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

By

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ABSTRACT

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

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Globally, mosquito-borne viral diseases such as dengue fever (DF) and dengue hemorrhagic fever (DHF) possess high annual incidence rate of infections. Chemical applications are the main control agents in many countries, but constant exposure to the same insecticide will cause resistance. Hence, this study was carried out to identify the seasonal distribution and to investigate the insecticide resistance status of the mosquito population in Taman Juloong, Kampar. Ovitrap surveillance was conducted for duration of 13 weeks using 60 ovitraps placed on 60 trees, 10 to 15 m apart and 1 m above ground. The total number of mosquitoes collected was 19,107 which comprised mostly of *Aedes albopictus* (99.57%) while *Aedes aegypti* (0.07%) and *Aedes albolateralis* (0.36%) were the minority species. Interspecific competition, temperature, humidity and rainfall were among the factors that affect the mosquito population in Taman Juloong. The distribution of mosquitoes was not significantly correlated to the rainfall. High rainfall floods the eggs resulting in stimulation and hatching of eggs. Therefore, the mosquito population was seen to increase one week after peaks of rainfall. *Aedes albopictus* was found to be a stronger competitor and survived better in wet and cool climate. Subsequently,

the WHO diagnostic test conducted on *Aedes albopictus* found them to be most susceptible to deltamethrin (KT_{50} = 16.18 minutes, KT_{95} = 49.44 minutes) and permethrin (KT_{50} = 17.52 minutes, KT_{95} = 54.54 minutes) as both belonged to the newer class of insecticides under the pyrethroid group. Fenitrothion (KT_{50} = 203.32 minutes, KT_{95} = 408.07 minutes) was the least effective insecticide towards *Aedes albopictus* and showed evidence of resistance. Insecticide resistance test could not be conducted on *Aedes aegypti* and *Aedes albolateralis* as they were too low in number. In this study, pyrethroids are still the most effective insecticide as compared to organophosphates against the mosquito population in Taman Juloong. Therefore, further evaluation on the effectiveness of organophosphates as vector control agents on the mosquitoes in Taman Juloong should be done.

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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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YAM LI-ANNE

APPROVAL SHEET

This project report entitled "**SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN JULOONG, KAMPAR**" was prepared by YAM LI-ANNE 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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PERMISSION SHEET

It is hereby certified that **YAM LI-ANNE** (ID No: **09ADB06983**) has completed this final year project entitled "**SURVEILLANCE AND INSECTICIDAL SUSCEPTIBILITY STATUS OF THE MOSQUITO POPULATION IN TAMAN JULOONG, KAMPAR**" supervised by Ms. Ho Lai Yee from the Department of Biomedical Science, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscripts of these research findings for publishing in any form, if I do not prepare it within six (6) months from this date, provided that my name is included as one of the authors for this article. The arrangement of the name depends on my supervisor.

Yours truly,

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(YAM LI-ANNE)

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CHIKV	Chikungunya virus
DDT	Dichloro-diphenyl-trichloroethane
DF	Dengue fever
DHF	Dengue hemorrhagic fever
EEE	Eastern Equine encephalitis
ITN	Insecticide impregnated bednets
IRS	Indoor residual spraying
KT_{50}	Knockdown time of 50% of test samples
KT_{95}	Knockdown time of 95% of test samples
LF	Lymphatic filariasis
MDK	Majlis Daerah Kampar
MOH	Ministry of Health
OI	Ovitrap index
ULV	Ultra low volume
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Mosquitoes belong to the family Culicidae of the order Diptera. Being nuisance biters and important vectors of diseases, they are still a persistent problem in Malaysia. *Aedes albopictus* and *Aedes aegypti* are the most important vectors of dengue in Southeast Asia (Estrada-Franco and Craig 1995), where the former is implied as a secondary vector of dengue while the latter as the principal vector (Harun 2007). Besides dengue, *Aedes* is also vector for Chikungunya virus (CHIKV), yellow fever, Eastern Equine encephalitis (EEE) and filariasis. On the other hand, *Culex* species are the dominant vector for lymphatic filariasis (LF) as well as Japanese encephalitis (Thomas et al., 2004). *Anopheles* transmit both malaria and filariasis (Rattanarithikul and Panthusiri 1994) while *Mansonia* are known vectors for filariasis (Chang 2002).

An estimated 2 billion people are at risk of dengue, with over 100 million infections a year and about 100,000 deaths occur globally (Harun 2007). In Malaysia, the first major national DF and DHF outbreak by *Aedes* species occurred in 1973 (Lee 1994), where both diseases continued to be endemic from then onwards. Although there was no death reported due to DF in Malaysia in 2011, the incidence rates of dengue was the highest among other vector-borne diseases, which were 63.75 per 100,000 (Table 1.1).Since CHIKV reemerged in 2004, millions of cases have been increasingly reported from tropical countries, especially in Africa, Southeast Asia and around the Indian Ocean (Moro et al., 2012). However, in Malaysia CHIKV showed a marked decrease in incidence rate from 2011 onwards (Hasan 2012).

Table 1.1: Incidence rate and mortality rate of vector borne diseases for year 2011(MOH 2012).

Vector borne diseases	Incidence rate (per 100,000 population)	Mortality rate (per 100,000 population)
Dengue	63.75	0
Dengue hemorrhagic fever	4.90	0.12
Malaria	18.32	0.06
Yellow fever	0	0

In order to control the spread of vector-borne diseases, several control measures have been applied. Chemical control remains the most widely used method by the government. Malathion and permethrin are the most frequently used adulticides in the vector-control programs in Malaysia (Chan et al., 2011). However, frequent usage of the same type of insecticides in fogging activities has caused rising of resistance among mosquito population (Loke et al., 2012). Therefore, it is necessary for constant monitoring to ensure these insecticides are still effective against the mosquitoes as fogging with insecticides is the major controlling method of vector-borne disease used in Malaysia.

Based on the report by Majlis Daerah Kampar (2013a), dengue cases in the Kampar district showed a decrease in incidence rate in the period of ten years from 2003 to 2012 by more than 50% (Figure 1.1). This may be due to the constant fogging activity carried out by local authorities (Appendix A). According to a report by the head officer of Vector Control Department at Klinik Kesihatan Kampar, Azrul (2012), Taman Juloong had dengue cases reported only in year 2008 and July 2012. Due to the existence of dengue cases, Taman Juloong was chosen to study the mosquito population in the area.

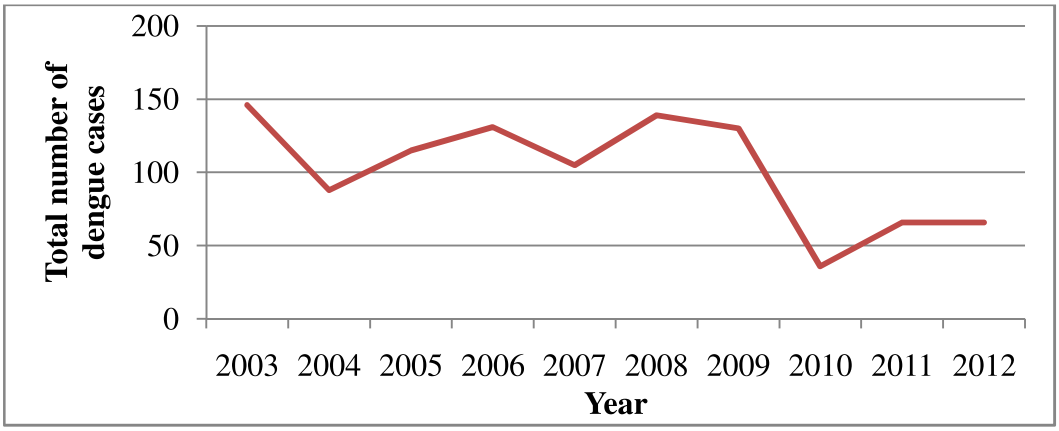


Figure 1.1: Number of dengue cases in Kampar District from 2003 to 2012 (MDK 2013a).

The first objective of this research was to identify the mosquito population found in the residential area of Taman Juloong, Kampar. Furthermore, the study was done to determine the seasonal distribution of the mosquito population within the months of October to December 2012. Lastly, it was to investigate the insecticide resistance status of the collected mosquito samples in this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Mosquitoes

In Malaysia, there are around 431 species representing 20 genera of mosquitoes (Harun 2007). *Aedes*, *Culex*, *Anopheles* and *Mansonia* mosquitoes are the commonly found mosquito genus in Malaysia of medical importance. *Aedes* and *Culex* are commonly found in urban and suburban areas whereas *Anopheles* and *Mansonia* are located mostly in rural areas. According to a study conducted in urban and suburban housing areas, it was found that *Aedes albopictus*, *Aedes aegypti* and *Culex quinquefasciatus* were found to be the most abundant species (Yap 1975).

Table 2.1: Taxonomy of mosquitoes.

Classification				
Kingdom	Animalia			
Phylum	Arthropoda			
Class	Insecta			
Order	Diptera			
Family	Culicidae			
Subfamily	Culicinae			
Genus	*Aedes*	*Culex*	*Anopheles*	*Mansonia*

Mosquitoes undergo complete metamorphosis in their life cycle which involves the hatching of eggs into larvae after oviposition, going through four larval instars and pupation, and finally emergence into adults. *Aedes* prefers breeding on clear water but not necessarily clean water, while *Culex* thrives in stagnant dirty water (Hamdan et al., 2005).

2.1.1 Eggs

After taking a blood meal for about two to four days, female mosquitoes start to lay eggs onto the water surface either singly or in batches. *Aedes* females deposit their eggs singly above the water surface. The eggs are well spread around the container at varying distances from the water surface (Estrada-Franco and Craig 1995) to avoid predation. *Culex* females however, lay their eggs in batches locked in a boat-shaped structure that may contain up to several hundreds of eggs (Becker et al., 2010).

Aedes albopictus, a container breeder, is found to lay eggs in urban, suburban, rural and forested area (Harun 2007) at any natural or artificial water-containing receptacle. Natural receptacles in favor for *Aedes albopictus* breeding are tree holes, bamboo holes and stumps, coconut shells, plant axils, ground pools and rock pools (Estrada-Franco and Craig 1995). Rubber tires, tin cans, bottles, flower pots and buckets are their favorite artificial habitats (Estrada-Franco and Craig 1995). *Aedes aegypti*, also container breeders, overlap with *Aedes albopictus*, as they prefer breeding in man-made containers such as tin cans and discarded tires. *Culex* however thrives abundantly in polluted and still waters in artificial containers in residential areas such as sewage treatment plants and catchment basins of drainage systems, public places like parks and preserves (Patrican et al., 2007).

The changes which occurred during the development of the mosquito eggs between fertilization and hatching is defined as embryonic development (Christophers 1960). Temperature and relative humidity are the main factors affecting the period of development of the embryo. According to Hawley (1988), eggs of Asian strains of *Aedes albopictus* embryonated at temperatures between 24°C and 27°C within two to four days.

Specific stimulus which is flooding is required for mosquito eggs to hatch. In order to survive desiccation, the eggs have to be dried slowly (Becker et al., 2010), thus ensuring their viability until they have been soaked in water and proceed to hatching. Besides egg flooding, water temperature and oxygen pressure are also contributing factors for hatching (Harun 2007). Low oxygen pressure due to colonizing of microorganisms on the egg surface, brings about an increase in the microbial activity and nutrients in the water, which in turn stimulates hatching (Edgerly et al., 1993).

2.1.2 Larvae

The larvae start to undergo four stages of larval instar once the eggs hatch. They possess strong adaptability to a wide range of water-retaining containers, especially *Aedes albopictus*, which have high tolerance to high organic content water conditions such as tree holes and septic tanks (Estrada-Franco and Craig 1995). Moreover, they are also capable of surviving in small amounts of water with a depth as low as 0.625 cm (Harun 2007).

In addition, *Aedes albopictus* develop ideally in water with an optimal pH between 6.8 and 7.6 (Ho et al., 1972), while *Culex* in pH 6.4 to 8.2 (Low et al., 2012). *Aedes aegypti* can adapt and survive in either extreme acidic or basic conditions ranging from pH 4 to pH 11 (Clark et al., 2004). Based on the study done in New Orleans, habitats of larvae such as tires and tree holes were shown to have pH ranges of 6.33 – 8.35 and 6.43 – 8.23 respectively (Estrada-Franco and Craig 1995).

Temperature, availability, crowding and sex are the factors that affect larval development (Estrada-Franco and Craig 1995). The duration of larval development affected by temperature is shown by Hien (1975) where six days were taken for larval growth at 30°C, 9 days at 25°C and 13 days at 20°C. This indicates that the lower the temperature, the longer it takes for the larvae to develop. Besides, the lack of food supply will also extend the larval development period to 42 days on average, resulting in an 80% mortality rate (Estrada-Franco and Craig 1995). Overcrowding among the larvae is another contributing factor too. In spite of that, the aggregation of mosquito larvae at the breeding sites may act as prevention for the predation of single larva

(Becker et al., 2010). *Aedes albopictus* was found to be more resistant to high larval density as compared to *Aedes aegypti* (Hien 1975). *Culex* however, avoid laying eggs in habitats containing cues of larval competition (Reiskinda and Wilson 2004). Apart from that, female mosquitoes have a longer larval period than males as reported by Livingstone and Krishnamoorthy (1982), with the former having 119 – 149 hours and the latter at 115 – 141 hours.

2.1.3 Pupae

Once it enters pupal stage, the pupae will no longer feed, instead, it prepares for adult emergence for about two days. Male mosquitoes reach adult stage first before female mosquitoes with a pupal development period of 32 – 36 hours and 49 – 52 hours respectively (Livingstone and Krishnamoorthy 1982).

In comparison to the pupae of most other insects, mosquito pupae are more mobile. This is because when they are disturbed, they dive down and float back to the water surface. Apart from that, mosquito pupae have higher tolerance for desiccation, still managing to emerge to adult even if the breeding sites dried out or left stranded (Becker et al., 2010).

2.1.4 Adults

After one to two days, the pupae emerge into adults which then complete the final stage of metamorphosis. The males will emerge one to two days earlier than females because they require more time to achieve sexual maturity (Becker et al., 2010) as compared to the females. Hence, once the females emerged, both males and females are able to achieve sexual maturity at the same period of time which in turn indicates the readiness of the mating process.

After mating, the female mosquitoes aggressively seek for blood meal which is a protein source from a large number of hosts including humans and animals for egg development. The blood obtained is mainly used for the production of eggs and not so much as a source of energy for females (Becker et al., 2010). Their energy source which is mostly for flying purposes are mainly from plant juices such as floral nectar, which is also the main food source for males. In general, female mosquitoes are able to survive up to two to three weeks but males can only live up to one week (Harun 2007).

According to Estrada-Franco and Craig (1995), there is a linear correlation between blood meal size and the number of eggs deposited, where more eggs are produced when a bigger blood meal is taken. There are several factors that influence the feeding activity of mosquitoes, such as temperature and rainfall. In tropical countries, rainfall is in abundance. For instance, in Singapore, adult population peaks are evident during March, June and July as well as November and December (Estrada-Franco and Craig 1995) when rainfall is high.

Based on Figure 2.1, mosquitoes are divided into few parts; the head, thorax and abdomen. The head consists of proboscis where the mosquitoes feed, a pair of antennae and compound eyes. Scutum is a useful feature commonly used for species identification, and a pair of wings is found on the thorax area. Besides, three pairs of jointed legs, fore, mid and hind legs are also located on the thorax with each pair on each segment (Becker et al., 2010). The abdomen of mosquitoes is composed of 11 segments for which they are used for mating, egg laying and feces disposal (Becker et al., 2010).

According to the World Health Organization (WHO) (2013), *Aedes* adults can be differentiated by the contrasting black and white rings on its legs. They have pointed abdomens in comparison to *Culex* adults which have blunt abdomens. The most significant feature of *Anopheles* adults lies at the length of the palps which is equal to that of the proboscis. Besides, their mouthparts and abdomen are in a straight line at an angle almost perpendicular to the resting surface when at rest. On the other hand, the body, including legs and wings of *Mansonia* adults, are covered with dark-brown and pale scales, giving them a rather dusty appearance, as if sprinkled with salt and pepper.

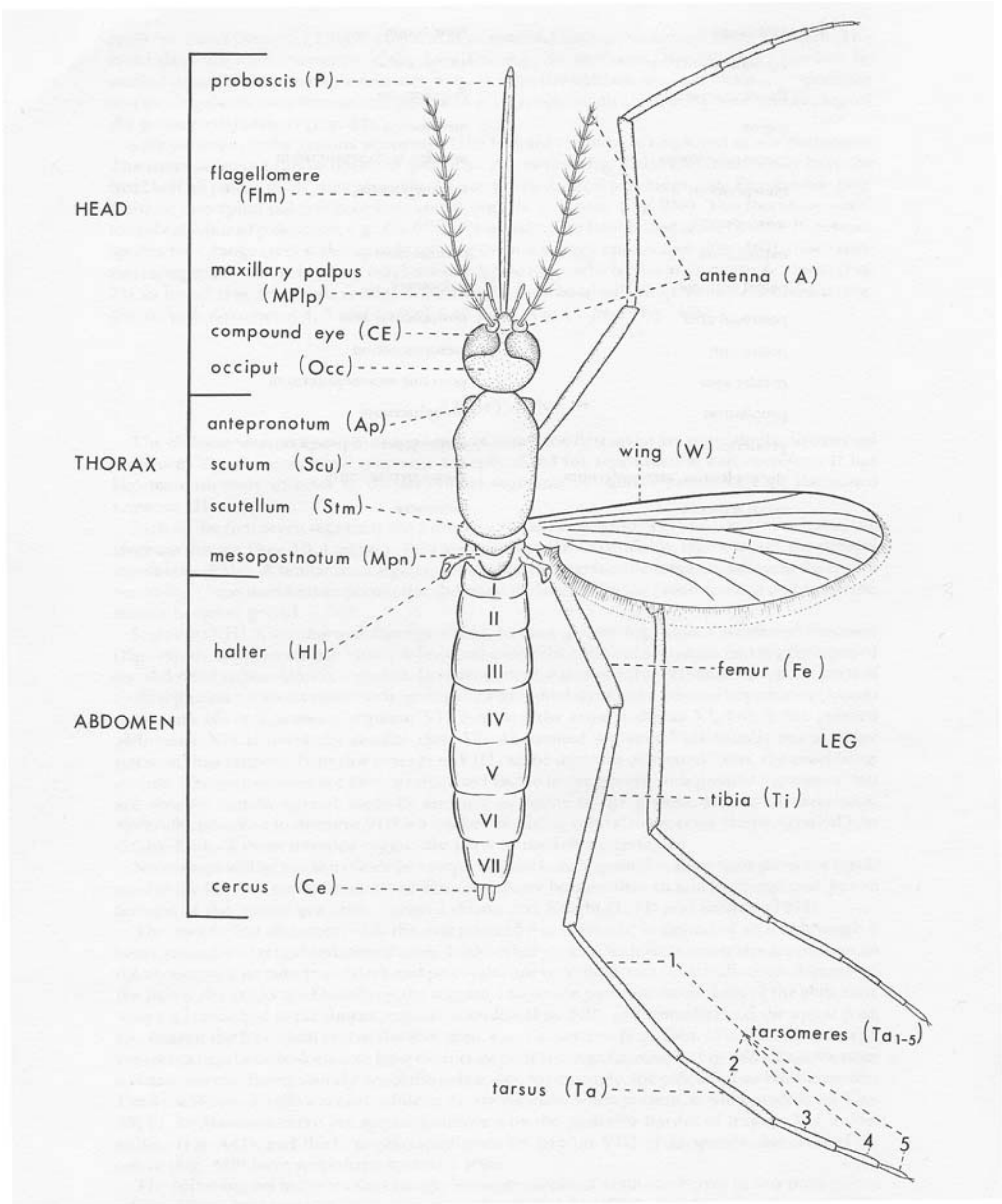


Figure 2.1: General outline of mosquito (Adapted from Nvmadil 2013).

2.2 Medical Importance of Mosquitoes

Mosquitoes are known as the most dangerous arthropod to mankind due to the ability to transmit many medically important arthropod-borne virus that cause serious diseases to human. Arboviruses are animal viruses capable of reproducing in an arthropod which can then be transmitted to a vertebrate host either vertically or horizontally (Estrada-Franco and Craig 1995). Vertical transmission also known as transovarial is the direct transfer of virus to the offspring by the parents. Mosquitoes hatched from the infected eggs contain the same virus as their mothers (Misbah et al., 2011). Horizontal transmission occurs when the arthropod becomes infected after ingesting blood from a viremic vertebrate, and after the multiplication of the virus in the arthropod tissues, the virus are then transmitted to new vertebrates by bite (Turell 1988).

Population of mosquitoes, especially *Aedes albopictus* which are widely found in human settlements, greatly increase the chances of contact between human and mosquitoes, thus leading to a potential increase in the rate of disease transmission. Being a successful vector, *Aedes albopictus* has been found to be correlated not only to dengue, but also other diseases such as Japanese encephalitis, Potosi, Keystone, Tensaw, Eastern Equine encephalitis (EEE), La Crosse (LAC) and West Nile (WN) viruses (Moore and Mitchell 1997). *Aedes* that transmits dengue virus has become the major concern in Malaysia as there are no specific dengue therapeutics available and prevention is currently limited to vector control measures (Loke et al., 2012). Dengue infection is predominant in urban areas where 61.8% of the total population lives (Harun

2007). Besides, many man-made opportunities for *Aedes* mosquito breeding were created due to the rapid industrial and economic development (Harun 2007).

Apart from *Aedes*, other species such as *Anopheles*, *Culex*, and *Mansonia* are also of medical importance because they have the habit of biting humans for blood meal. *Aedes* females feed aggressively in the morning and in the evening while *Anopheles* females are active between sunset and sunrise (WHO 2013). Both *Culex* and *Mansonia* females bite at night.

2.2.1 Dengue

Dengue is a mosquito-borne disease by the vector from the genus *Aedes*. Four serotypes of dengue virus designated DEN-1, DEN-2, DEN-3 and DEN-4 are circulating in Malaysia (Gubler and Clark 1996). According to Guha-Sapir and Schimmer (2005), the geographical spread of dengue is found to increase 20 fold since 1950s until now from five countries to more than 100 countries. Ministry of Health (MOH) Malaysia (2013) reported that currently there are 2,099 dengue cases reported in January 2013 where six states showed an increase in incidence rate as compared to the same period in 2012, with 1,868 cases. This showed an increase of 12% (231 cases). Rapid development and urbanization which increases potential breeding sites for *Aedes* may be reasons to the increase (Jamaiah et al., 2005). However, dengue fatality cases showed a slight decrease in number with a total of five deaths reported in January 2013 and six deaths in January 2012. This was probably due to the improvement of case management where early clinical diagnosis and careful clinical management by experienced physicians and nurses were implied (WHO 2009).

Aedes albopictus and *Aedes aegypti* are the main vectors of dengue in tropical and subtropical regions worldwide. Both of these species are well distributed in Peninsular Malaysia (Lee 1990). However, it is still difficult to determine the relative contribution of both species to disease transmission as *Aedes albopictus* breeding preferences often overlap with *Aedes aegypti* (Estrada-Franco and Craig 1995).

Aedes albopictus which originated in tropical Asia is now distributed worldwide (Rozilawati et al., 2007). There are several factors that led to the increase in dengue incidence associated with the abundance of vectors. One of them is due to the major increase in international travels. This was shown from studies that discovered the introduction of *Aedes albopictus* into the United States through shipping of tires from Asia (Estrada-Franco and Craig 1995). Apart from that, rainfall, temperature and relative humidity are also factors that contribute to the increase of mosquitoes, where wet seasons are found to have higher prevalence of *Aedes* mosquitoes (Rozilawati et al., 2007).

2.2.2 Chikungunya

The *Aedes* vectors for Chikungunya virus (CHIKV) are endemic in Malaysia, where the first confirmed outbreak occurred in Klang in 1998 and the second in Bagan Panchor (near Ipoh) in 2006 (Ayu et al., 2010). The MOH (2010) stated from April 2008 onwards Malaysia experienced a nationwide CHIKV outbreak that involved more than 10,000 cases, but with no fatalities reported. However, Sam et al. (2010) reported the first case of CHIKV-associated death in Kuala Lumpur in December 2008.

Conversely, Hasan (2012), reported there were rarely new CHIKV cases reported in Malaysia from November 2011 onwards. The total reported CHIKV cases demonstrated a 96% decrease in year 2011 with 30 cases as compared to year 2010 with 804 cases. Although CHIKV rarely occurs in Malaysia, it is still important to take prevention and precautionary steps as they have the same vector as dengue virus. Moreover, the absence of CHIKV may also be due to the failure to detect as the symptoms are similar to dengue fever and maybe mistakenly diagnosed as dengue (AbuBakar et al., 2007). Both *Aedes albopictus* and *Aedes aegypti* are the main vectors for CHIKV transmission (Becker et al., 2010).

2.2.3 Lymphatic Filariasis

An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis (LF), where approximately 66% of those at risk of infection live in the WHO Southeast Region and 33% in the African Region (WHO 2012a). By year 2020, the Global Program to Eliminate Lymphatic Filariasis targets global elimination of LF as a public health problem (WHO 2012b). LF is caused by *Wuchereria bancrofti* (Becker et al., 2010) which is a nematode worm. The infection is transmitted by various genera of mosquitoes, with the major vector being *Culex quinquefasciatus* and *Mansonia* species (Becker et al., 2010). The lack of adequate drainage and water stagnation (WHO 1992) due to the process of rapid urbanization and unplanned growth of cities led to the promotion of the breeding of *Culex quinquefasciatus* (Nazni et al., 2005).

In Malaysia, the first observed LF was in 1908 and *Culex quinquefasciatus* was the vector of the disease (Lee 2005). However, there has been no specific control program conducted for filariasis vectors, except through mass application of preventive chemotherapy (Nazni et al., 2005) with albendazole (WHO 2012b). Despite of that, it was found that *Culex quinquefasciatus* developed resistance to insecticides due to the indirect selection pressure of resistance to organophosphorous and pyrethroid compounds resulted from the extensive usage of malathion and permethrin for dengue control and agricultural pest control (Nazni et al., 2005). However, in comparison to insecticide-susceptible *Culex quinquefasciatus*, McCarroll et al. (2000) noted that their insecticide-resistant counterparts are less likely to transmit LF.

2.3 Ovitrap Surveillance

Ovitrap is a well-known economic tool in detecting the vector population in nature as it is sensitive and reliable. The ovitrap consists of a 300ml plastic container with straight, slight tapered sides with a height of 9.0cm. The outer wall of the container is painted black. This is because mosquitoes prefer to oviposit on ovitraps coated with red and black over other colors (Yap et al., 1995). The opening measures 7.8cm in diameter and 6.5cm for the base diameter. Paddles are placed diagonally into each ovitrap with the rough surface facing upward and filled with dechlorinated tap water to the level of 5.5cm (Wan-Norafikah 2009).This increases the oviposition of mosquitoes as they prefer to lay eggs on rough surfaces (Wong et al., 2011).

According to Williams et al.(2006), positive ovitrap collections at ground level are greater as compared to elevated ovitraps. However, in order to prevent the reach of children and pets, ovitraps are preferred to be elevated. When there is a choice of ovitraps at various elevations, 1.2m above ground level produces the greatest amount of *Aedes aegypti*. This is because the movement of mosquitoes to ovitraps may be influenced by wind, as the “flight boundary layer” is the height above ground that induces the maximum flight speed for an insect. Therefore, mosquitoes are able to fly in any direction within this layer, whereas anything above it will be susceptible to carriage by wind.

2.4 Control of Mosquitoes

There are several methods of vector control in combating mosquitoes, with personal protection being the most common type. Mosquito repellant and household insecticides are the favored personal protection used by residents as it is very convenient and easily available. Other types of vector control are chemical control, biological control and environmental management.

2.4.1 Chemical Control

In order to control and reduce the mosquito population, chemical applications are the main control agents in several countries. They are used in reducing vector abundance and infectious bites to prevent occurrence of mosquito-borne diseases. The major classes of insecticides used are pyrethroid, organophosphate, carbamate and organochlorine (Nauen 2007).

Dichloro-diphenyl-trichloroethane (DDT), is a broad spectrum organochlorine insecticide widely used for vector-borne disease control as well as in agriculture(CDC 2009). It is the first commercialized synthetic organochlorine used for malaria control as it is cost-effective and safe for indoor residual spraying (IRS) (Coleman and Hemingway 2007). The WHO however, only recommended pyrethroids for use on insecticide impregnated bednets (ITN) and malathion and carbamates on IRS due to the serious pollution DDT causes to the environment. DDT has a long half-life and remains persistent in plant and animal tissues, soil and aquatic environment (Becker et al., 2010), thus resulting in bioaccumulation. All use of DDT was banned since 1972,but some countries are still using DDT for endemic vector and malaria control (CDC 2009), especially Africa (Coleman et al., 2008).

Insecticide resistance has been induced due to the extensive application of the same chemicals on mosquitoes. *Aedes albopictus* and *Aedes aegypti* were found to be highly resistant to 4% DDT and completely susceptible to 5% malathion (Surendran et al., 2007).

Malathion is a broad spectrum non-systemic organophosphate insecticide. It became the insecticide of choice in the control of vector-borne diseases in several countries including Malaysia. This is because malathion possess fast action and low acute toxicity to both humans and animals (Becker et al., 2010) as compared to other organophosphates. In Khartoum state, Sudan, malathion has been used for mosquito control since 1990 (Jamal et al., 2011).

In Malaysia, malathion was introduced as an insecticide in the vector control program in 1986 by the MOH (Vythilingam et al., 1992). However, due to the foul smell and diesel-solvent which left oily residues on floors and walls of residents' houses, malathion was later replaced by pyrethroid (water-based formulation) in 1996 (Ang and Singh 2001).

Permethrin is a broad spectrum pyrethroid insecticide. It is currently the insecticide of choice in dengue vector control program in Malaysia (Wan-Norafikah et al., 2010). Resigen® and Aqua-resigen® are the water-based pyrethroid fogging formulations (Ang and Singh 2001) suitable to be used in many residential sites, both indoor and outdoor.

Deltamethrin is a synthetic pyrethroid that is commonly used to control malaria transmission in Asia (Potikasikorn 2005). It was chosen as a substitute for organochlorines and organophosphates in pest-control programs as they have low environmental persistence and toxicity (Sayeed et al., 2003). Since 1988, deltamethrin wettable powder (WP) has replaced DDT to be the main insecticide used in residual spraying (Rohani et al., 2006) in Malaysia's malaria control program. Based on the study conducted in Pahang, it was found that both wettable granules (WG) and WP formulations were able to reduce malaria cases by 90 to 100%. They were very effective against indoor resting Anophelines (Rohani et al., 2006).

Although it is effective against malaria vector, the *Aedes* population seemed to be less susceptible to deltamethrin as they were not reduced or suppressed by residual sprayed deltamethrin (Rozilawati et al., 2005). But in another study, deltamethrin was found to be effective against *Aedes albopictus* and *Aedes aegypti* (Sulaiman et al., 2000).

2.4.2 Resistance

Resistance as defined by WHO (1957) is the ability of an insect population to tolerate doses of insecticides which can kill majority of individuals in normal (susceptible) population of the same species. Insecticide resistance among insects develops when an insecticide is used repeatedly on them which results in a selection of insects that can survive a lethal dosage of insecticide (Loke et al., 2012). In tropical countries, resistance issue was found to be the highest as there were higher population of mosquitoes, shorter mosquito generation time and less effective vector control program (Head and Savinelli 2008). Besides that, over-dependence on chemical control will eventually lead to control failures. In Malaysia, malathion, being the cheapest, and permethrin, are the most commonly used insecticides in mosquito control (Chan et al., 2011). The usage of malathion and permethrin in the early 1970s and late 1990s in vector control program in Malaysia may be the contributing factors towards the occurrence of resistance for both insecticides.

In the 1950s, DDT was widely used in malaria control programs in Malaya, thus resulting in the development of natural tolerance of *Aedes* to DDT (Lee et al., 1998). In Colombia, although DDT usage has been terminated, DDT resistance was found to be sustaining in the population for 17 years (Fonseca-González et al., 2009).

When organophosphates such as malathion, fenitrothion and temephos were introduced in vector control program, resistance was found among *Culex quinquefasciatus* as well as *Aedes aegypti* (Lee et al., 1984). Likewise, when pyrethroids such as permethrin replaced organophosphates, *Culex quinquefasciatus* were found to be resistant to it too.

In a study conducted in Sudan, *Culex quinquefasciatus* were found to have developed resistance to permethrin, lambdacyhalothrin and malathion (Jamal et al., 2011). This is may be due to the extensive usage of aerosols and coils which were mostly pyrethroids, as well as the prolonged usage of DDT, malathion and pyrethroids in public health mosquito control. Household insecticide products which contained pyrethroid as an active ingredient (Low et al., 2011) also increased permethrin resistance among mosquitoes.

According to Low et al.(2011), malathion demonstrated the highest resistance ratio in Sitiawan, Perak, whereas permethrin faced the highest resistance ratio in Kota Bharu, Kelantan. Permethrin resistance was also detected in Shah Alam (Loke et al., 2012). Failure of insecticides in controlling the mosquito population may contribute to higher disease transmission rate (Eisen et al., 2009).

According to Chua et al. (2005), the ultra-low volume (ULV) chemical fogging of insecticides only marginally reduced the *Aedes* mosquitoes during fogging period, but surprisingly the number of immature *Aedes* mosquitoes collected in the immediate post-fogging period was higher than that in the immediate pre-fogging period. This supports studies made in Thailand where reduced numbers of adult mosquitoes after suscepting to malathion ULV fogging returned to pre-treatment level within two weeks (Pant et al., 1971). In addition, an American study showed a faster return by having a bounce back to the pre-treatment baseline within a few days (Chadee 1985).

Nazni et al. (2005) reported that resistance can be reversed if the mosquitoes are kept insecticide-free for a long period. This was evident in their study which showed that although *Culex quinquefasciatus* field strains were found to be highly resistant to malathion and DDT, the susceptible strain showed a low value of lethal time (LT_{50}) for malathion.

In order to measure mosquito resistance towards insecticides, the standard WHO diagnostic kit for adulticides, applying a single diagnostic dose, is a popular tool used. It is less sensitive because the dosage used is double that of the dose that would kill 99.9% of individuals in a population (Chan et al., 2011). Another bioassay known as topical application, as suggested by Chan et al. (2011) is a more sensitive and indicative bioassay because it is a dose-mortality assay in which the dosages of insecticide applied on the mosquitoes' prothorax can cause a fixed and comparable percentage of mortality. However, compared to WHO diagnostic kit which is rapid and convenient, topical application is more time consuming and fragile to be used. Besides, it also requires a large population sample in order to be effective. To date, the exact threshold of resistance level that would cause the failure of mosquito control and outbreak of disease has not yet been established.

Development of mosquito resistance to chemical insecticides represents a threat for the efficacy of vector control and hence, makes mosquito-borne diseases more difficult to control (Vythilingam et al., 1992). The main defense against resistance is close surveillance of the susceptibility of mosquito populations. If surveillance data were not sufficiently collected, resistance detection could interfere with disease control programs (Brogdon and McAllister 1998). Therefore, resistance monitoring is important to detect resistance at an early stage so that appropriate management can be further implemented. Furthermore, this provides baseline data for program planning and pesticide selection before the commencement of control operations (Brogdon and McAllister 1998).

2.4.3 Biological Control

Due to resistance problem encountered in mosquitoes and other urban pest, studies were shifting to biological control as it is more environmental friendly. As biological agents such as microbial agents, parasites and predators can survive and be recycled, thus biological control is able to demonstrate a longer lasting effect (Zairi and Lee 2005). However, specific microbial agents can only be targeted for certain pest species. For instance, *Bacillus thuringiensis* H-14 for *Aedes* and *Anopheles* which are clean water-breeders, while *Bacillus sphaericus* 2362 for polluted water-breeders, *Culex* and *Mansonia* (Zairi and Lee 2005).

Mermithid nematodes *Romanomermis culicivorax* parasitizing mosquitoes have substantial potential for vector control (Platzer 1981). Field studies have shown that they were able to reduce the mosquito larvae population upon release. However, instability of this control arose as successful controls require repeated introduction of mermithids into mosquito habitats (Juliano 2007). Larvivorous fish, which feeds on immature stages of mosquitoes are biological mosquito control agents which were important to malaria control programs in the 20th century in both developed and developing countries (Chandra et al., 2008). Natural predators of mosquitoes such as mosquitofish (*Gambusia affinis*) were used in many mosquito control programs due to their broad habitat tolerance and being perceived as an effective mosquito predator (Goodsell and Kats 2001).

2.4.4 Environmental Management

Besides insecticides, integrated vector control strategy is implied where it involves environmental modifications and manipulations (Surendran et al., 2007). Source reduction is a principle strategy that hinders the vector life cycle completion by draining or removing man-made water containers or natural water sources. This will eliminate all possible breeding sites for mosquitoes. In Malaysia, the best vector control strategy to prevent dengue was by applying four strategies which are anti-larval measures, anti-adult measures, health education and enforcement of the Destruction Disease Bearing Insects Act (DDBIA) (Harun, 2007).

The prevention of sewage effluents and soakage pits in domestic water requires proper environmental management such as adherence to basic architectural requirements (Okogun et al., 2003). Moreover, public awareness on applying personal protective measures was raised. Furthermore, many common trapping methods are available to significantly increase the catch counts and total number of target species sampled for testing (Newhouse et al., 1966).

Most countries in the dengue endemic areas only respond to an epidemic when it is at or near to the peak transmission (Gubler 2002). At that time, even if various mosquito control measures were effective, it will be too late to exert effect on the rate of transmission. Puerto Rico was known to have the best surveillance systems for DF and DHF, possessing good laboratory capability to support active surveillance with an early warning capability (Gubler 2002). However, due to lack of information gathered, this system had not been used effectively for planning and for emergency responses to prevent epidemics predicted, which defeated the objective to reduce epidemic transmission and to save lives.

According to Gubler (2002), Singapore was found to be the only one endemic country for dengue cases where surveillance was effectively used for planning, response, prevention and control. By using case definitions, surveillance information actively targeted the specific areas of the city for intensified control. This was in response to the epidemic outbreak of dengue cases in the late 1980s due to the importation of dengue viruses from high incidence rate countries into Singapore, although the level of *Aedes aegypti* and dengue virus were kept low at that time.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Site

The site chosen for this research project was a residential area known as Taman Juloong, located around 1 km away from the center of Old Town, Kampar, Perak. It is a moderately populated suburban residential area located on a hill. At the top of the hill are three bungalows, followed by six rows of double-storey semi-detached terraced houses (Figure 3.1). At the foot of the hill are three blocks of four-storey flats (Figure 3.2). There are neither shop houses nor playgrounds in this residential area. This site is surrounded by many lush tall trees and plants.

Based on a rough observation of the outdoor area of this residential site, it was found that the condition of the houses located towards the upper region of the hill is clean and well taken care of. There is no clogging of drains, water-filled containers or rubbish dumps around the area. Residents in this area seemed to be well aware of and are well educated on the importance of maintaining cleanliness of their house compound. However, the outer perimeters of their house compounds such as the stretch of trees along the road as well as the condition of the road are much neglected. Fallen leaves from these trees were left to rot and these may become good breeding grounds for the mosquitoes especially during rainy season.

Figure 3.1: Double-storey semi-detached terraced at the top of the hill at Taman Juloong.

Figure 3.2: Blocks of flat at the foothill of Taman Juloong.

Moreover, there is a stretch of road that is not well-tarred and many potholes are seen (Figure 3.3). When it rains, pools of water will be formed, thus attracting mosquitoes to breed.

On the other hand, cleanliness of the compounds surrounding the three blocks of flat houses is satisfactory. Overall the areas are well kept, but there is an overflowing of rubbish at a particular site, which may be the common area for the residents of these flats to dispose their rubbish (Figure 3.4). This practice may lead to an increase in the breeding of mosquitoes, resulting in a higher risk of disease infection carried by the mosquitoes. Furthermore, behind these flats is a slope full of matured trees growing close together forming a canopy (Figure 3.5). After a heavy downpour, clogged water is harder to evaporate and this may also be a potential breeding ground for mosquitoes (Figure 3.6).

3.2 Mosquito Sampling

Ovitraps were used to collect mosquito eggs. A total of 60 ovitraps were placed outdoors on 60 trees in Taman Juloong, with 15 ovitraps near the semi-detached terraces, 30 ovitraps near the flats and 15 ovitraps along the road which also included the bungalow area (Figure 3.7). The ovitraps set at a distance of 10 to 15 m apart, were marked and placed 1 m above ground on the tree. In this study, “outdoor” was referred to the outer area of the building or the gate of the house.

Figure 3.3: Potholes on the road.

Figure 3.4: Unmanaged sites –rubbish dumping site.

Figure 3.5: Trees forming canopy and branches left to dry and rot.

Figure 3.6: Unmanaged sites –branches left to be dried and rot.

Figure 3.7: Location of ovitrap positions in Taman Juloong, Kampar (Adapted from Google Map 2012).

Paddles with rough surface facing upward were placed individually into each ovitrap for oviposition of adult female mosquitoes (Figure 3.8). The ovitraps were filled with dechlorinated tap water until three quarter's full. The collections of paddles were carried out thrice a week on Mondays, Wednesdays and Fridays for three months (Table 3.1). Fresh paddles and water were replaced everytime.

Figure 3.8: Ovitrap and paddle hung on a tree.

Table 3.1: Date of paddles collected and replaced in 13 surveillance weeks.

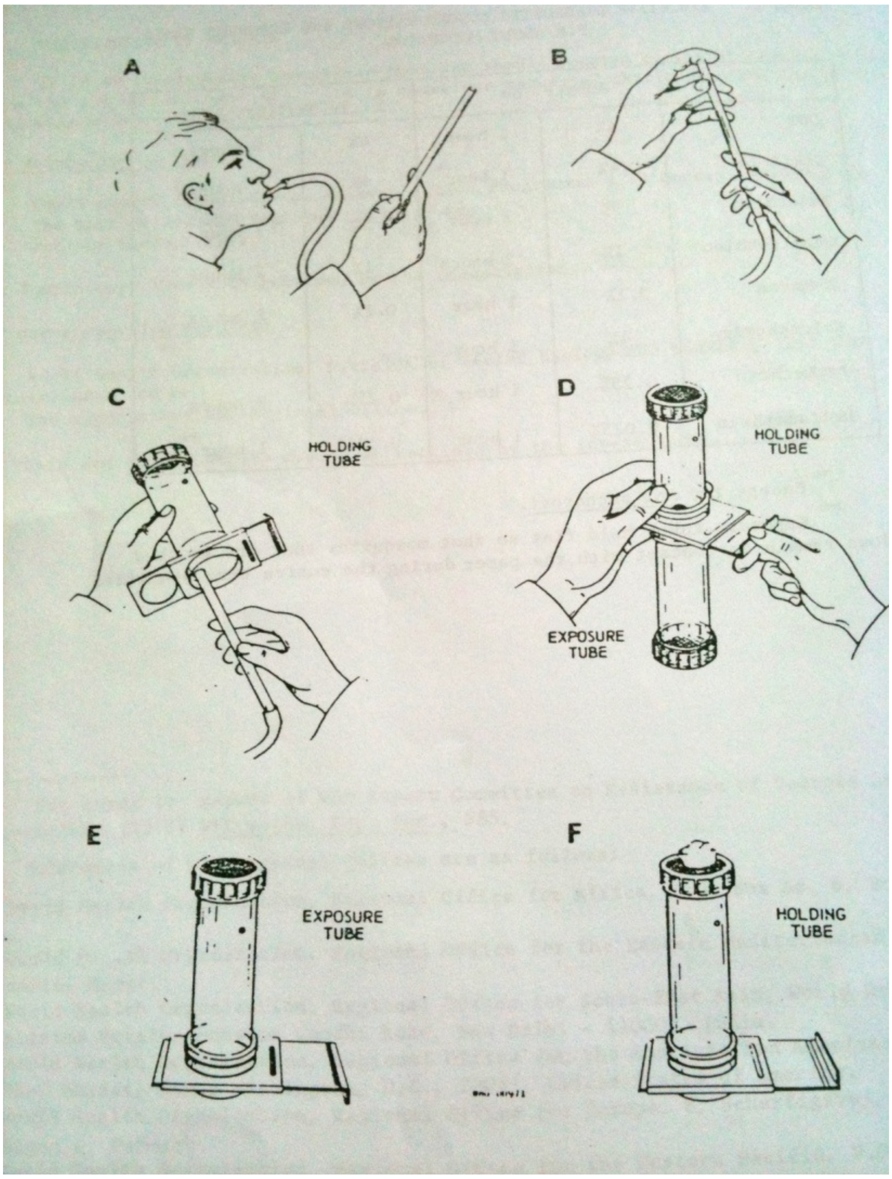
Week	Date of paddles collected and replaced
1	1, 3, 5 October 2012
2	8, 10, 12 October 2012
3	15, 17, 19 October 2012
4	22, 24, 26 October 2012
5	29, 31 October 2012, 2 November 2012
6	5, 7, 9 November 2012
7	12, 14, 16 November 2012
8	19, 21, 23 November 2012
9	26, 28, 30 November 2012
10	3, 5, 7 December 2012
11	10, 12, 14 December 2012
12	17, 19, 21 December 2012
13	24, 26, 28 December 2012

3.3 Mosquito Culturing

The collected paddles containing eggs were brought back to the laboratory to be air dried before being immersed in dechlorinated tap water the next day. After two days, emerged larvae were fed with ground cat food and allowed to grow until pupae stage. Then, emerged pupae were transferred into smaller containers and placed into mosquito cages in preparation for adult emergence. Ten percent sucrose solution was given as a source of nutrient for the emerged adults. With an aspirator, the emerged adults were transferred out and identified using dissecting microscope and taxonomy key. Subsequently, the identified females were collected and subjected to insecticide susceptibility tests.

3.4 WHO Diagnostic Test Kit Bioassay

The adulticidal procedures and methods used are as described in WHO (1976). Twenty sugar-fed female mosquitoes were first transferred into holding tubes using aspirator and then set upright with screen end up for 1 hour (Figure 3.9). This was to ensure that the transferred mosquitoes were in an optimal condition and not damaged. After the holding period, any damaged insects were removed. Sheets of impregnated paper with insecticides were introduced into the exposure tubes.



A: Mosquitoes were collected with the aspirator.

B: They were collected in lots of not more than ten.

C: They were transferred into the holding tube.

D: Mosquitoes were introduced into the exposure tube.

E: They were left standing upright for required exposure period.

F: The mosquitoes were transferred into the holding tube for 24 hour mortality count.

Figure 3.9: WHO Bioassay – Method for determining the susceptibility of adult mosquitoes to insecticides (Adapted from WHO 1976).

The mosquitoes were transferred from the holding tubes into the exposure tubes and the slides separating both tubes were closed to enable the holding tubes to be detached. The exposure tubes were left upright for the required exposure period where mortality was observed every 2 minutes for a period of 2 hours. In addition, the exposure period was extended for insecticides that require longer time. At the end of exposure period, the mosquitoes were transferred into paper cups covered with mesh cloth and provided with 10% sucrose solution. They were kept for 24 hours in a secluded shady area where temperature does not exceed 30°C. Mortality counts were made again after 24 hours. Dead mosquitoes including those which were unable to walk were counted as dead. Subsequently, mortality percentage was recorded.

The mosquitoes were tested with five types of insecticide impregnated papers which were malathion 5%, fenitrothion 1%, permethrin 0.75%, deltamethrin 0.05% and dichloro-diphenyl-trichloroethane (DDT) 4%. The olive oil and silicon oil treated papers were used as negative controls. Olive oil treated papers were used as control for organophosphate insecticides such as malathion and fenitrothion, whereas silicon oil treated papers were used for pyrethroid insecticides such as permethrin and deltamethrin. The insecticide susceptibility test was replicated six times. If the control mortality was between 5 to 20%, the percentage mortalities would be corrected using Abbott's formula:

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}}\ \text{X}\ 100$$

3.5 Data Analysis

The surveillance data which comprised of ovitrap number, gender and species of mosquitoes were tabulated and analyzed with meteorological data which consisted of parameters such as mean temperature, rainfall and relative humidity. Data obtained through paddles collection were analyzed and the Ovitrap Index (OI) was calculated using the following equation:

$$\frac{\text{Number of positive ovitraps}}{\text{Number of ovitraps collected from the specific area}} \text{ X } 100\%$$

Besides that, the mean number of each mosquito species per recovered ovitrap was also calculated using the following equation:

$$\frac{\text{Total number of mosquitoes}}{\text{Total number of ovitraps}}$$

The knockdown time of 50% and 95% of tested mosquito samples (KT_{50} and KT_{95}) on different insecticides were calculated using Probit analysis with SPSS software version 16.

CHAPTER 4

RESULTS

4.1 Species Identification

The collected mosquitoes were identified as *Aedes albopictus* (Figure 4.1; Figure 4.2), *Aedes aegypti* (Figure 4.3; Figure 4.4) and *Aedes albolateralis* (Figure 4.5; Figure 4.6).

Figure 4.1: *Aedes albopictus* – male.

Figure 4.2: *Aedes albopictus* – female.

Figure 4.3: *Aedes aegypti* – male.

Figure 4.4: *Aedes aegypti* – female.

Figure 4.5: *Aedes albolateralis* – male.

Figure 4.6: *Aedes albolateralis* – female.

4.2 Habitat Preferences in Relation to Mosquito Population

A total of 19,107 mosquitoes were collected in the study conducted for three months (October to December) in 2012 in Taman Juloong, out of which, 99.57% were *Aedes albopictus* (Table 4.1). *Aedes aegypti* and *Aedes albolateralis* took up a smaller percentage which was 0.07% and 0.36% of total number of mosquitoes respectively.

Table 4.1: Population of mosquito species identified in Taman Juloong, Kampar.

Species	Number of mosquitoes
Aedes albopictus	19,029
Aedes aegypti	13
Aedes albolateralis	65
Total	19,107

Aedes albopictus was found in all 60 ovitraps with the highest during Week 11 with 2,228 mosquitoes and lowest during Week 13 with 1,076 mosquitoes (Table 4.2). Both weeks also demonstrated the highest and lowest mean at 37.13 and 17.93 respectively. Overall, female mosquitoes outnumbered male mosquitoes. Females were the highest during Week 6 at 1,309 mosquitoes and lowest at 438 mosquitoes on Week 9. Conversely, the highest number of males was in Week 11 at 1,118 mosquitoes and the lowest number in Week 6 at 369 mosquitoes. During Week 11, both male and female mosquitoes showed the least difference which was 8 mosquitoes. The Ovitrap Index (OI) demonstrated 100% mosquito occupancy during Weeks 3 and 9. The lowest OI was in Weeks 1 and 13 at 85%.

Table 4.2: Number of *Aedes albopictus* and ovitrap index in 13 surveillance weeks in Taman Juloong, Kampar.

Week	Total mosquitoes	Number of mosquitoes		Ovitrap Index (%)	Mean number of mosquitoes
		Male	Female		
1	1396	728	668	85.00	23.27
2	1628	635	993	95.00	27.13
3	1132	488	644	100.00	18.87
4	1212	449	763	95.00	20.20
5	1589	714	875	96.67	26.48
6	1678	369	1309	88.33	27.97
7	1778	513	1265	98.33	29.63
8	1603	650	953	98.33	26.72
9	1178	740	438	100.00	19.63
10	1315	790	525	95.00	21.92
11	2228	1118	1110	95.00	37.13
12	1216	656	560	96.67	20.27
13	1076	491	585	85.00	17.93

On the other hand, *Aedes aegypti*, were only found in six ovitraps numbered 14, 15, 37, 45, 58 and 60 (Figure 4.7). The highest number of *Aedes aegypti* was recorded in Week 12 with 8 mosquitoes and a mean of 0.13 (Table 4.3). The lowest number was on Weeks 7 and 8 with only 1 *Aedes aegypti* at a mean number of 0.02. There were only 2 males collected on Week 6. The number of female *Aedes aegypti* was the highest on Week 12 in which also showed the highest OI which was 6.67%. The lowest OI was 1.67% on Weeks 7 and 8.

Aedes albolateralis were found in 11 ovitraps in Taman Juloong, numbered at 11, 14, 15, 24, 37, 38, 40, 41, 45, 46 and 60. The highest number of mosquitoes collected was on Week 7 at 28 mosquitoes with the highest mean of 0.47 (Table 4.4). Weeks 4 and 9 had the lowest amount of *Aedes albolateralis* with only 2 mosquitoes found and the lowest mean at 0.03. The highest OI fell on Week 7, which was at 13.33%, and the lowest OI was on Weeks 4 and 9 at 1.67%. In addition, Week 7 had the highest number of males and females at 9 and 19 respectively, whereas no males were found in Week 4 and no females in Week 9.

Figure 4.7: Location of ovitraps found with *Aedes aegypti* and *Aedes albolateralis* in Taman Juloong, Kampar.

Table 4.3: Number of *Aedes aegypti* and ovitrap index in 13 surveillance weeks in Taman Juloong, Kampar.

Week	Total mosquitoes	Number of mosquitoes		Ovitrap Index (%)	Mean number of mosquitoes
		Male	Female		
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	3	2	1	3.33	0.05
7	1	0	1	1.67	0.02
8	1	0	1	1.67	0.02
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	8	0	8	6.67	0.13
13	0	0	0	0	0

Table 4.4: Number of *Aedes albolateralis* and ovitrap index in 13 surveillance weeks in Taman Juloong, Kampar.

Week	Total mosquitoes	Number of mosquitoes		Ovitrap index (%)	Mean number of mosquitoes
		Male	Female		
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	2	0	2	1.67	0.03
5	0	0	0	0	0
6	3	1	2	3.33	0.05
7	28	9	19	13.33	0.47
8	0	0	0	0	0
9	2	2	0	1.67	0.03
10	0	0	0	0	0
11	19	7	12	1.67	0.32
12	11	5	6	8.33	0.18
13	0	0	0	0	0

4.3 Environmental Factors in Relation to Mosquito Population

The meteorological data were obtained from the Malaysian Meteorological Department stationed at Kampar Hospital at latitude 4° 18'N, longitude 101° 9'E and height above mean sea level of 37.5 m. The daily rainfall was the amount collected over 24-hour period beginning from 8 a.m. on that day according to Malaysian Standard Time (MST) (Appendix C).

Throughout the 13 surveillance weeks (1 October 2012 to 29 December 2012), the highest mean of rainfall was recorded in Week 5 at 32.9 mm while the lowest rainfall was in Week 1 at 0.5 mm (Figure 4.8). Secondary peaks were found on Weeks 3, 9 and 11. The temperature recorded during the surveillance period showed less fluctuation with a mean temperature of 26.6°C (**Figure 4.9**). Similarly, humidity was relatively constant throughout the three months with a mean of 83.9%. The increase in temperature correlated to the decrease in humidity where during Week 1, the highest recorded temperature was noted at 27.6°C while the humidity was lowest at 73.4%. Conversely, in Week 6 the temperature recorded was lowest at 25.8°C while the humidity was highest at 88.5%.

The distribution of *Aedes albopictus* was not significantly correlated to the rainfall (Pearson correlation: 0.241; p: 0.427) (Appendix D). It was noted that the highest numbers of *Aedes albopictus* were collected during Weeks 2, 7 and11, when there were moderate rainfalls.

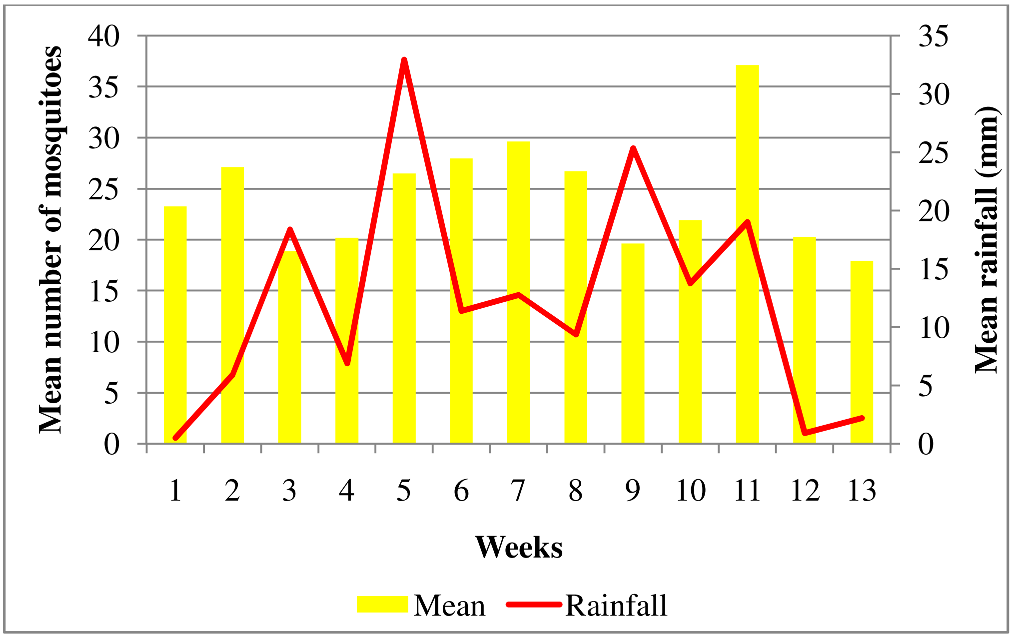


Figure 4.8: Mean number of *Aedes albopictus* against mean rainfall in 13 surveillance weeks in Taman Juloong.

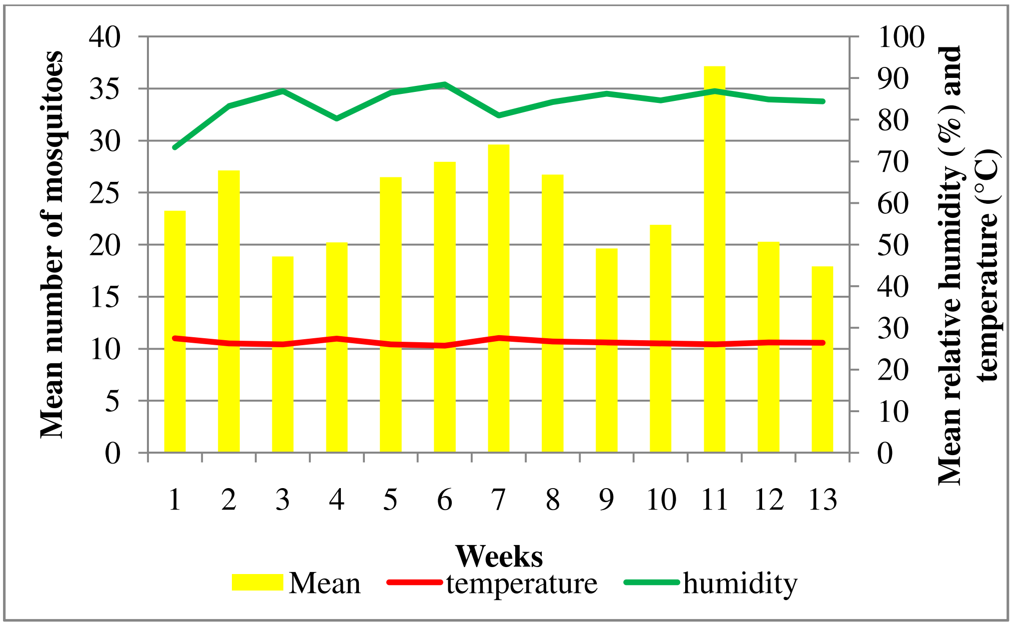


Figure 4.9: Mean number of *Aedes albopictus* against temperature (°C) and relative humidity (%) in 13 surveillance weeks in Taman Juloong.

In contrast, there was a negative correlation between the number of *Aedes aegypti* and rainfall (Pearson correlation: -0.363; p: 0.223). A primary peak of *Aedes aegypti* was shown in Week 12, whereas the secondary peak was at Week 6 (Figure 4.10; Figure 4.11) when the rainfalls were low. On the other hand, *Aedes albolateralis* showed similar trends as *Aedes albopictus* (Pearson correlation: 0.032; p: 0.917). Two peaks of *Aedes albolateralis* appeared during weeks of moderate rainfall which were in Weeks 7 and 11 (Figure 4.12; Figure 4.13).

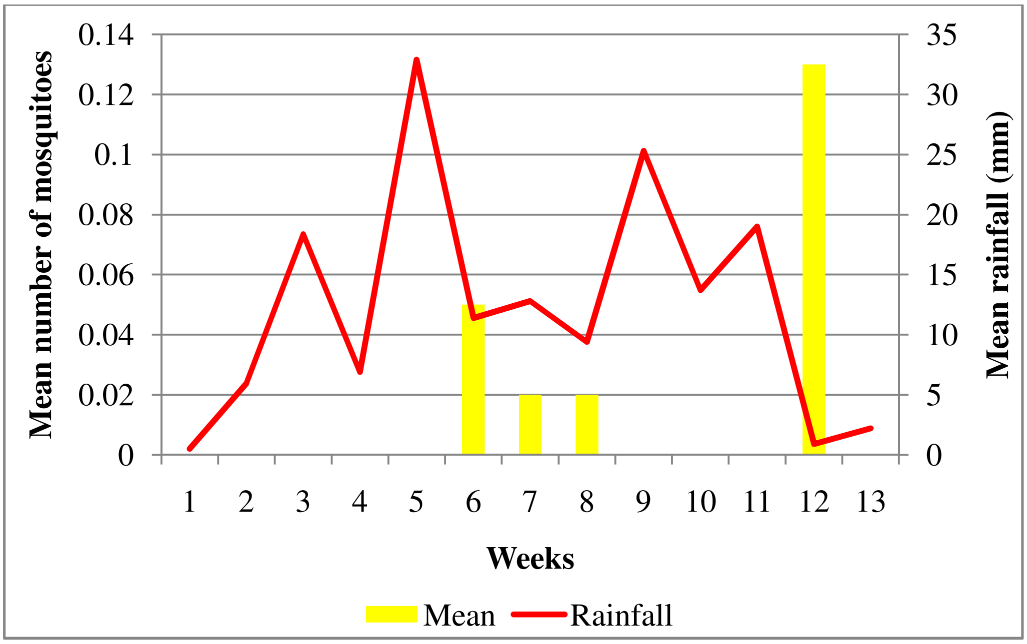


Figure 4.10: Mean number of *Aedes aegypti* against rainfall in 13 surveillance weeks in Taman Juloong.

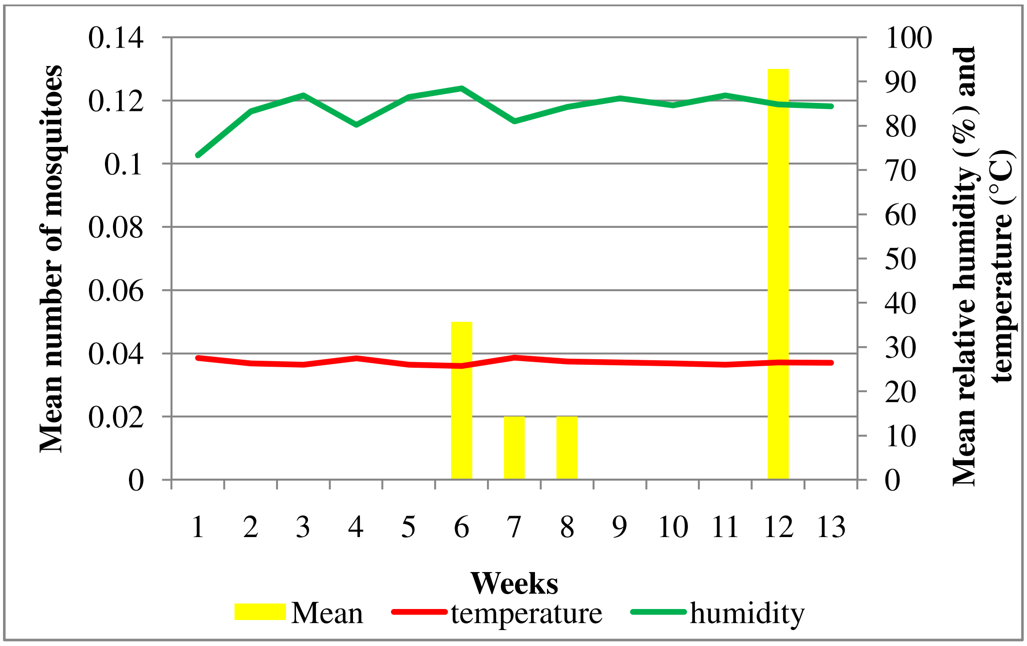


Figure 4.11: Mean number of *Aedes aegypti* against temperature (°C) and relative humidity (%) in 13 surveillance weeks in Taman Juloong.

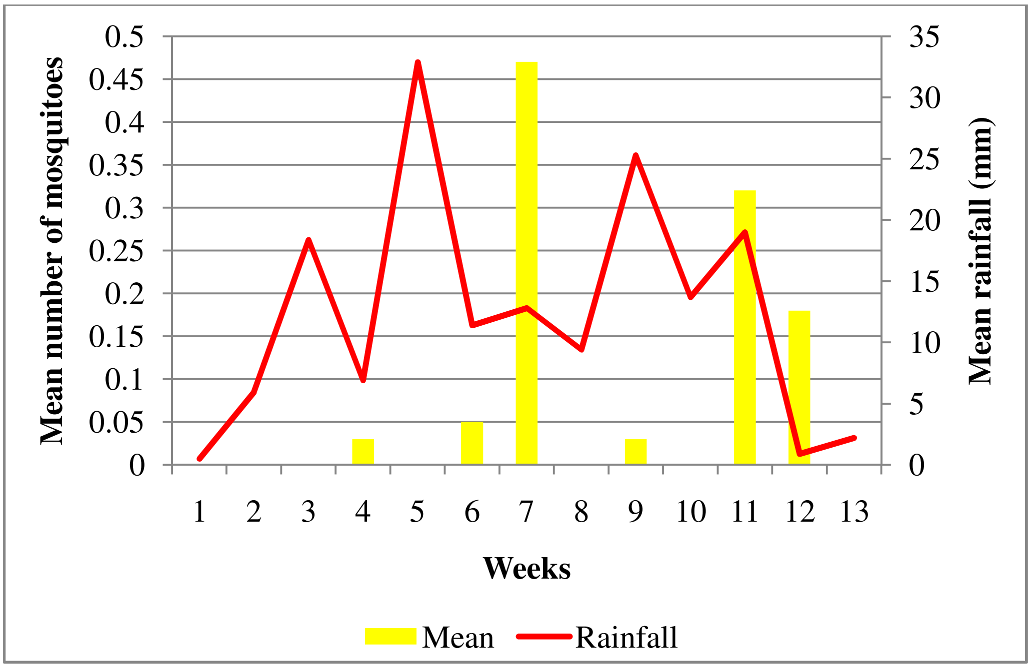


Figure 4.12: Mean number of *Aedes albolateralis* against rainfall in 13 surveillance weeks in Taman Juloong.

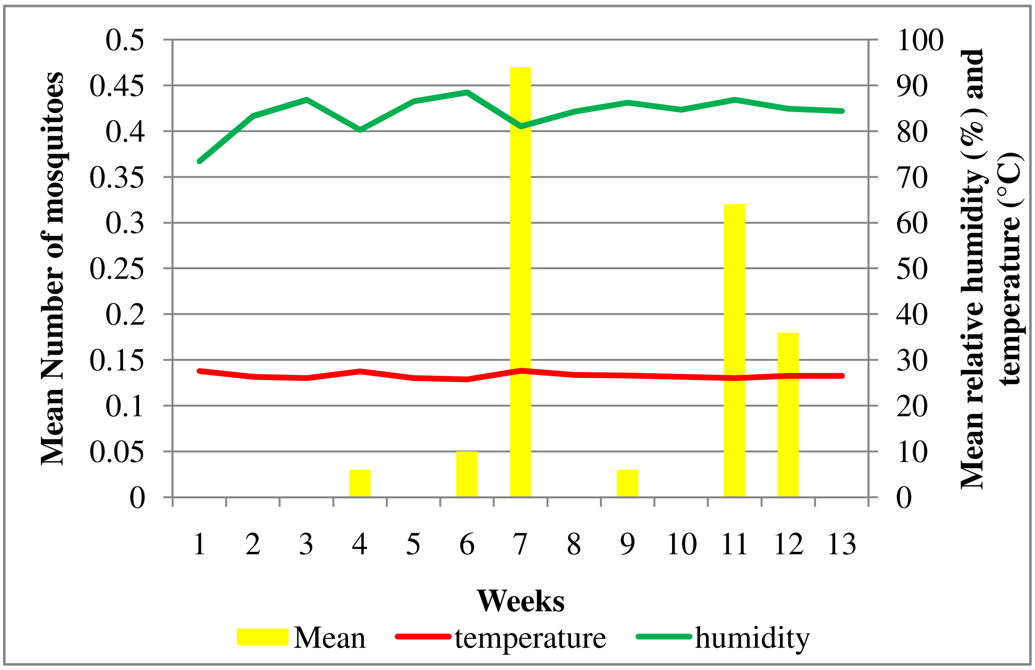


Figure 4.13: Mean number of *Aedes albolateralis* against temperature (°C) and relative humidity (%) in 13 surveillance weeks in Taman Juloong.

4.4 Mosquito Resistance

Fenitrothion (1%) had the highest knockdown time of 50% and 95% tested mosquitoes (KT_{50} and KT_{95}) of 203.32 minutes and 408.07 minutes respectively (Table 4.5). In addition, the regression slope for fenitrothion was the largest, at a value of 5.44 ± 0.06. Deltamethrin (0.05%) and permethrin (0.75%) showed the lowest knockdown time. The KT_{50} and KT_{95} of deltamethrin were 16.18 minutes and 49.44 minutes respectively, while the KT_{50} and KT_{95} of permethrin were 17.52 minutes and 54.54 minutes respectively. The regression slope for deltamethrin and permethrin were the lowest, at 3.39 ± 0.08 and 3.34 ± 0.08 respectively. It showed that the longer the time taken, the lesser the effectiveness of the insecticide.

All Chi-square values were significant ($p<0.05$), thus the heterogeneity factor was not taken into consideration in the calculation of confidence limit. All tested samples were homogenous as they were of the same age and sugar-fed.

No knockdown of mosquitoes was observed in the pyrethroid and organophosphate control. Since the control mortality for all the insecticide tests was less than 5%, hence they were not corrected with Abbott's formula. Mortality counts of the 24-hour post-exposure period showed 100% mortality rate.

Table 4.5: Susceptibility status of *Aedes albopictus* towards diagnostic dose of insecticides.

Class of insecticides	**Insecticides**	**KT_{50} (mins)**	**KT_{95} (mins)**	**Regression ± S.E.**
Organochlorine	DDT (4%)	62.63 (61.10 – 64.15)	174.03 (169.63 – 178.72)	3.71 ± 0.05
Organophosphate	Malathion (5%)	62.69 (61.39 – 63.98)	141.04 (137.71 – 144.61)	4.67 ± 0.07
	Fenitrothion (1%)	203.32 (194.60 – 212.00)	408.07 (381.62 – 441.64)	5.44 ± 0.06
Pyrethroid	Deltamethrin (0.05%)	16.18 (15.26 – 17.07)	49.44 (46.80 – 52.46)	3.39 ± 0.08
	Permethrin (0.75%)	17.52 (16.66 – 18.36)	54.54 (52.02 – 57.36)	3.34 ± 0.08

CHAPTER 5

DISCUSSION

5.1 Species Identification

Aedes were the only species identified in this study. Male mosquitoes differ from females by having plumose antennae and long, hairy maxillary palps. Females have shorter and less hairy antennae. *Aedes albopictus* can be differentiated by the presence of a single broad line of white scales situated in the middle of the thorax while *Aedes aegypti*, has the pattern of two straight lines surrounded by curved lyre-shaped lines on the side (Sucharit et al., 1993). As for *Aedes albolateralis*, they were identified by the presence of a pair of large lateral lens-form white patches connected anteriorly by a narrow white bridge on the scutum (Sasa and Nakahashi 1972).

5.2 Ovitrap Index (OI) and Mosquito Population

The Ovitrap Index (OI) obtained from this study was between 85to 100% for *Aedes albopictus*, 1.67 to 3.33% for *Aedes aegypti* and 1.67 to 13.33% for *Aedes albolateralis*. The OI were classified into four categories, where different actions were taken based on the indicated levels (Table 5.1). According to Cheung and Fok (2009), OI levels 1 and 2 can be controlled by source reduction through proper disposal of containers and eliminating potential breeding sites such as accumulated water in sauces underneath flower pots. Special control operations should be carried out by the vector control department in collaboration with the community when the OI reached level 3 or above. Once OI reaches level 4, insecticide space spraying must be carried out in order to contain the mosquito population.

Table 5.1: Classification of Ovitrap Index (OI).

Classification	Ovitrap Index (%)
Level 1	OI<5
Level 2	$5 \leq OI < 20$
Level 3	$20 \leq OI < 40$
Level 4	$OI \geq 40$

The population of *Aedes albopictus* in Taman Juloong belongs to the highest level of OI (Level 4). This showed the presence of persistent breeding grounds for *Aedes albopictus*, the secondary vector for dengue virus, which needed continuous attention from the local authorities. *Aedes aegypti*, the primary vector for dengue, is categorized under level 1 while *Aedes albolateralis* is categorized under level 2. This showed that both species were under control. However, further surveillance and prevention measures should be done as both habitats of *Aedes albopictus* and *Aedes aegypti* overlap.

5.3 Habitat Preferences and Mosquito Population

In this three-month study done in Taman Juloong, the ovitraps were mainly colonized by *Aedes albopictus* as they are container breeders. Other species such as *Aedes aegypti* and *Aedes albolateralis* being the minority species were found only in some parts of Taman Juloong.

Aedes albopictus were the majority species found because they are mostly outdoor breeders and the ovitrap surveillance conducted was also outdoors. This finding was similar to the study conducted by Rozilawati et al. (2007), where the number of *Aedes albopictus* exceeded that of *Aedes aegypti. Aedes albopictus* have a wide range of potential habitats, from natural receptacles to artificial containers. Natural containers found in abundance in Taman Juloong are tree holes and stumps, ground pools and plant axils whereas artificial containers found are flower pots, tin cans and earthenware containers. *Aedes albopictus* utilizes water-filled containers around or further away from households. They are aggressive biters that not only bite humans but also target a variety of available vertebrates such as cats and dogs that are mostly found in Taman Juloong. Therefore, their multiple feeding behavior patterns lower the capacity of transmitting dengue viruses. However, they remain dangerous as their wide host range may increase transmission of zoonotic viruses and potentially transmit them to humans (Knudsen 1995). *Aedes albopictus* prefers to breed in clean water, thus contributing to the large number of *Aedes albopictus* found in all ovitraps in which the water was replaced over two consecutive days.

Apart from that, concrete drainage system outside houses may also be a contributing factor towards the large population of *Aedes albopictus*, as clear stagnant water with fallen tree leaves were seen in some parts of the drains. These provided good breeding sites for them. This correlated to the findings of Chen at al. (2005), who suggested that concrete drainage system are good artificial breeding habitats although the residential area is clean and with no water storage. Besides, the habit of storing water in containers for plant watering purposes are also good breeding habitats (Rozilawati et al., 2007).

On the other hand, *Aedes aegypti* were least found because they are indoor breeders, which is in contrast with the ovitrap surveillance site. They mainly breed in man-made containers within or in close proximity to household areas which explains the occupancy of *Aedes aegypti* in six ovitraps that were situated nearer to the flats in Taman Juloong. Besides, small number of *Aedes aegypti* found might be due to their egg laying behavior in which they oviposit in batches indoors and might have randomly ovipositted at the ovitraps nearby, leading to the occasional presence of a few *Aedes aegypti*. They possess high preference in feeding on humans rather than vertebrates. This increases their capability in the transmission of dengue viruses leading to them being the primary vector worldwide. Apart from that, low number of *Aedes aegypti* collected in this area may be due to the shorter collection period (over two consecutive days) as recommended egg collection time from containers were over three to four consecutive days (Wong et al., 2011). This is because *Aedes aegypti* generations overlap in the field, thus some females would be excluded if collection period was short (Wong et al., 2011). Besides, *Aedes aegypti* are more persistent in urban area as compared to suburban and rural areas (O'Meara et al., 1995). Therefore, Taman Juloong, being a suburban area, had lower numbers of *Aedes aegypti*. Furthermore, non-urban environments are cooler than surrounding of urban areas (McIntyre 2000) which favored towards *Aedes albopictus*.

As both *Aedes albopictus* and *Aedes aegypti* shared same habitat, competition among both species might occur. Several studies have shown that the invasion of *Aedes albopictus* causes decline in the population of *Aedes aegypti*, sometimes even until the extent of local extinction (Juliano et al., 2002). In southeastern United States, displacement of *Aedes aegypti* by *Aedes albopictus* was due to the greater survivorship (Barrera 1996) and better resource competitor (Juliano 1998) of *Aedes albopictus* as compared to *Aedes aegypti.*

As for *Aedes albolateralis*, they are commonly found in high canopies and preferred to feed on man and monkeys (Choochote et al., 2001). Being a jungle vector, they were found in ovitraps along the roads in Taman Juloong which were located among matured trees grown close together forming a canopy. As Taman Juloong is located on a hilly area which was formerly a forest, thus wild animals such as monkeys and squirrels were occasionally seen among the trees. Choochote et al. (2001) stated that *Aedes albolateralis*, a species member of *Aedes niveus* group, was found to be a potential vector of DHF and *Wuchereria bancrofti* which leads to LF in Thailand. In Malaysia however, *Aedes albolateralis* was rarely reported as the vector of diseases.

5.4 Environmental Factors and Mosquito Population

The large number of mosquitoes collected from this study may also be related to abiotic environment factors such as rainfall, temperature and humidity (Juliano et al., 2002).Mosquitoes were known to breed more during wet seasons (high rainfall) which is also associated with higher prevalence levels of mosquito diseases (Okogun et al., 2003). It was found that during wet seasons, the daily biting rates of mosquitoes were 314 mosquitoes per person but reduced to 94 in dry seasons (Almeidaa et al., 2005). Besides, raindrops may also stimulate hatching of eggs that are already present in the habitat by the vibration of water that causes agitation (Roberts 2001). However, frequent increase in rainfall will in turn cause flushing from their habitats, resulting in death of immature stages of mosquitoes (Rohani et al., 2010) and loss of eggs. In order to prevent flushing or habitat overflow, the ovitraps in this study were only filled a quarter to half full instead of three quarters full. Hence, most eggs were preserved.

In Taman Juloong, high peak of mosquitoes were observed one week after the peak of rainfall. During weeks with high rainfall, the mosquito eggs were flooded, which subsequently led to stimulation and hatching of eggs. The high number of adult mosquito's emergence will lead to increased oviposition activity during the following week. This correlates to the studies conducted by Rozilawati et al. (2007) whereby a high number of eggs were found after one week of large rainfall. Moreover, high number of mosquitoes was also seen during the secondary peaks of rainfall which indicated that moderate rainfall encouraged growth of mosquitoes.

Rainfall is an important factor affecting the immature stages of mosquitoes. Sufficient amount of rain fills the artificial habitats as well as creating natural water bodies (Koenraadt and Harrington 2008), thus making them potential breeding grounds for mosquitoes. Artificial containers in the housing area such as garbage cans, cemetery vases and natural containers such as tree holes collect rainwater and detritus (Kesavaraju et al., 2011). In Taman Juloong, pools of water were observed in artificial habitats such as the rubbish dumping sites, plastic containers and sauces of flower pots. In addition, the flight activities of mosquitoes were negatively affected when heavy rain is accompanied by strong winds (Rozilawati et al., 2007). This will obstruct them from searching for host and oviposition.

High humidity and low temperature conditions improved the survivorship of *Aedes albopictus* and decreased occupancy of *Aedes aegypti*. In this study, temperature and humidity played a minor role towards the number of mosquitoes as both factors remained similar throughout the three months study, in which low temperature and high humidity were recorded generally. Overall, this condition encouraged growth of mosquitoes. A climate that is wet and cool facilitated invasion by *Aedes albopictus* and reduced the population of *Aedes aegypti* via interspecific competition. Findings from Juliano et al. (2002) stated that *Aedes aegypti* occupancy of containers was lowest at cool sites with minimum dry season, but was highest during long dry seasons and high mean temperatures. In contrast, *Aedes albopictus* occupancy of containers was highest at cool or shaded sites with little or no dry season, but lowest when mean temperature and months that are dry increased. The results were similar to the findings of this study where *Aedes aegypti* population peaked during the lowest rainfall period in Week 12 and showed no occupancy during weeks with the highest rainfall and humidity. *Aedes albopictus* were found to be low in number during weeks with low rainfall and increased during moderate rainfalls. Moderately high number of *Aedes albopictus* was shown in weeks with high humidity and low temperature.

Furthermore, mosquitoes have been seen to increase in both full and partial shades (Brant 2011) and reduced if the shade was completely removed (Vythilingam et al., 2005). Similarly, an increase in mosquitoes was found in heavily shaded area around a pond while a decrease in mosquito population in bright, sunny area (Gingrich et al., 2006). This can be associated with temperature whereby non-shaded area which has extreme high temperature (higher than 34°C) decreased the survival rate of mosquito larvae (Brant 2011) as compared to shaded area with lower and optimum temperature for growth.

Large mosquito population may be due to the large amount of matured trees that formed a canopy, thus providing an ideal place for mosquito breeding.

Brant (2011) found that number of leaves and amount of shade in ovitraps will affect amount of eggs present. The larger the amount of leaves, the larger the number of eggs and larvae found in ovitraps. In addition, with the abundance of trees in Taman Juloong, leaf litters were seen in most areas. These had provided nutritious breeding ground for mosquitoes as they were full of essential organic carbon for growth of mosquito larvae (Strand et al., 1999). Moreover, microbial growth (fungi and bacteria) resulted from the nitrogen and protein dissolved from leaf litter was food source for mosquito larvae. *Aedes albolateralis* which prefers high canopy areas increased in number during high humidity and low temperatures.

O'Neal and Juliano (2013) suggested that seasonal variation, acting as a fitness-equalizing factor, also contributed to the coexistence of *Aedes albopictus* and *Aedes aegypti*. In dry season, detritus, a food source for mosquitoes, were found to be three times greater than that in wet season. Large food availability reduced competition between both species. This corresponded to the findings of this study where frequent rainfall in Taman Juloong decreased the amount of detritus, thus increasing competitive displacement of *Aedes aegypti*. Besides, competition intensity also varied among sites where *Aedes aegypti* only persisted at sites where there were lesser intense interspecific competition (Juliano 1998). Therefore the number of *Aedes albopictus* outnumbered other species in this study.

5.5 Mosquito Insecticide Resistance

Deltamethrin and permethrin were found still to be effective as vector control insecticides as they had the fastest KT_{50} and KT_{95} towards *Aedes albopictus*. This is because both insecticides are pyrethroids, which are the newer class of insecticides. This study however, contradicted to the study done by Loke et al. (2012), in which they found the *Aedes* species in Shah Alam, an area which had 14 dengue hotspots in 2009 to be resistant to permethrin. Intensive usage of permethrin in thermal fogging operations by the Shah Alam Municipality during dengue outbreaks has led to the emergence of resistant mosquito populations. Permethrin resistance is found to develop at a faster rate as compared with other insecticides. However, since Taman Juloong is not endemic for dengue, pyrethroids were not used in the fogging sessions conducted by the Majlis Daerah Kampar (MDK). Therefore, resistance among mosquitoes would not have developed.

In contrast, fenitrothion was the least effective among the other insecticides and showed evidence of resistance as its KT_{50} and $KT_{95}$was the slowest. Adult susceptibility levels to fenitrothion were assessed by the method of continuous exposure, since two-hour exposure had been insufficient to give any mortality in resistant strains. Fenitrothion, an organophosphate insecticide, although not used in the Malaysian Vector Program, is a popular insecticide for the agricultural sector (Nazni et al., 2005). However, fenitrothion under the trade name of Sumithion® is currently used as the insecticide for fogging in Kampar by MDK (Table 5.2).

Table 5.2: Types of insecticides used in fogging activities in Kampar (Azrul 2012).

Month	Insecticides used	
	Taman Juloong	**Taman Kampar Jaya**
January	-	Sumithion®
February	Sumithion®	-
March	-	Aqua Resigen®
April	-	-
May	-	-
June	-	-
July	Sumithion®	Aqua Resigen®
August	-	-
September	-	-
October	-	-
November	Malathion	-
December	-	-

-No fogging was conducted

Malathion and DDT demonstrated similar slow response rate and might not be as effective as compared to pyrethroids. Thus, mosquitoes in Taman Juloong might have a chance to develop resistance against both insecticides if they were continually used. Malathion and fenitrothion (Sumithion®) used in fogging activities in Taman Juloong may prove to be unsuitable insecticides to control mosquitoes in this area. Although resistance was detected, complete mortality was achieved for all tested *Aedes albopictus* within 24 hours. Hence, this indicates that Sumithion® and malathion are still effective under operational field conditions. However, further evaluation on insecticide efficacy is still needed by the local authorities to delay development of resistance.

Similarly, *Aedes aegypti* were susceptible to malathion and fenitrothion with 100% mortality during a study conducted in Shah Alam but were resistant to permethrin, especially field strains (Wan-Norafikah et al., 2010). This is due to the standard vector control activity in a particular area has similar control methods, types, frequency and insecticide amount used (Wan-Norafikah et al., 2010). *Culex quinquefasciatus* on the other hand, showed high levels of resistance to organophosphates, malathion, fenitrothion as well as DDT in Kuala Lumpur (Nazni et al., 2005). Extensive indoor and outdoor house spraying of malathion in dengue prone areas may be one of the factors contributing to resistance. In the study conducted by Hidayati et al. (2011), mosquito resistance to malathion exhibited cross resistance to DDT and fenitrothion, to which they had never been exposed. This may be due to the fact that both malathion and fenitrothion belonged to the same class of insecticide.

Cross resistance happens when an insect population that has developed resistance to an insecticide appears to be resistant to another insecticide that they have never encountered (Hidayati et al., 2011). The rate of cross resistance increases especially when insecticides of the same group which shares the common mode of action are frequently used. Cross resistance can be observed when insecticide resistance is able to extend to other insecticides with similar mode of action. For instance, pyrethroid and DDT, acts on the sodium channels of nerve sheath while organophosphates and carbamates target acetylcholinesterase in nerve synapse (Brogdon and McAllister 1998).

Cross resistance were evident when *Aedes aegypti* and *Aedes albopictus* collected from Shah Alam (Loke et al., 2012) and Kuala Lumpur (Lee et al., 1998) respectively, were resistant to permethrin and DDT. In addition, it was found that malathion developed cross resistance to a wide range of organophosphates and carbamates (Kasap et al., 2000), which were widely used by pest control operators (Lee et al., 2013). This was evident when propoxur (carbamate) was found to be cross resistant to malathion-resistant strain (Selvi et al., 2005). Carbamate (propoxur and bendiocarb) resistance was detected in Malaysia although they were never introduced in vector control programs. Therefore, mosquito population in Taman Juloong may possibly have increased chances of developing resistance to carbamates as well since they were only exposed to organophosphates. But in this study, the rate of cross reactivity was low among *Aedes albopictus* in Taman Juloong towards DDT and pyrethroids as both were not applied in this area.

The data obtained from this study can be used in making timely management decisions about the judicious choice of pesticides in a vector control program. Routine surveillance on resistance status of field mosquito populations is important to implement suitable strategies in order to prevent outbreaks. As the indicator of insecticide efficiency, the susceptibility status of mosquitoes against insecticides should be evaluated from time to time for better insecticide resistance management and control. Moreover, the rotational use of insecticides in fogging activities has to consider the mode of action of insecticides. This is to avoid occurrence of possible cross resistance among mosquitoes.

5.6 Future Works

Further research should be carried out on the minority species, *Aedes aegypti* and *Aedes albolateralis*, on their seasonal distribution as well as insecticide susceptibility status, as both species were not tested in this study due to their scarcity. In addition, a comparison on the surveillance between outdoor and indoor mosquitoes could be done so as to give a more significant mosquito resistant status in the area studied. Moreover, future works should be done in collaboration with the MDK vector control team to conduct field insecticide surveillance during pre-fogging and post-fogging periods. This will provide much better results on the insecticide effectiveness by taking into consideration the effects of environmental factors. Apart from that, factors that play a role in transmission can be studied in future using computerized mapping with global positioning systems (GPS) and geographical information systems (GIS)

(Staedke et al., 2003). When environmental factors are coupled with clinical data, it may be possible to identify populations, households or areas that carry the heaviest burden of dengue or are the most important potential contributors to dengue transmission. Consequently, the impact of dengue control efforts can be maximized by implementing tailored control measures to carefully selected areas.

CHAPTER 6

CONCLUSION

In this study, a total of 19,107 mosquitoes were collected in Taman Juloong which comprised of *Aedes albopictus*, the majority species, together with *Aedes aegypti* and *Aedes albolateralis*, the minority species. The high OI of *Aedes albopictus* indicated the presence of persistent breeding sites of mosquitoes which can be associated with the abundance of natural and artificial containers in Taman Juloong. Rainfalls were higher from Weeks 3 to 11 (15 October to 15 December) and mosquitoes were seen to increase one week after peaks of rainfall. Temperature and humidity were relatively constant throughout the study which recorded low temperature and high humidity. These factors encouraged growth of mosquitoes.

Pyrethroids, belonging to newer classes of insecticides, were still effective against the mosquito population in Taman Juloong. In contrast, organophosphates and organochlorines showed evidence of resistance. Therefore, the application of malathion and fenitrothion for controlling *Aedes albopictus* should be used cautiously and alternative routes such as insecticide rotation and integrated vector management (IVM) can be conducted in order to delay the development of insecticide resistance. Besides, improving the understanding of insecticide resistance and cross resistance mechanisms will help to develop successful vector control program for minimizing or preventing resistance development.

Indoor residual spraying with a long-lasting insecticide is the classical method of mosquito control. Indoor residual application can be effective provided the spray is complete and correct dosages are used. In Malaysia, it is important to control vector populations, as effective vaccines and anti-viral drugs against dengue are still unavailable.

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APPENDICES

Appendix A

Number of fogging activities carried out in Kampar District in 2012 (MDK 2013b)

Month	Number of fogging activities
January	10
February	6
March	3
April	1
May	19
June	2
July	6
August	7
September	7
October	2
November	7
December	7
Total	77

Appendix B

Number of mosquitoes collected in 13 surveillance weeks in Taman Juloong

Ovitraps	Weeks											
	1		2		3		4		5		6	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
1	0	0	0	0	2	1	19	12	16	12	34	76
2	0	0	2	0	7	9	6	15	18	6	14	68
3	0	0	3	3	14	29	10	20	6	44	12	52
4	0	0	5	15	2	3	16	21	20	0	0	10
5	0	1	3	1	5	5	3	23	7	9	0	4
6	1	0	23	38	4	1	3	46	8	36	20	28
7	0	0	0	0	1	5	10	3	38	28	9	40
8	11	11	8	13	7	2	24	5	50	26	11	33
9	19	4	26	31	25	10	6	3	40	50	7	34
10	10	3	19	21	4	3	13	17	27	10	0	24
11	24	17	17	16	8	4	4	12	7	1	3	8
12	17	9	16	17	19	21	4	21	21	20	4	22
13	12	7	15	14	15	19	21	2	13	16	16	6
14	11	7	5	3	2	1	16	12	17	2	0	5
15	26	0	15	22	7	11	1	31	6	4	0	0
16	13	39	26	14	13	21	2	10	10	60	12	56
17	10	12	6	47	13	10	3	14	12	5	0	4
18	14	9	18	70	3	0	11	17	6	25	2	4
19	0	0	0	1	6	18	18	20	30	50	24	63
20	27	4	32	17	15	47	2	28	37	39	22	10
21	0	1	0	1	3	0	0	0	1	1	0	0
22	31	22	25	36	18	35	1	20	0	0	0	0
23	15	28	12	19	11	3	2	3	11	1	1	65
24	7	40	1	11	3	0	11	14	0	3	0	0
25	19	7	0	4	0	4	13	11	4	2	0	3
26	0	0	8	10	2	6	12	16	8	0	9	58
27	15	10	7	13	8	4	3	1	6	26	11	55
28	5	5	4	4	7	3	1	8	4	2	2	16
29	3	2	12	18	9	8	19	14	15	23	19	51
30	13	8	22	33	5	15	1	27	0	8	1	2
31	14	12	4	13	7	7	10	5	20	1	10	52
32	17	21	0	1	4	3	9	20	5	3	0	0
33	54	23	7	24	8	6	0	0	2	35	9	54
34	12	8	9	5	8	3	1	0	9	1	0	0
35	23	14	0	0	3	4	11	10	12	26	6	8
36	0	2	2	2	11	26	2	3	17	0	14	83
37	15	22	20	64	17	35	8	7	18	45	2	32
38	7	7	9	16	15	25	6	22	13	8	0	1
39	13	12	1	3	1	1	22	18	9	4	0	1
40	3	7	11	19	14	18	3	19	16	5	1	24
41	0	0	0	6	7	14	2	2	0	0	0	0
42	21	34	0	1	6	9	7	3	9	3	0	5
43	3	4	4	2	2	4	3	2	10	25	12	9
44	2	5	0	0	8	10	5	14	5	3	0	1
45	15	21	0	1	9	31	2	14	7	6	0	5
46	13	11	17	31	2	2	6	18	0	1	0	29
47	11	3	21	42	2	2	11	8	17	4	0	0
48	4	17	5	4	1	0	3	22	6	0	2	11
49	19	25	19	26	2	3	3	11	15	6	1	0
50	13	8	15	9	3	0	3	13	11	0	4	5
51	1	0	14	17	7	5	0	0	3	9	0	0
52	34	61	33	9	8	5	11	24	9	32	31	38
53	40	6	11	41	12	27	10	11	12	21	10	35
54	0	0	19	32	57	11	15	3	28	52	20	48
55	14	14	7	37	4	8	17	12	1	10	0	1
56	19	10	17	33	9	4	1	1	8	33	2	0
57	12	35	25	31	10	59	4	24	0	19	2	39
58	16	16	1	0	4	8	6	16	0	3	0	0
59	23	20	5	0	7	14	11	11	10	7	9	31
60	7	4	29	32	2	2	2	4	4	4	1	0

Ovitraps	Week 7		8		9		10		11		12		13	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	1	9	10	25	35	5	20	8	58	27	14	14	5	2
2	37	54	0	6	96	6	15	1	118	48	20	0	0	0
3	9	25	5	34	0	4	4	6	13	30	10	10	13	9
4	25	41	7	22	49	6	31	0	44	76	21	16	2	3
5	1	4	2	8	6	0	13	14	5	2	8	1	3	9
6	0	1	12	43	15	13	18	8	30	61	9	0	11	16
7	5	35	16	14	4	6	0	1	8	35	3	1	2	7
8	7	35	10	21	10	9	19	10	50	37	14	0	12	32
9	8	19	15	66	54	6	36	51	79	71	15	0	20	19
10	9	13	7	32	1	3	13	14	31	51	3	6	31	19
11	4	10	9	7	8	6	6	3	7	10	1	0	10	13
12	0	12	39	22	7	10	21	16	13	1	39	7	0	0
13	12	34	45	9	0	4	41	27	0	3	11	16	13	27
14	6	7	0	3	18	11	3	13	9	11	5	2	0	3
15	2	51	5	22	5	15	37	5	9	6	5	7	10	17
16	13	30	10	6	16	6	7	5	38	31	19	43	9	19
17	13	19	13	5	9	8	11	6	0	0	12	5	14	22
18	13	15	3	8	1	0	10	8	6	13	16	19	3	0
19	1	10	6	15	36	11	7	10	4	8	7	1	3	3
20	2	13	2	14	11	5	10	12	5	12	18	0	26	25
21	4	10	0	2	0	2	0	1	0	2	1	2	2	3
22	0	0	0	0	0	2	5	3	0	0	6	9	13	6
23	12	20	48	11	21	15	22	6	47	36	11	1	10	2
24	1	14	7	14	16	24	6	9	3	5	9	7	3	9
25	1	8	10	22	1	5	0	2	1	0	2	2	3	7
26	2	9	69	5	9	4	13	19	6	11	0	0	6	0
27	16	22	20	14	9	5	15	12	4	6	11	6	11	2
28	6	14	14	4	2	3	12	3	19	20	55	40	9	24
29	37	76	14	78	43	7	55	11	21	57	5	32	35	32
30	5	12	2	12	4	7	16	5	12	42	13	10	5	9
31	4	19	11	21	5	13	48	18	23	20	8	16	5	5
32	1	6	0	1	20	9	22	25	5	5	5	3	5	9
33	32	44	9	29	15	23	3	14	82	24	25	13	10	16
34	3	3	33	13	3	1	5	2	7	5	1	2	11	14
35	20	17	1	0	12	4	1	4	19	18	12	7	6	0
36	19	52	11	34	7	0	0	0	1	26	16	45	6	27
37	23	52	5	16	11	4	2	3	1	10	15	59	3	3
38	2	13	1	2	0	0	11	4	10	31	7	3	3	4
39	6	14	1	34	0	3	3	4	0	6	10	10	13	6
40	12	13	4	2	19	7	2	14	12	7	1	2	0	2
41	6	8	7	4	7	12	0	0	1	5	0	2	0	2
42	13	52	9	27	4	10	2	0	19	13	15	14	5	4
43	6	18	3	3	2	1	4	1	6	3	0	1	4	0
44	15	35	5	17	1	8	4	2	6	3	7	2	5	9
45	5	17	11	22	5	9	1	1	3	9	21	9	9	11
46	0	4	4	14	9	11	5	8	0	0	2	10	4	8
47	12	13	6	3	3	4	3	10	11	8	1	3	15	8
48	13	20	9	11	5	6	3	1	18	18	2	3	3	2
49	6	30	13	11	1	5	10	8	5	11	4	7	7	20
50	1	1	6	8	3	7	1	1	13	7	1	5	5	6
51	2	3	4	3	8	17	0	0	3	6	3	11	2	2
52	4	27	28	33	12	31	41	20	36	17	8	22	7	5
53	20	29	18	9	5	12	10	11	39	20	3	6	6	9
54	16	101	2	0	20	0	54	0	43	30	79	2	49	15
55	5	11	24	18	3	0	11	9	58	38	4	0	3	12
56	0	9	2	6	6	7	12	21	8	10	13	11	0	3
57	5	12	4	11	6	5	49	44	0	0	3	10	0	14
58	1	3	4	10	9	16	3	3	10	8	19	12	4	7
59	9	15	11	32	41	0	7	6	2	0	8	13	15	18
60	0	2	4	15	12	5	7	2	37	40	0	0	2	5

Appendix C

Meteorological Data for Year 2012

Month	Day	Rainfall (mm)	Mean Humidity (%)	Mean Temperature (°C)
10	1	0.2	76.7	27.8
10	2	0.0	70.5	27.7
10	3	0.0	73.1	28.1
10	4	0.0	75.2	27.2
10	5	0.0	69.4	27.5
10	6	3.2	68.7	28.1
10	7	0.2	79.9	26.5
10	8	0.0	75.7	27.0
10	9	5.2	75.7	27.6
10	10	3.2	85.1	26.3
10	11	1.0	86.4	25.8
10	12	1.2	85.3	26.2
10	13	30.2	94.0	24.6
10	14	0.8	80.5	26.8
10	15	14.8	86.6	26.4
10	16	61.6	86.2	26.1
10	17	15.0	84.0	26.4
10	18	0.4	86.7	26.2
10	19	26.2	90.6	25.1
10	20	5.6	81.0	26.9
10	21	5.0	92.9	25.2
10	22	0.2	84.8	26.2
10	23	0.0	78.7	27.7
10	24	0.0	75.0	28.6
10	25	9.8	84.7	26.8
10	26	37.8	83.4	27.2
10	27	0.0	78.6	28.0
10	28	0.4	76.5	27.9
10	29	16.6	82.7	27.4
10	30	18.0	91.9	25.7
10	31	25.8	90.8	25.6
11	1	0.4	89.1	25.8
11	2	59.4	84.5	26.3
11	3	18.6	79.8	26.3
11	4	91.8	86.5	25.3
11	5	11.4	85.9	25.9
11	6	14.6	89.1	25.4
11	7	2.6	83.8	26.4
11	8	2.2	84.7	26.4
11	9	9.2	91.5	25.6
11	10	13.0	89.9	25.8
11	11	26.8	94.3	24.9
11	12	11.6	87.3	26.1
11	13	50.8	85.5	26.1
11	14	4.2	78.6	27.7
11	15	0.0	78.1	28.7
11	16	0.0	74.2	29.4
11	17	0.0	79.5	27.9
11	18	22.8	84.0	27.3
11	19	0.8	84.7	26.4
11	20	8.6	81.5	27.4
11	21	7.8	85.0	26.6
11	22	4.2	84.5	27.1
11	23	43.6	93.0	25.9
11	24	0.4	85.5	26.0
11	25	0.2	75.6	27.7
11	26	25.2	87.1	26.2
11	27	49.8	84.4	26.6
11	28	2.8	82.7	27.3
11	29	27.4	89.7	26.2
11	30	0.6	84.4	27.3
12	1	61.6	88.0	26.0
12	2	10.0	87.2	26.3
12	3	3.6	89.8	25.6
12	4	0.4	79.4	27.3
12	5	0.2	84.7	26.5
12	6	41.8	82.7	26.5
12	7	3.0	86.1	26.0
12	8	1.6	81.2	26.9

12	9	45.6	88.6	25.5
12	10	67.8	90.2	25.2
12	11	54.0	90.2	25.1
12	12	0.4	86.5	26.1
12	13	0.2	83.2	26.9
12	14	4.2	85.1	26.6
12	15	0.2	86.9	26.3
12	16	6.2	86.0	26.3
12	17	0.6	84.5	26.7
12	18	0.8	87.6	26.2
12	19	0.0	83.3	26.5
12	20	0.0	81.1	27.2
12	21	2.8	84.6	26.7
12	22	0.2	87.2	26.1
12	23	2.0	85.7	26.3
12	24	0.0	84.3	26.3
12	25	0.2	86.4	25.9
12	26	1.4	80.6	26.8
12	27	9.6	89.7	26.0
12	28	0.6	85.8	26.2
12	29	1.0	80.1	27.3
12	30	2.6	84.0	26.9
12	31	2.4	93.5	24.3

Appendix D

Pearson Correlation

Correlation of *Aedes albopictus* with rainfall

Descriptive Statistics

	Mean	Std. Deviation	N
Rainfall	12.2549	9.69235	13
Mean	24.3954	5.45253	13

Correlations

		Rainfall	Mean
Rainfall	Pearson Correlation	1	.241
	Sig. (2-tailed)		.427
	N	13	13
Mean	Pearson Correlation	.241	1
	Sig. (2-tailed)	.427	
	N	13	13

Correlation of *Aedes aegypti* with rainfall

Descriptive Statistics

	Mean	Std. Deviation	N
Rainfall	12.2549	9.69235	13
Mean	.0169	.03706	13

Correlations

		Rainfall	Mean
Rainfall	Pearson Correlation	1	-.363
	Sig. (2-tailed)		.223
	N	13	13
Mean	Pearson Correlation	-.363	1
	Sig. (2-tailed)	.223	
	N	13	13

Correlation of *Aedes albolateralis* with rainfall

Descriptive Statistics

	Mean	Std. Deviation	N
Rainfall	12.2549	9.69235	13
Mean	.0831	.15008	13

Correlations

		Rainfall	Mean
Rainfall	Pearson Correlation	1	.032
	Sig. (2-tailed)		.917
	N	13	13
Mean	Pearson Correlation	.032	1
	Sig. (2-tailed)	.917	
	N	13	13

Appendix E

Probit Analysis of DDT

Data Information

		N of Cases
Valid		225
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	20	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Time	3.706	.049	75.339	.000	3.610	3.802
	Intercept	-6.659	.099	-67.078	.000	-6.758	-6.560

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

Chi-Square Tests

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	305.637	223	.000[b]

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

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

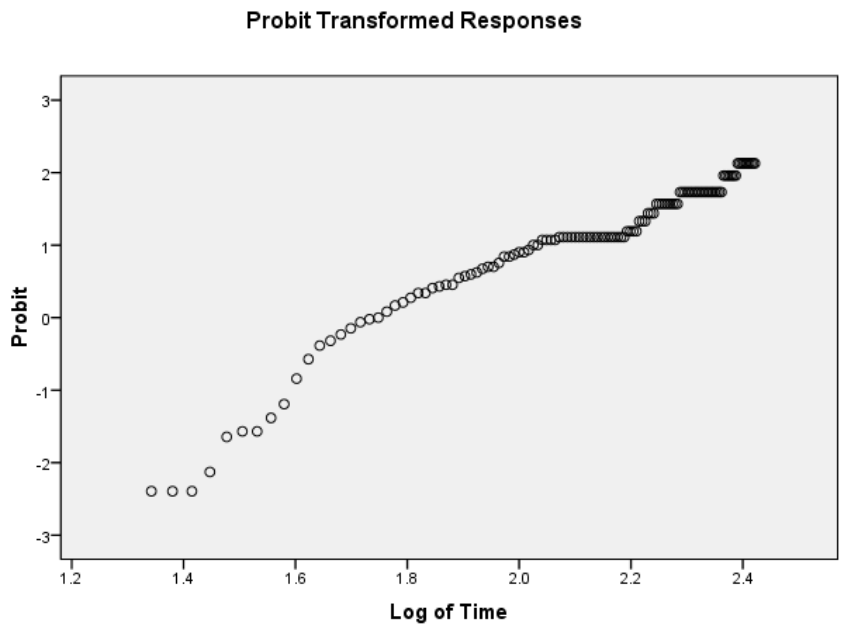
Confidence Limits

	Probability	95% Confidence Limits for Time			95% Confidence Limits for log(Time)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	0.01	14.759	13.850	15.672	1.169	1.141	1.195
	0.02	17.483	16.490	18.477	1.243	1.217	1.267
	0.03	19.467	18.419	20.511	1.289	1.265	1.312
	0.04	21.106	20.017	22.189	1.324	1.301	1.346
	0.05	22.540	21.418	23.655	1.353	1.331	1.374
	0.06	23.838	22.688	24.978	1.377	1.356	1.398
	0.07	25.037	23.863	26.200	1.399	1.378	1.418
	0.08	26.161	24.966	27.345	1.418	1.397	1.437
	0.09	27.228	26.014	28.429	1.435	1.415	1.454
	0.1	28.248	27.016	29.466	1.451	1.432	1.469
	0.15	32.895	31.594	34.177	1.517	1.500	1.534
	0.2	37.127	35.777	38.458	1.570	1.554	1.585

	0.25	41.190	39.799	42.558	1.615	1.600	1.629
	0.3	45.216	43.792	46.616	1.655	1.641	1.669
	0.35	49.297	47.844	50.726	1.693	1.680	1.705
	0.4	53.509	52.030	54.965	1.728	1.716	1.740
	0.45	57.927	56.422	59.411	1.763	1.751	1.774
	0.5	62.631	61.099	64.146	1.797	1.786	1.807
	0.55	67.717	66.152	69.268	1.831	1.821	1.841
	0.6	73.308	71.702	74.906	1.865	1.856	1.875
	0.65	79.572	77.909	81.235	1.901	1.892	1.910
	0.7	86.754	85.009	88.511	1.938	1.929	1.947
	0.75	95.233	93.364	97.131	1.979	1.970	1.987
	0.8	105.653	103.587	107.773	2.024	2.015	2.033
	0.85	119.248	116.850	121.735	2.076	2.068	2.085
	0.9	138.864	135.859	142.024	2.143	2.133	2.152
	0.91	144.067	140.878	147.430	2.159	2.149	2.169
	0.92	149.941	146.534	153.544	2.176	2.166	2.186
	0.93	156.676	153.008	160.567	2.195	2.185	2.206
	0.94	164.556	160.568	168.802	2.216	2.206	2.227
	0.95	174.028	169.634	178.723	2.241	2.230	2.252
	0.96	185.856	180.928	191.141	2.269	2.258	2.281
	0.97	201.504	195.828	207.619	2.304	2.292	2.317
	0.98	224.363	217.519	231.776	2.351	2.337	2.365
	0.99	265.771	256.621	275.754	2.425	2.409	2.441

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix F

Probit Analysis of Fenitrothion

Data Information

		N of Cases
Valid		225
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	17	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Time	5.437	.058	93.109	.000	5.322	5.551
	Intercept	-12.549	.138	-91.058	.000	-12.687	-12.411

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

Chi-Square Tests

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	5021.951	223	.000[b]

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

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

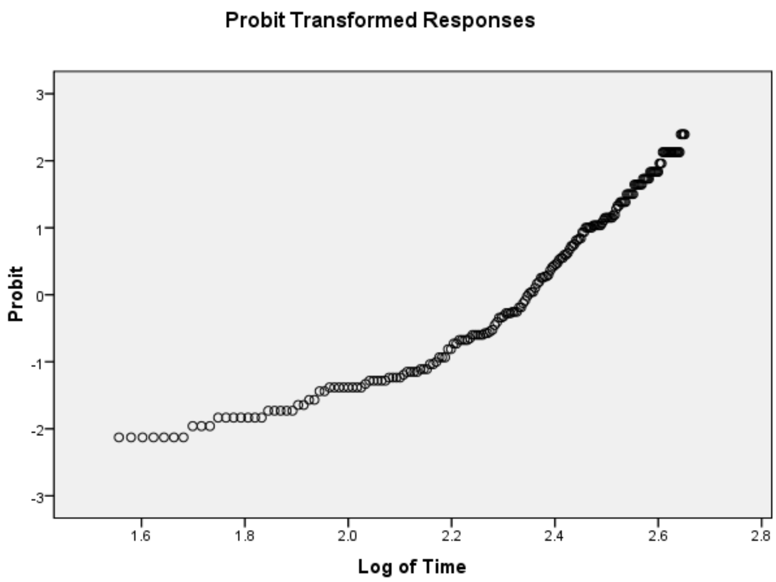
Confidence Limits

	Probability	95% Confidence Limits for Time			95% Confidence Limits for log(Time)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	0.01	75.910	66.712	84.479	1.880	1.824	1.927
	0.02	85.200	75.785	93.901	1.930	1.880	1.973
	0.03	91.675	82.163	100.427	1.962	1.915	2.002
	0.04	96.868	87.307	105.640	1.986	1.941	2.024
	0.05	101.309	91.724	110.083	2.006	1.962	2.042
	0.06	105.249	95.656	114.017	2.022	1.981	2.057
	0.07	108.829	99.238	117.585	2.037	1.997	2.070
	0.08	112.138	102.557	120.878	2.050	2.011	2.082
	0.09	115.234	105.669	123.956	2.062	2.024	2.093
	0.1	118.160	108.614	126.861	2.072	2.036	2.103
	0.15	131.086	121.674	139.671	2.118	2.085	2.145

	0.2	142.360	133.110	150.830	2.153	2.124	2.178
	0.25	152.802	143.719	161.174	2.184	2.158	2.207
	0.3	162.830	153.902	171.135	2.212	2.187	2.233
	0.35	172.710	163.912	180.991	2.237	2.215	2.258
	0.4	182.638	173.930	190.957	2.262	2.240	2.281
	0.45	192.786	184.110	201.225	2.285	2.265	2.304
	0.5	203.324	194.599	211.991	2.308	2.289	2.326
	0.55	214.439	205.553	223.475	2.331	2.313	2.349
	0.6	226.354	217.164	235.948	2.355	2.337	2.373
	0.65	239.366	229.680	249.761	2.379	2.361	2.398
	0.7	253.889	243.455	265.408	2.405	2.386	2.424
	0.75	270.552	259.027	283.637	2.432	2.413	2.453
	0.8	290.396	277.294	305.686	2.463	2.443	2.485
	0.85	315.372	299.931	333.878	2.499	2.477	2.524
	0.9	349.872	330.697	373.482	2.544	2.519	2.572
	0.91	358.755	338.542	383.785	2.555	2.530	2.584
	0.92	368.662	347.257	395.322	2.567	2.541	2.597
	0.93	379.871	357.079	408.430	2.580	2.553	2.611
	0.94	392.792	368.355	423.610	2.594	2.566	2.627
	0.95	408.067	381.624	441.644	2.611	2.582	2.645
	0.96	426.774	397.792	463.854	2.630	2.600	2.666
	0.97	450.950	418.565	492.747	2.654	2.622	2.693
	0.98	485.222	447.800	534.041	2.686	2.651	2.728
	0.99	544.604	497.943	606.430	2.736	2.697	2.783

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix G

Probit Analysis of Malathion

Data Information

		N of Cases
Valid		225
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	19	Yes

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Time	4.671	.068	68.579	.000	4.538	4.805
	Intercept	-8.395	.132	-63.366	.000	-8.528	-8.263

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

Chi-Square Tests

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	292.004	223	.001[b]

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

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

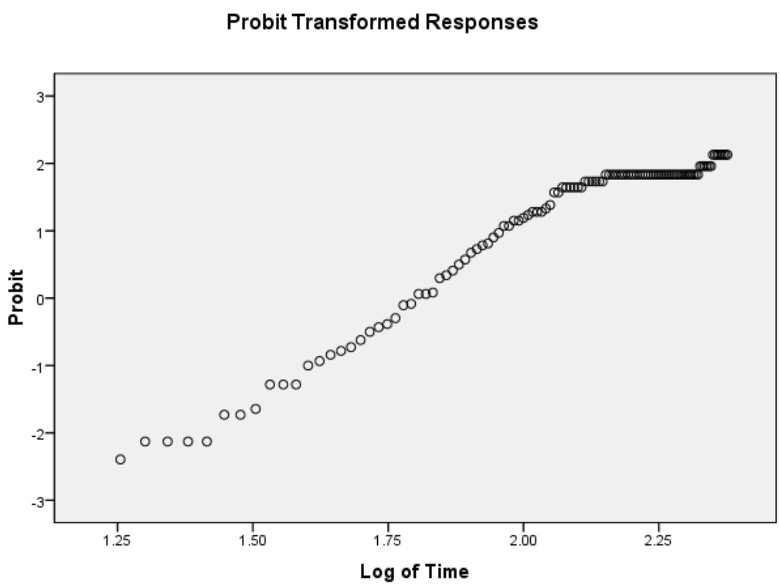
Confidence Limits

	Probability	95% Confidence Limits for Time			95% Confidence Limits for log(Time)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	0.01	19.915	18.893	20.927	1.299	1.276	1.321
	0.02	22.780	21.703	23.842	1.358	1.337	1.377
	0.03	24.807	23.698	25.899	1.395	1.375	1.413
	0.04	26.450	25.318	27.563	1.422	1.403	1.440
	0.05	27.866	26.716	28.996	1.445	1.427	1.462
	0.06	29.132	27.967	30.274	1.464	1.447	1.481
	0.07	30.288	29.112	31.442	1.481	1.464	1.498
	0.08	31.363	30.176	32.525	1.496	1.480	1.512
	0.09	32.373	31.178	33.544	1.510	1.494	1.526
	0.1	33.332	32.129	34.509	1.523	1.507	1.538
	0.15	37.613	36.383	38.815	1.575	1.561	1.589

	0.2	41.404	40.157	42.622	1.617	1.604	1.630
	0.25	44.959	43.702	46.188	1.653	1.641	1.665
	0.3	48.412	47.147	49.649	1.685	1.673	1.696
	0.35	51.847	50.576	53.093	1.715	1.704	1.725
	0.4	55.332	54.054	56.587	1.743	1.733	1.753
	0.45	58.927	57.641	60.193	1.770	1.761	1.780
	0.5	62.692	61.394	63.975	1.797	1.788	1.806
	0.55	66.698	65.382	68.006	1.824	1.815	1.833
	0.6	71.031	69.686	72.374	1.851	1.843	1.860
	0.65	75.806	74.418	77.200	1.880	1.872	1.888
	0.7	81.185	79.732	82.657	1.909	1.902	1.917
	0.75	87.420	85.866	89.006	1.942	1.934	1.949
	0.8	94.927	93.217	96.690	1.977	1.969	1.985
	0.85	104.495	102.535	106.539	2.019	2.011	2.028
	0.9	117.915	115.519	120.446	2.072	2.063	2.081
	0.91	121.407	118.883	124.079	2.084	2.075	2.094
	0.92	125.318	122.645	128.156	2.098	2.089	2.108
	0.93	129.763	126.914	132.798	2.113	2.104	2.123
	0.94	134.915	131.852	138.187	2.130	2.120	2.140
	0.95	141.041	137.712	144.608	2.149	2.139	2.160
	0.96	148.594	144.922	152.543	2.172	2.161	2.183
	0.97	158.437	154.293	162.910	2.200	2.188	2.212
	0.98	172.537	167.676	177.810	2.237	2.224	2.250
	0.99	197.351	191.128	204.148	2.295	2.281	2.310

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix H

Probit Analysis of Deltamethrin

Data Information

		N of Cases
Valid		225
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	20	No[a]

a. Parameter estimates did not converge.

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Tme	3.390	.083	40.721	.000	3.227	3.553
	Intercept	-4.098	.123	-33.271	.000	-4.221	-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[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	356.656	223	.000[b]

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

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

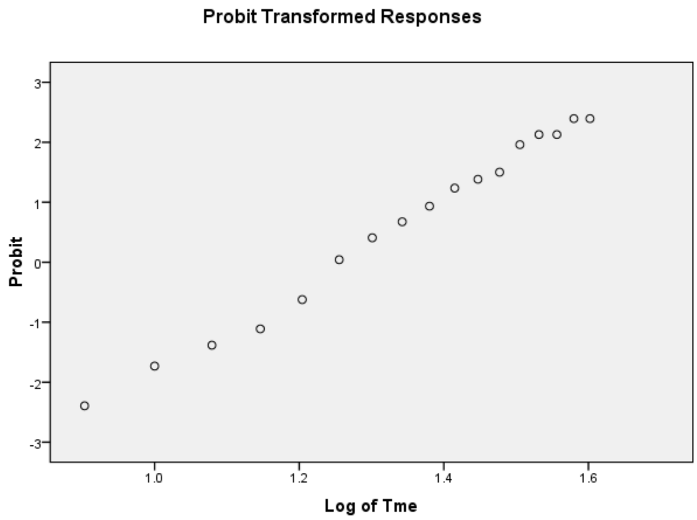
Confidence Limits

	Probability	95% Confidence Limits for Tme			95% Confidence Limits for log(Tme)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	0.01	3.331	2.878	3.793	.523	.459	.579
	0.02	4.008	3.504	4.520	.603	.545	.655
	0.03	4.508	3.969	5.051	.654	.599	.703
	0.04	4.925	4.359	5.492	.692	.639	.740
	0.05	5.292	4.704	5.879	.724	.672	.769
	0.06	5.626	5.019	6.230	.750	.701	.794
	0.07	5.936	5.313	6.555	.773	.725	.817
	0.08	6.228	5.590	6.861	.794	.747	.836
	0.09	6.506	5.855	7.151	.813	.768	.854
	0.1	6.773	6.110	7.429	.831	.786	.871
	0.15	8.000	7.286	8.702	.903	.862	.940

	0.2	9.132	8.379	9.870	.961	.923	.994
	0.25	10.230	9.444	10.998	1.010	.975	1.041
	0.3	11.328	10.514	12.123	1.054	1.022	1.084
	0.35	12.450	11.611	13.270	1.095	1.065	1.123
	0.4	13.618	12.754	14.462	1.134	1.106	1.160
	0.45	14.852	13.965	15.721	1.172	1.145	1.196
	0.5	16.176	15.264	17.071	1.209	1.184	1.232
	0.55	17.617	16.679	18.544	1.246	1.222	1.268
	0.6	19.213	18.244	20.177	1.284	1.261	1.305
	0.65	21.015	20.007	22.028	1.323	1.301	1.343
	0.7	23.098	22.036	24.176	1.364	1.343	1.383
	0.75	25.577	24.439	26.750	1.408	1.388	1.427
	0.8	28.652	27.396	29.971	1.457	1.438	1.477
	0.85	32.706	31.255	34.264	1.515	1.495	1.535
	0.9	38.631	36.822	40.628	1.587	1.566	1.609
	0.91	40.216	38.299	42.346	1.604	1.583	1.627
	0.92	42.012	39.965	44.300	1.623	1.602	1.646
	0.93	44.080	41.876	46.559	1.644	1.622	1.668
	0.94	46.509	44.113	49.225	1.668	1.645	1.692
	0.95	49.444	46.802	52.461	1.694	1.670	1.720
	0.96	53.129	50.162	56.546	1.725	1.700	1.752
	0.97	58.039	54.612	62.022	1.764	1.737	1.793
	0.98	65.274	61.122	70.156	1.815	1.786	1.846
	0.99	78.553	72.949	85.249	1.895	1.863	1.931

a. A heterogeneity factor is used.

b. Logarithm base = 10.



Appendix I

Probit Analysis of Permethrin

Data Information

		N of Cases
Valid		225
Rejected	Missing	0
	LOG Transform Cannot be Done	0
	Number of Responses > Number of Subjects	0
Control Group		0

Convergence Information

	Number of Iterations	Optimal Solution Found
PROBIT	20	No[a]

a. Parameter estimates did not converge.

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Time	3.335	.078	43.001	.000	3.183	3.487
	Intercept	-4.146	.118	-35.132	.000	-4.264	-4.028

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

Chi-Square Tests

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	287.502	223	.002[b]

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

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

Confidence Limits

	Probability	95% Confidence Limits for Time			95% Confidence Limits for log(Time)[b]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT[a]	0.01	3.514	3.100	3.937	.546	.491	.595
	0.02	4.242	3.778	4.711	.628	.577	.673
	0.03	4.780	4.284	5.280	.679	.632	.723
	0.04	5.229	4.708	5.752	.718	.673	.760
	0.05	5.626	5.084	6.168	.750	.706	.790
	0.06	5.987	5.427	6.545	.777	.735	.816
	0.07	6.322	5.747	6.895	.801	.759	.839
	0.08	6.639	6.049	7.225	.822	.782	.859
	0.09	6.940	6.338	7.538	.841	.802	.877
	0.1	7.230	6.616	7.839	.859	.821	.894
	0.15	8.563	7.901	9.217	.933	.898	.965

0.2	9.796	9.096	10.485	.991	.959	1.021
0.25	10.994	10.263	11.713	1.041	1.011	1.069
0.3	12.195	11.436	12.940	1.086	1.058	1.112
0.35	13.424	12.640	14.193	1.128	1.102	1.152
0.4	14.705	13.897	15.498	1.167	1.143	1.190
0.45	16.060	15.230	16.877	1.206	1.183	1.227
0.5	17.516	16.662	18.358	1.243	1.222	1.264
0.55	19.104	18.224	19.975	1.281	1.261	1.300
0.6	20.865	19.954	21.771	1.319	1.300	1.338
0.65	22.856	21.907	23.807	1.359	1.341	1.377
0.7	25.159	24.160	26.172	1.401	1.383	1.418
0.75	27.907	26.834	29.007	1.446	1.429	1.463
0.8	31.321	30.136	32.556	1.496	1.479	1.513
0.85	35.831	34.459	37.287	1.554	1.537	1.572
0.9	42.439	40.724	44.304	1.628	1.610	1.646
0.91	44.210	42.389	46.199	1.646	1.627	1.665
0.92	46.217	44.272	48.354	1.665	1.646	1.684
0.93	48.530	46.434	50.846	1.686	1.667	1.706
0.94	51.250	48.968	53.788	1.710	1.690	1.731
0.95	54.539	52.020	57.360	1.737	1.716	1.759
0.96	58.674	55.839	61.871	1.768	1.747	1.791
0.97	64.190	60.908	67.920	1.807	1.785	1.832
0.98	72.333	68.344	76.911	1.859	1.835	1.886
0.99	87.314	81.905	93.609	1.941	1.913	1.971

a. A heterogeneity factor is used.

b. Logarithm base = 10.