SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN KAMPAR JAYA, PERAK

By

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ABSTRACT

SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN KAMPAR JAYA, KAMPAR

LOH TAY SHIN

Due to its tropical climate, Malaysia is a conducive breeding ground for various mosquito species. At such, vector-borne diseases such as dengue fever, filariasis, malaria and Chikungunya have been of great concern to the country. This study aims to investigate the mosquito population in Taman Kampar Jaya and evaluate their insecticide susceptibility status. Ovitrap, a commonly used surveillance tool for monitoring mosquito's activity, was used for the sample collections in Taman Kampar Jaya, Kampar. Sixty ovitraps were randomly set outdoors around the residential area and the paddles containing mosquito eggs were collected and replaced three times a week from October to December 2012. The eggs were then cultured into adults for gender and species identification. In addition, a three-month seasonal distribution of the mosquito population was also evaluated. The most abundant species found was *Aedes albopictus* with a total number of 17,262 throughout the 13 weeks. Smaller numbers of other species were also found which included *Aedes aegypti*, *Aedes albolateralis*, *Aedes gardnerii imitator* and *Culex quinquefasciatus*. Majority of the Ovitrap Index (OI) exceeded 90% which is relatively high. Following that, the adult females of *Aedes albopictus* were tested for insecticides resistance following the World Health Organization (WHO)

standard diagnostic test kits and procedure. The insecticides tested were 1% fenitrothion and 5% malathion from the organophosphate group, and 0.05% deltamethrin and 0.75% permethrin from the pyrethroid group. Mortality counts were made every 2 minutes up to 2 hours and then analyzed using Probit analysis. Deltamethrin was found to be the most effective insecticide, giving the lowest KT_{50} of 15.84 minutes. Fenitrothion was found to be the least effective, giving the highest KT_{50} and KT_{95} values of 150.29 minutes and 293.41 minutes respectively. In conclusion, the *Aedes albopictus* population might have begun developing resistance towards malathion and fenitrothion which were currently used in the fogging activities conducted by the local district authorities. As pyrethroids were shown to be more effective than the organophosphates, they should be great alternatives as insecticides against the high number of mosquito population present in Taman Kampar Jaya.

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DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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LOH TAY SHIN

APPROVAL SHEET

This project report entitled **"SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN KAMPAR JAYA, PERAK"** was prepared by LOH TAY SHIN and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

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SUBMISSION OF FINAL YEAR PROJECT

It is hereby certified that **LOH TAY SHIN** (ID No: **09ADB04310**) has completed this final year project entitled "**SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN KAMPAR JAYA, PERAK**" under the supervision of Ms. HO LAI YEE from the Department of Biomedical Science, Faculty of Science.

I understand that University will upload softcopy of my final year thesis in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

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(LOH TAY SHIN)

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LIST OF ABBREVIATIONS

Ae.	*Aedes*
AMCA	American Mosquito Control Association
Bti	*Bacillus thuringiensis israelensis*
CDC	Centers for Disease Control and Prevention
Cx.	*Culex*
Dec	December
DEN	Dengue virus type
DF	Dengue fever
DHF	Dengue hemorrhagic fever
F	Female
GCGH	Grand Challenges in Global Health
KT	Knockdown time
KT_{50}	Time to knock down 50% mosquitoes
KT_{95}	Time to knock down 95% mosquitoes
M	Male
Nov	November
Oct	October
OI	Ovitrap index
OP	Organophosphate
PY	Pyrethroid

RH	Relative humidity
RNA	Ribonucleic acid
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Mosquitoes belong to the kingdom Animalia, phylum Arthropoda, class Insecta, and order Diptera by means of the true flies or two-winged flies (Klappenbach 2012). However, unlike flies, mosquito's wings have scales and long legs. Furthermore, the adult female mosquitoes possess a long proboscis which is used to puncture through the skin during blood feeding. Being in the family Culicidae, there are more than 3,500 recognized mosquito species at present. Due to the tropical climate, various mosquito species can be found. About 442 species from 20 genera of mosquitoes have been discovered in Malaysia. However, approximately 10% of them are involved in the transmission of diseases (Harbach 2012). Most commonly found mosquitoes belong to three genera which are *Aedes*, *Anopheles* and *Culex* (Freudenrich 2012).

The well known mosquito-transmitted diseases to human and animals are filariasis, yellow fever, dengue, malaria, chikungunya, and encephalitis which includes West Nile virus (WNV), Eastern Equine encephalitis (EEE), Japanese encephalitis (JE), LaCrosse encephalitis (LAC), Western Equine encephalitis (WEE) and St. Louis encephalitis (SLE) (AMCA 2011).

The Malaysian government is well aware of the hazardous diseases, with the implementation of vector control programs such as the Malaria Eradication Program in Peninsular Malaysia in 1960s, followed by Malaria Control Program in Sarawak in 1970, Malaria Control Program in Sabah in 1971, Malaria Control Program in Peninsular Malaysia in 1980, and finally the Vector Borne Disease Control Program in 1983 which was focused not only on malaria but six other vector-borne diseases as well, which are dengue, filariasis, Japanese encephalitis, plague, scrub typhus, and yellow fever. Dengue became the primary concern among the rest with the highest incidence and fatality rate (Figure 1.1) (MOH 2008).

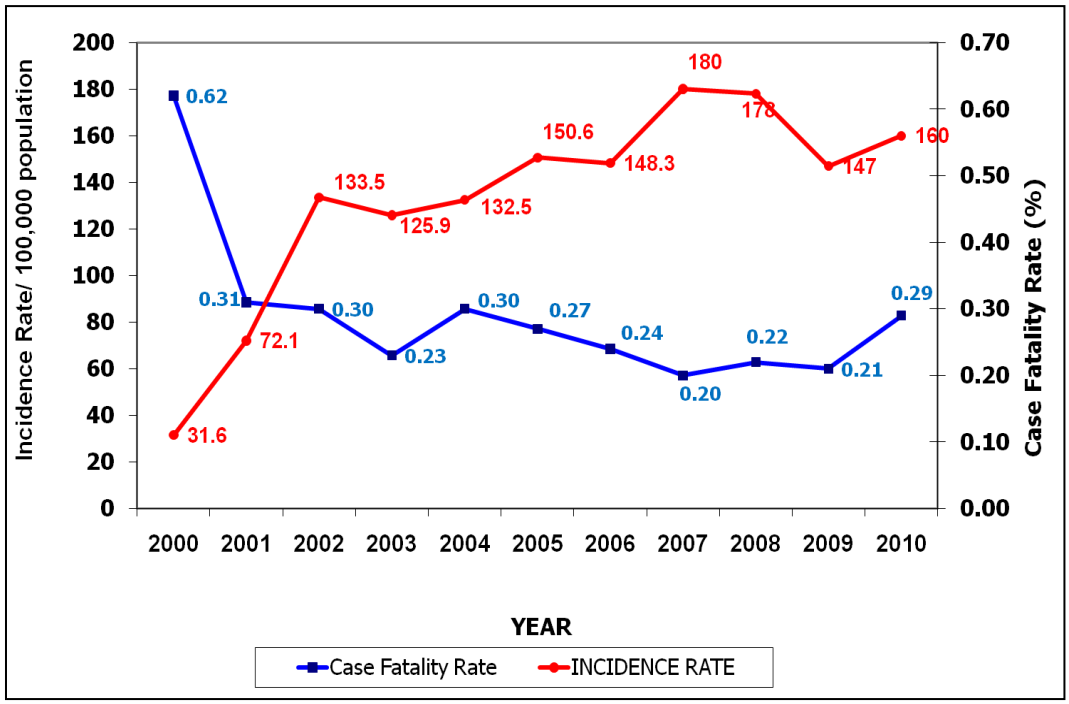

Figure 1.1: Dengue epidemiology in Malaysia from 2000 to 2010 (Adapted from Lokman 2012).

The process of rapid urbanization and growth of cities promotes the breeding of a variety of disease vectors. Many vector surveillance and control have been frequently carried out in Malaysia. Chemical control plays a major role in vector control but their effectiveness has been threatened by the development of resistance among vectors. There is a growing concern on the resistance towards insecticides which are commonly used during fogging in residential housing areas in Malaysia (Hidayati et al., 2011). Thus, there is a need for constant monitoring to keep the insecticidal resistance status in check while improving the implementation of vector control strategies.

1.1 Objectives

The objectives of the study are to:

1. Identify the mosquito population in Taman Kampar Jaya located in Old Town Kampar, Perak.

2. Evaluate a three-months seasonal distribution of the mosquito population of Taman Kampar Jaya.

3. Determine the insecticide susceptibility status of the mosquito population of Taman Kampar Jaya.

CHAPTER 2

LITERATURE REVIEW

2.1 Mosquito life cycle

Being in the order Diptera, mosquitoes undergo metamorphosis in their life cycle with four very distinctive stages that are the egg, larva, pupa and flying adult. Adult female mosquitoes lay eggs in water especially areas of collected standing water. The number of eggs that can be produced by a female mosquito such as *Aedes aegypti* and *Aedes albopictus* is approximately 102 and 79 respectively (Lee 2000). On the other hand, *Anopheles* mosquitoes can lay their eggs as many as 300 at one time and unlike *Aedes* and *Culex* mosquitoes, their eggs float on the water (Sulaiman 2000; Yap et al., 2000). Besides, eggs from *Culex* mosquitoes are laid in rafts which can be distinguished from *Aedes* and *Anopheles* eggs that are being laid singly (Yap et al., 2000).

Mosquito eggs are able to endure the winter and then hatch during spring. They hatch into larvae which live in water while they breathe through the siphon. Organic material in the surrounding will be filtered and consumed by the larvae. They can live for as long as several weeks depending on the species and environment factors such as nutrient availability and temperature. After the fourth and final instar, they metamorphose into pupae. Feeding on nothing, pupae

breathe through trumpets on the water surface. At the final stage of maturation, they break the pupal case using air pressure, crawl out and rest. While waiting for their external skeleton to harden, they spread their wings wide open for drying before taking off (Freudenrich 2012).

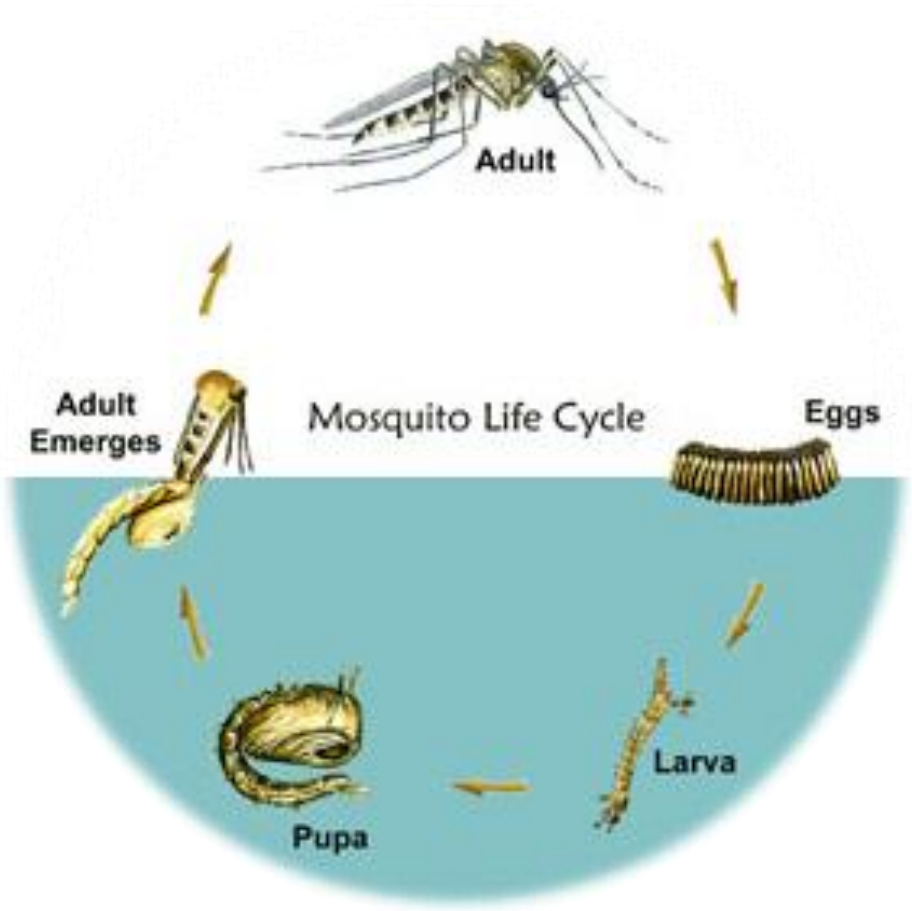

Figure 2.1: Mosquito life cycle (Adapted from Tallahassee 2010).

After emergence, the adult mosquitoes seek for mating and feeding. Male mosquitoes feed on plant nectar for sucrose while female mosquitoes feed on blood from humans and animals through their proboscis. The blood serves as good protein source for their eggs production. Female mosquitoes repeat the cycle and live up to few weeks. On the other hand, male mosquitoes usually survive for several days after mating. According to Freudenrich (2012), the life cycles of mosquitoes can be influenced by sex, time of year, species and environmental conditions which include temperature, rainfall and humidity.

At ambient temperature, the complete life cycle from egg to adult takes about nine to ten days for *Aedes* mosquitoes. Adult *Aedes albopictus* for instance, has a life span of 12 to 40 days for female while only 10 to 22 days for the male. The life span of *Aedes aegypti* is about 10 to 29 days for males and 12 to 56 days for females (Lee 2000). As for immature *Anopheles* mosquitoes, they take about 10 to 12 days for maturation and seek for blood meal from human and animals such as cattle, horses, goats and birds; 12 to 24 hours after emergence. The blood meal is then digested within 72 hours when the ovary is developing (Sulaiman 2000). Immature *Culex* mosquitoes take about 10 days for development to adult and engage in seeking for host 24 hours after emergence. They require four days for eggs development (Yap et al., 2000).

2.2 Mosquito species

Aedes, Anopheles and *Culex* are the most studied mosquito genera. As flooding is important for their eggs to hatch, *Aedes* mosquitoes are known as "floodwater" mosquitoes. These peridomestic mosquitoes breed in clean water containers and prefer to feed on human blood, being most active in the early morning and late afternoon (Whelan and Hurk 2003). The infamous mosquito *Aedes aegypti* served as primary vector for arboviruses which can cause yellow fever (Aitken et al., 1977), dengue (Coleman and McLean 1973) or chikungunya (Chua 2010) to humans. Besides the yellow-fever mosquito or *Aedes aegypti,* the other commonly known species which is responsible for transmitting such viruses is the Asian tiger mosquito or *Aedes albopictus*. Both domestic and peridomestic areas become their common breeding ground (Tilak et al., 2005).

Anopheles mosquitoes tend to breed in stationary fresh water, for example the *Anopheles farauti* which breeds in ponds, lagoons and swamps. It is predominantly a night biter especially at the first hour after sundown (Whelan and Hurk 2003). *Anopheles* species are capable of transmitting *Plasmodium* which accounts for malaria diseases, with *Anopheles maculatus* being the most commonly known as a major vector for human malaria in many countries in Southeast Asia (Reid 1968). *Anopheles culicifacies* and *Anopheles subpictus* are also known to be major vectors of both falciparum and vivax malaria (Surendran et al., 2006).

Culex mosquitoes tend to breed in quiet, standing water such as water puddles, ditches and paddy fields (Hassan et al., 2010). They usually attack at dawn or after dusk but preferring birds over humans. *Culex quinquefasciatus* are known to be vectors for Saint Louis encephalitis virus (Jones et al., 2002), Japanese encephalitis virus (Nitatpattana et al., 2005), West Nile virus (Godsey et al., 2005) and filarial parasite *Wuchereria bancrofti* that causes bancroftian filariasis in human (Samuel et al., 2004).

There are many ways to differentiate between the three renowned genera through their morphology. *Culex* mosquitoes have abdomens with blunt tips unlike *Aedes* and *Anopheles* which have pointed tips. Besides, *Culex* mosquitoes are generally brown and do not have any white band on their abdomen compared to *Aedes* mosquitoes. Female *Aedes* and *Culex* mosquitoes have maxillary palps which can be less than half the length their proboscis while female *Anopheles* mosquitoes have the same length between their maxillary palps and proboscis. In addition, they can be distinguished by their resting position. Adult *Culex* and *Aedes* mosquitoes rest almost parallel to the surface while adult *Anopheles* mosquitoes rest at an angle of approximately 45° from the surface with their bodies in linear to the proboscis (Yap et al., 2000).

In Malaysia, many surveillance activities on mosquitoes have been conducted and are still ongoing especially in Penang due to high mosquito population in the area. Based on the research by Hassan et al. (2010) in urban areas of Penang state, the presence of *Culex quinquefasciatus* can be found along drains while construction sites were to be blamed for high breeding activity of *Aedes albopictus* and *Aedes aegypti*. Another study was conducted in Selangor state by Dhang et al. (2005), in which they showed the mosquito eggs collected were either *Aedes albopictus* or *Aedes aegypti* with a minority of mixed breeding. *Aedes albopictus*, *Aedes aegypti* and *Culex quinquefasciatus* are the common species that are found in developing areas.

2.3 Mosquito-borne diseases

Mosquito-borne diseases such as malaria and dengue can be life-threatening to humans (Sachs and Malaney 2002). The flavivirus responsible for dengue are mainly transmitted by *Aedes aegypti* as the primary vector and also *Aedes albopictus* as secondary vector (Pethuan et al., 2007). Dengue hemorrhagic fever (DHF) and dengue fever (DF) are still a concern in tropical countries. Moreover, *Aedes* mosquitoes are important vectors of encephalitis virus and yellow fever. Under laboratory conditions, they can be competent vectors of more than 22 other arboviruses (Rosen et al., 1985). Dengue viruses infect about 50 million people and cause a high mortality rate of more than 20,000 each year (WHO 2006a). The common symptoms of dengue or dengue hemorrhagic fever are rash, severe eye,

joint, muscle and bone pain, high fever and mild bleeding manifestation. However, as body temperature declines three to seven days after symptoms began, patients may suffer from severe abdominal pain or persistent vomiting, difficulty in breathing, pale, cold and clammy skin, blood vomiting, drowsiness or irritability and bleeding from nose or gums (CDC 2009).

Dengue viruses are positive-sense, single-stranded RNA viruses of the *Flaviviridae* family (Da Silva and Richtmann 2006). Four serotypes for dengue viruses have been identified that are DEN-1, DEN-2, DEN-3 and DEN-4 and all of them are capable of causing great injury and fatality. Genetic variation can be found in each serotype where some of the genetic variants are more virulent and can cause epidemic outbreak (Malavige et al., 2011).

Chikungunya virus is another arbovirus which is transmitted to humans by *Culex quinquefasciatus* (Tan et al., 2011), *Aedes aegypti* and *Aedes albopictus* (Pialoux et al., 2007). It is a small envelope positive-sense RNA *alphavirus* from *Togaviridae*. According to Tilston et al. (2009), temperature plays a major role in effective arboviral transmission by mosquitoes. Their analysis conducted in Europe had shown that climates with mean monthly temperatures exceeding 20°C gave greater potential to Chikungunya outbreaks. Malaysia experienced the first Chikungunya outbreak in late 1998 (Lam et al., 2001) and reemerged in 2006

(Chua 2010). The clinical manifestations of Chikungunya virus infection include fever, joint pain, lymphadenitis and rash (Chua 2010).

Malaria is another infamous parasitic disease that is transmitted by *Anopheles* mosquitoes. Over three billion people are exposed to malaria infection and the disease causes one to three million deaths per year with morbidity reaching 515 million cases (Snow et al., 2005). The increasing insecticide resistance of the *Anopheles* mosquitoes and drug resistance of the parasite have contributed to the resurgence of the disease. Malaria has been proven as an obstacle in vaccine development due to the complication of immunoresponse and lack of political will (Breman et al., 2004). Most vaccines under development target the *Plasmodium falciparum*, which can cause severe malaria and deaths (Moorthy et al., 2004). Symptoms of malaria are muscle aches, diarrhea, vomiting, nausea, fatigue, headache, fever and flu-like illness. Jaundice and anemia could be present too due to the depletion of red blood cells. The clinical manifestations usually appear ten days after mosquito bite. Malaria can become fatal due to the disruption of blood supply to vital organs. At present, no effective vaccine has been developed against protozoa organisms due to their complexity (Engwerda and Good 2005).

Malaria infections are much lower than dengue in Malaysia. It is more prevalent in rural areas such as Malaysian Borneo which includes Sabah and Sarawak and to a lesser extent, in rural areas of Peninsular Malaysia rather than the urban areas (Gershman et al., 2011). According to Sibon (2012), Sarawak recorded a decline in reported malaria cases from 2,802 in 2010 to 1,761 cases in 2011 with two fatality cases reported. On the other hand, Malaysians are more exposed to dengue infections. Health Director-General Datuk Seri Dr. Hasan Abdul Rahman said from January to July 14, 2012, there were 12,518 dengue cases as opposed to 11,124 cases during the same period last year and up to date, there were 25 deaths recorded, an increase of six cases as compared to previous year's 19 (Ng 2012).

2.4 Vector control

Mosquitoes such as *Aedes*, *Culex*, *Anopheles* and *Mansonia* are anthropophilic which causes them to be responsible for many diseases. Hence, vector control is crucial especially in populated areas which are susceptible to high risk of exposure and infection. The vector control can be categorized into chemical control, biological control and environmental management (McCall and Kittayapong 2007).

2.4.1 Chemical control

Chemical control can be divided into larvicide and adulticide. There are five insecticides approved by the World Health Organization (2006b) for application to drinking water, which are temephos, methoprene, pyriproxyfen and novaluron. These insecticides target mainly *Aedes* mosquitoes such as *Aedes aegypti*, a typical indoor breeder species which often oviposit in drinking water. Temephos is an organophosphate compound which targets the nervous system of mosquito larvae by inactivating the enzyme acetylcholinesterase during nerve transmission. However, due to the resistance developed in mosquitoes in some areas and toxicity expression in non-target organisms such as crustaceans, temephos is applied only where high concentrations of late fourth instar larvae is found and no appreciable non-target animals are present (Whelan and Hurk 2003).

Pyriproxyfen is an insect juvenile-hormone analogue which is used as larvicides in drinking water where even at very low dosage can affect adults by decreasing their fertility. In addition, the chemical can be transferred to other breeding sites by the infected adult female (Chism and Apperson 2003). Pyriproxyfen can retain efficacy for up to six months after reformulation (Seng et al., 2006). Methoprene is another synthetic hormone used as larvicide. Functioning as insect growth regulator, it prevents the maturation of infected mosquito larvae into adults (McCall and Kittayapong 2007).

Meanwhile, to control the emerged adults, adulticides such as pyrethrins are used. Pyrethrins can be extracted from flowers of the chrysanthemum plant or synthetically produced. After entering the mosquito through the cuticle, they attack its nervous system by preventing the transmission of nerve impulses. The toxicity effect towards mosquitoes is rapid as the compounds can be broken down quickly but has very low dermal and oral toxicity to mammals. However, they do possess residual effect and may be toxic to other aquatic life. Some examples are bioresmethrin, deltamethrin and permethrin (Whelan and Hurk 2003). According to Chan et al. (2011), deltamethrin was shown to be more lethal than permethrin, by having a higher knockdown and mortality with lower doses.

Organophosphate compounds which also interrupt nerve impulse transmission in mosquitoes such as malathion and fenitrothion, are also used in adult mosquito control. Similar to pyrethrins, malathion develops rapid insecticidal properties with low toxicity to mammals. Unfortunately, residents are discomforted with malathion fogging as it releases strong odor (Whelan and Hurk 2003). The most common adulticides used by Ministry of Health in Malaysia are malathion and permethrin (Chan et al., 2011).

2.4.2 Biological control

Copepods have been known to be predators that prey on larvae, in particular, *Mesocyclops thermocyclopoides* and *Mesocyclops aspericornis* (Kay et al., 2002). They are small crustaceans that could be found in the sea and nearly every freshwater habitat. Even though copepods are capable of living up to six months, their life spans are usually shortened due to insufficient food present or when water is removed. Therefore, sustainable control by copepods may require constant reintroduction (Chansang et al., 2004). In Vietnam, this method has been implemented for a number of years as it was proven to be able to successfully control dengue transmission (Kay et al., 2002).

Water-treated with *Bacillus thuringiensis israelensis* (Bti) helps to control the population of mosquito larvae. The endotoxin produced by the bacteria has high larvicidal activity in mosquitoes but not to other beneficial organisms. Upon ingestion by the mosquito larva, the Bti products, Cry and Cyt proteins, lyse midgut epithelial cells causing paralysis and death within 24 hours (Whelan and Hurk 2003). A field trial in Thailand using slow-releasing, long-lasting Bti products showed that the toxicity effects could persevere up to three months (McCall and Kittayapong 2007). In addition, combination of strategies often gives greater impact. Based on the study by Chansang et al. (2004) in Thailand, the combined application treatments of Bti and copepods, with food provided for the

copepods, showed significant reductions of *Aedes aegypti* larvae in not more than three months.

Densonucleosis viruses or densoviruses belonging to the genus *Brevidensovirus* of the family *Parvoviridae* can be used as another form of biological control. Infected mosquito population suffers direct lethal effects or shortening of adult lifespan. The efficiency in vector control depends on the geographic origins of the mosquito and viral strains used (McCall and Kittayapong 2007).

One of the latest vector control method involved genetic engineering. Sample of mosquitoes can be genetically modified with a gene designed to kill them. Offspring of the genetically modified mosquitoes receive this same lethal gene which will kill the offspring before it reaches adulthood. The first trial of genetically modified mosquitoes have been set free in the open area in Cayman Islands in the year 2009, which resulted in a significant decrease in the mosquito population. The typical sterility levels reached 95 to 99% (Bakri et al., 2005) and produced 96.5% lethality (Harris et al., 2011). Being the first in Asia and second in the world, the Malaysian government released 6,000 of these genetically-modified *Aedes aegypti* in Bentong, Pahang (Daily Mail 2011).

2.4.3 Environmental management control

In addition to rejection of larviciding by communities, educational messages were often unsuccessful (Gubler and Clark 1994) and it is common to find houses in endemic areas infested with *Aedes* larvae (WHO 2000). Vector control can be carried out by the public through elimination of *Aedes* breeding sites such as broken pots, tires and other man-made containers (Tilak et al., 2005).

Personal protection can reduce exposure or infection with mosquitoes such as mosquito repellents and vaccination (Whelan and Hurk 2003). Besides, insecticide-treated materials have been proven to be highly effective in controlling *Aedes aegypti*. In Latin America, insecticide-treated domestic water container covers and insecticide-treated window curtains were demonstrated to be capable in reducing *Aedes* population (Kroeger et al., 2006).

2.5 Insecticidal resistance

Efforts to control dengue involved mainly insecticide spraying programs which included the most commonly, through indoor residual spraying (IRS), applicable also to malaria vector control (Coleman et al., 2006), although this strategy has proven ineffectual (Chua et al., 2005). Resistance is defined by the World Health Organization (WHO) as "the development of an ability or strain of some organisms to tolerate doses of a toxicant that would prove lethal to a majority of

individuals in a normal population of the same species" (Hemingway and Ranson 2000). Since the 1950s, development of potential resistance in vectors has been apparent but it was poorly documented. The common insecticide resistance mechanisms are alteration in acetylcholinesterases (AChE) and target sites including knockdown resistance (*kdr*), enhanced mixed function oxidases (MFO) or P450-mediated monooxygenases, enzyme activities of non-specific esterases and glutathione S-transferases (GST) (Oppenoorth 1985; Hemingway and Ranson 2000).

Pyrethroid resistance is widespread among *Aedes aegypti* (Bang et al., 1969; Malcolm and Wood 1982; Hemingway et al., 1989). Since the early 1980s, permethrin has been broadly used in house control (Taplin and Meinking 1987; Mumcuoglu et al., 1995). Resistance to synthetic pyrethroids has been developed in *Aedes aegypti* in many areas (Lima et al., 2003). The earlier reports on control failure by pyrethroids were in the early 1990s in France (Chosidow et al., 1995), the Czech Republic (Rupes et al., 1994) and Israel (Mumcuoglu et al., 1995). According to the findings from Sathantriphop et al. (2006) who tested the *Aedes aegypti* from Baan Suan community in Thailand, the adults were found resistant to permethrin and tolerant to deltamethrin, but were highly susceptible to fenitrothion and malathion. In Malaysia, the development of resistance began due to the fogging operations with malathion since early 1970s and permethrin since early 1996 against *Aedes* species (Hamdan et al., 2005). Due to frequent exposure

of mosquitoes to insecticides, understanding on insecticide resistance and the management is crucial for better control in future.

In 1955, global eradication of the most prevalent vector-borne disease, malaria, was proposed by the WHO by using the organochlorine dichlorodiphenyltrichloroethane (DDT) in residual house-spraying. However, WHO officially converted their policy from malaria eradication to malaria control in 1976 (Vontas et al., 2012). The development of DDT resistance in a wide range of mosquito species prompted this switch of policy. There were 256 million people reported living in areas where malaria control was undermined due to appearance of DDT resistance among the mosquitoes. As a result, newer insecticides such as organophosphates, carbamates and pyrethroids were introduced to replace DDT. The level and progression of resistance development in mosquito population are dependent on the frequency and volume of insecticides used against them. Besides, intrinsic characteristics of the species involved such as life cycles, generation time and the amount of offspring production affects the development of resistance. Having the characteristics of short life cycles and abundance of progeny, mosquitoes can develop resistance towards the insecticides rapidly (Hemingway and Ranson 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

Kampar is a town with the population range of between 20,000 and 50,000 with latitude of 4° 15' 39" N and longitude of 101° 09' 26" E (Figure 3.1) (Google Map 2012). Taman Kampar Jaya was the chosen residential area in Kampar for this research study. This area covers approximately 0.14 km^2. It is comprised of nine streets, namely Jalan Manggis, Jalan Limau Bali, Jalan Belimbing, Jalan Mempelam, Jalan Duku, Jalan Nenas, Jalan Kelub, Jalan Limau Kasturi, and Jalan Durian. There are eighteen rows of single-storey and double-storey houses, a field and two rows of shop lots.

The residents are mostly senior citizens and majority of them maintain a garden at the backyard or in front of their houses where some are not well-kept (Figure 3.2). Empty pots, uncovered trash bin and unorganized garbage sites can be seen around the residential area, which make good breeding sites for mosquitoes (Figure 3.3; Figure 3.4; Figure 3.5).

Figure 3.1: Satellite image of Taman Kampar Jaya (Adapted from Google Map 2012).

Figure 3.2: Front gardening area of houses in Taman Kampar Jaya.

Figure 3.3: Back lane of housing area in Taman Kampar Jaya.

Figure 3.4: Unorganized garbage disposal site in Taman Kampar Jaya.

Figure 3.5: Abandoned man-made container as good breeding site for *Aedes* mosquito in Taman Kampar Jaya.

3.2 Surveillance method

Sixty ovitraps measuring 7.8 cm in diameter and 9.0 cm in height each, and 300 hardboard paddles measuring approximately 10.0 cm X 2.5 cm X 0.3 cm with a rough surface on one side and smooth surface on the other side were prepared (Figure 3.6). Mosquito eggs were collected using ovitraps each filled with about a quarter of dechlorinated tap water. The ovitraps each were hung onto different tree trunks randomly around the residential area about 1 m above ground (Figure 3.7). The paddles with rough surface facing upward were placed into each ovitraps for oviposition of adult female mosquitoes. The paddles were collected and replaced three times a week on alternate days for three months from October until December 2012 (Table 3.1).

Figure 3.6: One of the ovitraps that hung onto tree trunks in Taman Kampar Jaya.

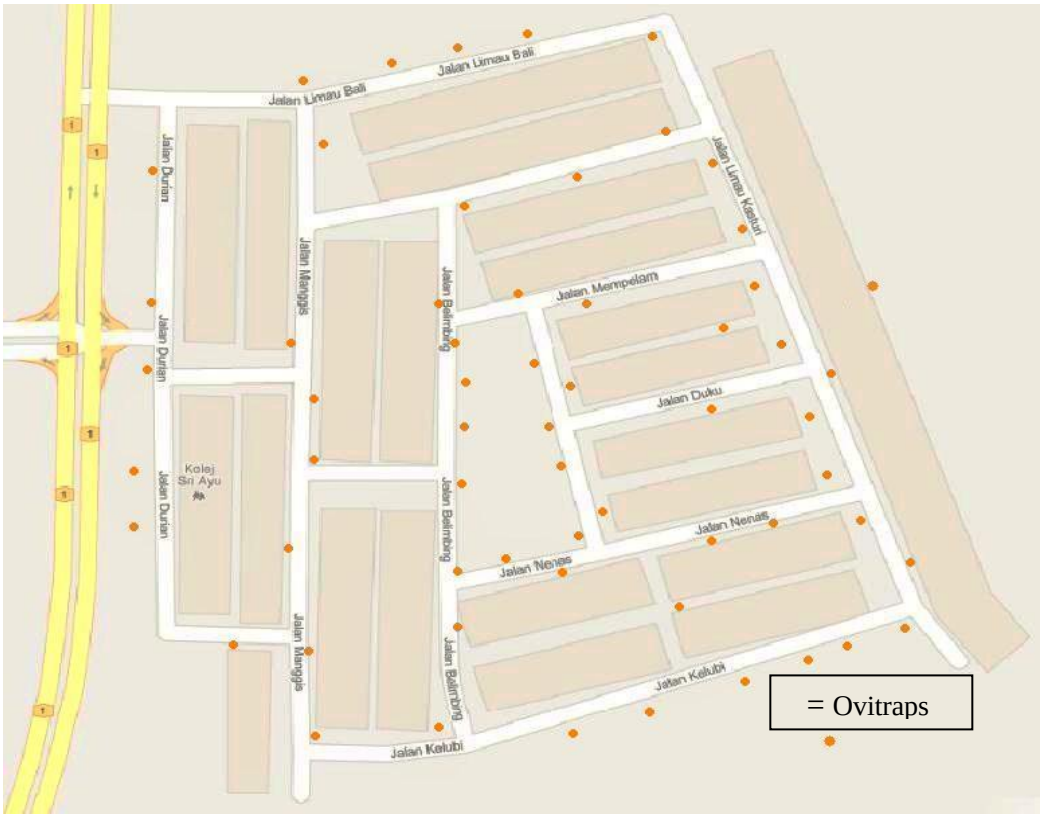

Figure 3.7: Locations of sixty ovitraps around Taman Kampar Jaya.

Table 3.1: Date of paddles collection and replacement in respective weeks.

Ovitrap surveillance week	Date of paddles collection and replacement
Week 1	1, 3, 5 October 2012
Week 2	8, 10, 12 October 2012
Week 3	15, 17, 19 October 2012
Week 4	22, 24, 26 October 2012
Week 5	29, 31 October; 2 November 2012
Week 6	5, 7, 9 November 2012
Week 7	12, 14, 17 November 2012
Week 8	19, 21, 23 November 2012
Week 9	26, 28, 30 November 2012
Week 10	2, 5, 7 December 2012
Week 11	9, 12, 14 December 2012
Week 12	17, 19, 21 December 2012
Week 13	23, 26, 28 December 2012

3.3 Mosquito culturing and identification

The collected paddles containing eggs were brought back to the laboratory to be air dried before immersing them in dechlorinated tap water the next day. Emerged larvae were fed with ground cat food and allowed to grow until pupae stage. Emerged pupae were then transferred into smaller containers and placed into mosquito cages in preparation for adult emergence. Ten percent sucrose solution was provided to serve as nutrient source for the emerged adults. They were transferred using aspirator to identify their gender and species using dissecting microscope and taxonomy key. Subsequently, the identified adult females were subjected to insecticides susceptibility tests.

3.4 WHO diagnostic test kit

The WHO diagnostic test kit was used to determine the insecticidal susceptibility of the female mosquito population collected from Taman Kampar Jaya. In each test, 25 sugar-fed female mosquitoes were transferred into holding tubes using aspirator, set upright and screen end up for 1 hour. Damaged insects were removed at the end of the holding period. A sheet of impregnated paper with insecticides was introduced into the exposure tube before transferring the mosquitoes from the holding tube into the exposure tube. The slide separating both tubes was then closed to enable the holding tube to be detached. The exposure tube was left upright for the required exposure period where mortality was observed every 2 minutes for a period of 1 hour. At the end of exposure

period, the mosquitoes were transferred into paper cups covered with mesh cloth and provided with cotton wool ball soaked with 10% sucrose solution. They were kept for 24 hours in a shaded place where temperature does not exceed 30°C. Mortality counts were made again after the 24 hours. Dead mosquitoes including those unable to walk are counted as dead. The mortality percentage was recorded.

The test was conducted using four types of insecticides and two types of control as listed in Table 3.2. If the control mortality was between 5 to 20%, the percentage mortalities were corrected using Abbott's formula:

$$\text{Abbott's formula} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

The insecticide susceptibility tests for each insecticide were replicated four times.

Table 3.2: Insecticide impregnated papers used with the corresponding control papers.

Insecticide Group	**Insecticide Used**	**Control**
Organophosphate	5% malathion	Olive oil
	1% fenitrothion	
Pyrethroid	0.75% permethrin	Silicon oil
	0.05% deltamethrin	

3.5 Data analysis

In order to analyze the abundance of mosquito species, the following parameters were included:

I. Total number of mosquitoes.

II. Ovitrap Index (OI).

$$\text{OI} = \frac{\text{Number of positive ovitraps}}{\text{Total number of ovitraps examines}} \times 100\%$$

III. Mean number of mosquito.

$$\text{Mean number} = \frac{\text{Total number of mosquitoes}}{\text{Total number of ovitraps examined}}$$

IV. Relationship between mean number of mosquitoes and meteorological parameters such as mean temperature, rainfall and relative humidity.

The knockdown time of 50% of total tested female mosquitoes (KT_{50}) and knockdown time of 95% of tested female mosquitoes (KT_{95}) for each insecticide were calculated using Probit analysis from the Statistical Package for the Social Sciences (SPSS) software.

CHAPTER 4

RESULTS

4.1 Mosquito population surveillance in the 13 weeks

Aedes albopictus was the most common species in Taman Kampar Jaya, found in all 60 ovitraps. The total sum of collected *Aedes albopictus* throughout the 13 weeks was 17,262 (Table 4.1). A total of 966 *Aedes albopictus* was collected on the first week, and subsequently doubled, reaching a peak of 2,031 in the following week. In the next six weeks, the number remained in the range of 1,100 to 1,700. On Week 9, only 677 *Aedes albopictus* were obtained which was the lowest among all weeks. The number then increased drastically to 1,231 in Week 10 and fluctuated in the last three weeks.

The ratio of male and female *Aedes albopictus* identified was approximately 50 : 50. The greatest difference between male and female percentage fell on Week 12 whereby 45.0% were males and 55.0% were females. As for the ovitrap index, all of them exceeded 90.0% except on Week 1 with only 83.3%. The highest percentage obtained was 98.3% in Weeks 4, 8, 10 and 13. The mean number of the mosquito ranged from 11.28 to 33.85 with an average of 22.13.

Table 4.1: Total number, percentage of male and female, ovitrap index (OI) and mean number of *Aedes albopictus* corresponding to the respective sample collection weeks.

Week	Total mosquito	Percentage (%)		Ovitrap index (OI)	Mean number
		Male	Female	(%)	of mosquito
1	966	47.4	52.6	83.3	16.10
2	2031	49.7	50.3	93.3	33.85
3	1560	49.0	51.0	96.7	26.00
4	1484	51.0	49.0	98.3	24.73
5	1508	51.1	48.9	95.0	25.13
6	1178	50.5	49.5	96.7	19.63
7	1687	51.0	49.0	91.7	28.12
8	1395	49.7	50.3	98.3	23.25
9	677	49.0	51.0	91.7	11.28
10	1231	48.7	51.3	98.3	20.52
11	865	52.0	48.0	91.7	14.42
12	1537	45.0	55.0	95.0	25.62
13	1143	52.0	48.0	98.3	19.05
Total:	**17262**			Average Mean:	22.13

Other species found in Taman Kampar Jaya were *Aedes albolateralis*, *Aedes aegypti*, *Aedes gardneri imitator* and *Culex quinquefasciatus* with a total of 274, 48, 15 and 11 respectively. *Aedes albolateralis* were found in 32 ovitraps set around Taman Kampar Jaya (Figure 4.1) and its highest number was recorded on Week 4 with a total of 64 mosquitoes (Table 4.2). The ovitrap index ranged from 1.7 to 18.3% and the average mean was 0.35.

Based on Table 4.3, *Aedes aegypti* were found on Weeks 6, 8, 10, 11, 12 and 13 with the highest total number of 33 on Week 8. They were found in ovitraps 1, 14, 17, 26, 38, 41 and 42 (Figure 4.2). The ovitrap indexes were at most 3.3% only and an average mean number of 0.06 (Table 4.3).

On the other hand, *Aedes gardneri imitator* were found on Weeks 4, 6, 9 and 10 and the highest number recorded was only eight on Week 4 (Table 4.4). They were found in ovitraps 7, 14, 16, 21, 43 and 57 (Figure 4.3) with maximum ovitrap index of 5.0% and average mean of 0.02.

Culex quinquefasciatus were least found with a total of 11. They were found in ovitrap 31 on Week 1, ovitrap 28 on Week 7 and ovitrap 22 on Week 10 (Figure 4.4). The ovitrap index was only 1.7% and the average mean was 0.01 (Table 4.5).

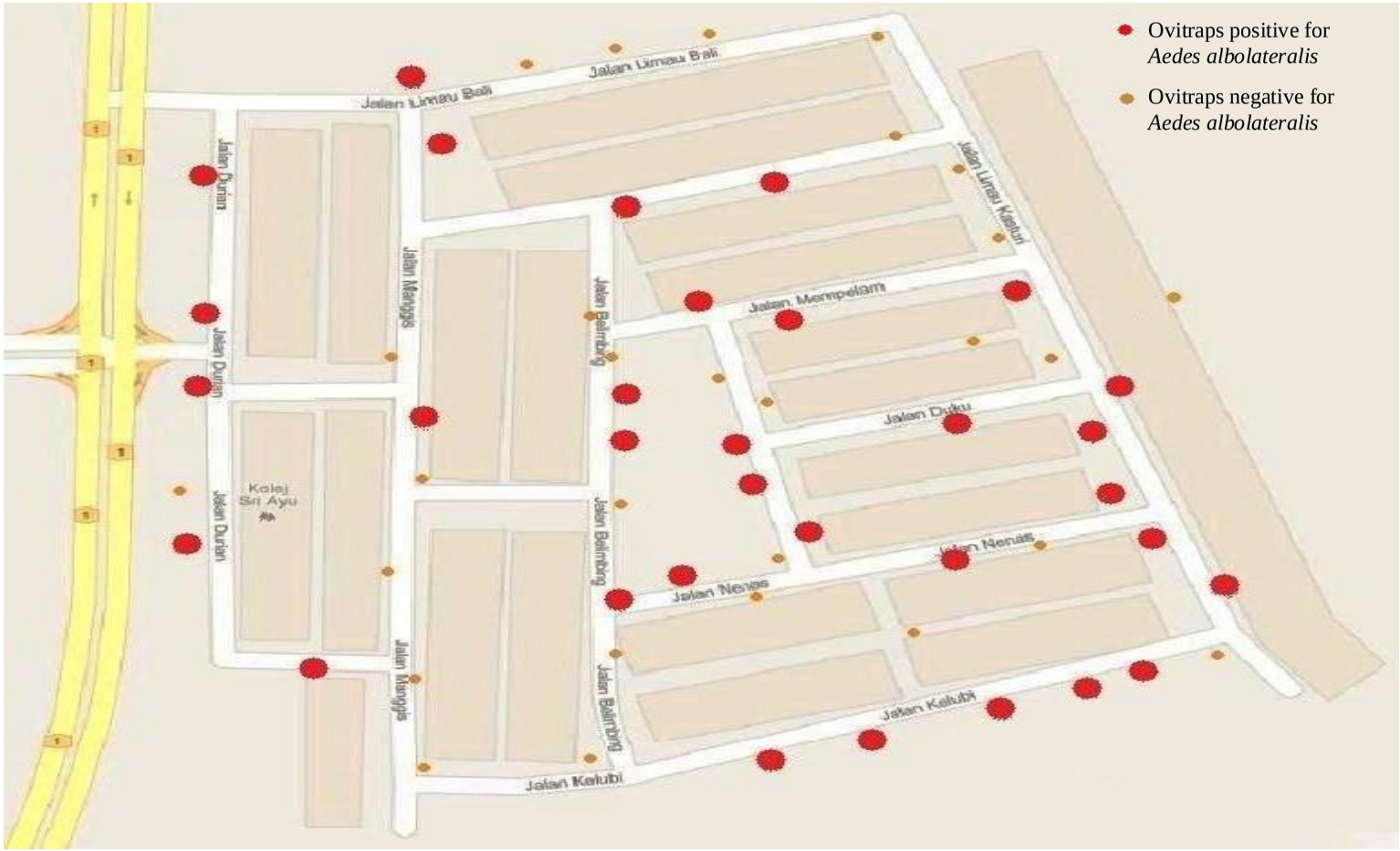

Figure 4.1: Locations of ovitraps positive for *Aedes albolateralis*.

Table 4.2: Total number, percentage of male and female, ovitrap index (OI) and mean number of *Aedes albolateralis* corresponding to the respective sample collection weeks.

Week	Total mosquito	Percentage (%)		Ovitrap index (OI)	Mean number
		Male	Female	(%)	of mosquito
1	1	0.0	100.0	1.7	0.02
2	5	20.0	80.0	3.3	0.08
3	2	0.0	100.0	1.7	0.03
4	64	31.2	68.8	18.3	1.07
5	46	45.7	54.3	13.3	0.77
6	31	58.1	41.9	11.7	0.52
7	29	44.8	55.2	18.3	0.48
8	8	75.0	25.0	3.3	0.13
9	8	50.0	50.0	1.7	0.13
10	13	38.5	61.5	10.0	0.22
11	39	41.0	59.0	15.0	0.65
12	10	60.0	40.0	10.0	0.17
13	18	27.8	72.2	8.3	0.30
Total:	**274**			Average Mean:	0.35

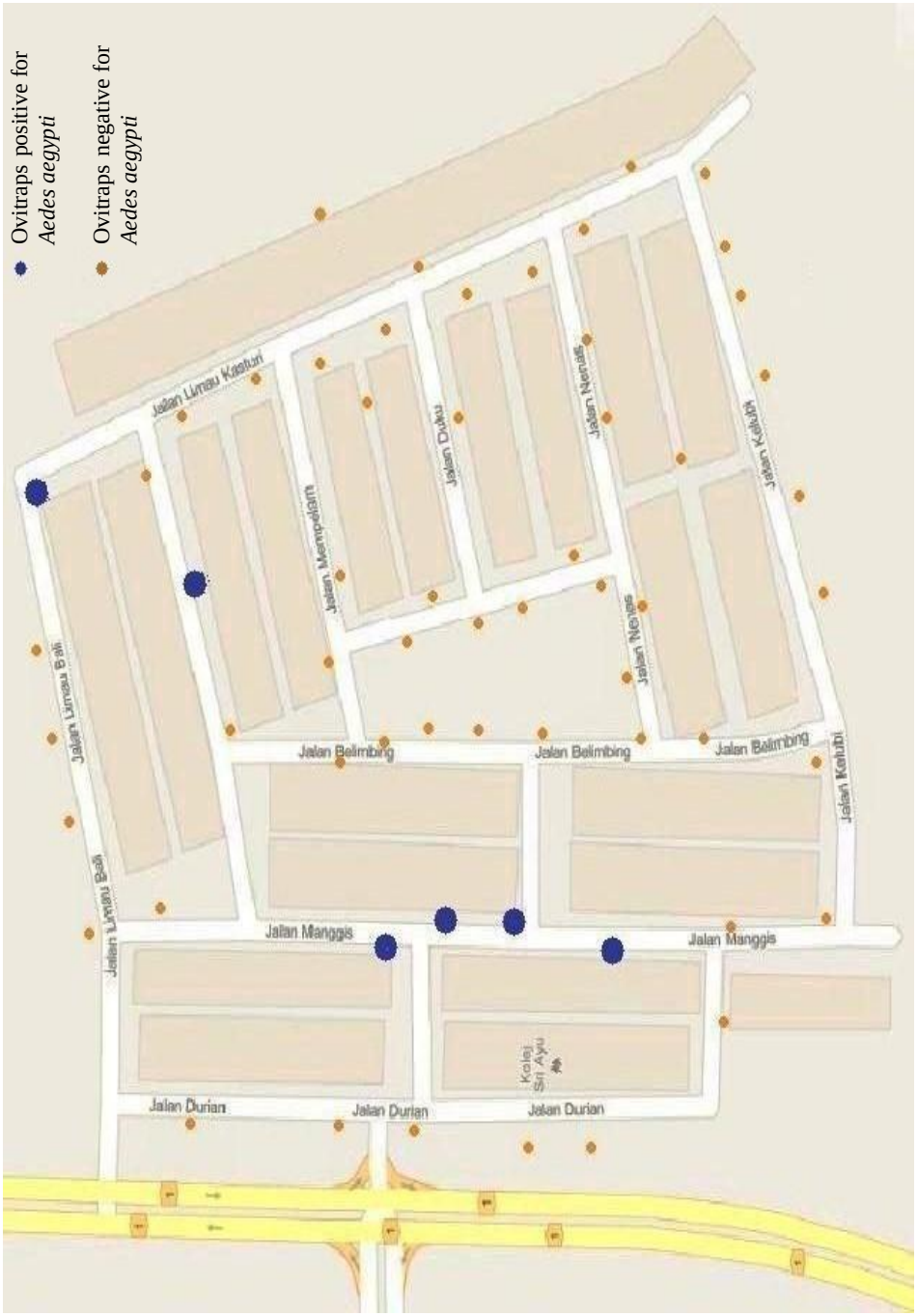

Figure 4.2: Locations of ovitraps positive for *Aedes aegypti*.

Table 4.3: Total number, percentage of male and female, ovitrap index (OI) and mean number of *Aedes aegypti* corresponding to the respective sample collection weeks.

Week	Total mosquito	Percentage (%)		Ovitrap index (OI)	Mean number
		Male	Female	(%)	of mosquito
1	0	0.0	0.0	0.0	0.00
2	0	0.0	0.0	0.0	0.00
3	0	0.0	0.0	0.0	0.00
4	0	0.0	0.0	0.0	0.00
5	0	0.0	0.0	0.0	0.00
6	2	50.0	50.0	1.7	0.03
7	0	0.0	0.0	0.0	0.00
8	33	39.4	60.6	3.3	0.55
9	0	0.0	0.0	0.0	0.00
10	2	50.0	50.0	1.7	0.03
11	4	50.0	50.0	3.3	0.07
12	6	33.3	66.7	3.3	0.10
13	1	0.0	100.0	1.7	0.02
Total:	**48**			Average Mean:	0.06

Figure 4.3: Locations of ovitraps positive for *Aedes gardneri imitator*.

Table 4.4: Total number, percentage of male and female, ovitrap index (OI) and mean number of *Aedes gardneri imitator* corresponding to the respective sample collection weeks.

Week	Total mosquito	Percentage (%)		Ovitrap index (OI)	Mean number
		Male	Female	(%)	of mosquito
1	0	0.0	0.0	0.0	0.00
2	0	0.0	0.0	0.0	0.00
3	0	0.0	0.0	0.0	0.00
4	8	37.5	62.5	1.7	0.13
5	0	0.0	0.0	0.0	0.00
6	1	0.0	100.0	1.7	0.2
7	0	0.0	0.0	0.0	0.00
8	0	0.0	0.0	0.0	0.00
9	2	0.0	100.0	1.7	0.03
10	4	50.0	50.0	5.0	0.07
11	0	0.0	0.0	0.0	0.00
12	0	0.0	0.0	0.0	0.00
13	0	0.0	0.0	0.0	0.00
Total:	**15**			Average Mean:	0.02

Figure 4.4: Locations of ovitraps positive for *Culex quinquefasciatus*.

Table 4.5: Total number, percentage of male and female, ovitrap index (OI) and mean number of *Culex quinquefasciatus* corresponding to the respective sample collection weeks.

Week	Total mosquito	Percentage (%)		Ovitrap index (OI)	Mean number
		Male	Female	(%)	of mosquito
1	1	0.0	100.0	1.7	0.02
2	0	0.0	0.0	0.0	0.00
3	0	0.0	0.0	0.0	0.00
4	0	0.0	0.0	0.0	0.00
5	0	0.0	0.0	0.0	0.00
6	0	0.0	0.0	0.0	0.0
7	6	50.0	50.0	1.7	0.10
8	0	0.0	0.0	0.0	0.00
9	0	0.0	0.0	0.0	0.00
10	4	25.0	75.0	1.7	0.07
11	0	0.0	0.0	0.0	0.00
12	0	0.0	0.0	0.0	0.00
13	0	0.0	0.0	0.0	0.00
Total:	11			Average Mean:	0.01

4.2 Relationship between mean number of mosquitoes and rainfall

Mean rainfall fluctuated heavily throughout the 13 weeks. As shown in Figure 4.5 and Figure 4.6, mean rainfall peaked at 32.9 mm on Week 5. The secondary peaks fell on Weeks 3, 9 and 11. The lowest mean rainfall was on Week 1 with only 0.5 mm. Although not significant ($p > 0.05$), there were negative correlations between mean rainfall and mean numbers of the *Aedes albopictus* (Pearson Correlation = -0.188, p = 0.538), *Aedes aegypti* (Pearson Correlation = -0.138, p = 0.653), *Aedes gardneri imitator* (Pearson Correlation = -0.044, p = 0.887) and *Culex quinquefasciatus* (Pearson Correlation = -0.013, p = 0.965). On the contrary, the seasonal distribution of *Aedes albolateralis* was positively correlated to the mean rainfall, although also not significant (Pearson Correlation = 0.281, p = 0.353) (Appendix C).

The highest mean number of *Aedes albopictus* of 33.85 was observed on Week 2 with a mean rainfall of 5.9 mm. The mean number of *Aedes albopictus* remained high for the subsequent six weeks where mean rainfall fluctuated in a wide range of 6.9 to 32.9 mm. On Week 9, the mean number of the mosquito was halved from 23.25 on Week 8 to 11.28 where its mean rainfall was second highest. The mean number of *Aedes albopictus* then recovered in the consequent weeks with mean rainfall of 13.7 mm and 19.0 mm. However, on Weeks 12 and 13, the mean rainfall diminished significantly to 0.9 mm and 2.2 mm while the mean number of mosquito remained high at 25.6 and 19.1 respectively.

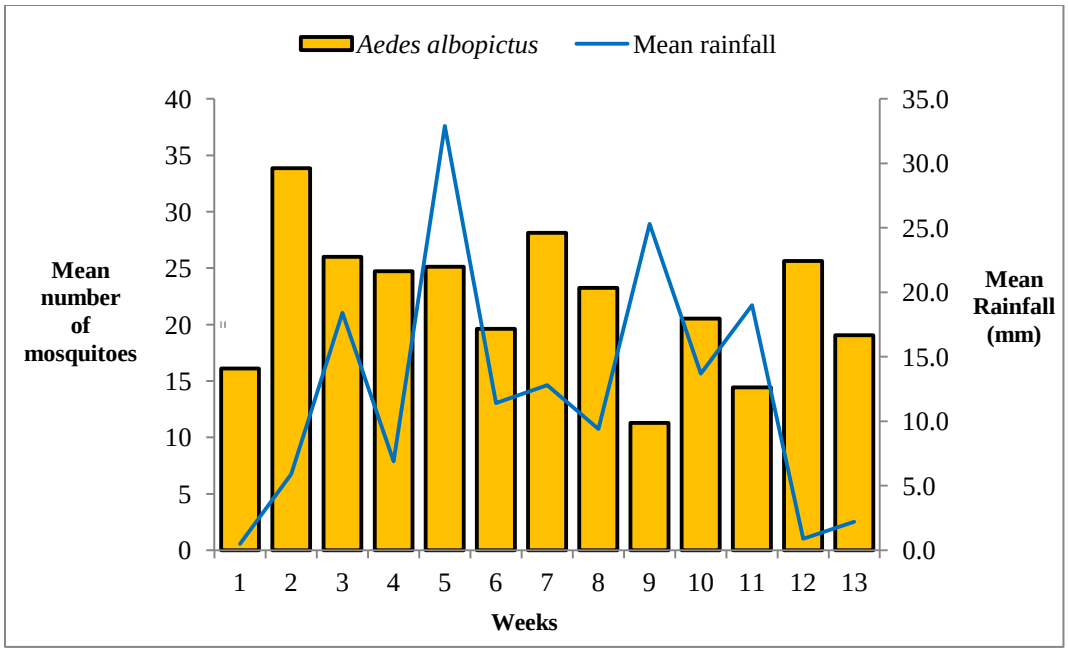

Figure 4.5: Mean number of *Aedes albopictus* and total rainfall (mm) against the period of sample collection.

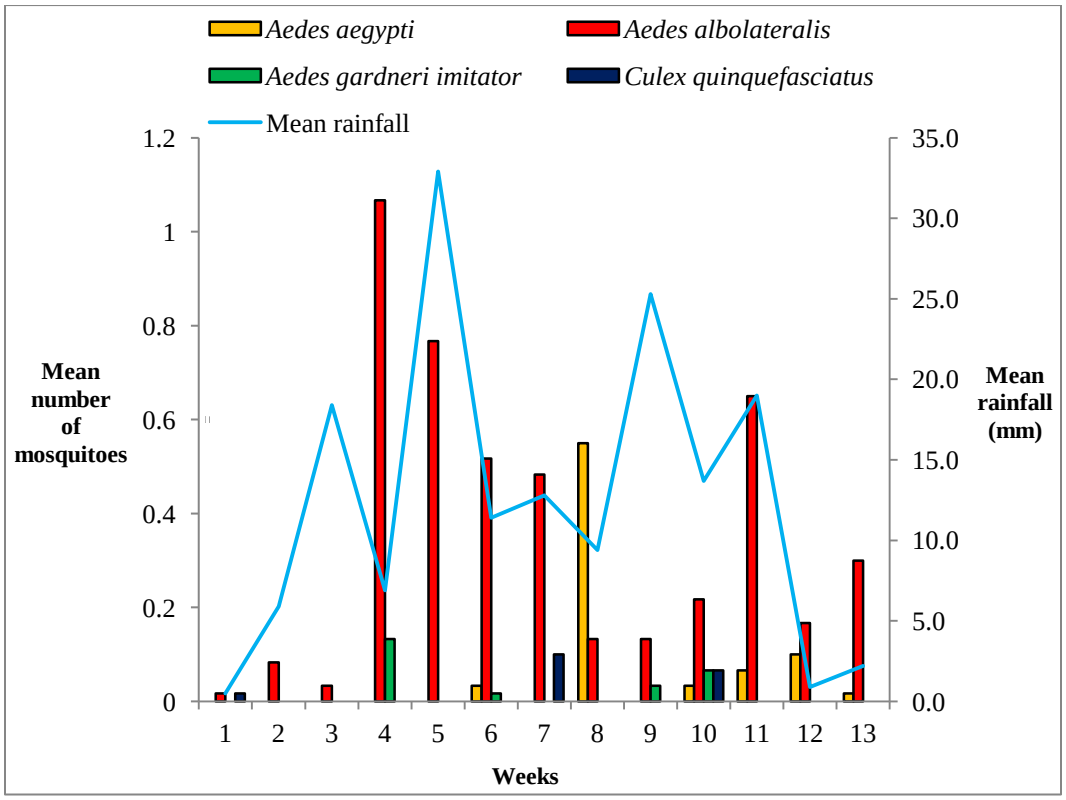

Figure 4.6: Mean number of other mosquito species and total rainfall (mm) against the period of sample collection.

On the other hand, based on Figure 4.6, *Aedes albolateralis* were found at lowest in mean number on Week 1 with only 0.017 with mean rainfall of 0.5 mm. The mean number remained low in the following weeks and then increased drastically and peaked to 1.07 in mean number on Week 4 after an increase of mean rainfall at 18.4 mm on the previous week. The mean number of mosquitoes decreased steadily since the mean rainfall peak on Week 5. The mean number remained low from Weeks 8 to 13 except a sharp peak at 0.65 and mean rainfall of 19.0 mm on Week 11. Mean number of *Aedes aegypti* peaked on Week 8 with mean rainfall of 9.4 mm. They were found on Weeks 6, 10, 11, 12 and 13 where mean rainfall were in between 0.9 to 19.0 mm. Meanwhile, *Aedes gardneri imitator* were found on Weeks 4, 6, 9 and 10 when mean rainfall were between 6.9 to 25.3 mm. *Culex quinquefasciatus* were found on Weeks 1, 7 and 10 when mean rainfall was below 13.7 mm.

4.3 Relationship between mean number of mosquitoes, temperature and relative humidity

The mean temperature was relatively steady throughout the 13 weeks (Figure 4.7; Figure 4.8). It ranged from 25.8 to 27.6°C. As the temperature rose, the mean relative humidity decreased. It can be illustrated on Week 1 where the highest mean temperature of 27.6°C was recorded while the mean relative humidity was at its lowest at 73.4%. Conversely, the mean temperature was found lowest at 25.8°C on Week 6 when the highest mean relative humidity value of 88.5% was recorded.

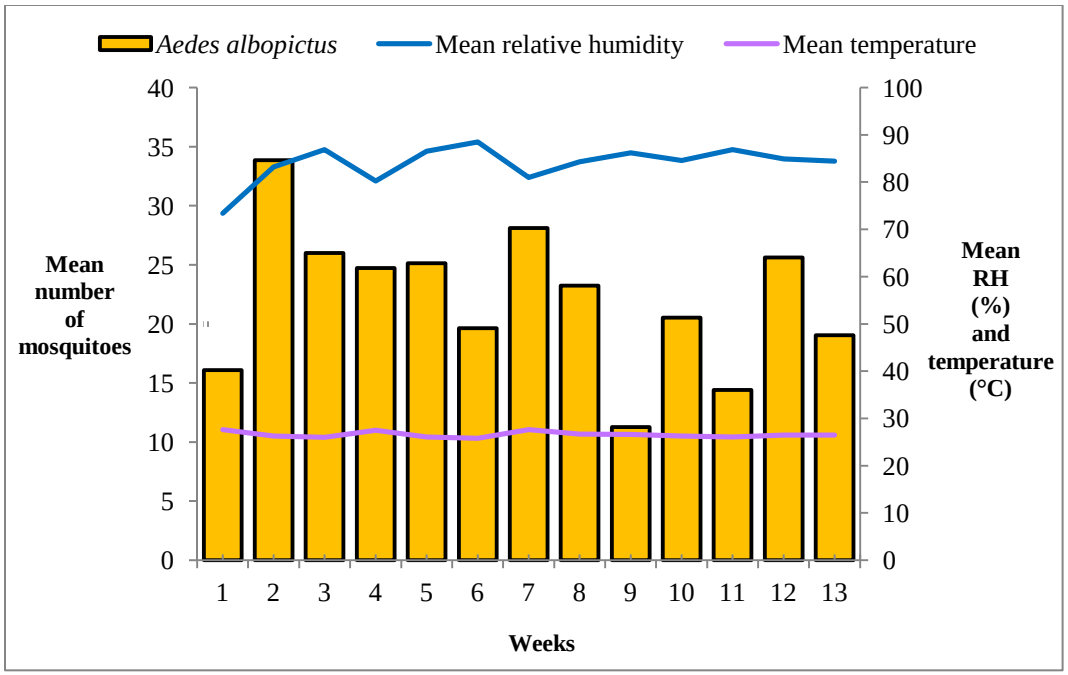

Figure 4.7: Mean number of *Aedes albopictus*, relative humidity (%) and temperature (°C) against the period of sample collection.

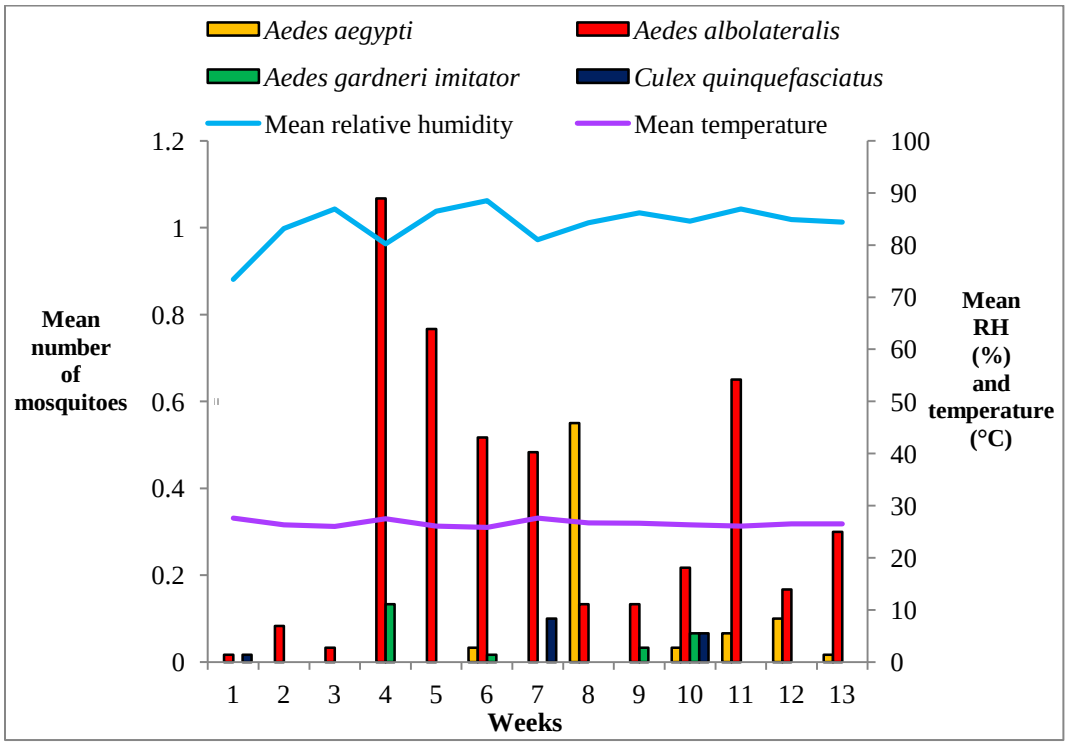

Figure 4.8: Mean number of other mosquito species, relative humidity (%) and temperature (°C) against the period of sample collection.

The highest mean number of *Aedes albopictus* was found on Week 2 where the mean relative humidity was 83.2% and temperature was 26.3°C. The mean number of the mosquitoes was found lowest on Week 9 when mean relative humidity and temperature was at 86.2% and 26.6°C respectively (Figure 4.7).

Based on Figure 4.8, the mean number of *Aedes albolateralis* was highest on Week 4 when mean relative humidity and temperature was at 80.4% and 27.5°C respectively. The lowest mean number of *Aedes albolateralis* was recorded on Week 1 with mean relative humidity and temperature at 73.4% and 27.6°C respectively. *Aedes aegypti* were found on Weeks 6, 7, 10, 11, 12 and 13 when mean relative humidity ranged from 84.3 to 88.5% and mean temperature ranged from 25.8 to 26.7°C. As for *Aedes gardneri imitator*, they were found on Weeks 4, 6, 9 and 10 when mean relative humidity were between 80.2 and 88.5% and mean temperature was between 25.8 and 27.5°C. *Culex quinquefasciatus* were found on Weeks 1, 7 and 10 when the mean relative humidity ranged from 73.4 to 84.6% and mean temperature was from 26.3 to 27.6°C.

4.4 Mortality test on *Aedes albopictus* using deltamethrin, fenitrothion, malathion and permethrin

Both pyrethroids, deltamethrin (0.05%) and permethrin (0.75%) have shown to be more effective than the organophosphates, malathion (5%) and fenitrothion (1%). Based on the result (Table 4.6), deltamethrin gave the lowest knockdown time,

Table 4.6: Knockdown time of *Aedes albopictus* against four different insecticides.

Insecticide	Knockdown time (min)		Regression slope ± Standard error
	KT_{50}	KT_{95}	
0.05% Deltamethrin	15.84 (14.99 – 16.68)	48.18 (45.70 – 51.00)	3.41 ± 0.760
1% Fenitrothion	150.29 (148.53 – 152.03)	293.41 (288.71 – 298.37)	5.66 ± 0.060
5% Malathion	48.46 (47.71 – 49.20)	87.72 (85.91 – 89.67)	6.382 ± 0.119
0.75% Permethrin	20.57 (20.17 – 20.96)	29.54 (28.89 – 30.27)	0.183 ± 0.006

KT_{50} of 15.84 minutes in the range of 14.99 – 16.68 minutes, followed by permethrin at the KT_{50} of 20.57 minutes. The KT_{50} for malathion was almost threefold at 48.46 minutes. Fenitrothion was the least effective and scored the highest KT_{50} of 150.29 minutes with range of 148.53 – 152.03 minutes.

Permethrin had also shown its effectiveness by scoring the lowest value of KT_{95} at 29.54 minutes. Deltamethrin came second with KT_{95} of 48.18 minutes with the range of 45.70 – 51.00 minutes. The KT_{95} for malathion was 48.46 minutes. Fenitrothion was proven again to be the least effective at KT_{95} of 293.41 minutes.

Mortality count was made again after 24 hours where 100% mortality was recorded for each test. No survival was observed indicating that they were susceptible to deltamethrin, permethrin, malathion and fenitrothion. The negative control results using both olive oil for organophosphate group and silicon oil for pyrethroid showed no mortality. Therefore, Abbott's formula was not used in correcting the percentage mortality of the insecticidal susceptibility tests.

Chi-square values were significant ($p < 0.05$). Thus, the heterogeneity factor was taken into consideration in the calculation for confidence interval because the mosquitoes tested for every tests were of the same age, gender, species and sugar-

fed. The regression slope ± standard error was highest in malathion with 6.382 ± 0.119 while the lowest value was obtained in permethrin with 0.183 ± 0.006.

CHAPTER 5

DISCUSSION

5.1 Mosquito surveillance and identification in Taman Kampar Jaya

Ovitrap surveillance method was used in the experiment as it is inexpensive, convenient and the ovitraps can be installed in large areas easily (Silver 2008; Chebabi et al., 2011). Most ovitrap index acquired exceeded 90%, indicating active mosquito breeding activity in Taman Kampar Jaya. The total *Aedes albopictus* collected in Taman Kampar Jaya throughout the 13 weeks was 17,262. It was the dominant species found and is considered exceptionally high for a small area that covers only approximately 0.14 km^2. The surveillance data was not influenced by any fogging activity as it was not conducted throughout the sample collection period.

Poor environmental management might be a major contributor for such great mosquito density. Garbage disposal was carried out frequently by the workers from the local authorities in the mornings. As such, the area is relatively clean with an exception at the side of the field where an abandoned air-conditioner, a cooler box and many disposed cardboard boxes with the largest one was once a shipping container for a plasma television, were seen left there the entire three months. When enquired, the residents said that the wastes belonged to one of the

local residents who lived nearby the dumpsite who makes a living from recycling materials. These artificial containers serve as water reservoir especially during rainy season, giving birth to more *Aedes* mosquito breeding sites (Tilak et al., 2005). In addition, there were gardening works done in almost every house in Taman Kampar Jaya and some garden accoutrements such as watering cans, vases, flower pots plates and flower pots were left unattended by irresponsible residents, providing more potential oviposition sites (Nyamah et al., 2010). The ratio of male and female *Aedes albopictus* identified was approximately 50 : 50 which showed no gender bias.

Aedes albopictus can be identified through the basal bands on its abdomen, one silvery-white stripe down the middle of the scutum, silvery-white scales at the tips of palps, dark wing scales, legs with white basal bands and tarsal segment 5 entirely white (Cutwa and O' Meara 2007). The dengue transmission by *Aedes albopictus* was first reported in 1958 and this mosquito was reported breeding in forest canopies. At present, *Aedes albopictus* is widely known to breed in urban or suburban populated areas accompanied by *Aedes aegypti*. Female *Aedes albopictus* was reported to be able to discriminate egg deposition sites to secure survivability of her offspring, which may be an explanation for the active breeding of the mosquitoes in the study site. *Aedes albopictus* showed a significant preference for feeding on humans and this may constitute a higher risk for the transmission of dengue viruses to human population (Dieng et al., 2012).

Sixty-six dengue cases were reported to the Majlis Daerah Kampar (Kampar District Council) in 2012 and only one of them occurred in Taman Kampar Jaya which was on March 22, 2012. Hosts such as birds and human infected with dengue virus become mobile carrier in which would aid in dengue viral transmission indirectly. Upon feeding on the infected host, a certain period of time is required for the ingested virus to enter and replicate in the midgut epithelium of *Aedes albopictus*. The viral particles are then transferred to the salivary glands via the hemolymph, 10 days after the ingestion of infectious blood meal. As a result, the *Aedes* mosquito will infect the subsequent hosts with the arbovirus (Aida et al., 2011; Luplertlop et al., 2011). Thus, the infection of dengue virus can easily spread especially to neighboring residential areas. Moreover, human transportation involving vehicles such as cars and town buses helps in increasing the reach of dengue virus infection (Huber et al., 2004). Hence, high dengue incidence rate in other areas in Kampar could pose a threat to Taman Kampar Jaya, especially with its high density of *Aedes albopictus* population which can hasten the viral transmission. Fortunately, no fatality case was reported out of the 66 dengue cases in 2012.

Aedes aegypti mosquitoes were also found from the paddles collected, but with a total of only 48, significantly much lower than that of *Aedes albopictus*. *Aedes aegypti* can be identified by the basal bands on abdomen, lyre-shaped silvery-white scales on scutum, white scales on clypeus, dark proboscis and wing scales,

legs with white basal bands and silvery-white scales at the tips of palps (Cutwa and O' Meara 2007). It was known that *Aedes albopictus* is more exophilic compared to *Aedes aegypti* which may account for the relatively lower number of *Aedes aegypti* obtained from outdoor ovitraps (Vontas et al., 2012). However, studies on other places such as Penang have demonstrated both *Aedes albopictus* and *Aedes aegypti* sharing preference in both indoor and outdoor breeding (Lee 2000; Chua et al., 2005; Hassan et al., 2005; Dieng et al., 2012). Based on the study by Hassan et al. (2005) in Penang, *Aedes aegypti* were found breeding in many similar sites with *Aedes albopictus* such as the basement flooded floor, drains, floor recessed opening and water tanks.

Besides *Aedes aegypti*, other smaller mosquito populations obtained were *Aedes albolateralis, Aedes gardneri imitator* and *Culex quinquefasciatus* with a total number of 274, 15 and 11 respectively. Based on the study conducted by Pemola and Jauhari (2007), *Aedes albolateralis* shared similar breeding preferences with *Aedes albopictus*. The immature *Aedes albolateralis* was mainly found in partially shady areas with slow flowing, clean or slightly turbid water such as ground water seepages, rice fields, tanks, streams and rock holes. Furthermore, this mosquito species showed great susceptibility towards *Wuchereria bancrofti* and dengue virus type 2, making it a potential vector as well. However, this species is poorly studied and further research is required (Choochote et al., 2001). *Aedes gardneri imitator* appears to prefer natural containers such as tree holes, hollow logs and

hollow stumps. This species can be expected to be found as Taman Kampar Jaya is situated at the edge of Kampar, surrounded by forest. It can be distinguished from *Aedes aegypti* and *Aedes albopictus* by its scutal median with broad white patch border or two lateral white patches on the anterior third of the scutum (Huang 1977).

On the other hand, *Culex quinquefasciatus* can be identified by its golden body, antenna of about the same size as the proboscis, dark legs without bands and broad M-shaped bands on abdomen with the most prominent on segments 4 and 5 (Cutwa and O' Meara 2007). Immature *Culex quinquefasciatus* can be found abundant in the drains due to its oviposition preference in dirty water. These drains can be clogged even by dried leaves and other wastes from the garden, obstructing the drainage flow, thus causing water to be stagnant. In addition, these dried leaves, branches, flowers and other organic matters can also serve as nutrient source for the immature *Culex quinquefasciatus* to survive and grow (Hassan et al., 2005).

Population sampling can be referred to as the means of estimating population size when a direct count of all the organisms constituting a population is impossible. As such, ovitrap surveillance technique was practiced in the experiment. However, there is a species-bias from such sampling methodology. The egg samples were

collected from paddles that favor mosquitoes which cultivate the oviposition strategy of laying eggs onto substances subjected to intermittent flooding. Such mosquitoes include *Aedes*, *Ochlerotatus* and *Psorophora*. Eggs of other species which are laid onto the water surfaces in the ovitrap will not attach to the paddles and thus, such species would be recorded absent or in a low quantity. Given the eggs are almost invisible under naked eye especially in a black-colored ovitrap, they can be easily overlooked. During the collection and replacement of paddles, the water was not collected and brought back into laboratory to check for any immature mosquitoes due to space constraint. In addition, ovitraps are artificial containers which favor breeding by *Aedes* mosquitoes (Lee 2000; Tilak et al., 2005; McCall and Kittayapong 2007; Saleeza et al., 2011). Moreover, ovitraps or paddles were often disturbed, damaged or missing, which was believed to be due to acts by curious residents especially children and stray animals. As such, data such as ovitrap index, total number of mosquito and mean of mosquitoes would be affected.

5.2 Effect of environmental factors on the mosquito population

The high population of mosquitoes can be related to the environmental factors such as the rainfall, relative humidity and temperature (Rozilawati et al., 2007). As such, these parameters were obtained from the Jabatan Meteorologi Malaysia (Malaysian Meteorological Department) to study their influences on the mosquito population in Taman Kampar Jaya. The data was collected from the meteorology

station in Hospital Kampar with latitude of 04° 18’ 00” N and longitude of 101° 09’ 00” E, situated at 37.5 m above the mean sea level.

In tropical countries where rainfall is abundant and relatively consistent, there is a low desiccation risk and most egg hatching happens rapidly (Ho et al., 1971). Many studies have been carried out in Southeast Asian countries such as Malaysia, Thailand and Myanmar, and have demonstrated that the seasonal dengue outbreaks due to high mosquito populations corresponded to rainy seasons (Rozilawati et al., 2007). This coincides with the current study as the sample collection was conducted in Taman Kampar Jaya during its rainy season in the end year of 2012.

5.2.1 Relationship between rainfall and mosquito population

The total number of eggs collected was abundant due to the constant rainfall that contributed to the increase of mosquito breeding sites and egg hatching (Rozilawati et al., 2007). This was correlated to the study by Ndiaye et al. (2006), which showed tremendous expansion of mosquito population size and mass egg hatching associated to rainy season. The peak of mean number of outdoor breeders, *Aedes albopictus*, was on Week 2 with mean rainfall of 5.9 mm. The mean number remained high in the weeks when mean rainfall were in a range of 6.9 to 32.9 mm.

The mean number of mosquitoes decreased after Weeks 5 and 11 when the mean rainfalls were at their peaks at 32.9 mm and 19.0 mm respectively. The research done by Foo et al. (1985) supported the current finding, whereby occurrence of heavy rainfall left negative impact to the aquatic stages of mosquito, as the excess water flushed out the eggs, larvae and pupae from their containers. This finding also correlated to the study by Hornby et al. (1994) in the United States where egg population surged at the beginning of rainy season but decreased after that due to heavy rain. In order to reduce the negative influence of rainfall, the ovitraps were only filled with dechlorinated tap water to about a quarter full.

On the other hand, during the secondary peak of mean rainfall at 25.3 mm on Week 9, the mean number of *Aedes albopictus* was halved from 23.25 to 11.28. This could be due to the influence of strong winds that accompanied the heavy rains, interfering with the mosquitoes' flight activity to seek for hosts and oviposit (Foo et al., 1985).

Aedes albolateralis showed a drastic decrease in mean number on Weeks 5 and 11 as well after the peaks of mean rainfall on the Weeks 4 and 12 respectively. On the contrary, the typical indoor breeders *Aedes aegypti* were found in the outdoor ovitraps when rainfall were within the moderate range of 0.9 and 19.0 mm. *Aedes gardneri imitator* which breed in natural container, were found on Week 4 after

the first peak of rainfall on Week 3. This may be due to the increase in their population which led to the competency for more alternative breeding sites. *Culex quinquefasciatus* were found during low mean rainfall and absent when mean rainfall exceeded 13.7 mm. High rainfalls may cause the flushing of the eggs, larvae and pupae along the clogged drains which are common breeding sites for *Culex* mosquitoes. However, the mean numbers of *Aedes albolateralis*, *Aedes aegypti*, *Aedes gardneri imitator* and *Culex quinquefasciatus* were too low to be further analyzed for the influence of rainfall towards their population.

Moreover, based on the result, there was a negative correlation between mean rainfall and mean numbers of the four mosquito species which included *Aedes albopictus*, *Aedes aegypti*, *Aedes gardneri imitator* and *Culex quinquefasciatus*. *Aedes albopictus* and *Aedes aegypti* have a life cycle from egg to adult which takes about nine to ten days (Lee 2000) while *Culex quinquefasciatus* takes nine to twelve days (Yap et al., 2000). When rain is frequent, mosquitoes oviposit continuously and a cycle can be completed within one week under ideal temperature (Rozilawati et al., 2007). Thus, the high rainfall led to an abundance of mosquito eggs which will then hatch and mature in the following weeks, and this explained for the negative correlation. On the other hand, although insignificant, there was a positive correlation between mean rainfall and mean number of *Aedes albolateralis*. Unfortunately, *Aedes albolateralis* and *Aedes*

gardneri imitator were poorly studied and documented as they were rarely reported as the cause for outbreaks of mosquitoes-borne diseases.

5.2.2 Relationship between temperature, relative humidity and mosquito population

The changes in mean temperature and relative humidity in Kampar were not significant due to the tropical climate in Malaysia. The highest and lowest mean temperature differed by only 1.8°C while in the case of mean relative humidity, the difference was only 15.1%. However, studies have shown that temperature and relative humidity played important roles in influencing the mosquito population (Gillies 1953; Ho et al., 1971; Rueda et al., 1990; Aida et al., 2011).

The mean temperatures in Kampar were high throughout the three months and this helped to increase the success rate of egg hatching by increasing the evaporation rates especially during rainy season (Ho et al., 1971). Furthermore, based on Aida et al. (2011), the growing period from egg to adult for *Aedes albopictus* was hasten to about 6.3 days under the mean relative humidity between 60 to 96% and daily temperature between 23 to 35°C. Reiskind and Zarrabi (2012) recorded that larvae could not survive at 8.6°C, and at 14.3°C, the survival rate was still low. Only when temperature reached 19.5°C, high survival rate was recorded. Based on the study conducted by Monteiro et al. (2007), they concluded that the maximum temperature limit for the development of immature *Aedes*

albopictus was close to 35°C, as immature *Aedes albopictus* could not survive when maintained at that temperature. In correlation, the temperature and relative humidity recorded in Kampar during the sample collection period fitted in the range for active development of *Aedes albopictus*. In addition, under rapid development, immature mosquitoes would be less subjected to the risk of desiccation, predation and parasite infections (Ho et al., 1971). However, the development period varies depending on many other environmental factors such as nutritional factor and number of predators.

In addition, adult female mosquitoes feed more frequently and digest blood quicker in warm climates (Gillies 1953). Thus, the oviposition activity would be increased. Based on Reiskind and Zarrabi (2012), higher temperatures were associated with heavier dry body mass and shorter wings of mosquitoes. Shorter wings provided more maneuverability which can be useful in mating, avoiding aerial predation and dodging host defenses. Larger size of *Aedes* mosquitoes can be associated with higher fecundity, longer lifespan and higher blood-feeding frequency (Reiskind and Zarrabi 2012). However, no variation in temperature and humidity was carried out to examine for their changes in morphology during the mosquito culturing period in this study.

5.3 Insecticide susceptibility test on female adult *Aedes albopictus*

The WHO test kit was used to determine the insecticidal susceptibility status of the *Aedes albopictus* in the study. It is fast, user-friendly, convenient and does not require any other equipment, making rapid diagnosis of susceptibility possible (WHO 2009; Chan et al., 2011). Both pyrethroids, deltamethrin and permethrin were shown to be more effective against the *Aedes albopictus* population in Taman Kampar Jaya as compared to the organophosphates, malathion and fenitrothion. Majority *Aedes albopictus* populations in many countries such as Malaysia, Thailand, India, Greece, Italy and Cameroon remained susceptible towards deltamethrin, permethrin, malathion and propoxur. It is likely to be due to lower exposure of insecticides towards exophilic *Aedes albopictus* compared to *Aedes aegypti*, and this was reflected in the findings from Vontas et al. (2012).

Organophosphates such as malathion, temephos and fenitrothion, and carbamates which include bendiocarb and propoxur were heavily used in vector chemical control for decades. However, the introduction of pyrethroids in vector chemical control, household insecticides and agriculture began replacing organophosphates and carbamate since 1992 (Chareonviriyaphap et al., 1999). The early exposure of mosquitoes to carbamates and organophophates longer than pyrethroid might have accounted for higher development of resistance towards the former than the latter chemicals. This could be an explanation for the *Aedes albopictus* in Taman

Kampar Jaya having higher knockdown time (KT_{50} and KT_{95}) towards fenitrothion and malathion as compared to permethrin and deltamethrin.

At present, the fogging activities in Kampar utilize malathion, Sumithion® and Aqua Resigen®. The active ingredient of Sumithion® is fenitrothion (Hassan et al., 2005) while Aqua Resigen® is a synthetic pyrethroid composed of S-bioallethrin and permethrin (Adanan et al., 1997). In 2012, fogging activities were carried in Taman Kampar Jaya using Sumithion® in January and Aqua Resigen® in both March and July (Table 5.1). Pyrethroids such as deltamethrin and permethrin target the sodium channels on the nerve membranes, immobilizing the vector before killing it. On the other hand, organophosphates and carbamates share a different mode of action. Infected vectors will have complications in their nervous system when these chemical compounds bind to the acetylcholinesterase at the nerve junction (Coleman and Hemingway 2007).

Table 5.1: Fogging activity in Taman Kampar Jaya in 2012.

Month	**Insecticide used**
January	Sumithion®
March	Aqua Resigen®
July	Aqua Resigen®

Moreover, cross-resistance between insecticides can develop between organophosphate and carbamate given that they share similar target sites but not between organophosphate and pyrethroids. Organochlorines such as dichlorodiphenyltrichloroethane (DDT) however, share the same mode of action with pyrethroids, causing development of cross-resistance possible, especially in *Aedes aegypti* as DDT was implemented as insecticide since 1955 by the WHO (Brogdon and McAllister 1998; Hemingway and Ranson 2000). However, this was not prevalent among *Aedes albopictus* in Taman Kampar Jaya as observed in this study.

Although currently, *Aedes albopictus* in Taman Kampar Jaya is still susceptible to the insecticides used in the experiment, insecticide resistance is likely to occur especially towards the active ingredients malathion and fenitrothion due to the expansion of the mosquito populations where insecticides are used regularly. This would eventually bring negative influences on vector chemical control in future.

The WHO diagnostic test kit was used to investigate insecticidal susceptibility status of mosquitoes in Taman Kampar Jaya because it is convenient, low cost, applicable to low sample quantity and allow rapid diagnostic result. However, the reliability of its diagnosis could be a shortcoming in this study. From the observation, mosquitoes tend to settle on the netting area when held in the

exposure tubes which may be caused by the irritation of the compound from the insecticide impregnated papers, and this was also shown in study conducted by Chareonviriyaphap et al. (2006). Sometimes, mosquitoes got themselves stuck in between the wall of exposure tube and the impregnated paper too. As such, the mosquitoes' exposure to the active compound became inconsistent, affecting the outcome of the bioassay. In addition, according to WHO (2009), the dose used in the test kit bioassay can be as high as twice the concentration that could eliminate 99.9% of a normal population. Mosquitoes with low level of resistance can be accidentally misjudged as susceptible. Hence, this would render the bioassay to be less sensitive (Coleman and Hemingway 2007).

Many studies have been conducted in assessing the susceptibility status of pests. However, indication of resistance in susceptibility test if any, does not necessarily imply the failure of field control because the exact threshold of resistance level that brings failure to field control leading to disease outbreak has yet to be established (Chan et al., 2011).

5.4 Future research work

5.4.1 Topical application

Topical application requires a large quantity of sample, a condition that is often unable to be fulfilled from field collection. Astonishingly, based on the ovitrap surveillance, high density of mosquito population was observed in Taman Kampar Jaya, making topical application bioassay possible to be utilized for insecticide susceptibility test. Topical application is an assay that relates insecticide dosage and mortality. Adult female mosquitoes are to be exposed to at least five different concentrations of insecticide covering a range of mortality from 10 to 90%, applied on their prothorax. The mortality will be recorded after 24 hours, which is similar to the WHO test kit used in this study (WHO 2009).

The downsides of topical application are, it is less convenient, time consuming, tedious and require specific equipments such as a chill plate and carbon dioxide tank as compared to WHO test kit. However, topical application would be more sensitive as the percentage of mortality by different dosages can be compared unlike the high dosages used in the WHO diagnostic test kit which can give a high risk of getting false positive results. Besides, topical application ensures mosquitoes are exposed to a constant amount of the active compound, enhancing the accuracy of the outcome (Chan et al., 2011). Thus, topical application would be a better indicative bioassay to confirm the effectiveness of the insecticide used in vector control while allowing prediction on possible outbreak.

5.4.2 Routine surveillance on mosquito population and in other residential areas

Dengue incidence rate in Kampar is relatively high ranging from 60 to more than 100 cases a year. Therefore, routine surveillance and insecticidal susceptibility status of mosquito population should be conducted for early detection of outbreak. From Chadee's (2009) findings, mosquitoes usually do not travel far away from their breeding sites but prefer to rest, blood feed and oviposit within a definite space such as a house. Moreover, the flight range of *Aedes* mosquito is only about 200 m (Lee 2000).

These suggested that there is a low marginal flow of mosquitoes between areas that are apart from one another where the mosquito population and insecticidal susceptibility status may differ. For instance, based on Pethuan et al. (2007), *Aedes aegypti* mosquitoes in Nakhon Sawan province were resistant towards fenitrothion and pyrethroid except those in Taklee where the *Aedes aegypti* were sensitive towards fenitrothion. Hence, surveillance and insecticidal susceptibility status of mosquito population should be conducted in nearby residential areas too, especially in areas with high dengue cases reported.

5.4.3 Study of resistance at molecular basis with associated genes

Studies in understanding insecticide resistance mechanisms in *Aedes* mosquitoes have been progressing significantly in elucidating resistance to insecticides at molecular basis with associated genes. The relative particular gene mutation that

contributes to the resistance phenotype is still poorly defined especially in cases involving multiple resistance mechanisms. Besides *Aedes aegypti*, studies on *Aedes albopictus* resistance mechanism have been growing due to its expansion and the increasing public health importance of this species (Vontas et al., 2012). By understanding the molecular mechanisms in insecticide resistance, vector control can be brought to a higher level by targeting the molecular mode of action or gene associated with the resistance and further analysis on cross-resistance between active compounds.

Grand Challenges in Global Health (GCGH) has aimed to perform genetic-based strategies on *Aedes aegypti* to reduce dengue viral transmission by eliminating their ability to transmit or resist dengue virus infection or by reducing density of mosquito population (McCall and Kittayapong 2007). Another research from GCGH aimed to reduce the lifespan of the population where the mosquitoes will not be able to reach the age capable of transmitting dengue virus. Genetic approach would be a great asset in reducing vector-borne disease outbreaks or perhaps creating a world free of the diseases in the near future.

CHAPTER 6

CONCLUSION

Despite the rapid development in medical science and technology in the 21st century, mosquito-borne diseases such as dengue, malaria, Japanese encephalitis and filariasis are still justifiably categorized as most terrified diseases. The number of dengue case is still a great concern in Malaysia, given an immense total of 17,262 *Aedes albopictus*, the secondary vector of dengue virus, were collected and bred throughout the 13 weeks of sample collection in a small residential area, Taman Kampar Jaya from October 2012 to December 2012. Other species that were found included *Aedes aegypti*, *Aedes albolateralis*, *Culex quinquefasciatus* and *Aedes gardneri imitator*.

The *Aedes albopictus* were examined their insecticidal susceptibility status using WHO test kit and procedure, and showed no resistance towards 0.05% deltamethrin, 1% fenitrothion, 5% malathion and 0.75% permethrin. Permethrin and deltamethrin proved to be highly effective towards the *Aedes albopictus* population in Taman Kampar Jaya with estimated KT_{50} value of 15.84 minutes and 20.57 minutes respectively. This was also supported by estimated KT_{95} value of only 29.54 minutes using permethrin while deltamethrin scored an estimated KT_{95} value of 48.18 minutes. With malathion as the third highest for both

knockdown time values, fenitrothion made up last in efficacy with significantly distinctive estimated KT_{50} value of 150.29 minutes and estimated KT_{95} value of 293.41 minutes.

Vector surveillance and monitoring should be conducted regularly in Taman Kampar Jaya due to its high mosquito population given that *Aedes albopictus* is the dominant species. This would help to alert the local government, Majlis Daerah Kampar (Kampar District Council) to be more aware of the potential risk of dengue viral outbreaks in the residential area and to implement any counter measures before it becomes endemic. Besides, such effort should be carried out in nearby residential areas in Kampar as construction and land development programs which give rise to mosquito breeding sites, are booming due to the escalating residential demand and business opportunities in Kampar, ever since Universiti Tunku Abdul Rahman (UTAR) was established. Temperature, relative humidity and rainfall were found capable in influencing the mosquito population. Thus, study of their relationship would help in the prediction of mosquito-borne disease outbreaks by analyzing the meteorological data.

Malathion and Sumithion® (containing fenitrothion as active compound) are currently being used by Majlis Daerah Kampar (Kampar District Council) in fogging activity and no resistance was manifested by *Aedes albopictus* based on

the insecticide susceptibility test conducted. However, their efficacy was not very convincing as the knockdown time obtained from the study was quite high even though the mosquitoes were constantly exposed to the chemical in the confined exposure tube of the WHO test kit. Toxicity effect and exposure toward field mosquitoes would be greatly reduced due to the open surrounding. Permethrin and deltamethrin should be considered to be implemented in fogging activities by the local government. Based on the experiment, permethrin and deltamethrin demonstrated high efficacy as insecticides. The study by Nazni et al. (2005) has shown that resistance towards insecticide can be reversed if mosquitoes are kept free from the particular insecticide for a long period. Therefore, constant monitoring on insecticidal susceptibility status of mosquito population allows recycling of effective active ingredient used in vector chemical control.

Moreover, as the control of mosquito populations depends heavily on chemical applications, over usage of chemical control will induce insecticide resistance in the near future and subsequently cause control failures. Thus, routine surveillance on insecticidal resistant status of mosquito populations is crucial in implementing suitable strategies in order to prevent outbreaks. Meanwhile, other approaches such as biological control, environmental management and genetic control should be further utilized and advanced, with the inevitable increase in chemical insecticide resistance in mosquito populations.

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APPENDICES

APPENDIX A

Total and Mean Number of *Aedes albopictus* and Ovitrap Index with a Total of 60 Ovitraps from Week 1 to Week 7

Week / Ovitrap	1		2		3		4		5		6		7	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	10	9	9	40	26	30	3	0	8	10	5	1	5	6
2	0	1	45	25	4	5	13	5	5	8	3	6	21	21
3	16	5	29	51	23	20	22	12	25	32	10	11	20	24
4	16	26	0	10	27	26	5	1	7	15	6	8	0	0
5	5	16	1	7	7	9	16	13	4	4	3	3	43	37
6	21	16	7	13	53	36	12	11	13	15	13	9	12	7
7	15	11	14	9	7	8	1	2	5	2	4	2	9	7
8	0	2	1	1	3	5	1	2	10	13	23	17	5	8
9	28	9	30	24	5	5	62	59	33	31	1	1	26	20
10	1	0	0	0	8	11	2	1	8	10	13	6	30	27
11	0	0	4	1	4	6	25	13	4	4	9	9	0	0
12	0	2	9	10	4	7	1	1	11	6	21	23	12	23
13	7	0	1	1	8	6	2	0	6	5	12	16	0	0
14	17	11	22	26	1	0	15	20	38	29	10	7	27	29
15	0	2	10	15	21	25	14	12	7	5	3	2	5	7
16	19	17	19	21	1	2	6	3	6	10	23	15	26	23
17	0	2	0	0	8	12	3	12	1	4	0	0	0	0
18	0	1	1	0	3	5	1	1	2	2	6	5	11	10
19	1	2	10	2	45	38	17	28	0	0	1	1	0	0
20	9	6	40	35	31	34	6	10	2	1	13	8	16	15
21	6	12	5	3	0	6	17	18	19	17	0	1	0	3
22	12	33	9	15	17	18	9	12	14	20	0	0	33	29
23	12	25	22	23	7	6	18	11	8	15	6	8	0	7
24	0	0	3	5	0	2	9	11	18	13	2	3	5	3
25	11	17	31	45	11	12	33	28	22	19	16	14	9	12
26	0	1	5	2	13	12	0	0	4	4	7	8	6	7
27	0	0	3	4	0	0	2	1	1	2	0	1	1	0
28	4	2	10	10	17	18	34	26	14	23	34	27	14	12
29	19	8	23	16	7	18	23	3	0	1	17	23	30	25
30	4	6	16	10	6	1	18	18	0	0	3	8	0	2
31	6	4	32	39	19	34	2	1	12	12	8	7	8	9

32	25	14	13	14	14	11	11	6	10	10	20	22	28	41
33	29	22	79	87	24	30	1	6	32	35	25	19	26	21
34	0	0	11	11	1	2	25	29	5	3	3	3	6	7
35	10	23	20	18	5	1	1	1	5	10	24	14	28	29
36	10	15	39	45	37	17	12	11	17	23	7	9	29	39
37	19	30	32	22	4	0	1	5	4	6	18	18	12	10
38	8	19	23	19	10	12	31	32	37	30	9	14	15	8
39	6	9	24	18	3	19	2	2	14	4	7	5	14	13
40	3	3	17	9	8	14	22	20	9	12	10	7	12	14
41	0	0	15	14	0	0	32	28	27	23	12	13	56	41
42	27	22	51	55	5	2	11	15	7	12	24	25	3	4
43	10	1	9	11	8	11	18	16	8	6	7	18	5	5
44	0	0	8	9	9	9	11	9	2	1	5	10	18	24
45	8	18	50	41	48	68	2	0	63	60	6	7	42	39
46	0	0	6	9	0	1	0	1	0	0	15	9	1	0
47	0	2	1	4	1	4	0	2	1	4	6	11	4	8
48	4	13	34	47	46	48	5	7	25	15	7	10	13	13
49	27	32	10	6	15	20	10	25	7	7	3	5	25	20
50	1	2	25	32	11	5	0	2	13	17	9	5	14	8
51	0	2	20	17	28	26	31	24	26	23	3	6	16	11
52	4	1	21	15	6	12	10	12	10	7	13	9	16	10
53	0	0	0	0	3	1	21	34	3	1	9	10	13	11
54	3	4	21	15	7	3	17	15	29	26	11	17	14	13
55	2	16	32	19	66	58	17	15	28	28	25	30	30	22
56	12	6	5	5	4	2	37	28	27	21	30	17	12	13
57	2	0	7	5	4	6	10	23	25	13	3	4	14	8
58	1	4	17	8	3	1	9	7	1	1	8	6	4	8
59	0	0	9	3	2	0	7	2	13	6	0	5	9	9
60	8	4	0	0	1	1	12	14	15	2	4	5	6	6
Total	966		2031		1560		1484		1508		1178		1687	
OI, %	83.3		93.3		96.7		98.3		95.0		96.7		91.7	
Mean	16.10		33.85		26.00		24.73		25.13		19.63		28.12	

*** M = Male**

*** F = Female**

APPENDIX B

Total and Mean Number of *Aedes albopictus* and Ovitrap Index with a Total of 60 Ovitraps from Week 8 to Week 13

Week / Ovitrap	8		9		10		11		12		13	
	M	F	M	F	M	F	M	F	M	F	M	F
1	12	13	4	2	6	0	1	4	7	6	4	4
2	11	8	1	3	21	12	13	12	9	13	5	9
3	19	36	17	14	12	13	10	8	27	27	13	10
4	0	3	8	7	4	14	7	6	16	18	13	12
5	11	9	29	25	19	25	8	5	13	16	4	2
6	7	7	7	11	3	0	8	8	15	8	8	6
7	13	9	2	1	6	3	1	3	18	18	16	9
8	1	4	1	0	1	0	12	5	0	2	5	7
9	14	20	5	6	11	11	12	6	14	17	22	19
10	8	6	3	3	1	0	5	6	5	7	1	1
11	3	7	2	1	9	10	12	12	15	31	11	13
12	2	3	1	3	6	3	4	4	10	7	5	1
13	3	8	5	8	0	0	0	0	8	11	8	8
14	22	18	1	2	19	25	5	7	8	5	10	12
15	6	8	2	1	13	9	17	18	5	2	3	2
16	0	0	5	5	16	27	21	17	6	12	15	16
17	19	18	1	1	0	0	1	1	2	2	3	3
18	1	2	0	0	12	7	1	1	16	22	11	7
19	2	0	0	1	5	11	9	10	11	11	17	14
20	18	16	0	1	5	3	1	0	22	21	10	16
21	14	9	8	7	1	4	2	0	2	0	5	5
22	2	3	21	23	12	13	7	13	0	0	3	0
23	19	10	2	1	5	9	0	0	26	25	9	10
24	21	23	4	4	10	13	3	1	5	7	2	7
25	36	42	6	5	13	16	18	15	21	17	19	17
26	5	1	1	2	1	3	1	0	17	16	0	1
27	5	4	0	0	6	4	1	0	0	0	1	1
28	4	8	3	3	34	33	14	7	10	5	6	4
29	3	2	10	2	19	27	4	9	25	33	27	22
30	9	10	0	1	3	3	0	0	0	0	0	1
31	6	7	2	2	5	3	0	1	0	1	4	5
32	15	15	0	2	14	13	5	2	12	16	20	22
33	7	11	5	11	15	17	17	11	36	38	13	13

34	1	6	1	1	6	1	0	0	3	4	8	2
35	26	25	3	3	18	14	12	7	14	18	9	10
36	0	1	25	23	13	14	10	9	16	18	13	9
37	25	18	0	1	15	7	5	4	16	25	18	17
38	14	13	10	5	39	35	35	33	30	38	32	29
39	6	6	0	0	5	9	3	2	2	0	2	2
40	25	21	1	6	15	13	16	23	12	17	8	15
41	7	2	0	0	11	23	4	4	26	58	25	23
42	19	21	15	12	7	3	7	9	9	18	16	14
43	20	14	3	3	5	4	5	5	16	21	27	20
44	11	17	2	2	4	2	0	1	43	36	12	17
45	35	32	13	13	20	16	6	7	17	31	26	33
46	0	2	0	0	1	0	0	0	6	4	0	0
47	2	5	1	4	12	8	0	3	12	19	4	2
48	8	3	2	1	16	4	7	4	8	6	1	0
49	17	13	0	1	5	12	2	0	4	8	2	1
50	12	9	15	15	11	16	9	14	9	18	14	13
51	17	12	7	4	6	6	5	1	2	2	5	10
52	4	3	14	15	15	26	12	16	12	12	15	9
53	1	5	8	11	1	7	4	3	5	3	1	4
54	37	47	13	16	9	9	32	32	0	2	2	0
55	20	19	8	6	11	10	22	13	22	24	30	19
56	9	7	9	15	15	17	3	3	8	7	5	5
57	35	29	1	6	12	15	3	5	3	7	12	10
58	12	16	4	3	2	2	7	6	10	12	3	6
59	5	10	22	17	10	12	16	15	10	16	7	2
60	8	5	0	3	9	15	4	5	1	2	1	1
Total	1395		677		1231		865		1537		1143	
OI, %	98.3		91.7		98.3		91.7		95.0		98.3	
Mean	23.25		11.28		20.52		14.42		25.62		19.05	

*** M = Male**
*** F = Female**

APPENDIX C

Data output generated from SPSS software for correlations between mosquitoes species and mean rainfall

Aedes albopictus

Descriptive Statistics

	Mean	Std. Deviation	N
Albopictus	22.1308	6.11873	13
Rainfall	12.2538	9.69619	13

Correlations

		Albopictus	Rainfall
Albopictus	Pearson Correlation	1	-.188
	Sig. (2-tailed)		.538
	N	13	13
Rainfall	Pearson Correlation	-.188	1
	Sig. (2-tailed)	.538	
	N	13	13

Aedes albolateralis

Descriptive Statistics

	Mean	Std. Deviation	N
Albolateralis	.3513	.32327	13
Rainfall	12.2538	9.69619	13

Correlations

		Albolateralis	Rainfall
Albolateralis	Pearson Correlation	1	.281
	Sig. (2-tailed)		.353
	N	13	13
Rainfall	Pearson Correlation	.281	1
	Sig. (2-tailed)	.353	
	N	13	13

Aedes aegypti

Descriptive Statistics

	Mean	Std. Deviation	N
Aegypti	.0615	.15006	13
Rainfall	12.2538	9.69619	13

Correlations

		Aegypti	Rainfall
Aegypti	Pearson Correlation	1	-.138
	Sig. (2-tailed)		.653
	N	13	13
Rainfall	Pearson Correlation	-.138	1
	Sig. (2-tailed)	.653	
	N	13	13

Aedes gardneri imitator

Descriptive Statistics

	Mean	Std. Deviation	N
Gardneri	.0192	.03943	13
Rainfall	12.2538	9.69619	13

Correlations

		Gardneri	Rainfall
Gardneri	Pearson Correlation	1	-.044
	Sig. (2-tailed)		.887
	N	13	13
Rainfall	Pearson Correlation	-.044	1
	Sig. (2-tailed)	.887	
	N	13	13

Culex quinquefasciatus

Descriptive Statistics

	Mean	Std. Deviation	N
Quinquefasciatus	.0141	.03171	13
Rainfall	12.2538	9.69619	13

Correlations

		Quinquefasciatus	Rainfall
Quinquefasciatus	Pearson Correlation	1	-.013
	Sig. (2-tailed)		.965
	N	13	13
Rainfall	Pearson Correlation	-.013	1
	Sig. (2-tailed)	.965	
	N	13	13

APPENDIX D

Data output generated from SPSS software using Probit analysis on *Aedes albopictus* as sample tested with deltamethrin

Data Information

		N of Cases
Valid		219
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		1

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Minute	3.405	.076	45.020	.000	3.257	3.553
	Intercept	-4.085	.111	-36.818	.000	-4.196	-3.975

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	393.140	217	.000[a]

a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

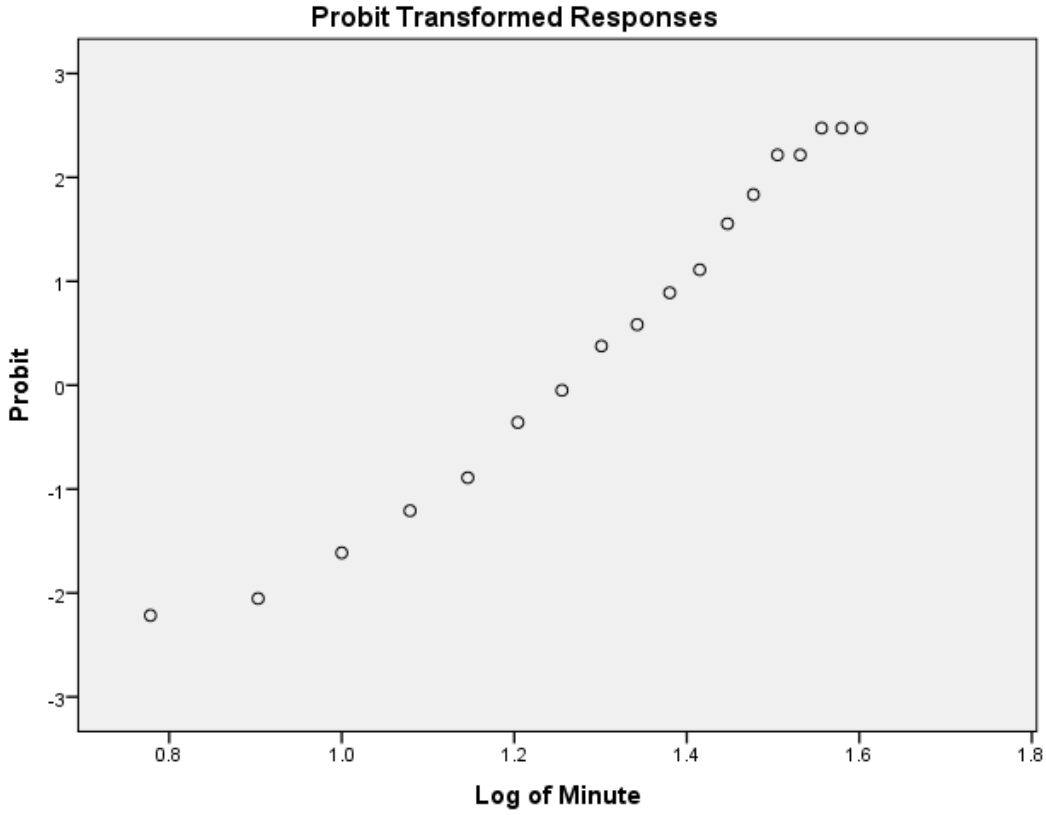
Confidence Limits

	Probability	95% Confidence Limits for Minute			95% Confidence Limits for log(Minute)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	3.286	2.858	3.721	.517	.456	.571
	.020	3.951	3.475	4.432	.597	.541	.647
	.030	4.441	3.933	4.952	.647	.595	.695
	.040	4.849	4.316	5.383	.686	.635	.731
	.050	5.209	4.656	5.761	.717	.668	.761
	.060	5.536	4.965	6.104	.743	.696	.786
	.070	5.840	5.254	6.422	.766	.720	.808
	.080	6.126	5.526	6.721	.787	.742	.827
	.090	6.398	5.786	7.004	.806	.762	.845
	.100	6.660	6.036	7.276	.823	.781	.862
	.150	7.860	7.190	8.519	.895	.857	.930
	.200	8.967	8.260	9.659	.953	.917	.985
	.250	10.040	9.303	10.760	1.002	.969	1.032
	.300	11.112	10.349	11.857	1.046	1.015	1.074
	.350	12.208	11.422	12.976	1.087	1.058	1.113
	.400	13.347	12.539	14.138	1.125	1.098	1.150

	.450	14.551	13.721	15.365	1.163	1.137	1.187
	.500	15.842	14.989	16.680	1.200	1.176	1.222
	.550	17.247	16.369	18.114	1.237	1.214	1.258
	.600	18.802	17.895	19.704	1.274	1.253	1.295
	.650	20.557	19.613	21.505	1.313	1.293	1.333
	.700	22.584	21.590	23.594	1.354	1.334	1.373
	.750	24.997	23.930	26.096	1.398	1.379	1.417
	.800	27.987	26.810	29.223	1.447	1.428	1.466
	.850	31.928	30.567	33.388	1.504	1.485	1.524
	.900	37.684	35.985	39.555	1.576	1.556	1.597
	.910	39.223	37.422	41.218	1.594	1.573	1.615
	.920	40.967	39.043	43.110	1.612	1.592	1.635
	.930	42.974	40.903	45.295	1.633	1.612	1.656
	.940	45.331	43.079	47.873	1.656	1.634	1.680
	.950	48.178	45.696	51.000	1.683	1.660	1.708
	.960	51.753	48.964	54.947	1.714	1.690	1.740
	.970	56.512	53.293	60.234	1.752	1.727	1.780
	.980	63.523	59.624	68.081	1.803	1.775	1.833
	.990	76.381	71.123	82.624	1.883	1.852	1.917

a. A heterogeneity factor is used.

b. Logarithm base = 10.



APPENDIX E

Data output generated from SPSS software using Probit analysis on *Aedes albopictus* as sample tested with fenitrothion

Data Information

		N of Cases
Valid		219
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		1

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Minute	5.661	.057	99.090	.000	5.549	5.773
	Intercept	-12.324	.129	-95.412	.000	-12.453	-12.194

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	348.436	217	.000[a]

a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

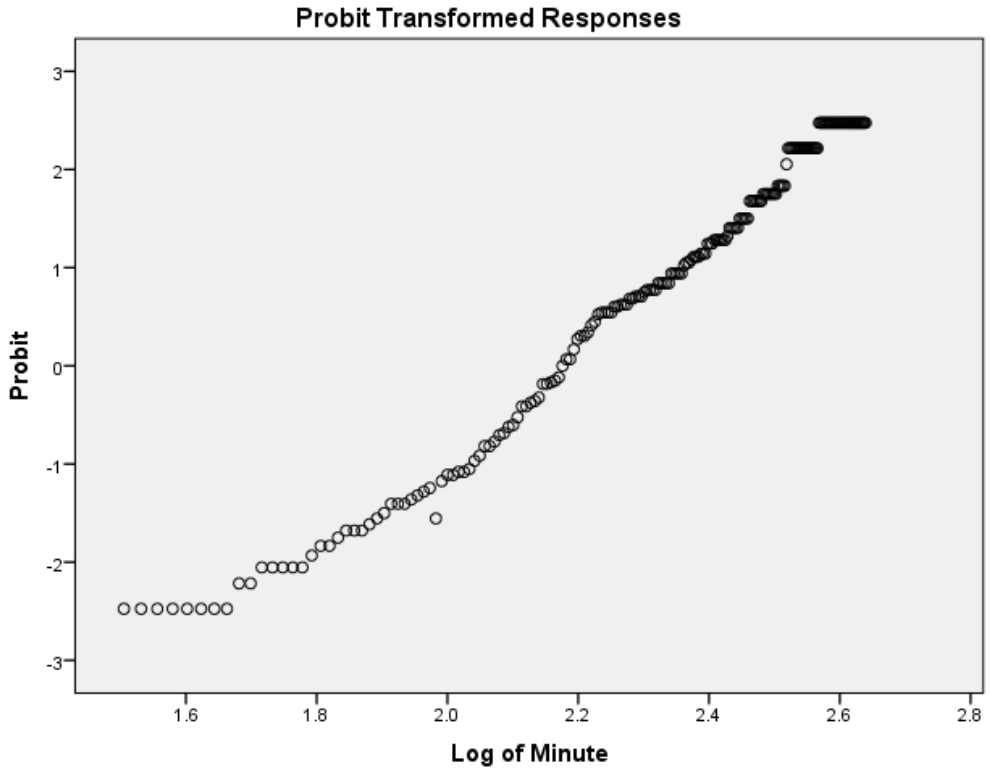
Confidence Limits

	Probability	95% Confidence Limits for Minute			95% Confidence Limits for log(Minute)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	58.341	56.561	60.092	1.766	1.753	1.779
	.020	65.182	63.362	66.968	1.814	1.802	1.826
	.030	69.933	68.094	71.736	1.845	1.833	1.856
	.040	73.733	71.884	75.545	1.868	1.857	1.878
	.050	76.977	75.121	78.794	1.886	1.876	1.896
	.060	79.849	77.990	81.669	1.902	1.892	1.912
	.070	82.456	80.596	84.277	1.916	1.906	1.926
	.080	84.863	83.002	86.683	1.929	1.919	1.938
	.090	87.112	85.252	88.931	1.940	1.931	1.949
	.100	89.235	87.376	91.053	1.951	1.941	1.959

	.150	98.590	96.746	100.394	1.994	1.986	2.002
	.200	106.721	104.895	108.506	2.028	2.021	2.035
	.250	114.228	112.423	115.995	2.058	2.051	2.064
	.300	121.418	119.634	123.168	2.084	2.078	2.090
	.350	128.485	126.718	130.222	2.109	2.103	2.115
	.400	135.570	133.815	137.299	2.132	2.127	2.138
	.450	142.797	141.048	144.527	2.155	2.149	2.160
	.500	150.286	148.531	152.027	2.177	2.172	2.182
	.550	158.167	156.391	159.937	2.199	2.194	2.204
	.600	166.598	164.782	168.418	2.222	2.217	2.226
	.650	175.785	173.900	177.687	2.245	2.240	2.250
	.700	186.016	184.022	188.041	2.270	2.265	2.274
	.750	197.726	195.567	199.934	2.296	2.291	2.301
	.800	211.635	209.226	214.116	2.326	2.321	2.331
	.850	229.087	226.295	231.985	2.360	2.355	2.365
	.900	253.104	249.679	256.689	2.403	2.397	2.409
	.910	259.273	255.668	263.052	2.414	2.408	2.420
	.920	266.145	262.333	270.148	2.425	2.419	2.432
	.930	273.912	269.858	278.176	2.438	2.431	2.444
	.940	282.854	278.512	287.431	2.452	2.445	2.459
	.950	293.410	288.714	298.370	2.467	2.460	2.475
	.960	306.316	301.170	311.763	2.486	2.479	2.494
	.970	322.963	317.210	329.067	2.509	2.501	2.517
	.980	346.501	339.846	353.583	2.540	2.531	2.548
	.990	387.132	378.813	396.021	2.588	2.578	2.598

a. A heterogeneity factor is used.

b. Logarithm base = 10.



APPENDIX F

Data output generated from SPSS software using Probit analysis on *Aedes albopictus* as sample tested with malathion

Data Information

		N of Cases
Valid		219
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		1

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Minute	6.382	.119	53.763	.000	6.150	6.615
	Intercept	-10.757	.209	-51.461	.000	-10.966	-10.548

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	107.774	217	1.000[a]

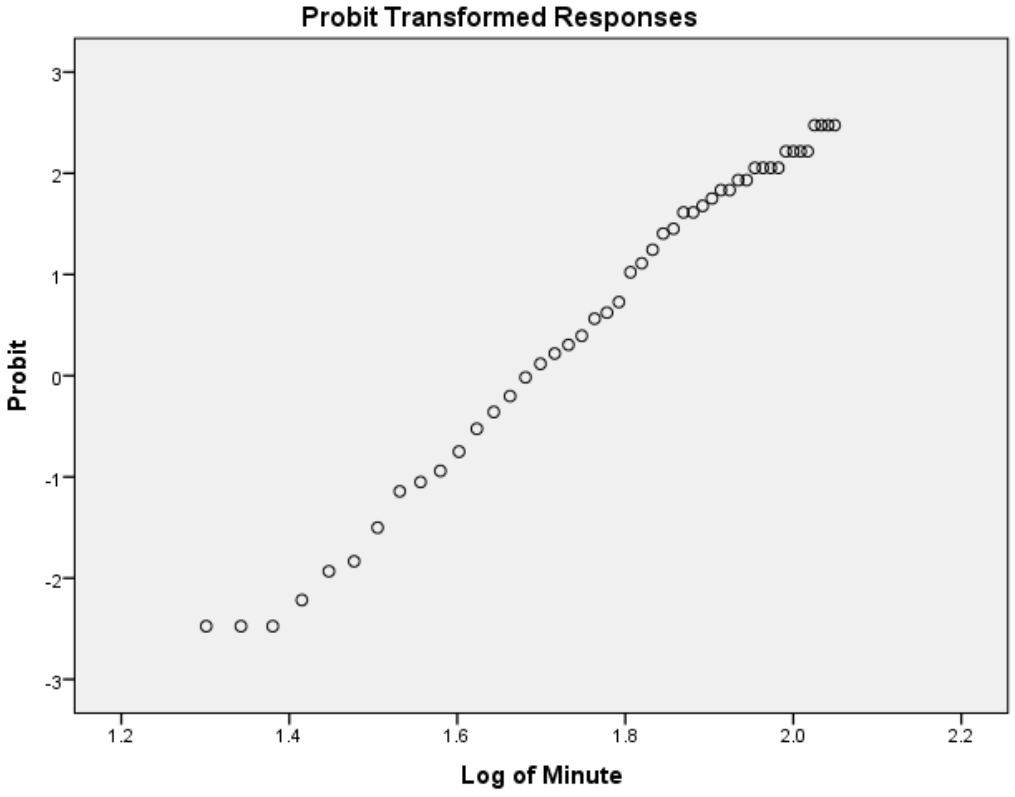
a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

Confidence Limits

	Probability	95% Confidence Limits for Minute			95% Confidence Limits for log(Minute)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	20.935	20.108	21.739	1.321	1.303	1.337
	.020	23.099	22.263	23.909	1.364	1.348	1.379
	.030	24.586	23.747	25.398	1.391	1.376	1.405
	.040	25.767	24.928	26.579	1.411	1.397	1.425
	.050	26.770	25.932	27.581	1.428	1.414	1.441
	.060	27.655	26.818	28.463	1.442	1.428	1.454
	.070	28.454	27.619	29.260	1.454	1.441	1.466
	.080	29.189	28.357	29.993	1.465	1.453	1.477
	.090	29.874	29.044	30.676	1.475	1.463	1.487
	.100	30.519	29.692	31.319	1.485	1.473	1.496
	.150	33.341	32.528	34.127	1.523	1.512	1.533
	.200	35.769	34.970	36.542	1.554	1.544	1.563

	.250	37.992	37.206	38.753	1.580	1.571	1.588
	.300	40.106	39.333	40.857	1.603	1.595	1.611
	.350	42.170	41.407	42.912	1.625	1.617	1.633
	.400	44.226	43.472	44.964	1.646	1.638	1.653
	.450	46.311	45.562	47.047	1.666	1.659	1.673
	.500	48.459	47.711	49.198	1.685	1.679	1.692
	.550	50.706	49.953	51.456	1.705	1.699	1.711
	.600	53.097	52.330	53.866	1.725	1.719	1.731
	.650	55.686	54.895	56.486	1.746	1.740	1.752
	.700	58.551	57.721	59.399	1.768	1.761	1.774
	.750	61.809	60.919	62.728	1.791	1.785	1.797
	.800	65.651	64.669	66.674	1.817	1.811	1.824
	.850	70.431	69.308	71.614	1.848	1.841	1.855
	.900	76.943	75.589	78.385	1.886	1.878	1.894
	.910	78.604	77.186	80.119	1.895	1.888	1.904
	.920	80.450	78.956	82.048	1.906	1.897	1.914
	.930	82.529	80.948	84.225	1.917	1.908	1.925
	.940	84.914	83.229	86.727	1.929	1.920	1.938
	.950	87.719	85.907	89.673	1.943	1.934	1.953
	.960	91.133	89.161	93.267	1.960	1.950	1.970
	.970	95.513	93.326	97.887	1.980	1.970	1.991
	.980	101.662	99.158	104.393	2.007	1.996	2.019
	.990	112.169	109.086	115.549	2.050	2.038	2.063

a. Logarithm base = 10.



APPENDIX G

Data output generated from SPSS software using Probit analysis on *Aedes albopictus* as sample tested with permethrin

Data Information

		N of Cases
Valid		220
Rejected	Missing	0
	Number of Responses > Number of Subjects	0
Control Group		1

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Minute	.183	.006	30.399	.000	.171	.195
	Intercept	-3.768	.130	-28.941	.000	-3.898	-3.638

a. PROBIT model: PROBIT(p) = Intercept + BX

Chi-Square Tests

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	45.108	218	1.000[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

Confidence Limits

	Probability	95% Confidence Limits for Minute		
		Estimate	Lower Bound	Upper Bound
PROBIT	.010	7.868	6.894	8.736
	.020	9.356	8.474	10.144
	.030	10.300	9.475	11.038
	.040	11.010	10.227	11.712
	.050	11.588	10.839	12.261
	.060	12.079	11.359	12.728
	.070	12.511	11.814	13.138
	.080	12.897	12.222	13.506
	.090	13.248	12.592	13.841
	.100	13.571	12.932	14.149
	.150	14.909	14.339	15.430
	.200	15.972	15.452	16.453
	.250	16.884	16.401	17.335
	.300	17.703	17.250	18.131
	.350	18.462	18.032	18.874
	.400	19.183	18.769	19.584
	.450	19.879	19.477	20.275
	.500	20.565	20.170	20.960
	.550	21.251	20.857	21.650
	.600	21.948	21.550	22.356

.650	22.668	22.262	23.091
.700	23.427	23.007	23.871
.750	24.247	23.805	24.718
.800	25.159	24.689	25.666
.850	26.222	25.713	26.777
.900	27.560	26.995	28.182
.910	27.883	27.304	28.522
.920	28.234	27.639	28.892
.930	28.620	28.007	29.300
.940	29.051	28.417	29.755
.950	29.543	28.885	30.274
.960	30.120	29.434	30.885
.970	30.831	30.108	31.638
.980	31.775	31.003	32.638
.990	33.262	32.412	34.218