COMPARATIVE TOXICITY AND PERSISTENCE OF PYRIPROXYFEN, DIFLUBENZURON, AND TEMEPHOS AGAINST *Aedes aegypti* (L.).

by

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LIST OF ABBREVIATIONS

Bsp	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis israelensis*
CSIs	Chitin Synthesis Inhibitors
DDT	Dichlorodiphenyltrichloroethane
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DNA	Deoxyribonucleic acid
DSS	Dengue Shock Syndrome
EI	Emergence Inhibition
GST	Gluthathione-S-transferases
IVM	Integrated Vector Management
JHAs	Juvenile Hormone Analogues
Kow	Octanol-Water Partition Coefficient
LC	Lethal Concentration
OPs	Organophosphates
SC	Serosal Cuticle
SIT	Sterile Insect Technique
TEPP	Tetraethyl Pyrophosphate
VCRU	Vector Control Research Unit
YF	Yellow Fever

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PERBANDINGAN KETOKSIKAN DAN KESAN SISA PYRIPROXYFEN, DIFLUBENZURON DAN TEMEPHOS TERHADAP *Aedes aegypti* (L.).

ABSTRAK

Kesan analog hormone juvenil, pyriproxyfen dan perencat sintesis chitin, diflubenzuron berbanding dengan racun serangga konvensional organofosfat, temephos telah dikaji terhadap larva *Aedes aegypti* strain VCRU. Gred teknikal telah digunakan sepanjang kajian ini. Bioasai makmal telah dijalankan mengikut kaedah yang dicadangkan oleh World Health Organisation (WHO) tahun 2005. Dalam bioasai makmal, pyriproxyfen didapati paling berkesan terhadap *Ae. aegypti* larva, dengan menunjukkan nilai EI_{50} yang paling rendah, iaitu 0.00031 (0.00026 – 0.00036) ppm. Keberkesanan diflubenzuron pula adalah sederhana dengan memberikan nilai EI_{50} 0.00144 (0.00129 – 0.00159) ppm. Nilai LC_{50} yang diperolehi daripada temephos pula paling tinggi, iaitu 0.00363 (0.00349 – 0.00375) ppm. Ini menunjukkan bahawa keberkesanan temephos adalah paling tidak memuaskan berbanding dengan pyriproxyfen dan diflubenzuron dalam keadaan makmal. Dengan membandingkan nilai-nilai EI_{50} atau LC_{50} masing-masing, pyriproxyfen adalah 11.7 kali ganda lebih berkesan daripada temephos. Sebaliknya, keberkesanan diflubenzuron adalah 2.52 kali ganda lebih tinggi daripada temephos. Di samping itu, kajian lapangan tersimulasi juga telah dijalankan untuk mengkaji kesan residu ketiga-tiga larvisid tersebut bawah keadaan penukaran air atau tanpa penukaran air. Dalam kajian ini, setiap larvicid digunakan pada kadar dua kali ganda LC_{99} masing-masing, terhadap 40 larva instar ketiga dalam bekas plastik yang mengandungi 40 liter air. Kepekatan diflubenzuron pada 0.0035 ppm, temephos pada 0.015 ppm dan pyriproxyfen pada 0.013 ppm telah diuji. Larva yang telah dirawat digantikan dengan kumpulan larva yang baru bagi setiap minggu. Sejumlah 10 kumpulan larva telah

dikaji sepanjang kajian tersebut. Bawah keadaan tanpa penukaran air, diflubenzuron menunjukkan kesan sisa yang paling memuaskan di mana 100 % EI telah tercapai selama 5 minggu selepas dirawat. Sebaliknya, bekas yang dirawat dengan pyriproxyfen hanya menunjukkan 100 % EI terhadap larva yang menerima rawatan pada hari pertama. Temephos pula memberikan 100 % EI selama 4 minggu. Walau bagaimanapun, kedua-dua temephos dan diflubenzuron memberikan 100 % EI selama 3 minggu bawah keadaan penukaran air. Pyriproxyfen hanya memberikan 100 % EI terhadap larva yang didedahkan pada hari pertama. Kesan ketiga-tiga larvisid yang diuji terhadap aktiviti bertelur juga telah dikaji dalam keadaan makmal. Analisis statistik menunjukkan bahawa tiada kesan yang ketara bagi ketiga-tiga larvisid yang diuji terhadap *Ae. aegypti*. Sebagai kesimpulan, diflubenzuron berpotensi sebagai pengganti temephos dalam kawalan nyamuk *Aedes* kerana ia menunjukkan keberkesanan yang memuaskan serta memberi kesan residu yang panjang.

COMPARATIVE TOXICITY AND PERSISTENCE OF PYRIPROXYFEN, DIFLUBENZURON AND TEMEPHOS AGAINST *Aedes aegypti* (L.).

ABSTRACT

The effects of the juvenile hormone analogue (JHA), pyriproxyfen, and chitin synthesis inhibitor (CSI), diflubenzuron, in comparison with the conventional organophosphate insecticide, temephos were tested against Vector Control Research Unit (VCRU) strain of *Aedes aegypti*. Technical-grades were used throughout the study. A laboratory bioassay was conducted following the standard method recommended by World Health Organization (WHO) year 2005. According to the study, pyriproxyfen was found to be the most effective against *Ae. aegypti* larvae, with the lowest EI_{50} value of 0.00031 (0.00026 – 0.00036) ppm. While diflubenzuron showed a more tolerant effect with an EI_{50} value of 0.00144 (0.00129 – 0.00159) ppm. Temephos recorded the highest LC_{50} value, which is 0.00363 (0.00349 – 0.00375) ppm, showing a lower larvicidal efficacy against *Ae. aegypti* larvae under laboratory condition. By considering the EI_{50} or LC_{50} value for each larvicide, the efficacy of pyriproxyfen was 11.7 times greater compared to temephos while the efficacy of diflubenzuron was 2.52 times greater. A simulated field trial was also conducted to evaluate the residual activity of the insecticide under both replenished and non-replenished regimes. In this study, each larvicide was applied at their respective diagnostic concentration, which is two times that of their LC_{99} value, against 40 3rd instar larvae placed in a 50 liter plastic container holding 40 liter of seasoned water. A concentration of 0.035 ppm diflubenzuron, 0.015 ppm temephos and 0.013 ppm pyriproxyfen were assessed. Exposed larvae were removed weekly and a new batch of larvae was introduced into the treated container. A total of 10 batches of larvae were tested throughout the study. Under a non-replenished

condition, diflubenzuron provided an excellent residual activity where 100 % EI was obtained for 5 weeks post-treatment. On the other hand, containers treated with pyriproxyfen showed complete emergence inhibition only for the 1st batch of larvae. While in the treatment with temephos, complete emergence inhibition was observed which lasted for 4 weeks post-treatment. However, both temephos and diflubenzuron showed complete emergence inhibition up to 3 weeks post treatment under a replenished condition. While pyriproxyfen provided 100 % emergence inhibition only on the first batch of larvae. The oviposition-deterrent and ovicidal effects of all the test larvicides were also evaluated under laboratory conditions. Statistical analysis showed that there was no significant effect on oviposition – deterrent and ovicidal activity of all the test larvicides against *Ae. aegypti.* As a conclusion, diflubenzuron has the potential to serve as a substitute for temephos due to its good larvicidal efficacy and excellent residual activity against *Ae. aegypti*.

CHAPTER ONE

GENERAL INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are currently one of the major international health problems worldwide, where Southeast Asia and the Western Pacific are most seriously affected (WHO, 2002). In recent years, transmission of these diseases has increased dramatically where half of the world's population in 124 endemic countries is at risk. It was also estimated that, there may be around 50 - 100 million cases and thousands of death every year as a consequence of the viral infection. The only way to reduce or prevent the transmission of these diseases depends solely on an effective vector control measure in which *Aedes aegypti* is the primary vector for the viral infection (WHO, 2012a).

Chikungunya fever is another arthropod-borne disease transmitted by *Ae. aegypti*. Between December, 1998 and February, 1999, there was an outbreak of this infection in Klang, Malaysia (Lam *et al*., 2001). After 7 years of nondetection, there was a second known outbreak in Bagan Panchor, northwest of Malaysia, from March through April of year 2006. It infected at least 200 villagers, but no death was reported (Abu Bakar *et al*., 2007). Besides, another arthropod-borne disease spread by *Aedes* mosquito is yellow fever (YF) where tropical areas of Africa and South America are more seriously affected. However, it is believed that there are some areas with no reported cases due to the availability of vaccine in the population or poor surveillance of the disease (WHO, 2012b).

In general, there are several approaches to vector control where the use of chemical is the most effective and widely used method. In chemical control, adulticides and larvicides are used to control the adult and larval stages of the

mosquitoes, respectively (Yap *et al*., 2003). However, larvicides have appeared to be more important due to their high efficacy in controlling mosquito by larval reduction (Darriet and Corbel, 2006). There are various active ingredients such as temephos (Mulla *et al*., 2004; Thavara *et al*., 2004; Tawatsin *et al*., 2007), novaluron (Mulla *et al*., 2003; Arredondo-Jimenez and Valdez-Delgado, 2006), diflubenzuron (Thavara *et al*., 2007; Chen *et al*., 2008) and *Bacillus thuringiensis israelensis* (*Bti*) (Mulla *et al*., 2004; Lee *et al.,* 2008) which are used widely in Asia including Malaysia against *Ae. aegypti* larvae in water storage containers.

In Malaysia, temephos (1% sand granule) has been widely used as a major larvicide during the past 30 years to control *Ae. aegypti* due to its good larvicidal efficacy for several weeks and its affordable price (Mulla *et al*., 1986). Unfortunately, there is a decrease of susceptibility of mosquito to temephos due to the development of resistance in recent years. Temephos resistance among *Aedes* population has been reported in Americas (WHO, 1992), Brazil (Lima *et al*., 2003; Macoris *et al*., 2003; Braga *et al*., 2004), Bolivia, Argentina (Biber *et al*., 2006), Clorinda, and Iquazu (Seccacini *et al*., 2008). Also, temephos resistance has been found previously among the population of *Ae. aegypti* and *Ae. albopictus* in some areas around Kuala Lumpur and in Selangor, Malaysia (Chen *et al*., 2005). Besides temephos, resistance against DDT, dieldrin, malathion, fenitrothion, fenthion and pyrethroids in *Ae. aegypti* and *Ae. albopictus* in Malaysia has also been reported. (WHO, 1980; WHO, 1992).

Recently, one potential method for environmental-friendly and species specific population control, which is the Sterile Insect Technique (SIT) was proposed to control *Aedes* population in Malaysia. However, it has not been widely accepted as there are various practical difficulties in rearing, sterilization and distribution of the engineered mosquitoes. Besides, the population dynamics of mosquitoes will be

another problem for mosquito SIT as the population-reducing effect of induced sterility will tend to be offset by reduced density-dependent mortality (Hoang *et al*., 2007).

Insect growth regulators (IGRs) which are the third generation insecticides can be considered as a new approach to mosquito control. In general, there are two groups of IGRs, namely Chitin synthesis inhibitors (CSIs) and Juvenile hormone analogues (JHAs). IGRs offer a good alternative for a selective insect pest control due to their species or stage-specificities that are higher than those of conventional insecticides. Besides, there are no serious field resistance to JHAs reported (Tunaz and Uygun, 2004). Since IGRs have a low mammalian toxicity and do not persist in the environment, it offers a potential advantage as a substitute for temephos in controlling mosquitoes (Mulla, 2005).

Therefore, the objectives of this study are:

1. To evaluate the efficacy of both IGRs, pyriproxyfen (JHA) and diflubenzuron (CSI), in comparison with temephos against *Ae. aegypti* larvae in the laboratory.
2. To evaluate the residual activity of pyriproxyfen and diflubenzuron, in comparison with temephos at their respective diagnostic concentration under simulated field trial.
3. To determine the oviposition-deterrent and ovicidal effects of pyriproxyfen, diflubenzuron and temephos against *Ae. aegypti*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Aedes aegypti*

Aedes aegypti is a species of mosquito that originated from Africa. In the year 1930, it had expanded with a pantropical distribution around the tropical world (Womack, 1993). Those artificial containers such as tyres, earthen jars and plastic containers that contain relatively clean water can serve as breeding habitats for *Ae. aegypti* (Hasanuddin *et al*., 1997). *Aedes aegypti* is capable of surviving in a variety of environment due to their special adaptation mechanisms (Becker *et al*., 2010a). However, they do not exist in extreme cold or dry regions in the world (Marianne and Jonathan, 2001).

In general, *Ae. aegypti* lives in and around human dwellings. The adult females are day-biting mosquitoes in which the biting activity peaks at dawn and dusk (Abu Hassan and Yap, 2003) and human blood will be their preferential choice (Marianne and Jonathan, 2001). *Aedes aegypti* is a holometabolous insect that undergoes complete metamorphosis through the egg, larval, pupal and adult stages. Their survivorship, growth and development are always influenced by climatic variables (Bliss and Gill, 1933; Christophers, 1960; Focks *et al*., 1993a, b; Macfie, 1920; Rueda *et al*., 1990).

2.1.1 Biology of *Ae. aegypti*

Aedes aegypti eggs are laid singly and can withstand desiccation for up to several months. They can remain viable in dry condition and will hatch when flooded by water (Abu Hassan and Yap, 2003). They are black in color, ovoid, and small with a length of around 1 milimeter (Christophers, 1960).

Aedes aegypti larvae are legless. Their body can be divided into 3 parts: head, thorax and abdomen in which their head consists of mouth-part, eye and antennae while their abdomen composed of seven identical segments and three modified posterior segments. There are four anal papillae found in these three modified posterior segments which function to regulate electrolyte levels (Becker *et al*., 2010a). *Aedes aegypti* larvae have a short siphon which is blunt with a pair of subventral tuft at the abdominal segment VIII for the intake of oxygen (Abu Hassan and Yap, 2003). *Aedes aegypti* larvae are considered as filter feeders, as they hang their head downward from the water surface, filtering and collecting the food particles suspended in the water column by beating their lateral palatal brushes in order to generate water currents which carry the food particles into their preoral cavity (Dahl *et al*., 1988).

Pupa is the stage where metamorphosis takes place. At this stage, the head and thorax of the pupae are fused to form the cephalothorax, giving it a comma-shape (Abu Hassan and Yap, 2003). In tropical areas, the non-feeding pupal stage usually lasts for 2 days. Pupa can withstand dry environment where the temperature is 26°C and relative humidity is 87% for around 2 days (Del Rosario, 1963).

At the end of metamorphosis, the abdomen of the pupa will be straightened and thus allow the swallowing of air which increases the internal pressure between the pupa and pharate adult cuticle. This leads to the split of the cephalothoracic cuticle of the pupa along the ecdysial line and the emerging of the adult from the pupal skin. Their legs and wings will stretch after emergence due to the increase in their haemolymph pressure. The adult is able to fly within a few minutes after emergence when the soft cuticle has sclerotized (Becker *et al*., 2010a). Only adult female bites as they require blood meal for egg development whereas male only feed

on plant juice as their food. Adult male can mate many times compared to adult female, which mates only once in their life time but eggs can be produced whenever a blood meal is taken (Rozendaal, 1997). Since the time to be sexually matured are different between adult male and female where adult male needs to take about 1 day to rotate their hypopygium through 180°, the adult males in a population will emerge 1-2 days earlier before the emergence of females. Thus, both adult males and females will be sexually matured at the same time (Becker *et al*., 2010a). The adult male mosquito is positioned below the female during copulation as they fly "face to face". Copulation can occur either in flight or when drop to the ground (Clements, 1992).

2.2 Oviposition

Aedes aegypti produces around 50 – 500 eggs at each oviposition every 2-4 days. Eggs are not laid on water surface, but onto a moist substrate such as moist soil or between particles of moss where rise in water levels will occur subsequently and thus promote hatching. The eggs do not hatch immediately after oviposition, but will hatch when flooded by water (Barr and Azawi, 1958). Moisture on a certain substrate is very important to ensure successful embryogenesis. Besides, a subsequent and sufficient flooding is crucial for the growth and development from hatching all the way to adult emergence. In addition, low number of mosquito predators is also one of the decisive factors in determining the choice of breeding site (Clements, 1992). The ability of mosquito in selecting a favorable breeding site is an adaptation which has developed through evolution, yet it has not been fully understood (Becker *et al*., 2010a). It is possible that a suitable substrate produces pheromone-like odour which can be recognized by gravid females. Moreover, the presence of mosquitoes' eggs or plant in a certain substrate which indicate the occurrence of regular floods could also produce odour that attract oviposition activity. It is also believed that gravid females

are able to differentiate between various soil types (Ikeshoji and Mulla, 1970; Strickman, 1980a, b; Becker, 1989). It has been observed that gravid females are able to evaluate the suitability of water before depositing eggs by alighting and hovering around a substrate, touching the water surface with its mid and hind leg tarsi as well as its ovipositor (Seenivasagan *et al*., 2009). There are several factors that influence breeding site selection by mosquito, such as visual, tactile, and olfactory (Bantley and Day, 1989).

2.3 Embryonic Development

Embryogenesis of *Ae. aegypti* takes around 61.5 hours at 28 °C (Gustavo *et al*., 2008). Mosquito eggshell that consists of exochorion and endochorion is formed upon oviposition (Valle *et al*., 1999). The eggs are soft and whitish in colour at the beginning. However, they will sclerotize and darken at 1 – 2 hours after egg laying (Becker *et al*., 2010a). This darkening process starts during oogenesis in which dopa decarboxylase deposit at the chorion oocyte (Schlaeger and Fuchs, 1974). Embryonic rudiment which originated from the cells of the developing eggs will form the embryo and amnion (Handel, 2000). The external morphological characters of an embryo include a separated head, three fused thoracic segments, eight abdominal segments and respiratory siphon (Christophers, 1960). The development of the hatching spine indicates the completion of embryogenesis. Hatching spine assists eggshell opening when the eggs are exposed to hatching stimulus (Raminani and Cupp, 1978).

Serosa that originated from the anterodorsal portion of the eggs encloses the entire embryo after gastrulation. Serosal cuticle (SC), a layer that consists of chitin, formed by extra-embryonic cell will be secreted by the serosa. It is lying below the

endochorion and surrounding the embryo. It is synthesized at early embryogenesis, becoming the third eggshell layer (Clements, 1992). Serosal cuticle is the main component for egg desiccation resistance by impeding water loses from the embryo. The SC is completely formed at 15 hours after eggs laying. Complete blockage of hatching will occur if the eggs were exposed to desiccation or dry conditions before the complete formation of SC (Gustavo *et al*., 2008).

2.3.1 Interspecific Variation in Egg Desiccation Resistance

There are variations in desiccation tolerance among subgenus *Stegomyia* eggs. *Aedes aegypti* eggs were found to be the most tolerant to desiccation. However, *Ae. albopictus* eggs are more sensitive to desiccation compared to *Ae. aegypti* eggs, but it survives longer in dry condition than forest species, such as, *Ae. riversi*, *Ae. galloisi*, and *Ae. flavopictus* (Sota and Mogi, 1992). These differences may be due to the differences in habitat selection. For example, since *Ae. aegypti* is a water container breeder, their eggs are frequently exposed to severe desiccation due to the absorption of water by some artificial containers. Thus, its excellent ability to withstand desiccation is an adaptation to keep the embryo viability inside the eggs under extended dry condition (Hong *et al*., 1971; Eshita and Kurihara, 1979; Mogi, 1990). Open area breeding site are always more likely to be exposed to intensive dry condition compared to forest areas (Machado-Allison and Craig, 1972). It is believed that the effects of interspecific competition on *Ae. aegypti* will be reduced by reducing *Ae. albopictus* population via egg mortality during the dry season in which more containers without *Ae. albopictus* will be created (Steven *et al*., 2001).

2.4 Hatching

The main hatch stimulus for several species of floodwater mosquito, including *Ae. aegypti*, is a decline of the oxygen level after the submersion of eggs in a liquid medium (Charles and Hassan, 1967). A reduction of oxygen level indicates the presence of bacteria which are able to decompose organic matters, thus ensuring adequate larval food at the time of their hatching. Besides, it is also a signal showing that the water will remain stagnant where no predator will be found (Becker *et al*., 2010a). In natural breeding sites such as tree holes, the reduction of oxygen level are due to the growth of bacteria caused by nutrients that flow down to the tree trunk into tree hole during a rainfall (Walker and Merritt, 1988; Walker *et al*., 1991). Hatchings of eggs can also be induced by using chemical agent such as ascorbic acid or bubbling nitrogen to reduce the oxygen concentration in a medium. Besides, adding mouse pellets or larval food into a breeding site can be served as another method to stimulate hatching in which this media leads to a bacterial bloom that cause reduction of oxygen level (Horsfall, 1956; Mogi, 1976; Novak and Shroyer, 1978; Livdahl *et al*., 1984). Besides reduction of oxygen level, several pre-hatch factors such as temperature, photoperiod, relative humidity, and moisture also have major effects on hatching (Borg and Horsfall, 1953). The increase of the time period between oviposition and hatching may increase the sensitivity of the eggs to hatch stimulus, thus eggs' vulnerability to desiccation can be minimized (Christopher and Todd, 2006).

Multiple hatching, the variability in hatching time has been detected in many *Aedes* species (Buxton and Breland, 1952; Service, 1970; Cooney *et al*., 1981; Logan *et al*., 1991). There is a spectrum of hatching threshold within a population of eggs in which some eggs do not hatch upon first submersion, but they require subsequent

hatch stimuli to hatch progressively (Judson, 1960). Erratic hatching is a way to prevent total reproductive failure under unfavorable condition at the time of their hatching (Seger and Brockmann, 1987). It is an evolutionary response or adaptation to unpredictable environmental conditions such as competition (Livdahl *et al*., 1984) and desiccation (Khatchikian *et al*., 2009). This strategy is the main reason for the increase in the size of *Aedes* population (Hamady *et al*., 2006).

2.5 Medical Importance of *Ae. aegypti*

Aedes mosquitoes play an important role in transmitting many viral diseases in the world (Gubler, 2002). *Aedes aegypti* is the principle epidemic vector of dengue fever where the disease has become the major endemic arboviral in the past 20 years (Chen and Sudderuddin, 1978; Gubler, 1998). It includes dengue fever, dengue haemorrhagic fever or dengue shock syndrome (Lo and Narimah, 1984). Besides, yellow fever and chikungunya are other arthropod-borne diseases that can be transmitted by *Ae. aegypti* (Ross, 1956).

2.5.1 Dengue Fever

Dengue viruses which belong to the genus *Flavivirus* (Mawlouth *et al*., 2003) are classified according to the biological and immunological criteria. According to the classification, there are four serotypes of dengue virus, such as DEN-1, DEN-2, DEN-3 and DEN-4. Normally, dengue infection gives rise to dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) in which DF can cause severe bleeding symptom while DHF or DSS can cause mortality. DHS or DSS is always considered as the most severe form of infection (Malayige *et al*., 2004).

According to WHO, a DF infected person will normally experience high fever (40 °C / 104 °F) coupled with any two of the following symptoms: severe headache, red eyes, pain behind the eyes, deep muscle and joint pains, nausea, vomiting, enlarged lymph node, swollen glands, or rashes. Typically, the appearance of the first symptom will only occur at 5 days after being bitten by an infected mosquito. These symptoms normally last for 2 – 7 days. During this period, the high level of dengue virus in the blood is enough to transmit the infection via *Aedes* mosquito. While an infected *Aedes* mosquito is able to transmit the virus for the rest of its life after the virus incubation takes place, which takes around 4 – 10 days.

Dengue haemorrhagic fever or dengue shock syndrome is potentially fatal due to plasma leaking, fluid accumulation, severe bleeding, organ impairment or respiratory distress. Dengue haemorrhagic fever normally occurs when other serotypes of dengue virus infect a person who has experienced DF. Their symptoms include high fever, persistent vomiting, rapid breathing, bleeding of the nose and gum, severe bleeding, fatigue, abdominal pain, restlessness, and blood in vomit. A patient's condition may be worsened 3 – 7 days after the appearance of the first symptom in which warning sign will be shown in conjunction with a sudden drop of body temperature (below 38 °C/ 100 °F) (WHO, 2012c).

2.5.2 Yellow Fever

Yellow fever virus is an arbovirus which belongs to the genus *Flavivirus.* Sylvatic cycle and urban cycle in tropical Africa and America cause the transmission of Yellow fever (YF) where *Ae. aegypti* serves as the main vector through an urban cycle (WHO, 2003). Monkeys are the main reservoir in the jungle and forest areas in which the infection is transmitted from monkey to monkey, and occasionally, to humans (WHO, 2012b). Jaundice is apparent in infected patients, thus the word

"yellow" in the name of the disease. The virus is transmitted by horizontal transmission and the infection will usually cause a wide spectrum of disease, from mild symptoms to severe illness and death (WHO, 2003).

There are two phases for the infection where 15 % of patients who suffered from the first phase of YF will progress to a second phase after a few days, and 50 % of them will die 10 – 14 days after the onset of the illness. The symptoms of the first phase include fever, headache, muscular pain, chills, nausea, vomiting, anorexia, often with bradycardia. While the symptoms of second phase are resurgence of fever, abdominal pain, vomiting, development of jaundice, and haemorrhagic manifestation (WHO, 2012b).

2.5.3 Chikungunya

Chikungunya fever is caused by the chikungunya virus which belongs to the genus *Alphavirus* (Ross, 1956). According to previous studies, *Ae. albopictus* are shown to be more susceptible to this virus and also more efficient in transmitting them compared to *Ae. aegypti* although both are well known as vectors of chikungunaya disease (Mangiafico, 1971; Turell *et al*., 1992; Yamanishi, 1999; Schufferenecker *et al*., 2006; Vazeille *et al*., 2007, 2008). However, chikungunya is milder and rarely life-threatening, but more acute and predominant in high fever when compared to dengue infection (Nimmannitya and Mansuwan, 1966). The clinical symptoms include high fever, headache, muscle pain, severe arthralgia, arthritis, and the appearance of rash (Thaikruea *et al*., 1997).

Chikungunya fever has re-occurred periodically after the first outbreak in few countries such as Thailand, India and Malaysia (Thavara *et al*., 2009). Between December, 1998 and February, 1999, there was an outbreak of this infection in Klang,

Malaysia (Lam *et al*., 2001). After 7 years of nondetection, there was a second known outbreak in Bagan Panchor, northwest of Malaysia, from March through April of year 2006 (Abu Bakar *et al*., 2007). It was believed that broken transmission cycle between infected humans and vector mosquitoes may lead to this phenomenon. This is because the presence of chikungunya virus in the blood circulation will only last for a few days, thus the transmission cycle will easily be broken if there is no blood meal taken by the vector mosquito during the viremic period (Thavara *et al*., 2009).

A mutant strain of chikungunya virus (E1: A226V) has been reported in Thailand in a previous study. Mutation of the virus leads to a shorter incubation period in its vector, causing the transmission to be initiated as early as two days after an infected-blood is taken (Vezeille *et al*., 2007). As seen in dengue virus, chikungunya virus has also been detected in both male *Ae. aegypti* and *Ae. albopictus*, illustrating the phenomenon of transovarial transmission under natural habitats (Thavara *et al*., 2006). It was also found that chikungunya virus can be vertically transmitted by a vector to their offspring for more than three generations (Zhang *et al*., 1993).

2.6 Mosquito Control

There are several mosquito control measures such as, chemical, biological, genetic, physical, environmental management and educational components. The combination of all available control methods in a cost-effective and safe manner will be a wiser way to control a mosquito population (Becker *et al*., 2010b). In general, there are four categories in controlling mosquito, first, source reduction and

environmental management, second, biological control, third, chemical control and the last, physical barriers and personal protection (Yap *et al*., 2003).

2.6.1 Source Reduction and Environmental Management

Source reduction, a physical reduction of breeding sources is always conducted by minimizing unwanted containers such as artificial containers that serve as a breeding site for *Aedes* mosquito. While improving basic infrastructure including better drainage or sub-soil piping which are highly effective in controlling *Anopheles maculatus* are the examples of environmental management (Yap *et al*., 2003). Besides, water and vegetation management that can create an unfavourable condition for mosquito breeding will be other examples of environmental management (Becker *et al*., 2010b).

2.6.2 Biological Control

There are several biological agents used in controlling mosquitoes, such as parasites, pathogens and predators. Pathogens that include bacteria, fungi, virus and microsporidia are usually considered as microbial-control agent (Woodring and Davidson, 1996). VectoBac which consists of *Bacillus thuringiensis* var. *israelensis* (*Bti*) and VectoLex that contains *Bacillus sphaericus* (*Bsp*) are used widely in controlling clean water breeders and polluted water breeders, respectively. In general, *Aedes* and *Anopheles* are clean water breeders while *Culex* and *Mansonia* are polluted water breeders. *Romanomermis culicivorax* and *Romanomermes iyengari* are two mermethid nematodes that parasitized *Culex* mosquitoes. Since mass-production of these parasites is manpower intensive and its application is inundative, their usage is always limited. While *Poecilia reticulata*, *Aplocheilus spp*. and larvae

of *Toxorynchites* are some of the predators used in controlling mosquito (Yap *et al*., 2003).

2.6.3 Chemical Control

Chemical control involves the use of insecticides in controlling mosquitoes. Basically, they can be divided into two groups based on the targeted stage of the mosquito, which is adulticide and larvicide (Yap *et al*., 2003). Conventional insecticides are usually broad-spectrum, in which non-target organisms can be affected (Becker *et al*., 2010b). In chemical control, the most frequently practised method is residual applications in which chemical are sprayed on the interior walls in houses. While space applications control mosquitoes for only a short time due to its low residual effects (Lacey and Lacey, 1990).

According to their chemical structures, insecticides are generally divided into two major groups, which are the organic insecticides and inorganic insecticides. Synthetic organic insecticides, botanical insecticides and Insect Growth Regulators (IGRs) are organic insecticides while those inorganic insecticides consist of arsenic, mercury chloride and boric acid. IGRs include both juvenile hormone mimics (JHs) and chitin synthesis inhibitors (CSIs) (Yap *et al*., 2003).

2.6.4 Physical Barriers and Personal Protection

Physical barriers are always being applied for reduction of human-mosquito contact. They include window meshed screen and bed nets (Becker *et al*., 2010b). While personal protections include the usage of household insecticide products such as aerosols, mosquito coils, mosquito mats, liquid vaporisers and repellent (Yap *et al*., 2003).

2.6.5 Genetic Control

Genetic technique in controlling mosquitoes has limited success although it has been widely used successfully in controlling agricultural and veterinary pest. There are few genetic techniques such as sterile-male release technique, cytoplasmic incompatibility and chromosomal translocations (Becker *et al*., 2010b). Recently, the lead strain of genetically modified *Ae. aegypti* OX513A created in the laboratories of Oxford University and Oxitec, a biotechnology company in England has been transferred to Institute for Medical Research, Malaysia for independent evaluation. OX513A is developed by inserting a single gene into *Ae. aegypti*'s deoxyribonucleic acid (DNA) in which mating between OX513A male mosquitoes with wild female mosquitoes will produce offspring that die at the pupa stage, thus reducing the population (WHO, 2009a).

2.6.6 Integrated Vector Management

A variety of vector-borne diseases in developing countries represents a heavy burden on human population and gives an obstacle to socioeconomic development (WHO, 2002). Integrated vector management (IVM) is a recent management principle promoted by World Health Organization (WHO) to combat vector-borne diseases. According to WHO, IVM is defined as a rational decision-making process for the optimal use of resources for vector control. IVM is not simply combining different vector-control interventions, it requires institutional arrangements, regulatory frameworks, decision-making criteria and skills, and procedures that can be applied at the lowest administrative level. There are several important attributes of IVM, such as cost-effectiveness, intersectoral actions, regulatory and operational measures, subsidiary, decision-making and sustainability (WHO, 2008a). The

collaboration within public and private agencies, as well as with households and community are important for the implementation of IVM, It is intended to address several diseases concurrently and improve the efficacy, cost-effectiveness, ecological soundness, and sustainability in vector control by using a range of interventions of proven efficacy, separately or in combination (WHO, 2012d).

2.7 Insecticide Resistance

Extensive usage of insecticide is the main factor that leads to the development of insecticide resistance. This phenomenon is due to the insecticide selection pressure on a population of insect, causing them to have an increased tolerance towards a lethal concentration of insecticide (Lee *et al.*, 1999). Cross-resistance will also occur in which other insecticides with similar mode of action become ineffective to control the population (Yap *et al*., 2003). Previous reports have shown that resistance against DDT, malathion, fenitrothion, dieldrin, fenthion, and pyrethroids were detected among *Aedes* population in Malaysia (WHO, 1980; WHO, 1992). Besides, temephos resistance has also been found previously among the population of *Ae. aegypti* and *Ae. albopictus* in some areas in Kuala Lumpur and Selangor, Malaysia (Chen *et al*., 2005).

Basically, there are three types of resistance mechanism in insect, such as reduced cuticular penetration, target site insensitivity, and elevated activity of detoxification enzymes (Lee *et al*., 2003).

2.7.1 Reduced Cuticular Penetration

This mechanism is a minor factor in the contribution to insecticide resistance. It is usually present along with other mechanisms as it does not provide significant resistance to the lethal effects of insecticide on its own. It happens when insect's

outer cuticle develops barrier which in turn slows down the absorption of the chemicals into their bodies (Ahmad and McCaffery, 1999).

2.7.2 Target Site Insensitivity

Basically, target site insensitivity is caused by the alterations of amino acids responsible for insecticide binding at its site of action. Due to this mechanism, insecticide will no longer bind to its target site and thus causing them to be less effective or even ineffective. Acetylcholinesterase in nerve synapses is the target site for organophosphate and carbamate insecticide, while sodium channel of the nerve sheath is the target site for organochlorines and synthetic pyrethroids (Miyazaki *et al*., 1996; Williamson *et al*., 1996).

2.7.3 Elevated Activity of Detoxification Enzymes

In general, there are three groups of enzymes that are involved in insecticide metabolism, such as esterases, monooxygenases, and gluthathione-S-transferases (GSTs). However, the major family of detoxification enzyme that plays an important role in insecticide resistance in insect is GSTs (Lee and Chong, 1995). They are commonly found in most organisms such as plant, vertebrate, insect, nematode, yeast and aerobic bacteria (Hayes and Pulford, 1995; Sheehan *et al*., 2001; Ketterer, 2001). GSTs catalyze the conjugation of glutathione (GSH) to diverse hydrophobic toxic compounds such as drugs, insecticides and endogenous substrate. The binding of GSH with toxins will lead to the formation of a soluble compound which will then be excreted out from the body (Lomaestro and Malone, 1995).

2.8 Insect Growth Regulators

Insect growth regulators (IGRs) are the third generation insecticide that can be considered as a new approach to insect pest control. They do not regulate the development of an insect, but adversely affect their growth and development by interfering with their physiological processes which may lead to various morphogenetic aberrations that impair their survival (Siddall, 1976). Some treated insects may develop into adult with a reduced reproductive potential in which their genitalia will be abnormally developed. This will lead to the failure in mating and also affect their fertility (Tunaz and Uygun, 2004). Besides, it was also found that IGRs are highly potential in sterilizing effect and ovicidal activity (Williams, 1967). Since mosquito larvae are beneficial, they are always ideal target for IGRs as IGRs do not induce quick mortality during the larval stage, but induce abnormalities beyond that was treated (Mulla, 1991). Their species or stage-specificities, high degradability and non-lethal and reversible effects on most aquatic arthropod making it acceptable as an alternative for insect pest control (Tunaz and Uygun, 2004).

There are two major groups of IGRs, which are chitin synthesis inhibitors (CSIs) and Juvenile hormone analogs (JHAs). In general, they are classified according to their mode of action. Bistrifluron, buprofezin, chlorfluazuron, cyromazine, diflubenzuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, novaluron, noviflumuron, teflubenzuron, and triflumuron are some examples of chitin synthesis inhibitors. While JHAs substances include fenoxycarb, hydroprene, kinoprene, methoprene, pyriproxyfen, triprene, juvenile hormone I, juvenile hormone II, and juvenile hormone III (Tunaz and Uygun, 2004).

2.8.1 Chitin Synthesis Inhibitors (CSIs)

Insect cuticle that consists primarily of protein and chitin fractions is important in forming exoskeleton and served as a supportive shell and lining for the tracheal system, gut, genital ducts and some gland ducts in insect. Due to the inhibition of chitin biosynthesis, larvae treated with CSIs will usually develop until molting, but cannot ecdyse and die eventually (Tunaz and Uygun, 2004).

However, species-specificity to CSIs is less pronounced when compared to JHAs as crustacean and some aquatic arthropod share a similar molting process (Miyamoto *et al*., 1993) and contain a similar molting hormone causing sensitivity to CSIs application (Nimmo *et al*., 1980).

2.8.1.1 Diflubenzuron

Diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea is a direct acting insecticide that normally used to treat mosquito and noxious fly larvae. According to WHO Pesticide Evaluation Scheme, maximum dosage of diflubenzuron in portable water is 0.25 ppm (WHO, 2008b). It is the first chitin synthesis inhibitor introduced as a novel insecticide into the market (Miyamoto *et al*., 1993). It is suggested that diflubenzuron functions by interfering with the transport system of UDP-*N*-acetylglucosamine across the biomembrane into the chitin of the peritrophic membrane, preventing the formation of chitin (Eto, 1990). In other words, diflubenzuron inhibits the release of UDP-*N*-acetylglucosamine from the epithelial cell which in turn inhibits chitin biosynthesis (Mitsui *et al*., 1984). It has been used to treat larvae of common cutworm, Spodoptera litura, Cydia pomonella (Miyamoto et al., 1993), some Coleoptera and Diptera (Goktay and Kismali, 1990).

2.8.1.2 Persistence of Diflubenzuron

Even though the effect of diflubenzuron on terrestrial non target organisms is considered minimal when compared to conventional insecticides, yet, it is still highly toxic to both aquatic invertebrates and crustaceans. It was found that the LC_{50} for crustacean is about 0.1 – 1.0 ppm (Miura and Takahashi, 1974). Diflubenzuron is very stable when applied on leaf surface. However its half-life in alkaline water and acidic water is 1 day and > 16 days respectively, suggesting that it degrades more rapidly in alkaline water. When applied in soil, its half-life is dependent on the particle size as degradation in soil involves breaking down of the particles into 4-chloroaniline (PCA) followed by its binding with the soil (ETN, 1996).

2.8.1.3 Ovicidal Activity of Diflubenzuron

Diflubenzuron possesses both larvicidal and ovicidal activities. It has been used in managing insect pest problems and applied against mite infesting field crops (Asai *et al*., 1985; Ellsworthip and Martinez, 2001). Ovicidal activity of diflubenzuron has been observed on *Pieris brassicae, Leptinotarsa decemlineata, Delia brassicae, Spodoptera littoralis* (Ascher and Nemny, 1974), and *Tribolium castaneum* (Carter, 1975).

2.8.2 Juvenile Hormone Analogues (JHAs)

In insects, control of hormone is important in growth and development in which any disturbance in the hormonal balance may lead to disorder or abnormalities. Juvenile hormone is secreted from the corpora allata in insects, and responsible in initiating molting process, together with ecdysone. Metamorphosis will occur in the absence of juvenile hormone. Thus, there is no juvenile hormone found in the pupae (Miyamoto *et al*., 1993). However, in adult stage, juvenile hormone is usually

present to serve some functions in reproduction. Besides, embryogenesis, diapause, communication, migration or dispersal, caste differentiation, pigmentation, silk production and phase transformation of an insect are also controlled by the level of the secretion of juvenile hormone (Eto, 1990). JHAs are much more stable compared to juvenile hormone although both act in the same manner (Riddiford, 1994).

Timing of application is crucial for JHAs in which the initial stage of metamorphosis and embryogenesis in insects are more susceptible. When newly deposited eggs are exposed to JHAs, embryogenesis will be disrupted. While development of supernumerary instar will occur when a freshly ecdysed last larval instar is treated with JHAs. Abnormalities during pupation will be observed when freshly ecdysed pupae are exposed to the treatment (Tunaz and Uygun, 2004). Besides inhibiting growth, egg production and brood care, other social interactions will also be affected after being exposed to JHAs due to hormonal imbalance. Overloading the hormonal system of the target insect will often lead to death eventually (Glancey *et al*., 1990).

2.8.2.1 Pyriproxyfen

Pyriproxyfen, 2-[1-methyl-2-(4-phen-oxyphenoxy)ethoxy]pyridine ($C_{20}H_{29}NO_3$) is one of the juvenile hormone analogs which works well against several vectors and pests, such as mosquito larvae, housefly and ant (Worthing, 1991). Pyriproxyfen has no significant adverse effect on mayfly, dragonfly, ostracod, cladoceran, copepod or beetle, and bumblebee colony, but crustacean, aquatic insect larvae and predator bug are sensitive to it (Ishaaya and Degheele, 1998). However, a study showed that 0.01 ppm treatment will not result in any significant adverse effect on nontarget aquatic organisms that coexist in mosquito breeding sites (Schaefer *et al*., 1988).

Currently, pyriproxyfen has been applied to treat German cockroach, *Blatella germanica* (Reid *et al*., 1994), pear psylla, *Casopsylla pyricola* (Higbee *et al*., 1995), tobacco whitefly, *Bemisia Tabaci* (Ishaaya and Horowitz, 1992), horn fly, *Haematobia irritans* (Bull and Meola, 1993), grain psocid, *Liposcelis entomophila* (Ding *et al*., 2002), mosquito larvae (Miyamoto *et al*., 1993), red imported fire ant, *Solenopsis invicta* , green peach aphid, arrowhead scale, and greenhouse whitefly (WHO, 2008c). Treated German cockroach will exhibit morphogenetic wing twisting and they will usually fail to reproduce (Reid *et al*., 1994). Treatment of mosquito larvae with pyriproxyfen will result in morphogenetic abnormalities in the pupal stage or during larval-pupal transformation which inhibit adult emergence and lead to the eventual death in the pupal stage (Invest and Lucas, 2008).

2.8.2.2 Persistence of Pyriproxyfen

Pyriproxyfen is a relatively stable aromatic compound that functions like a juvenile hormone (JHs), in which it gives rise to an abnormal level of JHs in an insect which usually leads to the eventual death of the insect (Glancey *et al*., 1990). Besides, it is moderately volatile and has low water solubility (Katagi and Takahashi, 1994). Previous report has shown that pyriproxyfen does not readily adsorb onto soil surface, but it does adsorb onto suspended organic matter (Schaefer *et al*., 1988).

2.8.2.3 Ovicidal Activity of Pyriproxyfen

Pyriproxyfen has been shown to be able to produce ovicidal effect and prevent egg hatching in cat fleas (Meola, 1993). Besides, egg hatch of pear psylla (Higbee *et al*., 1995), both egg hatch and adult formation of *B. tabaci* (Ishaaya and Horowitz, 1992) and *Haematobia irritans* (Bull and Meola, 1993) were suppressed

after being exposed to pyriproxyfen. Moreover, ovicidal activity has been observed in *Liposcelis entomophila* treated with pyrirpoxyfen (Ding *et al*., 2002).

2.9 Organophosphate

Organophosphates (OPs) are nerve poisons that derived from phosphoric acid. The first OPs insecticide is Tetraethyl pyrophosphate (TEPP) which was developed as a by-product of nerve gas development. Hostathion (triazophos), Metasystox-R (oxydemeton-methyl), Dursban and Lorsban (chlorpyrifos), Sumithion (fenitrothion) and Actellic (pirimiphos-methyl) are some of the marketed agricultural products (Minton and Murray, 1988). While the organophosphate temephos has been widely used in controlling *Aedes aegypti* larvae in Brazil since 1980s (Andrighetti *et al*., 2008).

2.9.1 Temephos

Temephos, C16H20O6P2S3, with the IUPAC name of O,O,O',O'-tetramethyl O, O'-thiodi-*p*-phenylene bis (phosphorothioate) (WHO, 2009b) is a non-systemic organophosphorus insecticide which is commonly used as a larvicide. It is mainly used to treat the larvae of mosquito, black fly, and flea by affecting their central nervous system through the inhibition of cholinesterase, which in turn results in the death of the larvae (Lima *et al*., 2003). It has been widely used in controlling *Ae. aegypti* larvae during the past 30 years despite a relatively low persistency in the field (Pinheiro and Tadei, 2002; Thavara *et al*., 2004). According to the WHO Pesticides Evaluation Scheme, the maximum dosage of temephos applied in drinking-water sources and containers is 1mg/l, which is equivalent to 1 ppm (WHO, 2009b).