single factor is used when the sample size is more than 30. To investigate the repellent activity of *Artemisia argyi* towards mosquitoes and identify the type of t-test to use. This research did fifteen (15) trials to acquire essential data, including the number of minutes a mosquito die. These fifteen trials resulted in fifteen different data, which were then evaluated and analyzed using the ANOVA single factor analysis.

An ANOVA (analysis of variance) is used to see if there is a statistically significant difference between three or more groups' means. In practice, the one-way ANOVA or Anova single factor test is the most widely employed ANOVA test.

## CHAPTER 4

### RESULT AND DISCUSSION

#### **4.1 The difference between organic treatment, chemical treatment and no treatment: First round treatment.**

The difference between organic treatment, chemical treatment, and no treatment in the first treatment is shown as the summary in the table 4.1 the ANOVA test statistic was less than the critical region, therefore, accepting the null hypothesis ( $H_0$ ) because the value of P (P-Value = 9.257) was greater than the significance level ( $\alpha$ ) is 0.05 signifying that the variances are equal. Therefore, these findings confirm the difference between organic treatment, chemical treatment, and no treatment as in Figure 4.1 the time taken by a mosquito to die. The three treatments carried out showed a very significant difference between the treatments i.e. the organic mosquito repellent takes five (5) to eight (8) minutes for dead mosquitoes while, the chemical mosquito repellent takes eight (8) to nineteen (19) minutes, and no repellent takes ten thousand (10,000) to twenty-eight thousand (28,000) minutes. This relationship further strengthens the Mosquitoes repellent theory by Singhamahapatra (2020) these repellents create a virtual barrier

between human beings and mosquitoes; reducing the biting of mosquitoes, thus minimizing the probability of various diseases.

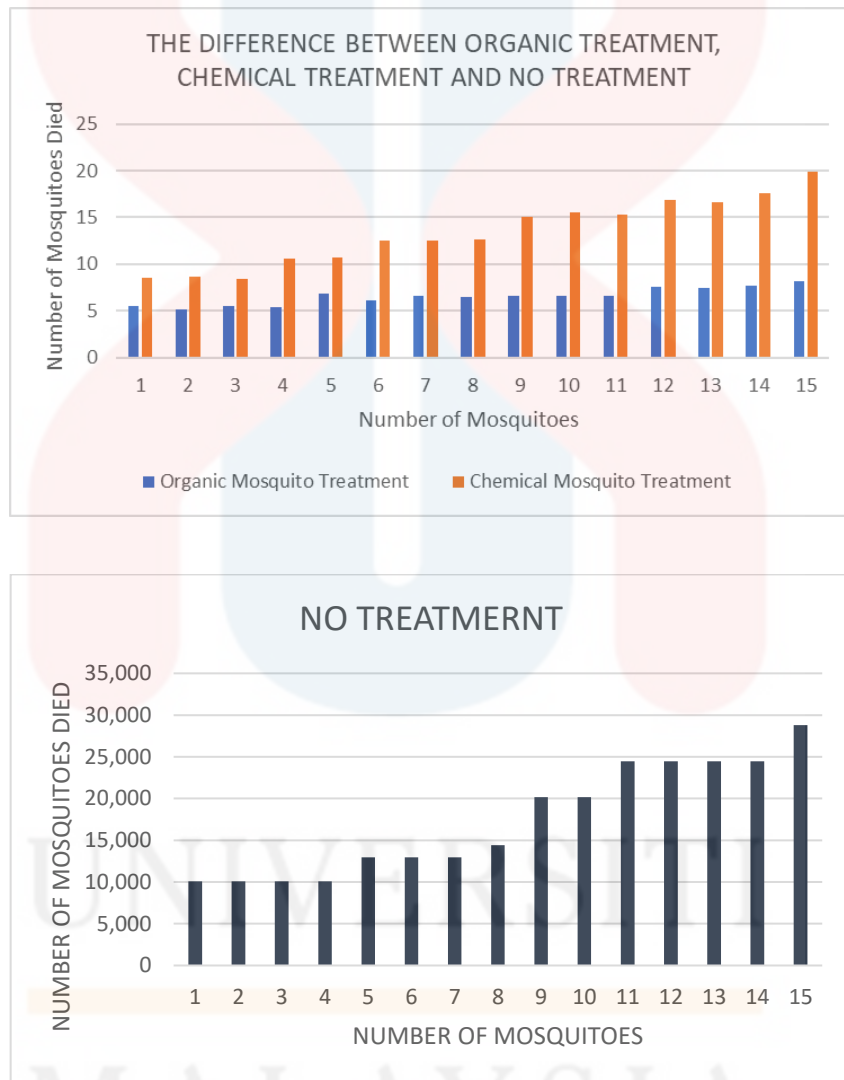


Figure 4.1. organic treatment, chemical treatment and No treatment. The figure show differences between the three treatment. (Round 1)



#### **4.2 The difference between organic treatment, chemical treatment and no treatment: Second round treatment.**

The difference between organic treatment, chemical treatment, and no treatment in the second, round treatment. As seen in the summary table 4.3 and Table 4.4, show P-Value = 1.385 more than the significance level ( $\alpha$ ) is 0.05 thus rejecting the alternative hypothesis ( $H_1$ ) and accept the null hypothesis ( $H_0$ ) because the null hypothesis show the mean number of minutes the mosquitoes change its designated place with the presence of the organic repellent is more than that of the chemical repellent and no repellent. Based on figure 4.2, show that the significant differences according to the type of treatment carried out that is, the organic mosquito repellent takes four (4) to eight (8) minutes for mosquitoes die, while the chemical mosquito repellent takes six (6) to eleven (11) minutes, and no repellent takes seven thousand (7000) to twenty-eight thousand (28,000) minutes for mosquitoes die. The findings of this study show that the organic mosquito repellent repels the mosquito more than the chemical mosquito repellent and no treatment since the mosquitoes change position faster and away from the mosquito repellent as presented by Ngo, J. K.(2020) The study has proven that *Artemisia* has biologically active components that show insecticidal activity, making it applicable as a mosquito controlling agent. Song (2019) *A. argyi* have potential antimicrobial and insecticidal properties.

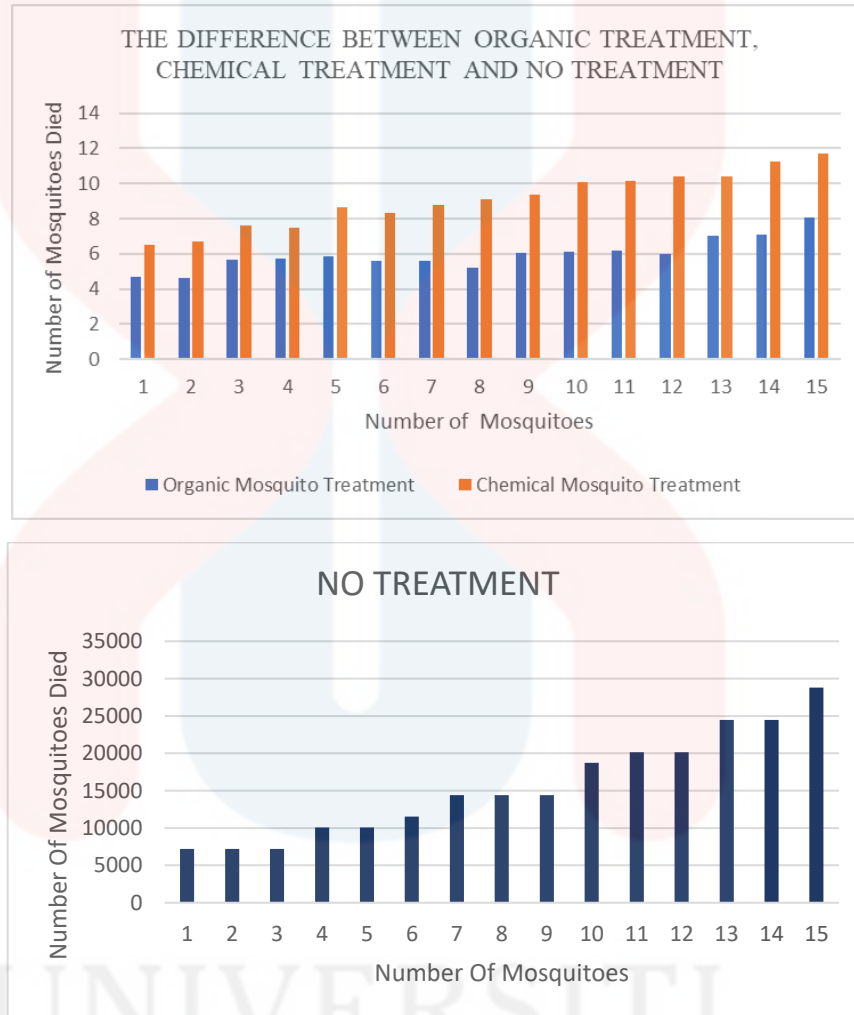


Figure 4.2 organic treatment, chemical treatment and No treatment. The figure show differences between the three treatment. (Round 2)

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### 4.3 Flammability of Mosquito Repellent

In order to measure the flammability of the mosquito repellent, with the use of a stopwatch and recorded the number of minutes it takes the mosquito repellent to light up. Based on Table 4.3 show the flammability of mosquito repellent, it is seen that the organic mosquito repellent takes four (4) to eight (8) minutes, the chemical mosquito repellent takes six (6) to nineteen (19) minutes, while no repellent takes seven thousand (70,000) to twenty-eight thousand (28,000) minutes. In conclusion, the organic mosquito repellent lights up faster than the chemical mosquito repellent, and no repellent.

**Table 4.3:** Flammability of Mosquito repellent

<b>Organic Mosquito Repellent</b>	<b>Chemical Mosquito Repellent</b>	<b>No Repellent</b>
4-8 Minutes	6-19 Minutes	7,000-28,000 Minutes

### 4.4 Cost per box mosquito repellent

In addition to that, the cost per box of both organic and chemical mosquito repellent. Showing in Table 4.4 is the average cost of one (1) box containing ten (10) mosquito repellent.

**Table 4.4** Cost per box mosquito repellent

<b>Organic Mosquito Repellent</b>	<b>Chemical Mosquito Repellent</b>
RM 2.50	RM 2.10

## CHAPTER 5

### 5.1 CONCLUSION

In conclusion, Usage of *Artemisia Argyi* for the production of mosquito repellent has not been reported before. The developed organic herbs are quite efficient in repelling and killing mosquitoes without harming human health. This study resulted in the development of eco-friendly organic herbal repellent with long-lasting protection, safe for human life, animal skin, and humans with no side effects, as an alternative to commercially available synthetic chemical repellents. The results of this investigation indicated that the *Artemisia Argyi* could be beneficial for the control of mosquito-borne diseases.

### 5.2 LIMITATIONS

The limitation in conducting this study is the lack of journals and books in making references.

### 5.3 RECOMMENDATION

As for improvement recommendations for this study, the dose quantity for organic mosquito repellent needs to be increased or mixed with other plants to get a significant effect because natural organic additives might be useful like Neem leaves consist of metabolites with strong and dominant effects on insects as presented by Leyva (2020) because the statistical tests show, that both rounds of treatment that have been carried out are the same, which is 3.22 for critical region and accepting the null hypothesis. Therefore, research on organic mosquito repellents should be continued in the future, as organic mosquito repellents have many benefits for long-term human health. In addition, organic mosquito repellent does not contain harmful chemicals and is safe to use. Organic insect repellents are recommended by health authorities to avoid mosquito bites and prevent mosquito-borne disease. The provision of recommendations on commercially available formulations in light of current research is critical to the effectiveness of topical insect repellents. Lastly, the result of the study could be used as references.



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**APPENDIX**

**Round (1) Treatment**

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Organic Mosquito Repellent	15	98.07	6.538	0.808302857
Chemical Mosquito Repellent	15	201.2	13.41333333	12.93108095
No Repellent	15	260640	17376	44868754.29

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3015788366	2	1507894183	100.8202977	9.25747E-17	3.219942293
Within Groups	628162752.4	42	14956256.01			
Total	3643951118	44				

Table 4.1: Anova Single Factor for Variances

Table 4.1.1: Analysis of the Result

H <sub>0</sub>	The population variance for the organic mosquito repellent is equal to that of the chemical repellent and no repellent
H <sub>1</sub>	The population variance for the organic mosquito repellent is not equal to that of the chemical repellent and no repellent
$\alpha$	0.05
p-value	9.257
Conclusion	Accept H <sub>0</sub> since p-value > $\alpha$ therefore, the variances are EQUAL

Table 4.1 show the Anova result and Table 4.1.1 shows the analysis of the result (Round 1). An Anova single factor was used in Microsoft Excel to determine if the raw data gathered has equal or unequal variances.



### Round (2) Treatment

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Organic Mosquito Repellent	15	89.59	5.972666667	0.802135238
Chemical Mosquito Repellent	15	136.6	9.106666667	2.515895238
No Treatment	15	233280	15552	48344502.86

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2416302544	2	1208151272	74.9713691	1.38557E-14	3.219942293
Within Groups	676823086.5	42	16114835.39			
Total	3093125631	44				

Table 4.2: Anova Single Factor for Variances

Table 4.2.1: Analysis of the Result

H <sub>0</sub>	The mean number of minutes the mosquito died from it using organic repellent is greater than or equal than that of the chemical repellent and no repellent.
H <sub>1</sub>	The mean number of second the mosquito die using organic repellent is less than that of the chemical repellent and no repellent
$\alpha$	0.05
p-value	1.385
f crit	3.219
Conclusion	Accepted H <sub>0</sub> since P-Value > $\alpha$ therefore, the mean number of minutes the mosquitoes change its designated place with the presence of the organic repellent is less than that of the chemical repellent and no repellent.


Table 4.2 show the Anova result and Table 4.2.1 shows the analysis of the result, (round 2). An Anova single factor was used in Microsoft Excel to determine if the raw data gathered has equal or unequal variances.

### Organic Mosquito Repellent



### Treatment box



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3. No. untuk dihubungi/ Contact No: H/P: 01161047270 Email: asma.f18b0306@siswa.umk.edu.my
4. Program Akademik/ Academic Programme: IJAZAH SARJANA MUDA SAINS GUNAAN (AGROTEKNOLOGI) DENGAN KEPUJIAN
5. Fakulti/Faculty, Pusat /Centre: FAKULTI INDUSTRI ASAS TANI

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 PENYELIA : PM. DR KUMARA THEVAN A/L KRISHNAN  
 JENIS PENYELIDIKAN : Penyelidikan (MOSQUITO CONTROL)

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Tarikh: 20/12/2021

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