THE SURVEILLANCE AND RESISTANCE STATUS OF *AEDES* MOSQUITOES AGAINST INSECT GROWTH REGULATORS IN MALAYSIA

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ABSTRACT

Dengue is the most important mosquito-borne disease in Malaysia and the principal vector of dengue are *Aedes aegypti* and *Aedes albopictus*. Little information was available of Insect growth regulators (IGRs) in Malaysia. This study was attempted to (1) determine the distribution and abundance of *Aedes* mosquitoes in multiple storey buildings in Selangor and Kuala Lumpur; (2) evaluate the susceptibility status of nationwide collected *Aedes* mosquitoes against IGRs; and (3) investigate the residual efficacy of IGRs in indoor and outdoor conditions.

The vertical distribution and abundance of *Aedes* mosquitoes were determined in 4 high rise apartments located in Selangor [Kg. Baiduri (KB)] and Kuala Lumpur [Student Hostel of University of Malaya (UM), Kg. Kerinchi (KK) and Hang Tuah (HT)] using ovitrap surveillance. The results implied that *Aedes* mosquitoes could be found from ground floor to highest floor of multiple storey buildings but no significant difference was found. Ovitrap indices obtained from all sites were 8.33 to 69.09%. *Aedes aegypti* and *Ae. albopictus* were found breeding in HT, KK and KB; while only *Ae. albopictus* was obtained from UM. The study suggests that the invasion of *Aedes* mosquitoes in high-rise apartments could enhance the transmission of dengue virus, and approach on vector control in this type of residential areas should be developed.

The susceptibility status of *Ae. aegypti* and *Ae. albopictus* obtained from 12 states in Malaysia was evaluated against 5 insect growth regulators, namely pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron according to the protocol by WHO published in 1981. Field populations of *Ae. aegypti* exhibited moderate and low resistance against methoprene (Resistance Ratio, RR = 12.65) and pyriproxyfen (RR = 1.35), respectively; and susceptible to diflubenzuron, cyromazine and novaluron. On the other hand, field populations of *Ae. albopictus* only exhibited low resistance against diflubenzuron (RR = 2.08) and susceptible to other tested IGRs.

Although field populations of *Aedes* mosquitoes have developed some degree of resistance toward certain groups of IGRs such as methoprene, pyriproxyfen and diflubenzuron; cyromazine and novaluron still provide promising effect towards field populations of *Ae. aegypti* and low resistance was shown for populations of *Ae. albopictus* from several states. The use of IGRs should be considered as an alternative control agent when larvae had developed resistance to conventional insecticides.

The residual activities of 5 insect growth regulators (IGRs) were studied and compared to operational dosage of temephos (1 mg/L) and *Bacillus thuringiensis israelensis* (Bti) (0.008 mg/L). The IGRs, temephos and Bti were introduced into plastic containers containing 5 litres of water. Thirty *Aedes aegypti* larvae were added into each container weekly. The indicators of effectiveness of each control agent for these studies were duration of effectiveness of each dosage and the percentage of emergence inhibition (EI). An end-point of EI/mortality $\geq$ 50% was considered to be effective. Pyriproxyfen possessed the longest residual activity in both indoor (43 weeks) and outdoor (26 weeks) conditions, followed by temephos (26 weeks in indoor and 16 weeks in outdoor). The residual activity of Bti in indoor lasted 8 weeks which was longer than cyromazine and diflubenzuron; however, it was least effective in outdoor, lasting only 2 weeks. This study revealed that pyriproxyfen possessed good residual effect among five IGRs when compared to temephos and Bti. The use of IGRs can be an alternative long-term control measure against dengue vector mosquitoes in stagnant waters.

ABSTRAK

Denggi merupakan penyakit jangkitan nyamuk yang paling penting di Malaysia dan *Aedes aegypti* dan *Aedes albopictus* merupakan vektor denggi yang utama. Hanya sedikit maklumat mengenai penggunaan perencat pertumbuhan serangga di Malaysia. Kajian ini bertujuan untuk (1) menentukan penyebaran dan kelimpahan nyamuk *Aedes* di bangunan berbilang tingkat di Selangor dan Kuala Lumpur; (2) menilai tahap kerintangan nyamuk *Aedes* yang dikumpul dari seluruh Negara terhadap perencat pertumbuhan serangga; dan (3) mengkaji keberkesanan residu perencat pertumbuhan serangga di bawah keadaan dalam dan luar bangunan.

Penyebaran menegak dan kelimpahan nyamuk *Aedes* telah dikaji di 4 pangsapuri bertingkat tinggi yang berlokasi di Selangor [Kg. Baiduri (KB)], dan Kuala Lumpur [Asrama Pelajar Universiti Malaya (UM), Kg. Kerinchi (KK) dan Hang Tuah (HT)] dengan menggunakan kaedah peninjauan ovitrap. Keputusan menunjukkan nyamuk *Aedes* boleh dijumpai dari tingkat bawah sehingga ke tingkat teratas dalam bangunan berbilang tingkat. Indeks ovitrap yang diperolehi dari semua tapak kajian adalah di antara 8.33 hingga 69.09%. *Aedes aegypti* dan *Ae. albopictus* didapati membiak di HT, KK dan KB. Akan tetapi, hanya *Ae. albopictus* didapati membiak di UM. Kajian ini mencadangkan pembiakan nyamuk *Aedes* di pangsapuri bertingkat tinggi dapat meningkatkan penyebaran virus denggi dan pendekatan mengenai kawalan vektor di kawasan perumahan ini perlu diperkembangkan.

Tahap kerintangan *Ae. aegypti* dan *Ae. albopictus* yang diperolehi dari 12 negeri di Malaysia telah dikaji terhadap 5 jenis perencat pertumbuhan serangga berdasarkan kaedah yang diterbitkan oleh WHO pada tahun 1981. Perencat pertumbuhan serangga yang dikaji adalah pyriproxyfen, methoprene, diflubenzuron, cyromazine dan novaluron. *Aedes aegypti* menunjukkan kerintangan sederhana terhadap methoprene (nisbah kerintangan, NK = 12.65), kerintangan rendah terhadap pyriproxyfen (NK = 1.35), dan rentan terhadap

diflubenzuron, cyromazine dan novaluron. Di simpang itu, *Ae. albopictus* hanya menunjukkan kerintangan rendah terhadap diflubenzuron (NK = 2.08) dan rentan terhadap perencat pertumbuhan serangga lain. Walaupun nyamuk *Aedes* yang dikumpulkan dari lapangan menunjukkan sedikit kerintangan terhadap perencat pertumbuhan serangga tertentu, seperti methoprene, pyriproxyfen dan diflubenzuron, tetapi cyromazine dan novaluron masih memberi kesan yang meyakinkan terhadap *Ae. aegypti* dan *Ae. albopictus*. Penggunaan perencat pertumbuhan serangga perlu dipertimbangkan sebagai agen kawalan alternatif apabila jentik-jentik telah menghasilkan kerintangan terhadap insektisid konvensional.

Aktiviti residu perencat pertumbuhan serangga juga telah dikaji dan dibandingkan dengan dos operasi temephos (1 mg/L) dan *Bacillus thuringiensis israelensis* (Bti) (0.008 mg/L). Perencat pertumbuhan serangga, temephos dan Bti telah dimasukkan ke dalam bekas plastik yang mengandungi 5L air. Sebanyak 30 ekor jentik-jentik *Ae. aegypti* telah dimasukkan ke dalam setiap bekas setiap minggu. Penunjuk keberkesanan setiap agen kawalan dalam kajian ini adalah tempoh keberkesanan setiap agen kawalan dan peratusan perencatan kemunculan (EI) atau peratusan kematian. Tahap EI/kematian yang melebihi atau sama dengan 50% adalah dianggap sebagai berkesan. Pyriproxyfen menunjukkan aktiviti residu yang paling panjang dalam keadaan dalam (43 minggu) dan luar (26 minggu) bangunan, diikuti dengan temephos (26 minggu di dalam bangunan dan 16 minggu di luar bangunan). Walaupun aktiviti residu Bti di dalam bangunan berlangsung selama 8 minggu dan lebih panjang daripada cyromazine dan diflubenzuron; akan tetapi ia adalah paling kurang berkesan di luar bangunan, iaitu hanya selama 2 minggu. Kesimpulannya, pyriproxyfen menunjukkan kesan residu yang baik di antara semua perencat pertumbuhan serangga dan dibandingkan dengan temephos dan Bti. Kajian ini menunjukkan perencat pertumbuhan serangga boleh digunakan sebagai langkah kawalan jangka panjang alternatif di kawasan air bertakung.

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LIST OF ABBREVIATIONS

≈	About
&	and
°	Degree
°C	degree Celsius
=	Equal
≤	less or same
>	more than
≥	more or same
%	Percent
±	plus minus
Ae.	*Aedes*
ANOVA	analysis of variance
Bti	*Bacillus thuringiensis israelensis*
cm	Centimeter
C.L.	confidence limit
CSI	chitin synthesis inhibitor
Div.	Division
DF	dengue fever
DHF	dengue hemorrhagic fever
EI	emergence inhibitor
et al.	et alia (“and others”)
E	East
EC	emulsifiable concentrate
EI_{50}	50% of emergence inhibition

EI_{90}	90% of emergence inhibition
F1	filial generation 1
g	Gram
GR	granule sand formulation
HT	Hang Tuah
IGRs	insect growth regulators
IMR	Institute for Medical Research
IVM	Integrated Vector Management
JHA	juvenile hormone analogue
Kg.	“Kampung”
KB	Kampung Baiduri
KK	Kampung Kerinchi
L	Liter
mg/L	milligram per liter (concentration)
mm	Millimeter
mL	Milliliter
N	North
NK	nisbah kerintangan
No.	Number
OI	ovitrap index
P	possibility value
r	r value
RR	resistance ratio
sp.	species (singular)
SPSS	statistical snalysis software
th	suffix

UM	University of Malaya
v	Version
VBDCP	Vector Borne Disease Control Program
w/w	weight per weight
WP	wettable powder

CHAPTER 1

INTRODUCTION

1.1 SCOPE OF STUDY

Dengue is the most important mosquito-borne disease in Malaysia. Dengue was first reported as an epidemic in Penang, Malaysia by Skae (1902) and become nationwide outbreak in 1973 due to the massive infrastructure development creating man-made environment for *Aedes* mosquitoes breeding. *Aedes aegypti* and *Aedes albopictus* have been recognized as the principal vector of dengue (Boromisa *et al*., 1987; Gubler, 1988).

Chikungunya is an emerging mosquito-borne disease in Malaysia since an outbreak occurred in Klang, Malaysia, between December 1998 and February 1999 (Lam *et al.*, 2001). Chikungunya virus is transmitted from primates to humans by *Ae. aegypti* in Asia (Rohani *et al.*, 2005). In Thailand, *Ae. aegypti* and *Ae. albopictus* have been associated with chikungunya outbreaks in 1995 (Thaikruea *et al*., 1997). Although the vector of chikungunya remains unknown in Malaysia, *Ae. aegypti* has been considered a potential vector as its vector competence has been proven experimentally by Rohani *et al*. (2005).

As long as no effective and affordable vaccine is available, no adequate prevention other than control of vector is effective approach. Mosquito control can be divided into four categories namely source reduction and environmental management, biological control, chemical control and personal protection (Yap *et al*., 2003). Chemical insecticides still play an important role in the control of dengue vectors

especially during epidemics of the disease. Unfortunately, *Ae. aegypti* and *Ae. albopictus* resistance against major classes of chemical insecticide has been reported and increasingly become a worldwide problem in the past decades. Weill *et al*. (2003) reported that mosquitoes will rapidly develop resistance to insecticide especially in urban areas where the same insecticides were frequently applied. Thus, alternate choice of insecticide should be considered in vector control.

Insect growth regulators (IGRs) are diverse group of chemical compounds that are highly active against larvae of mosquitoes and other insects. The IGRs in general have a good margin of safety to most non-target biota. In Malaysia, the current baseline data of mosquito larvae against IGRs is incomplete. Due to the insufficient data, IGRs are seldom used in mosquito control programs.

1.2 OBJECTIVES OF STUDY

This study updates the current baseline data of susceptibility status of mosquito larvae against IGRs and promotes the usage of IGRs in mosquito and other pest control programs. The objectives of the present study are:

1. To determine the distribution and abundance of *Aedes* mosquitoes in multiple storey buildings in Selangor and Kuala Lumpur.
2. To evaluate the susceptibility status of field collected *Ae. aegypti* and *Ae. albopictus* against insect growth regulators (IGRs).
3. To investigate the residual efficacy of different IGRs in indoor and outdoor conditions.

A schematic flow of the proposed study is illustrated in Figure 1.1

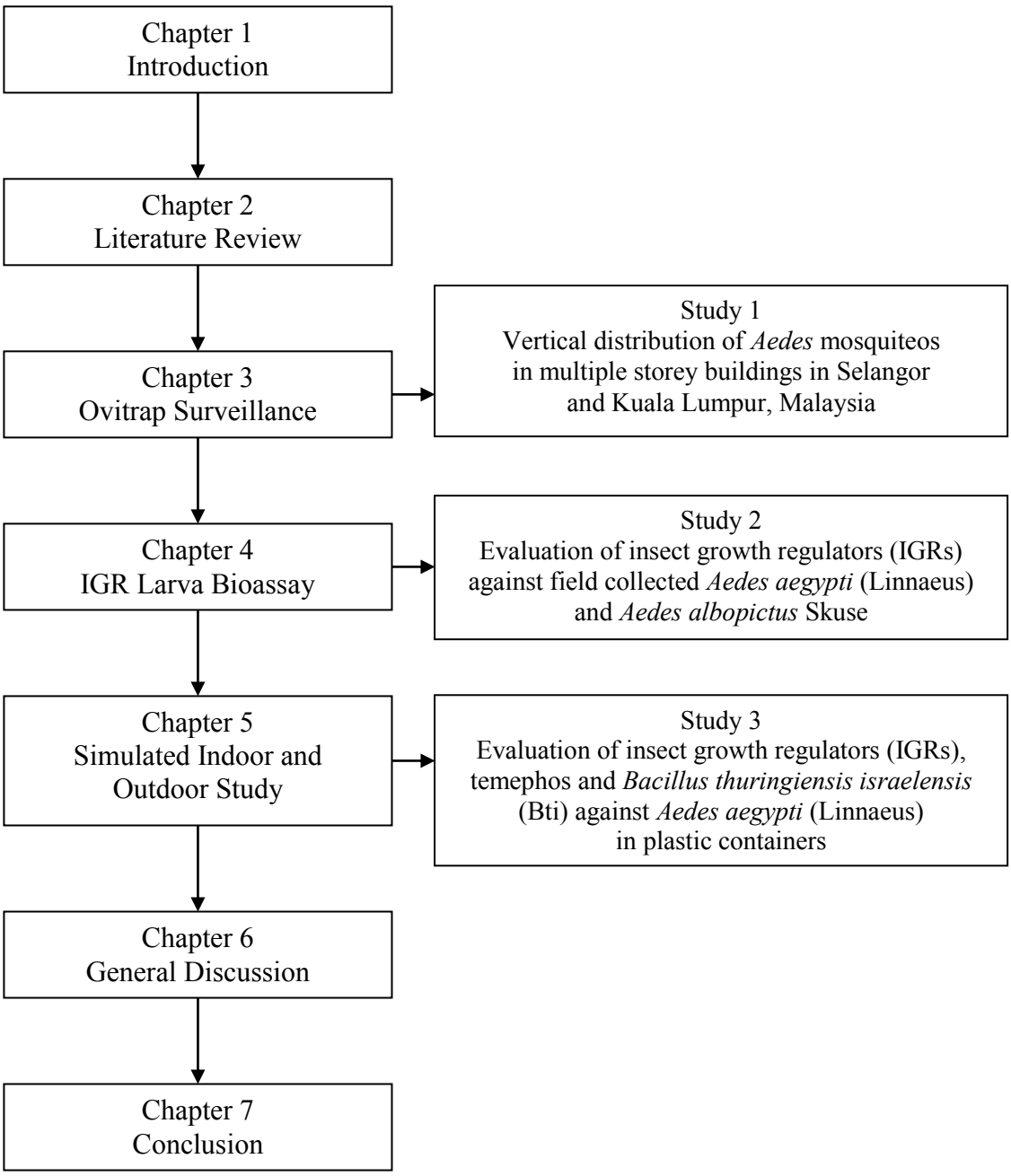

Figure 1.1 Schematic diagram of "The Surveillance And Resistance Status Of *Aedes* Mosquitoes Against Insect Growth Regulators In Malaysia"

CHAPTER 2

LITERATURE REVIEW

2.1 *AEDES* MOSQUITOES

Mosquitoes belong to the family Culicidae, one of the families in the insect order Diptera. Diptera is one of the largest orders in insects, and its members are abundant in individuals and species almost everywhere. Most Diptera can be readily distinguished form other insects by the fact that they have one pair of wings, while the hind wings are reduced to small, knobbed structures called halteres, which function as organs of equilibrium. Mosquitoes are among the best known groups of arthropods because of their importance as pest as well as vector of diseases. *Aedes* mosquitoes are placed in the subfamily Culicinae, family Culicidae, Suborder Nematocera of the order Diptera. There are about 500 species from genus *Aedes* in Malaysia and the most important species are *Aedes aegypti* and *Aedes albopictus* (Abu Hassan and Yap, 2003).

Aedes aegypti and *Aedes albopictus* are the vectors for dengue fever and dengue haemorrhagic fever. *Aedes albopictus* has been repeatedly incriminated as a vector during dengue outbreak, particularly in Southeast Asia (Shroyer, 1986). Jumali *et al*. (1979) compared the efficiency in transmission of dengue-3 virus by oral route of *Aedes aegypti* and *Aedes albopictus* and found that both species were equally efficient. In some regions, *Aedes* species transmit filariasis (Rozendaal, 1997).

2.2 THE LIFE CYCLE OF *AEDES* MOSQUITOES

The mosquitoes undergo a complete metamorphosis during the life cycle, passing through 4 stages: egg, larva, pupa and adult. The immature stages are always associated with free water, which may occur in wide range of location.

The eggs are small, intensely black, elongate oval, seed-like bodies under a millimeter in length (Christophers, 1960). The eggs are laid singly on damp surfaces just above or at the edge of water surface in temporary pools and other habitats such as tree holes, mud, leaves on pond edge, rock pools and wet earthen jars where water level rises and falls (Abu Hassan & Yap, 2003). The eggs can withstand desiccation for many months and hatch only when flooded with water. Some of the species breed in coastal salt marshes and swamps that are flooded at intervals by usually high tides or heavy rains, while others have adapted to agricultural irrigation practices (Rozendaal, 1997).

Egg hatch giving rise to the first-instar larva. This is followed by three successive ecdysis, leading to the second, third and fourth instar larva (Christophers, 1960). The first instar is about 1.5 mm in length while the fourth instar is 8-10 mm. The larva processes no legs but has well developed head and body covered with hairs, and swim with sweeping movements of the body. They feed on yeast, bacteria and small aquatic organisms using paired mouth brushes on the head. Air is taken in by larvae using siphon located at the tip of the abdomen when they come to the water surface to breathe. They dive to the bottom for short periods in order to feed or escape danger (Rozendaal, 1997). Vision is rudimentary but larvae react rapidly to light intensity changes, moving actively with a wriggling or darting motion through water (Burgess & Cowan, 1993).

The larval period lasts about 7 days or longer if there is shortage of food. The fourth instars will develop into a comma-shaped pupa, the head and thorax having fused

to form a cephalothorax, with the abdomen hanging down form it. The pupal stage is mobile, using a pair paddled located on the hind end of the abdomen to progress in a tumbling motion through the water. Pupa is a non-feeding stage and spends most of its time at the water surface to obtain oxygen through a pair of dorsal trumpets on the cephalothorax (Burgess & Cowan, 1993).

When metamorphosis is complete and the adult is fully formed within the pupal cuticle, the pupa swallows air to increase internal pressure, and the cuticle splits along the cleavage lines. The adult slowly emerges from pupal cuticle then stands on the water surface while the exoskeleton hardens and dries, and pupal period lasts 1-3 days.

The adult has a globular head in which large part of the surface is taken up by the compound eyes. The antennae, which are about three times as long as the head, are somewhat hairy in the female and quite bushy in the male; this provides a ready means of distinguishing the sexes with the naked eye. In both sexes the mouthparts are elongated into a proboscis, but those of the male do not include elements capable of piercing skin to suck blood. A pair of palps is present, one on each side of the proboscis (Busvine, 1980).

The events that characterize the life of an adult mosquito are mating, feeding and oviposition. Both male and female mosquitoes become sexually mature approximately 2 days after adult emergence. Male mosquitoes may mate many times, whereas females generally mate only once but produce eggs at intervals throughout their life. Female mosquitoes require blood meal for egg development while male mosquitoes do not suck blood but feed on plant juices. The digestion of a blood meal and the simultaneous development of eggs take 2-3 days in the tropics but longer in temperate zones. The gravid females search for suitable places to deposit their eggs, afterward another blood meal is taken and another batch of eggs is laid. This process is repeated until the mosquito dies (Rozendaal, 1997).

2.3 *AEDES ALBOPICTUS*

Ae. albopictus is believed to have originated in the forest since larvae of most members of the *Albopictus* Subgroup occur in tree holes in Southeast Asia. The ability of *Ae. albopictus* to colonize man-made containers is unknown, but this ability is the key to its present widespread and expending distribution (Hawley, 1988).

According to the review paper by Gratz (2004), the mosquito *Ae. albopictus* originally indigenous to Southeast Asia, islands of the Western Pacific and Indian Ocean, has spread during recent decades to Africa, the mid-east, Europe and the America (north and south) after extending its range eastwards across Pacific islands during the early 20th century. The majority of introductions are apparently due to transportation of dormant eggs in tyres.

The evolution of *Ae. albopictus* provides an interesting contrast with that of *Ae. aegypti*, which purportedly has its origins in Africa (Mattingly, 1957). Both species have spread worldwide as a consequence of their ability to colonize man made containers. *Ae. aegypti* has evolved a closer association with man, preferring to live inside his house in parts of its range, while *Ae. albopictus* seems to have retained a greater ability to recolonize tree holes in forests after transport to the new region (Hawley, 1988).

2.4 *AEDES AEGYPTI*

Ae. aegypti is clearly a non-indigenous species in Malaya. It probably oringinated in Africa (Mattingly, 1957) and was introduced via India, but owing to the scarcity of records prior to 1900 even an approximate time-sequence for its spread would be largely guesswork (MacDonald, 1956). Since *Ae. aegypti* was present in southeast Asia

by 1850, though from until 1900, it was probably confined to seaports and coastal areas (MacDonald, 1956). In 1904, it was still very largely a mosquito of ports in Malaya and absent in inland. By 1913, it had been introduced into Kuala Lumpur and later it replaced *Ae. albopictus* as the common *Aedes* species in the town (MacDonald, 1956). However, from that period onwards, *Ae. aegypti* has been steadily spreading within the country.

According to Smith (1956), *Ae. aegypti* was found only on the coast in Malaya at the beginning of the century and that it has since been gradually moving further inland and becoming more common. The inference is that it was introduced to seaports by shipping, then spread along the coast by fishing boats and local shipping towards the end of the 19th century.

According to the review paper done by MacDonald (1956), there were three tentative conclusions which may therefore be drawn concerning the dispersal of *Ae. aegypti*. Firstly, mechanical transportation of one or other of the life-stages is the principal means by which the distribution of *Ae. aegypti* is extended. Secondly, dispersal is relatively slow, since *Ae. aegypti* has difficulty in becoming established in new locality. And thirdly, once the species is established in a town or village the rate of spread depends on the houses and the habits of the human population, which mean the poorer the living conditions, the more suitable is the area for *Ae. aegypti*.

2.5 DIFFERENCES BETWEEN *AEDES* (STEGOMYIA) *AEGYPTI* AND *AEDES* (STEGOMYIA) *ALBOPICTUS*

The mosquitoes of this subgenus are small to medium size, black to dark in color and highly ornamented with patches, spots or lines of snow white scales. Two or more basal white bands on tarsi of at least one pair of legs or one or more tarsal segments

completely white. In all *Stegomyia* the tarsi are never completely dark or with both apical and basal bandings together. The proboscis is black in color (Div. of Medical Entomology, IMR, 2000).

Table 2.1. Differences between *Ae. aegypti* and *Ae. albopictus* (Div. of Medical Entomology, IMR, 2000).

Ae. aegypti	***Ae. albopictus***
Adult	
➢ Dark brown with characteristic lyre-shaped marking on the mesonotum, covered with silvery white scales, pleurae with several patches of snow white scales. ➢ Scutellum with broad flat scales. ➢ Fore and mid pairs of legs with white narrow bands at the bases of tarsi, hind pair with five broad white basal bands; the last segment being wholly or almost white. ➢ Abdomen dark with white basal bands in the dorsum of segments and also laterally. ➢ All tibiae without white scales or spots. ➢ Two dots of white scales on the clypeal present.	➢ Dark brown with a single longitudinal medium silvery white narrow stripe on the mesonotum. ➢ Scutellum with broad flat scales. ➢ Pleurae with irregular patches of snow white scales. ➢ Fore and mid tarsi with narrow white bands, hind tarsi with broad white bands, 5th segment white. ➢ A line of silvery white scales on border of mesonotum in front of wing-root but continued over wing-root. ➢ Basal bands on the dorsum and laterally on the abdominal segments. ➢ All tibiae without dots of white scales. ➢ Clypeal without white scale dots.
Larval	
➢ Comb on the eighth segment of *Ae. aegypti* abdomen with 8 -12 teeth which have well developed lateral denticles. ➢ Spine on the *Ae. aegypti* thorax is longer and ending in a single point.	➢ Comb on the eighth segment of *Ae. albopictus* abdomen with 8 -12 strong teeth without lateral denticles. ➢ Spine on the *Ae. albopictus* thorax is shorter and ending in several points.

2.6 MEDICAL IMPORTANCE OF *AEDES* MOSQUITOES

2.6.1 Dengue

Dengue ranks the most important mosquito borne viral disease in the world. In the past 50 years, it is incidence has increased 30 fold with significant outbreaks occurring in five of six World Health Organization (WHO) regions. At present, dengue is endemic in 112 countries in the world (Malavige *et al.*, 2004). Dengue has remained endemic in Malaysia since the first case was documented in 1902. The disease was made noticeable in 1973 and the first outbreak of dengue fever was reported in 1962 (Lam, 1993).

There are four serotypes (DEN 1 – 4) of dengue virus, classified according to biological and immunological criteria (Malavige *et al.*, 2004). Studies on the genetic relatedness of strains of dengue virus serotypes 1 – 4 have revealed similarities among strains of serotype recovered from the same geographical region (Halstead, 1990).

Mosquitoes belonging to the genus *Aedes* play an important role in transmission of dengue. The primary and the most important vector is *Aedes aegypti*, but *Aedes albopictus* and *Aedes polynesiensis* may act as vectors depending on the geographic location (WHO, 1999). Dengue infections may be asymptomatic or give rise to dengue fever, dengue haemorrhagic fever or dengue shock syndrome.

Dengue fever (DF) is characterized as an acute viral disease that is recognized by a sudden onset of fever for 3 to 5 days, which often is diphasic, associated with an intense headache, anorexia, abdominal discomfort and rash. Minor bleeding phenomena, such as petechiae and epistaxis may occur at any time during the febrile phase (Kundsen, 1994).

Dengue haemorrhagic fever (DHF) is characterized by high fever, haemorrhagic phenomena and feature of failure (Malavige *et al.*, 2004). The clinical features of DHF

more or less same as dengue fever but with severe bleeding manifestations such as bleeding from gums, haematemesis and maelena.

Dengue shock syndrome (DSS) is associated with very high mortality. Severe plasma leakage leading to dengue shock syndrome is associated with cold blotchy skin, circumoral cyanosis and circulatory disturbances. Some early warning signs of impeding shock are acute abdominal pain and persisting vomiting. Sudden hypotension may indicate the onset of profound shock. Prolonged shock is often accompanied by metabolic acidosis, which may precipitate disseminated intravascular coagulation or enhance ongoing disseminated intravascular coagulation, in turn lead to massive haemorrhage. DSS may be accompanied by encephalopathy due to metabolic or electrolyte disturbances (Malavige *et al.*, 2004).

2.6.2 Yellow Fever

Yellow fever (YF) is a disease caused by an arbovirus which was isolated from human case in West Africa in 1927 (Gubler, 2004). Yellow fever is endemic in tropical Africa and America and transmitted through two major cycles: the sylvatic cycle restricted to wild and rural areas, and the urban cycle. *Aedes aegypti* is the main vector of urban cycle yellow fever which is characterized by large epidemics that may quickly spread from city to city, covering wide areas (WHO, 2003).

Yellow fever usually occurs in endemics. Many patients suffer only a short feverish illness for 3 to 4 days with headache and muscle pains and sometimes jaundice (which gives the patient a yellow color). A minority will have a brief respite, then become seriously ill with high fever, vomiting, severe headache and finally death from gastrointestinal haemorrhage or liver or kidney failure. Death may occur within 3 days after the onset of the disease (Rozendaal, 1997; Burgess and Cowan, 1993).

Immunization is the best prevention of yellow fever, which is recommended for all persons working in or visiting forests where yellow fever occurs. Vaccination normally provides protection for at least 10 years and revaccination is required every 10 years by the port or frontier health authorities in number of tropical countries (WHO, 2005).

2.6.3 Chikungunya

Chikungunya virus belongs to genus *Alphavirus* in family *Togaviridae*. This virus was first isolated from the serum of a febrile human in Tanganyika (Tanzania) in 1953 (Powers *et al.*, 2000). Alphavirus consists of 30 species of arthropod borne viruses, which are futher subgrouped into seven serocomplexes based on serological data (Khan *et al.*, 2002). Between the 1960s and 1980s, the virus was isolated repeatedly from numerous countries in central and southern Africa as well as in Senegal and Nigeria in western Africa. During the same period, the virus was also identified in many areas of Asia. Since 1953, Chikungunya virus has caused numerous well-documented outbreaks and epidemics in both Africa and Southeast Asia, involving hundreds of thousands of people (Halstead *et al.*, 1969a, Halstead *et al.*, 1969b). According to Powers *et al.* (2000) chikungunya virus probably originated in tropical Africa and subsequently was imported into southern Asia. In Africa, evidence that the virus circulates continually in sylvatic cycles has been documented for decades.

Ae. aegypti and *Ae. albopictus* are the only vector species known to transmit chikungunya virus in Asia. These are urban and peridomestic, anthropophilic mosquitoes that maintain close associations with humans. It is therefore not surprising that outbreaks of chikungunya virus infection are noted more frequently in Asia than in Africa (Powers *et al.*, 2000).

In Malaysia, chikungunya was never reported until a group of population Taman Kem, Port Klang came down with symptom like fever, joint pain and rash in January 1999. The infection was later confirmed to be due to chikungunya virus by the WHO Collaborating Centre for arbovirus, UM University Hospital and The Western Australia Centre for Pathology and Medical Research, Australia (Asmad and Satwant, 2000).

Malaysia is heavily dependent on migrant workers from neighboring countries, including those in which chikungunya is endemic. It is speculated that the virus has been introduced into the country through the movement of these workers (Lam *et al*., 2001). Recently, Apandi *et al*. (2009) isolated chikungunya virus (CHIKV) from non-human primates suggested that a CHIKV sylvatic transmission cycle may exists in Malaysia and possibly contributes to the outbreaks. Mohd *et al*. (2011) reported that CHIKV strains circulating in Malaysia during the outbreak in 2008 to 2009 were from Central/East African genotype and were different from CHIKV strains previously isolated in 1998 to 1999 and 2006 outbreaks.

Chikungunya virus infection produces an illness in humans that is characterized by nausea, vomiting, fever, headache, myalgia, rash and arthralgia. Due to the clinical symptoms of chikungunya infection often mimic those of dengue fever and at the same time chikungunya virus circulates in regions where dengue virus is endemic, it has been postulated that many cases of dengue virus infection are misdiagnosed and that the incidence of chikungunya virus infection is much higher than reported (Powers *et al.*, 2000). Chikungunya and dengue viruses are difficult to differentiate because of the clinical symptoms of the two viral diseases are similar and both are transmitted by same mosquito species in Asia. Moreover, there have been documented cases of simultaneous coinfection with chikungunya and dengue viruses (Halstead, 1966).

2.7 MOSQUITOES CONTROL

Since ancient time, various ways had been attempted to control the mosquitoes in order to reduce the man-mosquito contact. At that time only several approaches were used mainly source reduction, environmental management and personal protection. In 1940s and 50s, the invention of synthetic insecticides changes the earlier methods to over reliance on chemical insecticides. Other alternative such as biological control, insect growth regulator (IGR), a revival of the concept of environmental management and re-emphasis on personal protection as a mean of mosquito control since there exists insecticide resistance and environment problem in the 1960s and 70s (Yap *et al.*, 2003).

According to Yap *et al.* (2003), the mosquito control can be categorized into 4 groups:

(1) Source reduction and environmental management,

(2) Biological control,

(3) Chemical control and

(4) Physical barrier and personal protection.

The best approaches that provide long-term solutions to mosquito problem are source reduction and environmental management (Yap *et al.*, 2003). Those effective measures have been reviewed by Mitchell (1996), Rozendaal (1997) and Lee (2000b), and concluded as: (a) stream improvement to promote water flow, (b) filling, to remove depressions that collect water, (c) drainage, to remove water favorable to mosquito breeding, (d) vegetation control, (e) relocation of human settlements to mosquito-safe areas, (f) use of mosquito nets, (g) mosquito-proofing of houses, and (h) better management of containers.

Biological control can be briefly defined as the control of pests using biological agents such as pathogens, parasites and predators. Mermethid nematodes as parasites,

Romanomermis culicivorax and *Romanomermis iyengari* are effectively used to control mosquito in open field. For predators, indigenous fish species such as *Poecilia reticulata* and *Aplochelus* species are used to control mosquitoes. Another successful biological agent, *Bacillus thuringiensis* H-14 (*Bti*), is also used to control mosquitoes (Yap *et al.*, 2003).

Chemical control is the control of pest involving the use of insecticide. The insecticide can be divided into two groups based on the targeted stage of mosquito, adulticide and larvicide. Adulticides are the insecticide used to control adult mosquitoes whether they are flying or resting while larvicides are used to control the immature stages of mosquito especially the larvae (Yap *et al.*, 2000).

Physical barrier and personal protection involve preventing or lessening the man-mosquito contact with insecticide (Yap *et al.*, 2000). Among the personal protect measures, household insecticide products (aerosols, mosquito coil, vaporizing mat and electric liquid vaporizers) are considered as the most active form of community participation because most of the active ingredient used are synthetic pyrethroids (57.6%) which are considered less hazardous to humans (Yap *et al*., 2000).

2.8 RESISTANCE STATUS IN *AEDES* MOSQUITOES

Insecticides have play an important role in the control of insect vectors of diseases since early 20th century. Although important advances continue to be made in the development of alternative control measures, insecticides will remain a vital part of integrated control program for the foreseeable future. Unfortunately, the remarkable ability of insect population to evolve resistance to every class of insecticide that has been developed often leaves control programs with few insecticides option (Ferrari, 1996).

In 1992, WHO redefined resistance "as an inherited characteristic that imparts an increased tolerance to a pesticide, or group of pesticides, such that the resistant individuals survive a concentration of compound(s) that would normally be lethal to the species". On the basis of this definition, the proportion of survivors (heterozygotes in the first place, but including homozygotes as selection progresses) can be looked upon as reflecting the frequency of the gene or genes that code for particular resistance mechanisms and thus confer resistance (WHO, 1992).

Resistance to one or more insecticides has been documented in more than 504 species of arthropods (Georghiou and Lagunes, 1991). Of these, about 41% are considered of medical or veterinary importance. The status of resistance in arthropod vectors has been reviewed (WHO, 1992). However, the presence of resistant individuals in one population of species does indicate the potential for resistance to spread to other populations (Ferrari, 1996).

Resistance results in increased pesticides application frequencies, increased dosages, decreased yields, environmental damage and outbreaks of arthropod-borne human and veterinary diseases (Mullin and Scott, 1992).

The first documented case of insecticide resistance in arthropods was 1908 in Washington for the San Jose scale *Quadraspidiotus perniciosus* to lime-sulfur. Incidence of resistance in the "field" has generally correlated with the length of time an insecticide has been used, hence the trend among insecticide classes is organochlorines > organophosphates > carbamates > pyrethroids > insect growth regulators, microbials etc (Mullin and Scott, 1992).

2.8.1 Resistance studies on *Aedes* mosquitoes in Malaysia

Since 1970s, Thomas (1970, 1976) had reported malathion-resistant *Ae. aegypti* larvae in Malaysia. In year 1978, toxicological studies of insecticides against *Ae. aegypti* hace

been conducted by Yan and Sudderuddin (1978). They found that *Ae. aegypti* was generally more tolerant against the organophosphorus compounds (and carbaryl) showing higher CarE activity. Toxicity tests carried out on the larvae of *Ae. aegypti* showed that the order of toxicity was temephos > DDT > DDVP > malathion > lindane > carbaryl. They also found that the second-instar larvae were more susceptible than fourth-instar larvae.

The insecticide susceptibility status of field-collected *Ae. albopictus* against DDT, permethrin, malathion and temephos were conducted by Lee *et al.* in 1998. Their results indicated that the *Ae. albopictus* larvae were highly susceptible to both malathion and temephos, while the adult mosquitoes were highly susceptible to malathion but multiple resistance to permethrin and DDT. In this study, non-specific esterase did not appear to play a role in the multiple resistance of the adults of *Ae. albopictus* to both permethrin and DDT. Rohani *et al.* (1998) also found multiple resistance to both permethrin and DDT in an urban strain of *Ae. albopictus* in Kuala Lumpur city. In addition, Lee and Chong (1995) reported that DDT susceptibility status of Malaysian mosquito was not correlated with GST activity.

Nazni *et al.* (2000) reported that in *Ae. aegypti* mosquitoes, which are slightly tolerant to permethrin, oxidases are involved in the resistance mechanism. They also found that resistance to malathion and temephos could also be due to oxidase in larval stage.

A similar study was also conducted by Rohani *et al.* (2001) in the major towns in Malaysia. All *Ae. aegypti* strains collected from the study areas showed resistance to DDT and permethrin. All *Ae. albopictus* strains collected from all areas only showed resistance to DDT, but strains from Selangor and Kedah were also resistant to malathion. According to Rohani *et al.* (2001), the effectiveness of insecticides to adults of *Ae. aegypti* and *Ae. albopictus* indescending order was malathion > permethrin > DDT,

while that to larvae was temephos > malathion > permethrin > DDT. The enzyme microassay data revealed that the field strains had 2 – 5 folds elevated levels of esterases compared to the laboratory strain in both adults and larvae. This explains the high level of insecticides tolerance in the field strains compared to the laboratory strain.

Lee *et al.* (1987) reported that *Ae. aegypti* and *Ae. albopictus* larvae collected from the major towns in Kuala Lumpur were resistance to malathion and permethrin. In contrast, they were susceptible in the adult stage. The reason to this could be the larval stages could detoxify the malathion at the faster rate than at the adult stage (Lee *et al.*, 1998).

Futhermore, resistance against DDT, dieldrin/HCH, malathion, fenitrothion, fenthion, temephos and pyrethroid, of *Ae. aegypti* and *Ae. albopictus* in Malaysia has also been reported by WHO (1980, 1992).

2.8.2 Resistance Studies on *Aedes* mosquitoes in other countries

In Thailand, resistance to temephos, fenitrothion and malathion has been reported by Chareonviriyahpap *et al.* (1990). Somboon *et al.* (2003) reported field-collected *Ae. aegypti* and *Ae. albopictus* were highly resistant to DDT. At present, pyrethriods are widely used for controlling adult mosquitoes at household (aerosal canisters) and community level (fogging and ULV). *Ae. aegypti* from some areas was also resistant to permethrin, deltamethrin and etofenprox, but susceptible to lambda-cyhalothrin or fenitrothion. *Ae. albopictus* only showed to be susceptible to permethrin and fenitrothrin.

Prapanthadara *et al.* (2002) found that DDT resistance in both *Ae. aegypti* strains, R^dS^p (resistance to DDT and susceptible to permethrin) and R^dR^p (resistance to DDT and permethrin), was due to increased DDTase activity and cytochrome P^{450} content whereas permethrin resistance in R^dR^p strain probably involved a non-metabolic kdr mechanism.

Paeporn *et al*. (2003) detected the temephos resistance in microplate by biochemical assay and reported that the main mechanism is based only on EST detoxification. Paeporn *et al.* (2004) conducted enzymes biochemical assay to detect the emergence of insecticide resistance and defined the mechanisms involved in pyrethroid resistance of *Ae. aegypti*, selected strains against permethrin and deltamethrin. The results revealed significant increase of EST activity and MFO levels in both strains, but GST were associated with permethrin resistance in *Ae. aegypti*.

Yaicharoen *et al.* (2005) reported that adult *Ae. aegypti* mosquitoes collected from Bangkok and Pathum Thani provinces, showed low resistant to deltamethrin (resistance ratio = 8 – 17.2) and cross-resistance to DDT. Biochemical analysis also showed a significant elevation of MFO and EST enzyme activity in the population. Sealim *et al.* (2005) also reported that insensitive acetylcholinesterase (AChE) was not found to be responsible for the resistance in the field-collected *Ae. aegypti* mosquitoes from Roi Et, Thailand. Their study suggests that EST detoxification is the primary cause of resistance in the *Ae. aegypti* population.

In Singapore, Ong *et al.* (1981) reported that the susceptibility (LC_{50} value) of *Ae. aegypti* to the nine insectivides was Abate® > bioresmethrin & dursban > fenthion > fenitrothion > deldrin > DDT > malathion > BHC; while susceptibility (LC_{50} value) of *Ae. albopictus* was dursban > bioresmethrin > Abate® > fenthion > fenitrothion > dieldrin > DDT > malathion > BHC. He found that *Aedes* mosquitoes were resistant to organochlorines and were becoming more resistant to the organophosphate compound malathion, but were susceptible to pyrethroid and bioresmethrin.

In 1994, Liew *et al.* reported that the resistance ratios of larval populations collected in 1993 compared to data from 1979 indicated a 3.5 hold increase in LD_{50}'s for both *Ae. aegypti* and *Ae. albopictus* against temephos. The LD_{90} values had increased proportionately. *Ae. albopictus* was slightly more resistant than *Ae. aegypti*.

They also reported that adult *Ae. aegypti* were found to be more tolerant to pirimiphos-methyl than *Ae. albopictus*, with the ratios of LD_{50} and LD_{90} of *Ae. aegypti* to *Ae. albopictus* being 4.73 and 4.45 respectively.

In 2001, Lai *et al.* reported that *Ae. aegypti* and *Ae. albopictus* still susceptible to pirimiphos-methyl, with resistance ratio for LC_{50} 1.5 and 1.4 respectively. However, *Ae. aegypti* showed resistant to permethrin (RR for LC_{50} = 12.9) but *Ae. albopictus* was still susceptible to peemethrin. They concluded that the Singapore control of dengue vectors using pirimiphos-methyl was still effective.

2.9 INSECT GROWTH REGULATORS (IGRs)

Insect growth regulators (IGRs) are potent insecticides containing substances with growth retarding and growth inhibiting properties (Mulla, 1995). The IGRs are divided into 2 groups, juvenile hormone analogues (JHAs) and chitin synthesis inhibitors (CSIs). Juvenile hormone analogues were chemically related to the natural juvenile hormones of insect and commonly known as juvenoids (Slama *et al*., 1974). The chemicals disrupt the hormonal control of larval development, cause hormonal imbalance, and eventually suppress insect embryogenesis, metamorphosis and adult emergence. Chitin synthesis inhibitor prevents chitin formation of the insect, thus treated insect fail to molt or have soft cuticle that cannot protect them and die soon after ecdysis.

Pyriproxyfen and methoprene belong to the juvenile hormone analogue group. Methoprene was the most successful early compound found to be nontoxic to vertebrates (Hendrick *et al*, 1973) and the chemical was registered in 1974. Other IGRs developed were generally similar in structure to methoprene but have a wider insect spectrum of effectiveness compared to methoprene which is physiology unique to targeted insects (Dhadialla and Carlson, 1998). Pyriproxyfen is another juvenile

hormone analogue that has been used against a range of pests since its introduction to the market in early 1990s. Over the past decades, many studies have been examined the utility of pyriproxyfen as a valuable tool to control dengue vectors, *Ae. aegypti* and *Ae. albopictus*. In general, pyriproxyfen is effective in inhibiting adult emergence of *Ae. aegypti* and *Ae. albopictus* at concentrations ≤ 1 mg/L (Estrada and Mulla, 1986; Hatakoshi *et al*., 1987; Loh and Yap, 1989; Itoh, 1994; Vythilingam, 2005). In addition to its larvicidal activity, it has been reported to decrease fertility and fecundity of *Ae. aegypti* female that developed from sublethally exposed larvae, and can act as vehicles for the dissemination of pyriproxyfen to previously uncontaminated environment (Loh and Yap, 1989). Pyriproxyfen also shows considerable potential for control of *Ae. aegypti* in water storage under field conditions (Nayar *et al*., 2002).

Among the chitin synthesis inhibitors, several compounds have been evaluated against mosquitoes, for example, diflubenzuron, hexafluron, triflumuron and cyromazine (Mulla, 1995; Chen *et al*., 2008). These compounds are highly active against mosquito larvae and treated individuals die during ecdysis. The larvae do not have the rigidity to get out of the old cuticle due to inhibition of chitin deposition caused by CSI. The larvae may survive for some period but eventually die. In past decade, Lam (1990), Mulla (1995), Seccacini *et al*. (2008) and Chen *et al*. (2008) have reported studies on laboratory evaluation and field efficacy of a number of IGRs against mosquito larvae.

The common characteristic of these chemicals is that they do not induce instant mortality in the treated larvae. The active ingredients enter the insect body either through the cuticle or by ingestion. Larvae received lethal doses do not die instantly, the larvae survive and suffer mortality in the pupal stage or adult stage.

The IGRs in general have good margin of safety to bird, wildlife and aquatic organisms including fish and also possess low mammalian toxicity. However, some of

the IGRs do adversely affect some aquatic crustaceans and species of insects closely related to mosquitoes or sharing the same environment (Mulla, 1995). The IGRs are safely used without any noticeable impact on non-target organisms and there are indications that this pattern of usage will continue into the future. It is reasonable to assume that IGRs will be employed in mosquito and other vector control programmes.

CHAPTER 3

VERTICAL DISTRIBUTION OF *Aedes* MOSQUITOES IN MULTIPLE STOREY BUILDINGS IN SELANGOR AND KUALA LUMPUR, MALAYSIA

3.1 INTRODUCTION

Mosquito-borne diseases such as dengue heamorrhagic fever and dengue fever (DF) are the most important arthropod borne viral diseases of public health in Malaysia. In year 2011, a total of 19,884 DF cases were reported with 36 deaths in Malaysia (Ministry of Health Malaysia, 2011). *Aedes aegypti* and *Aedes albopictus* are the two major vectors involved in these infections. (Lam, 1993; Chen *et al*., 2005a, 2005b, 2005c).

Aedes aegypti is a domestic mosquito in urban area exclusively breeding in artificial containers such as earthen jars and plastic containers which contain relatively clear water near human dwellings (Hasanuddin *et al*., 1997), while *Aedes albopictus* was reported breeding in artificial containers and natural containers near human dwellings (Hawley, 1988). Both species were adapted to urban and suburban area (Chen *et al*., 2006). The close association between human and *Aedes* mosquitoes has provided the mosquitoes with breeding sites, shelter, and blood meals, which can increase the risk of dengue transmission.

Ovitrap surveillance is the commonest sampling method to monitor *Aedes* mosquitoes populations (Service, 1992; Cheng *et al.*, 1982). According to Lee (1992b),

ovitrap surveillance has been shown to be a more effective and sensitive technique especially when the *Aedes* infestation rates were low.

Many studies had been done in Malaysia to determine the population and abundance of *Aedes* mosquitoes (Lee, 1992a, 1992b; Chen *et al*., 2005c, 2006; Rozilawati *et al*., 2007; Wan-Norafikah *et al*., 2009). However, little information is available on the distribution of *Aedes* mosquitoes at different level of high-rise buildings. A preliminary study on the vertical dispersal of *Aedes* population in high-rise apartments was conducted by Wan-Norafikah *et al*. (2010) in Putrajaya. Their study indicated the possibility of lower *Aedes* population to be found at higher level of high-rise apartments. However, their study was conducted in high-rise apartments with 10 levels in one study site only.

The present study was conducted in high-rise apartments located in the 4 selected urban residential areas in Kuala Lumpur and Selangor. This study provides more comprehensive information regarding the vertical distribution and abundance of *Aedes* mosquitoes in high-rise apartments in Kuala Lumpur and Selangor, Malaysia.

3.2 MATERIALS AND METHODS

3.2.1 Description of study sites

Ovitrap surveillance was conducted in high-rise apartments located in 4 residential areas namely, Kg. Baiduri (KB), Student Hostel's University of Malaya (UM), Kg. Kerinchi (KK) and Hang Tuah (HT). The geographical and ecological description of the study sites was given in Table 3.1.

3.2.2 Ovitrap surveillance

Ovitrap as described by Lee (1992a) was used in this study. The ovitrap consists of 300 ml plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter is 6.5 cm, and the container is 9.0 cm in height. The outer wall of the container is coated with a layer of black oil paint. An oviposition paddle made from hardboard with measurement of 10.0 cm (Length) x 2.5 cm (Width) x 0.3 cm (Thick) was placed diagonally into each ovitrap which was filled with tap water to the level of 5.5 cm.

Ovitraps were placed randomly in each floor of the apartment from ground level to highest level. Ovitraps were placed in not less than 10% of the rooms/houses in each level of the apartments in all study sites. Ovitraps were placed indoor along a corridor near stairways, near the ornamental plants and under the shoe rack. In this study, "indoors" refers to the interior of the apartments (Wan-Norafikah *et al*., 2010)

All ovitraps were collected after 5 days and replaced with fresh ovitraps and paddles. Four continuous weekly ovitrap surveillance was conducted in each study site.

Table 3.1 Geographical and ecological description of study sites

Study site	Geographical Description	Physical Description	Ecological Description
12th Student College, University of Malaya (UM)	• 3°07'N, 101°35'E • Located in Kuala Lumpur	• The building consists of 9 floors. • 34 units of rooms each floor. • Each floor is about 3.0 meter in height • The building is about 10 years old.	• High vegetation in the study site. • Tree and shrubs planted around the student college. • The environment is generally clean and well managed.
Vista Angkasa Apartment, Kampung Kerinchi (KK)	• 3°06'N, 101°39'E • Located in Kuala Lumpur near the border of Selangor state.	• The building consists of 15 floors. • 10 units of houses each floor. • Level height is 3.0 meter. • The building is about 15 years old.	• Scattered vegetation around the apartment. • Proper waste management and drainage system.
Sri Sarawak Apartment, Hang Tuah (HT)	• 3°08'N, 101°42'E • located in the city center of Kuala Lumpur.	• The building consists of 16 floors. • 16 units of houses each floor. • Level height is 3.0 meter. • The building is more than 20 years old.	• Sparse vegetation and artificial pond around the apartment. • Poor waste management and sanitation. • Some of the households have ornamental plants placed around the corridor in front of their house.
Impian Baiduri Apartment, Kampung Baiduri (KB)	• 3°05'N, 101°37'E • Located in Selangor.	• The building consists of 16 floors. • 20 units of houses each floor. • Each floor is about 3.0 meter in height. • The building is about 3 years old.	• Scatted vegetation around the building. • Proper waste management and drainage system.

3.2.3 Identification of larvae

The collected ovitraps were brought back to laboratory and the contents were poured into plastic containers, together with the paddles. Fresh water was added into the container and a small piece (10 mm) of fresh beef liver was added as larval food. The larvae were allowed to hatch and colonize in the laboratory for another 9 days. The hatched larvae were subsequently counted and identified at 3rd instar. The larval numbers were recorded for each positive ovitrap.

3.2.4 Data analysis

All data obtained from this study was analysed as follow:

1. Ovitrap Index (OI), the percentage of positive ovitrap against the total number of ovitraps recovered from each site.
2. Mean number of *Ae. aegypti* and/or *Ae. albopictus* larvae per recovered ovitrap.

All levels of statistical significance were determined at $p \leq 0.05$ by using the statistical programme, student t-test and one-way ANOVA (SPSS v 11.5).

3.3 RESULTS

Table 3.2 shows the ovitrp index (OI) and the mean number of larvae per ovitrap of *Ae. aegypti* and *Ae. albopictus* obtained from ovitrap surveillance conducted in 4 high-rise apartments located in Kuala Lumpur and Selangor. The highest ovitrap index was obtained from Hang Tuah (HT) (45.08%), followed by Kg. Kerinchi (KK) (37.48%), Kg. Baiduri (KB) (21.43%) and University of Malaya (UM) (11.43%). There was significant difference between OI obtained from apartments in all study sites ($p < 0.05$). Mean number of *Ae. aegypti* larvae per ovitrap obtained from HT (9.26 ± 0.93) was significant higher than KK (6.20 ± 3.21) and KB (2.64 ± 0.42). There was no *Ae. aegypti* reported in UM. On the other hand, UM (1.50 ± 0.57) had higher mean number of *Ae. albopictus* larvae per ovitrap than KK (0.55 ± 0.27), HT (0.30 ± 0.11) and KB (0.24 ± 0.13), but this was not significantly different ($p > 0.05$).

Table 3.3 shows the OI of each level in all apartments. *Aedes* were found breeding from ground floor to highest floor in KK and HT. Two out of 9 floors and 6 out of 16 floors in apartments located in UM and KB showed no *Aedes* breeding, respectively. However, there was no significant difference of OI in each floor within the apartment ($p > 0.05$). The OI obtained from KK, HT, KB and UM ranged from 0 – 91.67%, 8.33 – 83.33%, 0 – 55.56% and 0 – 29.17%, respectively.

The mean number of larvae per ovitrap of *Ae. aegypti* and *Ae. albopictus* obtained from ovitrap surveillance in each floor in 4 high-rise apartments are shown in Table 3.4. The mean number of larvae per ovitrap indicated that *Ae. aegypti* was significantly dominant than *Ae. albopictus* for HT, KK and KB ($p<0.05$) by 11 to 31 folds. In contrast, *Ae. albopictus* was the only principal dengue vector found in UM. The mean number of larvae per ovitrap of *Ae. albopictus* obtained from UM, KB, KK and HT ranged from 0 – 9.63, 0 – 2.89, 0 – 2.75 and 0 – 2.13, respectively. On the other

hand, mean number of larvae per ovitrap of *Ae. aegypti* obtained from KK, HT and KB ranged from 0.33 – 34.50, 0.42 – 28.00 and 0 – 11.67, respectively. Generally, *Ae. aegypti* was found breeding up to the highest floor (16th floor, 45.1 – 48.0 m), while *Ae. albopictus* was only up to fourteenth floor (39.1 – 42.0m). Although the highest mean number of larvae were found in first level of each apartment, there was no significant correlation between the mean number of *Aedes* larvae collected with the height of the apartment (UM: $r = -0.471$, $p = 0.193$; KK: $r = -0.036$, $p = 0.893$; KB: $r = -0.293$, $p = 0.263$) except HT ($r = -0.682$, $p = 0.004$), indicating that *Aedes* could be found breeding in every level of the apartment and not restricted by the height of the apartment.

Table 3.5 shows the percentage and ratio of *Ae. aegypti* and *Ae. albopictus* mixed breeding in ovitrap surveillance conducted in high-rise apartments in Kuala Lumpur and Selangor. The percentage of mixed breeding in HT, KB and KK accounted for 10.77%, 15.00% and 26.56% from the total collected ovitraps, respectively. In addition, the numbers of *Ae. aegypti* larvae found in mixed breeding ovitrap were 1.50 – 3.44 folds more than those of *Ae. albopictus*.

Table 3.2 Comparative ovitrap index (mean ± S.E.) and larval number (mean ± S.E.) per ovitrap obtained from four high-rise apartments located in Kuala Lumpur and Selangor, Malaysia.

Site	No. of Ovitrap Surveillance conducted	No. of collected ovitrap	Ovitrap Index (%)	Collected Larvae						*Ae. aegypti* : *Ae.albopictus* in the population	Comparison of the mean number larvae per ovitrap of *Ae. aegypti* & *Ae.albopictus* within the study site
				Aedes aegypti			*Aedes albopictus*				
				Total number of larvae	%	Mean number of larvae per ovitrap	Total number of larvae	%	Mean number of larvae per ovitrap		
University of Malaya (UM)	4	104 / 108	11.43 ± 1.26	0	0	0.00 ± 0.00	150	100	1.50 ± 0.57	Nil	T = -2.632 P = 0.039
Kg. Kerinchi (KK)	3	175 / 180	37.48 ± 15.80	1054	91.41	6.20 ± 1.21	99	8.59	0.55 ± 0.27	11.27 : 1	T = 4.557 P = 0.010
Hang Tuah (HT)	4	145 / 192	45.08 ± 3.80	1347	96.77	9.26 ± 0.93	45	3.23	0.30 ± 0.11	30.87 : 1	T = 9.568 P = 0.000
Kg. Baiduri (KB)	3	108 / 144	21.43 ± 9.43	276	91.39	2.64 ± 0.42	26	8.61	0.24 ± 0.13	11.00 : 1	T = 5.459 P = 0.005
One way ANOVA			F = 3.98 P= 0.042			F = 34.43 P= 0.000			F = 2.84 P= 0.092		

Table 3.3 Ovitrap index at each level of four high-rise apartments

Level	Height (meter)	University of Malaya (UM), Kuala Lumpur	Kg. Kerinchi (KK), Kuala Lumpur	Hang Tuah (HT), Kuala Lumpur	Kg. Baiduri (KB), Selangor
		Ovitrap Index (%)	Ovitrap Index (%)	Ovitrap Index (%)	Ovitrap Index (%)
1	0.0 –3.0	29.17 ± 17.18	91.67 ± 8.33	83.33 ± 16.67	55.56 ± 11.11
2	3.1 – 6.0	25.00 ± 25.00	66.67 ± 16.67	50.00 ± 28.87	27.78 ± 14.70
3	6.1 – 9.0	8.33 ± 8.33	33.33 ± 8.33	77.78 ± 22.22	0.00 ± 0.00
4	9.1 – 12.0	8.33 ± 8.33	41.67 ± 16.67	41.67 ± 15.96	44.44 ± 29.40
5	12.1 – 15.0	0.00 ± 0.00	25.00 ± 14.43	58.33 ± 15.96	27.78 ± 14.70
6	15.1 – 18.0	8.33 ± 8.33	58.67 ± 21.80	62.50 ± 14.23	11.11 ± 11.11
7	18.1 – 21.0	8.33 ± 8.33	25.00 ± 14.43	29.17 ± 10.49	0.00 ± 0.00
8	21.1 – 24.0	0.00 ± 0.00	25.00 ± 25.00	33.34 ± 19.25	38.89 ± 5.56
9	24.1 – 27.0	16.67 ± 9.62	25.00 ± 25.00	16.67 ± 16.67	0.00 ± 0.00
10	27.1 – 30.0		16.67 ± 16.67	79.17 ± 12.50	16.67 ± 16.67
11	30.1 – 33.0		19.44 ± 10.01	54.17 ± 15.78	0.00 ± 0.00
12	33.1 – 36.0		25.00 ± 0.00	50.00 ± 28.87	25.00 ± 25.00
13	36.1 – 39.0		16.67 ± 16.67	16.67 ± 9.62	0.00 ± 0.00
14	39.1 – 42.0		41.67 ± 16.67	45.83 ± 20.83	0.00 ± 0.00
15	42.1 – 45.0		41.67 ± 30.05	37.50 ± 23.94	22.22 ± 22.22
16	45.1 – 48.0			8.33 ± 8.33	33.33 ± 33.33
One Way ANOVA		F = 0.72	F = 1.31	F = 1.44	F = 1.50
		P = 0.672	P = 0.252	P = 0.196	P = 0.144

Table 3.4 Mean number of larvae (mean ± S.E.) per ovitrap obtained from four high-rise apartments located in Kuala Lumpur and Selangor, Malaysia.

		University of Malaya (UM), Kuala Lumpur			Kg Kerinchi (KK), Kuala Lumpur			Hang Tuah (HT), Kuala Lumpur			Kg. Baiduri (KB), Selangor		
Level	Height (m)	*Ae. aegypti*	*Ae. albopictus*	*Aedes* spp.*	*Ae. aegypti*	*Ae. albopictus*	*Aedes* spp.*	*Ae. aegypti*	*Ae. albopictus*	*Aedes* spp.*	*Ae. aegypti*	*Ae. albopictus*	*Aedes* spp.*
1	0.0 –3.0	0.00 ± 0.00	9.63 ± 5.96	9.63 ± 5.96	34.50 ± 1.62	2.75 ± 1.52	37.25 ± 1.91	28.00 ± 10.40	0.00 ± 0.00	28.00 ± 10.40	11.67 ± 6.17	2.89 ± 1.85	14.56 ± 7.98
2	3.1 – 6.0	0.00 ± 0.00	1.25 ± 1.25	1.25 ± 1.25	3.33 ± 1.12	1.50 ± 1.25	4.83 ± 1.45	16.58 ± 10.72	0.17 ± 0.17	16.75 ± 10.75	2.83 ± 2.59	0.00 ± 0.00	2.83 ± 2.59
3	6.1 – 9.0	0.00 ± 0.00	0.17 ± 0.17	0.17 ± 0.17	4.25 ± 2.92	0.75 ± 0.75	5.00 ± 2.51	22.39 ± 10.86	0.00 ± 0.00	22.39 ± 10.86	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	9.1 – 12.0	0.00 ± 0.00	1.67 ± 1.67	1.67 ± 1.67	7.83 ± 4.04	0.42 ± 0.42	8.25 ± 4.05	6.25 ± 2.59	0.00 ± 0.00	6.25 ± 2.59	0.78 ± 0.40	0.00 ± 0.00	0.78 ± 0.40
5	12.1 – 15.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.08 ± 0.74	0.00 ± 0.00	1.08 ± 0.74	14.92 ± 6.34	1.00 ± 0.59	15.92 ± 5.94	4.00 ± 3.51	0.00 ± 0.00	4.00 ± 3.51
6	15.1 – 18.0	0.00 ± 0.00	0.33 ± 0.33	0.33 ± 0.33	3.08 ± 1.50	0.83 ± 0.51	3.91 ± 1.23	12.08 ± 5.82	0.33 ± 0.33	12.41 ± 6.13	0.56 ± 0.56	0.00 ± 0.00	0.56 ± 0.56
7	18.1 – 21.0	0.00 ± 0.00	0.42 ± 0.42	0.42 ± 0.42	2.92 ± 2.55	0.08 ± 0.08	3.00 ± 2.63	3.50 ± 1.75	0.88 ± 0.88	4.38 ± 2.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8	21.1 – 24.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 0.67	0.00 ± 0.00	0.67 ± 0.67	6.09 ± 3.57	0.25 ± 0.25	6.34 ± 3.75	5.56 ± 1.37	0.00 ± 0.00	5.56 ± 1.37
9	24.1 – 27.0	0.00 ± 0.00	1.09 ± 0.88	1.09 ± 0.88	7.33 ± 7.33	0.00 ± 0.00	7.33 ± 7.33	1.25 ± 1.25	0.00 ± 0.00	1.25 ± 1.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	27.1 – 30.0				1.50 ± 1.50	0.00 ± 0.00	1.50 ± 1.50	14.33 ± 7.76	2.13 ± 2.13	16.46 ± 6.82	4.84 ± 4.84	0.00 ± 0.00	4.84 ± 4.84
11	30.1 – 33.0				5.72 ± 4.53	0.00 ± 0.00	5.72 ± 4.53	5.84 ± 1.38	0.00 ± 0.00	5.84 ± 1.38	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12	33.1 – 36.0				4.67 ± 3.33	0.67 ± 0.55	5.33 ± 2.96	4.00 ± 2.45	0.00 ± 0.00	4.00 ± 2.45	0.75 ± 0.75	0.00 ± 0.00	0.75 ± 0.75
13	36.1 – 39.0				0.33 ± 0.33	0.00 ± 0.00	0.33 ± 0.33	6.84 ± 4.27	0.00 ± 0.00	6.84 ± 4.27	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
14	39.1 – 42.0				5.42 ± 3.34	1.25 ± 0.66	6.67 ± 2.68	13.42 ± 7.45	0.00 ± 0.00	13.42 ± 7.45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	42.1 – 45.0				12.75 ± 1.13	0.00 ± 0.00	12.75 ± 1.13	0.63 ± 0.38	0.00 ± 0.00	0.63 ± 0.38	2.56 ± 2.56	0.00 ± 0.00	2.56 ± 2.56
16	45.1 – 48.0							0.42 ± 0.42	0.00 ± 0.00	0.42 ± 0.42	0.67 ± 0.67	0.00 ± 0.00	0.67 ± 0.67
Spearman's rank correlation		–	–	r = -0.471 p = 0.193	–	–	r = -0.036 p = 0.893	–	–	r = -0.682 p = 0.004	–	–	r = -0.293 p = 0.263

*Mean number of *Ae. aegypti* and *Ae. albopictus* larvae per ovitrap

Table 3.5 Mixed breeding of *Ae. aegypti* and *Ae. albopictus*.

Study site	No. of collected ovitrap	Total no. positive ovitrap	No. Ovitrap with mixed breeding of *Ae. aegypti* and *Ae. albopictus*	Percentage of positive ovitrap (%)			Ratio of *Ae.aegypti* : *Ae. albopictus* in mixed breeding
				Ae. aegypti only	*Ae. albopictus* only	Mixed breeding of *Ae. aegypti* and *Ae. albopictus*	
University of Malaya, Kuala Lumpur	104	12	0	0.00	100.00	0.00	Nil
Kg. Kerinchi, Kuala Lumur	175	64	17	70.31	3.13	26.56	3.44 : 1.00
Hang Tuah, Kuala Lumpur	145	65	7	87.69	1.54	10.77	1.91 : 1.00
Kg. Baiduri, Selangor	108	20	3	85.00	0.00	15.00	1.50 : 1.00

3.4 DISCUSSION

According to Tham (2000), ovitrap surveillance is to obtain information on *Aedes* larval densities in terms of time and space to determine the major breeding sources as well as early forecast of impending outbreaks of dengue. Among the 4 high-rise apartments, HT showed significantly higher OI than other apartment. However, mean numbers of larvae in each ovitrap were less than 10. This phenomenon may due to avoidance of "superoviposition" by female as reported by Chadee *et al*. (2004). In other word, the female mosquitoes preferred to lay eggs in ovitraps having small number of pre-existing eggs to ensure the survival of their progeny. There was significant difference between the number of larvae per ovitrap of *Ae. aegypti* and *Ae. albopictus* obtained from 4 apartments (Table 3.2). *Aedes aegypti* population was dominant in KB, KK and HT and these results were similarly reported by Lee (1992a) and Chen *et al.* (2006) in *Aedes* surveillance conducted in Selangor state.

Aedes aegypti is a domestic mosquitoes in urban area and breed exclusively in artificial containers containing relatively clean water near human dwelling (Hasanuddin *et al*., 1997). The present results suggest that the high-rise apartment creates a complete ecosystem and provides an ecological niche with biotic and abiotic components. Biotic components comprised humans, plants and pet animal in houses, while abiotic factors are temperature, humidity and house structure. All the components provide blood meals, water for aquatic stage in house with aquatic plant or unclean rubbish and resting place for adults at various elevations in high-rise apartments. Chadee (2004) reported that the adaptive quality of *Ae. aegypti* to house design had improved from ground floor to higher elevation apartment buildings. Tinker (1974) suggested that the movement of *Ae. aegypti* above the ground level may result from the insecticide pressure on breeding sites at ground level.

Aedes albopictus was domimant in UM, similarly reported by Wan-Norafikah (2009) and Chen *et al.* (2009). The typical habitats of *Ae. albopictus* to breed are natural containers, tree holes and bamboo stumps near human dwellings (Foo *et al*., 1985; Hawley, 1988). Rudnick *et al*. (1986) reported that *Ae. albopictus* has a preference for forest-fringe habitats and well-vegetated habitats with trees. Similarly, in this study the 12th Residential College was surrounded by trees and vegetations. The absence of *Ae. aegypti* in UM may be due to the lack of preferred breeding condition. The environment of 12th Residential College was generally clean with minimal potable containers since piped water supply is also available.

In Table 3.4, the results showed that *Ae. aegypti* can be found in highest floors in KB, KK and HT and *Ae. albopictus* in UM. The highest building in this study is HT which is 16 floors in height (45.1 – 48.0 m). The results suggested that *Aedes* mosquitoes could have been transported by human either by way of elevators or stairs. These results were similar to studies by Liew & Curtis (2004) who reported that ovitraps with rubidium (Rb^{-}) marked eggs of *Ae. aegypti* and *Ae. albopictus* recovered from the third level until the twenty first level (60.0 m) while Chadee (2004) reported *Ae. aegypti* can be found in a high-rise apartment up to 60.0 m.

Among 4 high-rise apartments, the waste managements and sanitation status of HT are poor compared to other apartments. Rubbish can be seen everywhere and the rubbish dumpsite was improper where the rubbish was placed outside instead inside the big container. Moreover, the drainage system of the apartment was poor where stagnant water accumulated on corridor and in the drain after raining which can provide breeding site for *Aedes* mosquitoes. This was supported by Chen *et al.* (2005), who reported that drainage system with stagnant water served as a good artificial breeding site for *Aedes* mosquitoes. According to Knudsen & Slooff (1992), garbage collection services and surface-water drainage system combined create favourable habitats for vectors and may

lead to vector-borne disease outbreak. This support the finding that HT obtained the highest OI compared to other apartment, while UM is generally clean with minimal natural container which leads to low OI. Ho *et al*. (2004) in Hong Kong reported that cleanliness is among the 8 key environmental qualities that contributed to good health and hygienic apartment which subsequently guarantee occupants' health. Ho *et al*. (2004) also stated that unhygienic environment not only created nuisance to occupants, but was also conducive to pest problem and growth of micro-organism, which led to infectious diseases outbreak.

This study confirmed that ovitrap surveillance is still a reliable and sensitive tool for detecting the presence of dengue vectors. This study showed that the *Aedes* mosquitoes had invaded and adapted to the high-rise ecosystem and this invasion can enhance the transmission of dengue especially when little or no vector control effort is conducted at the higher elevations. Integrated vector management (IVM) comprising surveillance, source reduction, education and public awareness, biological control, chemical control as well as personal protection should be carried out to suppress the *Aedes* populations, especially when the ovitrap index is 10% or higher (Lee, 1992b). In Trinidad, West Indies, Chadee (1988) reported that for security reasons, many apartments are closed for most parts of the day and vector control is difficult to execute. This phenomenon also can be seen in Malaysia. Thus, the IVM should be developed to educate households on the potential breeding sites around the high-rise apartment as well as suitable vector control measures in order to prevent future threats of dengue transmission. To prevent breeding of *Aedes*, operations and maintenance are crucial. Operations refers to standards of cleaning, pest control and refuse handling conditions, whereas maintenance refers to the inspection and maintenance of various building service such as water supply and drainage system.

CHAPTER 4

EVALUATION OF INSECT GROWTH REGULATORS (IGRS) AGAINST FIELD COLLECTED *Aedes aegypti* (Linnaeus) AND *Aedes albopictus* Skuse

4.1 INTRODUCTION

Dengue fever and dengue haemorrhagic fever have been reported as the most important arboviral diseases in Malaysia since the first description of dengue in 1902 by Skae (Lee, 2000a). Despite the control efforts in suppressing mosquito populations in Malaysia, cases of both dengue fever and dengue haemorrhagic fever are on the rise. Container-breeding *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) serve as the primary and secondary vectors, respectively (Lee, 2000b).

Control and elimination of the dengue vectors remain the most important option in long-term dengue control programme. In the situation without an effective vaccine and specific treatment, integrated vector management (IVM) approaches such as source reduction, use of insecticide, biological control, education and public awareness, as well as personal protection offer the most promising result. The use of insecticide remains a major component in vector control strategy especially during an outbreak. The insecticides frequently used are organophosphate and pyrethroid classes (WHO, 2007).

It is expected that the use of insecticide will be intensified due to the current increasing trend of dengue incidence and outbreaks. This practice will likely lead to selection of resistant strains among the exposed vector populations and subsequently

rendering current insecticide less effective (Vythilingam *et al*., 2005). Insecticide resistance is a major problem encountered in control programs both medically and agriculturally, resulting in increased insecticide usage which eventually causes environmental and health problems (Cetin *et al*., 2009). In Malaysia, temephos (Abate® 1% sand granules) has been widely used for control of immature of *Aedes* sp. since 1970s. However, *Aedes* larvae tolerance against temephos has been reported by Lee & Lime (1989) and Chen *et al* (2005b). Thus, an alternative group of larvicides with different mode of action should be used to control the immature of *Aedes* species.

Insect growth regulator (IGR) is a group of chemicals containing substances that possess growth-retarding and growth-inhibiting properties (Mulla, 1995). In general, IGR is highly effective against mosquito larvae and known to have low mammalian toxicity and good margin of safety to most nontarget biota (Vythilingam *et al*., 2005). Due to scarity of information on the susceptibility status of dengue vectors against IGR, this study was designed to evaluate the effectiveness of 5 IGRs, namely, pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron against *Ae. aegypti* and *Ae. albopictus* obtained from all states in Malaysia. This study provides comprehensive information regarding the susceptibility status of *Aedes* mosquitoes against IGRs.

4.2 MATERIALS AND METHODS

4.2.1 Study sites

Ovitrap surveillance was conducted in twelve states of Malaysia to collect representative strain of *Ae. aegypti* and *Ae. albopictus* for larval susceptibility bioassay. The coordinations of the ovitraps placed in all study sites were shown in Figure 4.1 and Table 4.1.

4.2.2 Collection using ovitrap

Ovitraps as described by Lee (1992a) were used to obtain the *Aedes* larvae. The ovitrap consists of 300 ml plastic container with straight, slightly tapered sides. The opening measures 7.8 cm in diameter, the base diameter is 6.5 cm and the container is 9.0 cm in height. The outer wall of the container is coated with a layer of black oil paint. An oviposition paddle made form hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally into each ovitrap. Each ovitrap was filled with tap water to a level of 5.5 cm. A total of 40 ovitraps were placed in indoor and outdoor in selected residential areas, respectively. In this study, “indoor” is referred to the interior of the house while “outdoor” is referred to the outdoor of the house but confined to the immediate vicinity of the house (Lee, 1992b). All ovitraps were collected after 5 days and transported to laboratory for identification and rearing purpose.

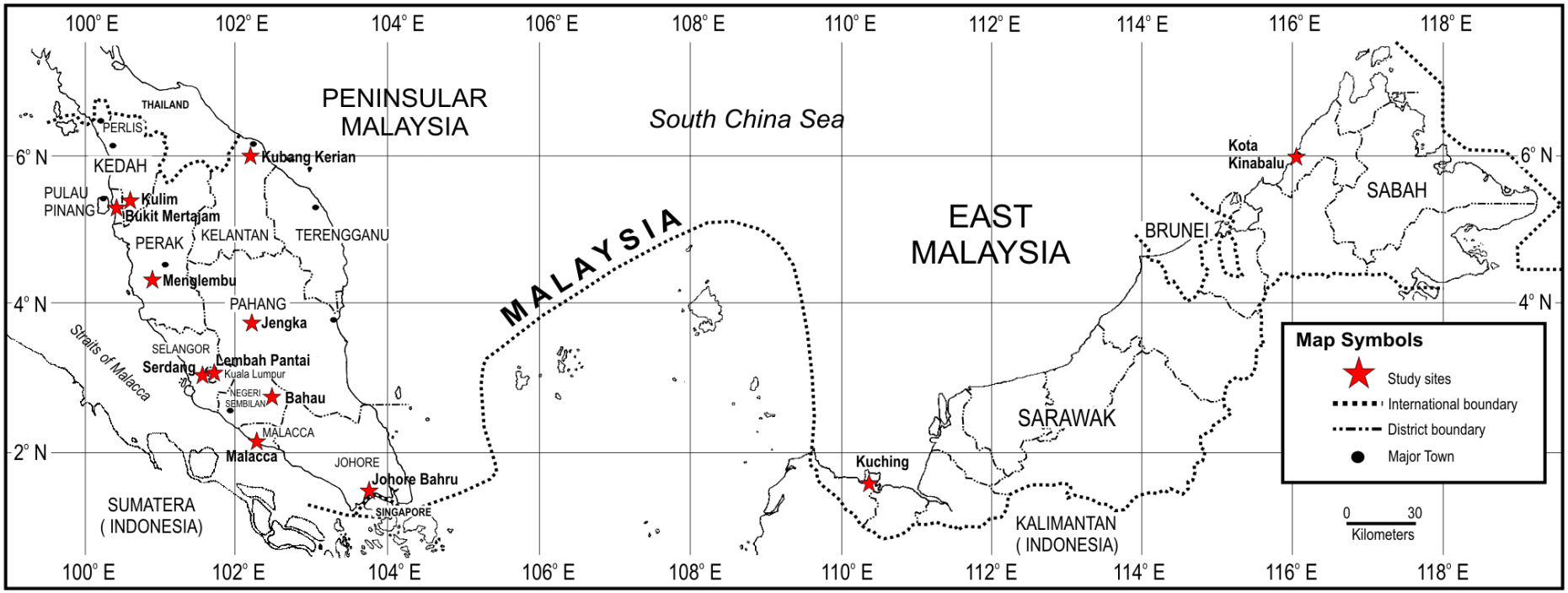

Figure 4.1 Map of Malaysia. Ovitrap surveillance was conducted in 12 states in Malaysia (as indicated by the star symbols in the map) to obtain *Aedes aegypti* and *Aedes albopictus* for larval bioassay.

Table 4.1 Geographical description of study sites in 12 states in Malaysia.

Malaysia	Region	State	Study Sites	Geographical Coordination	Landscape
Peninsular	Northern	Kedah	Kulim	5º 22’ 34.83” N, 100º 34’ 29.59” E	Urban
		Penang	Bukit Mertajam	5º 22’ 19.76” N, 100º 28’ 52.32” E	Urban
		Perak	Menglembu	4º 34’ 14.49” N, 101º 02’ 42.07” E	Urban
	Central	Selangor	Serdang	3º 01’ 40.03” N, 101º 42’ 27.23” E	Urban
		Kuala Lumpur	Lembah Pantai	3º 06’ 40.18” N, 101º 40’ 0.92” E	Urban
	Southern	Negeri Sembilan	Bahau	2º 49’ 12.54” N, 102º 25’ 00.53” E	Sub-urban
		Malacca	Malacca City	2º 17’ 41.12” N, 102º 12’ 58.56”E	Urban
		Johore	Johor Bahru	1º 29’ 5.24” N, 103º 43’ 25.10” E	Urban
	East Coast	Pahang	Jengka	3º 45’ 17.30” N, 102º 32’ 50.08” E	Sub-urban
		Kelantan	Kubang Kerian	6º 05’ 3.22” N, 102º 16’ 31.20” E	Urban
East Malaysia	East	Sabah	Kota Kinabalu	5º 57’ 57.69” N, 116º 05’ 29.19” E	Urban
	West	Sarawak	Kuching	1º 29’ 50.50” N, 110º 21’ 1.95” E	Sub-urban

4.2.3 Mosquito rearing

The collected ovitraps were brought back to the laboratory and the contents were poured into a plastic container, together with the paddle. Fresh water was added into the container and a small piece (10 mm) of fresh beef liver was added into each container as larval food. All larvae were allowed to reach adulthood and identified in the laboratory. The adult mosquitoes were transferred into mosquito cage (30 x 30 x 30 cm) for rearing purpose. Three days after emergence, female mosquitoes were blood-fed using a white mouse. Ovipostiton cup was provided for engorgement mosquitoes three days after blood-feeding. The hatched larvae, designated as first generation (F1), were subsequently used for susceptibility bioassay. For comparison purposes, two laboratory reference strains of *Ae. aegypti* and *Ae. albopistus* from Medical Entomology Unit, Institute for Medical Research, Kuala Lumpur, which have been cultured under insecticide-free condition for 1347 and 13 generations, were used, respectively.

4.2.4 Insecticides

Five insecticides used in the larval susceptibility bioassay were pyriproxyfen 0.5% w/w GR (granules), methoprene 1.3% w/w GR (granules), diflubenzuron 25% w/w WP (wettable powder), cyromzine 75% w/w WP (wettable powder) and novaluron 10% w/w EC (emulsifiable concentrate).

4.2.5 Larval susceptibility test

This test was conducted according to WHO (1981) larval susceptibility bioassay procedure for determining the susceptibility or resistance of mosquito larvae to insect development inhibitiors. A series of range finding concentrations were first prepared by diluting the stock solution into 250 mL water in a paper cup. For juvenile hormone

(pyriproxyfen and methoprene), 25 early fourth-instar larvae were introduced into each cup. After 6 hours of exposure, the pupae were removed and discarded and the remaining larvae were poured through a screen and lightly rinsed with water. They were then transferred into a paper cup filled with clean water and labeled. For chitin synthesis inhibitor (novaluron, diflubenzuron and cyromazine), 25 third-instar larvae were introduced into each cup and the larvae were continuously exposed to the insecticide. Beef liver powder was provided as larvae food. Mortality of larvae, pupae and adults was assessed daily where live and dead larvae, pupae and adults were counted until all individual died or emerged as adults. An untreated (control) was similarly set up without any insecticide.

4.2.6 Data analysis

Windows SPSS program version 11.5 was used to analyse all the data in this study. Interpretation of the results obtained from bioassay was pooled and analysed using a probit analysis software with 95% confident level. According to WHO (1970), dead larvae are those that cannot be induced to move when probed with a needle in the siphon or the cervical region. On the other hand, moribund larvae are those with characteristic diving reaction when the water is disturbed, and they may show discolouration, unnatural positions, tremors, incoordination or rigour. Both moribund and dead larvae were combined for data analysis. If emergence inhibition (EI) percentage of control was > 5%, the EI percentage of treated was corrected by Abbott's formula:

$$\frac{\%\ \text{treated EI} - \%\ \text{control EI}}{100 - \%\ \text{control EI}} \times 100\%$$

The EI_{50} (50% emergence inhibition) values for each species and their treatments were considered to be significantly different from one another when their 95% confidence limits failed to overlap. The following formula was used to calculate the resistance ratio:

$$\text{Resistance ratio (RR)} = \frac{EI_{50} \text{ of tested field strain}}{EI_{50} \text{ of tested laboratory strain}}$$

RR value of 1 – 10, 10 – 40, 40 – 160 and > 160 were classified as low, moderate, high, and extremely high resistance respectively (Kim *et al*., 2004). Values of RR less than or equal to 1 is considered susceptible. The associations between the RR values of 5 tested IGRs were assessed by Spearman rank-order correlation.

4.3 RESULTS

The emergence inhibition and resistance ratio of *Ae. aegypti* against 5 tested IGRs are summarized in Table 4.2 and 4.3. *Aedes aegypti* collected from all the 12 states in Malaysia were susceptible against diflubenzuron, cyromazine and novaluron with RR ranged between 0.07 – 0.58, 0.52 – 0.77 and 0.08 – 0.16, respectively (Table 4.2). *Aedes aegypti* obtained from 12 states exhibited low to moderate resistance to methoprene with resistance ratio (RR) ranging between 1.92 – 35.76 and low resistance to pyriproxyfen was detected with RR ranging from 0.11 – 6.06 (Table 4.3). *Aedes aegypti* from Malacca and Kuala Lumpur had highest resistance ratio to methoprene (RR = 35.76) and pyryproxyfen (RR = 6.06), respectively. In terms of mean RR, *Ae. aegypti* was moderately resistant to methoprene with RR value at 12.65 and exhibited low resistance to pyriproxyfen with RR value at 1.35. Field collected *Ae. aegypti* was highly susceptible to diflubenzuron, cyromazine and novaluron with mean RR at 0.22, 0.63 and 0.12, respectively.

The resistance levels of *Ae. albopictus* against 5 tested IGRs are shown in Table 4.4 and Table 4.5. Among all tested IGRs, field collected *Ae. albopictus* showed low resistance against diflubenzuron with RR ranged between 0.50 – 6.00, and susceptible against cyromazine, novaluron, pyriproxyfen and methoprene with RR ranging between 0.67 – 1.31, 0.75 – 1.50, 0.04 – 0.87 and 0.12 – 1.87, respectively. Mean RR value of 12 strains of *Ae. albopictus* showed that resistance against diflubenzuron was developing (2.08 folds), but susceptible to cyromazine (RR=0.91), novaluron (RR=0.98), pyriproxyfen (RR=0.22) and methoprene (RR=0.65).

Table 4.6 showed the correlation between the RR of different kind of IGRs tested against *Ae. aegypti* and *Ae. albopictus*. There is a significant correlation within the chitin synthesis inhibitor (CSI) group (diflubenzuron and novaluron, r = 0.829, P = 0.000; novaluron and cyromazine, r = 0.854, P = 0.000; cyromazine and diflubenzuron, r = 0.748, P = 0.000) and within the juvenile hormone analogue (JHA) group (pyriproxyfen and methoprene, r = 0.809, P = 0.000). However, the results showed negative correlation between all insecticides in CSI and JHA group.

In general, the results showed that field strain *Ae. aegypti* was resistant to methoprene and pyriproxyfen, but susceptible to diflubenzuron, cyromazine and novaluron. *Aedes albopictus* was only resistant to diflubenzuron, but susceptible to cyromazine, novaluron, pyriproxyfen and methoprene.

Table 4.2 Emergence inhibition (EI) and resistance ratios (RR) of *Ae. aegypti* obtained from all states in Malaysia against chitin synthesis inhibitors.

State (Strain)	EI_{50} (mg/L) (95% C.L.*)	EI_{90} (mg/L) (95% C.L*)	Regression Line	RR
Diflubenzuron				
Laboratory	0.00074 (0.00051–0.00134)	0.01112(0.00415–0.10334)	y = 1.09x – 2.50	–
Kedah	0.00012 (0.00008–0.00016)	0.00139 (0.00085–0.00299)	y = 1.20x + 4.71	0.16
Penang	0.00009 (0.00006–0.00012)	0.00117 (0.00071–0.00262)	y = 1.13x + 4.60	0.12
Perak	0.00023 (0.00002–0.00282)	0.00160(0.00047-755884.4)	y = 1.52x + 5.54	0.31
Selangor	0.00030 (0.00020–0.00042)	0.00363 (0.00188–0.01337)	y = 1.19x – 2.72	0.41
Negeri Sembilan	0.00005 (N. D.)	0.00103 (N.D.)	y = 0.94x + 4.09	0.07
Malacca	0.00013 (0.00000–0.00070)	0.00089 (0.00028–1.30139)	y = 11.52x + 5.94	0.18
Johore	0.00015 (0.00011–0.00020)	0.00166 (0.00101–0.00359)	y = 1.23x + 4.69	0.20
Pahang	0.00026 (N.D.)	0.00236 (N.D.)	y = 1.40x + 4.80	0.35
Sabah	0.00007 (0.00004–0.00011)	0.00159 (0.00085–0.00484)	y = 0.95x + 3.93	0.09
Sarawak	0.00004 (0.00001–0.00007)	0.00128 (0.00067–0.00455)	y = 0.85x + 3.73	0.05
Kelantan	0.00008 (0.00005–0.00012)	0.00166 (0.00091–0.00472)	y = 0.99x + 4.04	0.11
Kuala Lumpur	0.00043 (0.00021–0.00104)	0.04617 (0.00672–199.687)	y = 0.63x + 0.82	0.58
Mean ± S.E.	0.00016 ± 0.00003	0.00537 ± 0.00371	–	0.22 ± 0.05
Cyromazine				
Laboratory	0.15941 (0.12709–0.20580)	0.66357 (0.45829–1.13944)	y = 2.07x – 14.04	–
Kedah	0.08842 (0.07890–0.09782)	0.22577 (0.18967–0.29251)	y = 3.15x + 3.32	0.55
Penang	0.08286 (0.07338–0.09189)	0.21367 (0.18004–0.27573)	y = 3.12x + 3.37	0.52
Perak	0.12216 (0.11230–0.13379)	0.25976 (0.22157–0.32630)	y = 3.91x + 3.57	0.77
Selangor	0.08647 (0.05250–0.13323)	0.17926 (0.12153–1.41196)	y = 4.05x – 31.19	0.54
Negeri Sembilan	0.09500 (0.07504–0.11625)	0.18906 (0.14660–0.33592)	y = 4.29x + 4.38	0.60
Malacca	0.10650 (0.09572–0.11861)	0.28480 (0.23118–0.39212)	y = 3.00x + 2.92	0.67
Johore	0.10079 (0.09244–0.10966)	0.21405 (0.18604–0.26039)	y = 3.92x + 3.90	0.63
Pahang	0.10863 (0.09780–0.12108)	0.28824 (0.23379–0.39680)	y = 3.02x + 2.92	0.69
Sabah	0.10927 (0.09944–0.12025)	0.25757 (0.21650–0.33213)	y = 3.44x + 3.31	0.68
Sarawak	0.10244 (0.09268–0.11308)	0.25306 (0.21117–0.33063)	y = 3.26x + 3.23	0.64
Kelantan	0.08981 (0.08020–0.09940)	0.23120 (0.19342–0.30176)	y = 3.12x + 3.27	0.56
Kuala Lumpur	0.10593 (0.09418–0.13167)	0.21115 (0.15888–0.39610)	y = 4.28x – 33.61	0.66
Mean ± S.E.	0.09986 ± 0.00334	0.23397 ± 0.01012	–	0.63 ± 0.02
Novaluron				
Laboratory	0.00038 (0.00029–0.00059)	0.00327 (0.00157–0.01400)	y = 1.37x – 4.01	–
Kedah	0.00004 (0.00004–0.00005)	0.00011 (0.00009–0.00014)	y = 3.13x + 13.66	0.11
Penang	0.00004 (0.00003–0.00005)	0.00012 (0.00010–0.00017)	y = 2.64x + 11.66	0.11
Perak	0.00004 (0.00003–0.00005)	0.00012 (0.00010–0.00018)	y = 2.68x + 11.75	0.11
Selangor	0.00005 (0.00005–0.00006)	0.00010 (0.00009–0.00012)	y = 4.33x – 19.69	0.13
Negeri Sembilan	0.00005 (0.00004–0.00005)	0.00010 (0.00009–0.00012)	y = 4.22x + 18.18	0.13
Malacca	0.00004 (0.00004–0.00005)	0.00010 (0.00009–0.00013)	y = 3.42x + 14.95	0.11
Johore	0.00005 (0.00004–0.00005)	0.00011 (0.00009–0.00013)	y = 3.59x + 15.55	0.13
Pahang	0.00006 (0.00003–0.00009)	0.00013 (0.00008–0.00330)	y = 3.41x + 14.50	0.16
Sabah	0.00005 (0.00004–0.00006)	0.00016 (0.00013–0.00027)	y = 2.47x + 10.64	0.13
Sarawak	0.00004 (0.00003–0.00004)	0.00012 (0.00009–0.00017)	y = 2.62x + 11.58	0.11
Kelantan	0.00003 (0.00003–0.00004)	0.00012 (0.00009–0.00018)	y = 2.37x + 10.60	0.08
Kuala Lumpur	0.00004 (0.00000–0.00006)	0.00008 (0.00005–0.00082)	y = 3.82x – 16.28	0.11
Mean ± S.E.	0.00004 ± 0.000008	0.00011 ± 0.000006	–	0.12 ± 0.01

N.D. = Not determined

* Confidence Limit

Table 4.3 Emergence inhibition (EI) and resistance ratios (RR) of *Ae. aegypti* obtained from all states in Malaysia against juvenile hormone analogues.

State (Strain)	EI_{50} (mg/L) (95% C.L.*)	EI_{90} (mg/L) (95% C/L/*)	Regression Line	RR
Pyriproxyfen				
Laboratory	0.01537 (0.01223–0.01975)	0.07614 (0.05063–0.14438)	y = 1.84x – 10.09	–
Kedah	0.00228 (0.00034–0.00506)	0.19024 (0.07989–1.59148)	y = 0.67x + 1.76	0.15
Penang	0.00272 (0.00060–0.00549)	0.15398 (0.07171–0.83209)	y = 0.73x + 1.88	0.18
Perak	0.00173 (0.00017–0.00425)	0.17266 (0.07211–1.60693)	y = 0.64x + 1.77	0.11
Selangor	0.02297 (0.01752–0.02955)	0.21138 (0.14400–0.35601)	y = 1.33x – 6.12	1.49
Negeri Sembilan	0.02615 (0.02037–0.03385)	0.19474 (0.12617–0.36791)	y = 1.47x + 2.33	1.70
Malacca	0.01424 (0.01019–0.01907)	0.16303 (0.09884–0.35706)	y = 1.21x + 2.23	0.93
Johore	0.01963 (0.01398–0.02717)	0.29701 (0.15848–0.84338)	y = 1.09x + 1.85	1.28
Pahang	0.02683 (0.01949–0.03777)	0.39454 (0.20329–1.18959)	y = 1.10x + 1.72	1.71
Sabah	0.01755 (0.00030–0.12945)	0.23367 (0.05664–15.6468)	y = 1.14x + 2.00	1.14
Sarawak	0.01305 (0.00865–0.01825)	0.22359 (0.12112–0.63243)	y = 1.04x + 1.96	0.85
Kelantan	0.00931 (0.00472–0.01454)	0.37893 (0.15983–2.17261)	y = 0.80x + 1.62	0.61
Kuala Lumpur	0.09312 (0.06932–0.13195	1.24344 (0.67396–3.08850)	y = 1.14x – 5.22	6.06
Mean ± S.E.	0.02080 ± 0.00706	0.32143 ± 0.08692	–	1.35 ± 0.46
Methoprene				
Laboratory	0.00026 (0.0001–0.00093)	0.02451 (0.01134–0.12878)	y = 0.65x + 0.84	–
Kedah	0.00195 (0.00069–0.00347)	0.06103 (0.02989–0.25460)	y = 0.86x + 2.32	7.5
Penang	0.00132 (N.D.)	0.04035 (N.D.)	y = 0.86x + 2.49	5.08
Perak	0.00050 (0.00000–0.00192)	0.28747 (0.05952–500.762)	y = 0.46x + 1.53	1.92
Selangor	0.00494 (0.00285–0.00752)	0.10493 (0.05092–0.39975)	y = 0.97x – 2.46	19.00
Negeri Sembilan	0.00207 (0.00081–0.00355)	0.05435 (0.02774–0.19989)	y = 0.90x + 2.42	7.96
Malacca	0.00933 (0.00675–0.01283)	0.08320 (0.04964–0.18657)	y = 1.35x + 2.74	35.76
Johore	0.00643 (0.00448–0.00886)	0.06115 (0.03719–0.13342)	y = 1.31x + 2.87	24.73
Pahang	0.00264 (N.D.)	0.19388 (N.D.)	y = 0.69x + 1.77	10.15
Sabah	0.00242 (0.00090–0.00422)	0.08730 (0.03933–0.45983)	y = 0.82x + 2.15	9.31
Sarawak	0.00150 (0.00050–0.00271)	0.03724 (0.02003–0.12221)	y = 0.92x + 2.59	5.77
Kelantan	0.00093 (0.00012–0.00219)	0.06447 (0.02727–0.54086)	y = 0.70x + 2.11	3.58
Kuala Lumpur	0.00546 (0.00266–0.00915)	0.26347 (0.09168–2.73189)	y = 0.76x – 0.88	21.00
Mean ± S.E.	0.00329 ± 0.00077	0.11157 ± 0.02514	–	12.65 ± 2.96

N.D. = Not determined
*Confidence Limit

Table 4.4 Emergence inhibition (EI) and resistance ratios (RR) of *Ae. albopictus* obtained from all states in Malaysia against chitin synthesis inhibitors.

State (Strain)	EI_{50} (mg/L) (95% C.L.*)	EI_{90} (mg/L) (95% C.L.*)	Regression Line	RR
Diflubenzuron				
Laboratory	0.00006 (0.00003–0.00010)	0.00180 (0.00090–0.00674)	y = 0.87x + 3.66	–
Kedah	0.00010 (0.00006–0.00014)	0.00136 (0.00081–0.00311)	y = 1.13x + 4.51	1.67
Penang	0.00007 (0.00004–0.00010)	0.00103 (0.00062–0.00239)	y = 1.08x + 4.50	1.17
Perak	0.00019 (0.00015–0.00024)	0.00107 (0.00076–0.00171)	y = 1.71x + 6.37	3.17
Selangor	0.00036 (0.00029–0.00046)	0.00194 (0.00134–0.00326)	y = 1.76x + 6.07	6.00
Negeri Sembilan	0.00018 (0.00013–0.00024)	0.00222 (0.00129–0.00529)	y = 1.18x + 4.40	3.00
Malacca	0.00009 (0.00005–0.00012)	0.00134 (0.00078–0.00327)	y = 1.07x + 4.36	1.50
Johore	0.00015 (0.00011–0.00020)	0.00154 (0.00096–0.00317)	y = 1.28x + 4.88	2.50
Pahang	0.00018 (0.00000–0.00800)	0.00222 (0.00052–99179.1)	y = 1.19x + 4.43	3.00
Sabah	0.00003 (0.00001–0.00005)	0.00058 (0.00035–0.00135)	y = 0.99x + 4.47	0.50
Sarawak	0.00002 (0.00000–0.00004)	0.00055 (0.00032–0.00145)	y = 0.89x + 4.19	0.33
Kelantan	0.00009 (0.00006–0.00012)	0.00105 (0.00066–0.00222)	y = 1.17x + 4.78	1.50
Kuala Lumpur	0.00004 (0.00002–0.00007)	0.00090 (0.00052–0.00238)	y = 0.96x + 4.20	0.67
Mean ± S.E.	0.00013 ± 0.00003	0.00312 ± 0.00026	–	2.08 ± 0.46
Cyromazine				
Laboratory	0.10746 (0.09982–0.11569)	0.20095 (0.17901–0.23478)	y = 4.71x + 4.57	–
Kedah	0.09472 (0.08515–0.10465)	0.23951 (0.20010–0.31293)	y = 3.18x + 3.26	0.88
Penang	0.08708 (0.07847–0.09564)	0.20502 (0.17563–0.25623)	y = 3.45x + 3.65	0.81
Perak	0.14101 (0.12455–0.16551)	0.44000 (0.32295–0.74155)	y = 2.59x + 2.21	1.31
Selangor	0.10592 (0.09535–0.11770)	0.27775 (0.22708–0.37710)	y = 3.06x + 2.98	0.99
Negeri Sembilan	0.10830 (0.09552–0.12327)	0.35280 (0.26767–0.55869)	y = 2.50x + 2.41	1.01
Malacca	0.07160 (0.05753–0.08340)	0.28195 (0.21397–0.46069)	y = 2.15x + 2.47	0.67
Johore	0.10882 (0.10050–0.11797)	0.21855 (0.19121–0.26294)	y = 4.23x + 4.08	1.01
Pahang	0.08829 (0.07013–0.10573)	0.52021 (0.32472–1.43203)	y = 1.66x + 1.75	0.82
Sabah	0.10351 (0.08840–0.12103)	0.44461 (0.30623–0.89259)	y = 2.02x + 1.99	0.96
Sarawak	0.09615 (0.08039–0.11309)	0.45801 (0.30674–1.00122)	y = 1.89x + 1.92	0.89
Kelantan	0.09619 (0.08244–0.11085)	0.37520 (0.27178–0.66694)	y = 2.17x + 2.20	0.90
Kuala Lumpur	0.07180 (0.05652–0.08447)	0.31444 (0.23018–0.56270)	y = 2.00x + 2.29	0.67
Mean ± S.E.	0.09778 ± 0.00535	0.34400 ± 0.03009	–	0.91 ± 0.05
Novaluron				
Laboratory	0.00004 (0.00003–0.00004)	0.00009 (0.00008–0.00013)	y = 3.49x + 15.38	–
Kedah	0.00003 (N.D.)	0.00018 (N.D.)	y = 1.65x + 7.43	0.75
Penang	0.00003 (0.00002–0.00004)	0.00012 (0.00010–0.00020)	y = 2.27x + 10.15	0.75
Perak	0.00006 (0.00005–0.00006)	0.00012 (0.00010–0.00015)	y = 4.01x + 17.02	1.50
Selangor	0.00005 (0.00004–0.00006)	0.00017 (0.00013–0.00029)	y = 2.47x + 10.57	1.25
Negeri Sembilan	0.00005 (0.00004–0.00006)	0.00016 (0.00012–0.00025)	y = 2.57x + 11.04	1.25
Malacca	0.00003 (0.00002–0.00004)	0.00010 (0.00008–0.00015)	y = 2.52x + 11.31	0.75
Johore	0.00005 (0.00004–0.00005)	0.00018 (0.00013–0.00034)	y = 2.17x + 9.41	1.25
Pahang	0.00003 (0.00001–0.00004)	0.00022 (0.00013–0.00105)	y = 1.40x + 6.40	0.75
Sabah	0.00004 (0.00003–0.00005)	0.00018 (0.00012–0.00037)	y = 1.94x + 8.56	1.00
Sarawak	0.00003 (0.00002–0.00004)	0.00013 (0.00010–0.00021)	y = 2.12x + 9.53	0.75
Kelantan	0.00004 (0.00003–0.00005)	0.00017 (0.00012–0.00030)	y = 2.20x + 9.61	1.00
Kuala Lumpur	0.00003 (0.00002–0.00004)	0.00011 (0.00009–0.00017)	y = 2.34x + 10.54	0.75
Mean ± S.E.	0.00004 ± 0.000003	0.00015 ± 0.00001	–	0.98 ± 0.08

N.D. = Not determined

* Confidence Limit

Table 4.5 Emergence inhibition (EI) and resistance ratios (RR) of *Ae. albopictus* obtained from all states in Malaysia against juvenile hormone analogues.

State (Strain)	EI_{50} (mg/L) (95% C.L.*)	EI_{90} (mg/L) (95% C.L.*)	Regression Line	RR
Pyriproxyfen				
Laboratory	0.04422 (0.03028–0.07334)	1.08576 (0.41443–6.56175)	y = 0.92x + 1.25	–
Kedah	0.00302 (0.00081–0.00577)	0.13475 (0.06638–0.59575)	y = 0.78x + 1.96	0.07
Penang	0.00175 (0.00043–0.00348)	0.03827 (0.02391–0.08616)	y = 0.96x + 2.64	0.04
Perak	0.00121 (0.00008–0.00277)	0.02096 (0.01294–0.06626)	y = 1.03x + 3.01	0.03
Selangor	0.00607 (0.00381–0.00845)	0.05955 (0.04014–0.10890)	y = 1.29x + 2.86	0.14
Negeri Sembilan	0.01736 (0.01199–0.02426)	0.29837 (0.15560–0.90204)	y = 1.04x + 1.83	0.39
Malacca	0.00579 (0.00190–0.01027)	0.43441 (0.15733–4.67813)	y = 0.68x + 1.53	0.13
Johore	0.01104 (0.00606–0.01688)	0.41650 (0.17474–2.35401)	y = 0.81x + 1.59	0.25
Pahang	0.00860 (0.00000–0.03043)	0.10790 (0.03046–14936.3)	y = 1.17x + 2.41	0.19
Sabah	0.00703 (0.00375–0.01061)	0.15864 (0.08530–0.48445)	y = 0.95x + 2.04	0.16
Sarawak	0.00256 (0.00043–0.00551)	0.21135 (0.08745–1.78760)	y = 0.67x + 1.73	0.06
Kelantan	0.01198 (0.00819–0.01634)	0.15636 (0.09312–0.35916)	y = 1.15x + 2.21	0.27
Kuala Lumpur	0.03838 (0.02688–0.05990)	0.80220 (0.33777–3.81482)	y = 0.97x + 1.37	0.87
Mean ± S.E.	0.00957 ± 0.00296	0.23661 ± 0.06470	–	0.22 ± 0.07
Methoprene				
Laboratory	0.00531 (0.00402–0.00684)	0.02685 (0.01905–0.04378)	y = 1.82x + 4.14	–
Kedah	0.00113 (0.00022–0.00238)	0.05204 (0.02429–0.28403)	y = 0.77x + 2.27	0.21
Penang	0.00062 (0.00007–0.00152)	0.02728 (0.01396–0.11414)	y = 0.78x + 2.50	0.12
Perak	0.00062 (N.D.)	0.03510 (N.D.)	y = 0.73x + 2.35	0.12
Selangor	0.00619 (0.00405–0.00890)	0.08425 (0.04633–0.22991)	y = 1.13x + 2.50	1.17
Negeri Sembilan	0.00144 (0.00052–0.00253)	0.02800 (0.01611–0.07544)	y = 1.00x + 2.83	0.27
Malacca	0.00711 (0.00498–0.00982)	0.06944 (0.04153–0.15629)	y = 1.30x + 2.78	1.34
Johore	0.00992 (0.00696–0.01412)	0.11509 (0.06297–0.31009)	y = 1.20x + 2.41	1.87
Pahang	0.00122 (0.00033–0.00233)	0.03323 (0.01780–0.11321)	y = 0.89x + 2.60	0.23
Sabah	0.00077 (0.00000–0.00289)	1.30385 (0.13480–5.22267)	y = 0.40x + 1.24	0.15
Sarawak	0.00106 (0.00031–0.00198)	0.01999 (0.01180–0.05107)	y = 1.00x + 2.99	0.20
Kelantan	0.00232 (0.00113–0.00366)	0.03719 (0.02151–0.09611)	y = 1.06x + 2.80	0.44
Kuala Lumpur	0.00883 (0.00574–0.01325)	0.16364 (0.07714–0.63368)	y = 1.01x + 2.08	1.66
Mean ± S.E.	0.00351 ± 0.00101	0.16409 ± 0.10435	–	0.65 ± 0.19

N.D. = Not determined
* Confidence Limit

Table 4.6 Correlation between RR of 5 tested insect growth regulators (IGRs) against *Ae. aegypti* and *Ae. albopictus*.

Insect Growth Regulator (IGR)		Juvenile Hormone Analogue		Chitin Synthesis Inhibitior		
		Pyriproxyfen	Methoprene	Novaluron	Diflubenzuron	Cyromazine
Juvenile Hormone Analogue	Pyriproxyfen	-	r = 0.809 *P* = 0.000	r = - 0.416 *P* = 0.043	r = - 0.428 *P* = 0.037	r = - 0.489 *P* = 0.015
	Methoprene	r = 0.809 *P* = 0.000	-	r = - 0.707 *P* = 0.000	r = - 0.619 *P* = 0.001	r = - 0.705 *P* = 0.000
Chitin Synthesis Inhibitior	Novaluron	r = - 0.416 *P* = 0.043	r = - 0.707 *P* = 0.000	-	r = 0.829 *P* = 0.000	r = 0.845 *P* = 0.000
	Diflubenzuron	r = - 0.428 *P* = 0.037	r = - 0.619 *P* = 0.001	r = 0.829 *P* = 0.000	-	r = 0.748 *P* = 0.000
	Cyromazine	r = - 0.489 *P* = 0.015	r = - 0.705 *P* = 0.000	r = 0.845 *P* = 0.000	r = 0.748 *P* = 0.000	-

4.4 DISCUSSION

Insect growth regulator (IGR) is a group of insecticides containing compounds that possess growth retarding and inhibiting behaviour against larvae of mosquitoes (Mulla, 1995). Methoprene and pyripoxyfen are a juvenile hormone mimic that prevents adult emergence and are available in slow-release formulations (McCarry, 1996; Sihuincha *et al*., 2005). Diflubenzuron, cyromazine and novaluron belong to another group of IGR, namely chitin synthesis inhibitor (CSI) which inhibits the larvae to synthesis chitin during ecdysis and affecting the larval development at all larval instars and other stages.

Developments of resistance toward insecticide due to frequent use in vector control have been reported in different countries (Chen *et al*., 2005; Bisset *et al*., 1997; Georghiou *et al*., 1987). *Aedes* mosquitoes resistance has been previously reported in Malaysia for organochlorine, organophosphate, and pyrethroid insecticides (Lee *et al*., 1998; Nazni *et al*., 2000; WHO, 1980; 1992) but so far no IGR resistance in Malaysian *Aedes* populations.

In this study, we found that moderate levels of resistance toward methroprene and low resistance toward pyriproxyfen in field populations of *Ae. aegypti* by 12.65-folds and 1.35-folds, respectively. On the other hand, field populations of *Ae. albopictus* only exhibited low resistance toward diflubenzuron by 2.08-folds. According to Schoeppner (1978) and Estrada and Mulla (1986), methoprene and pyriproxyfen have been introduced and evaluated in the past decades. Mullin and Scott (1992) reported that increased insecticide application frequencies and increased dosage resulted in development of resistance. Resistance built up by field populations of *Aedes* mosquitoes may be due to prolonged usage of the same insecticide since these chemicals exist in market for more than a decade. The lack of information and consistent surveys on

monitoring the susceptibility status of *Aedes* mosquitoes to IGR was another factor contributing to the development of resistance. Without the baseline data for programme planning and insecticide selection obtained from surveillance, early detection of resistance was neglected and replacement of the insecticides may not be available. Consequently, resistance was developed without any consciousness by users. It is important to conduct regular monitoring to determine the level of susceptibility in order to maintain the effectiveness of existing insecticides and delay the development of resistance resulting in control failures (Cetin *et al*., 2009).

Hatakoshi *et al.* (1987), Loh and Yap (1989), Itoh (1994), and Seccacini *et al*. (2008) reported that EI_{50} of pyriproxyfen against *Ae. aegypti* ranged from 0.000011 to 0.000214 mg/L. Our study found that the EI_{50} of pyriproxyfen ranged from 0.00121 to 0.09312 mg/L for both *Aedes* species. Our EI_{50} values were higher indicating that our *Aedes* species were more resistant than the above mentioned studies by 110.00 – 435.14 folds.

The EI_{50} of diflubenzuron and methoprene against *Ae. aegypti* and *Ae. albopictus* obtained from this study ranged from 0.00002 – 0.00036 mg/L and 0.00026 – 0.00933 mg/L, respectively. Silva and Mendes (2007) reported that the LC_{50} of dliflubenzuron and methoprene were 0.00519 mg/L and 0.01995 mg/L, respectively, while Seccacini *et al*. (2008) reported the EI_{50} of diflubenzuron was 0.00159 mg/L. Although our result showed that Malaysian strain of *Aedes* mosquitoes developed a certain level of resistance toward diflubenzuron and methoprene; however, the concentrations used to obtain EI_{50} in this region is much lower than EI_{50} reported by Silva and Mendes (2007) and Seccacini *et al*. (2008) by 4.42 – 259.50 folds and 2.14 – 76.73 folds in both IGRs, respectively. This indicated that both compounds still can be used in this region when compared to other countries.

There are significant correlations within JH group and within CSI group indicating cross-resistance between insecticides within the same group. On the other words, resistance developed by targeted vector of one insecticide of the group may resist to other chemical within the same group of insecticides or same mode of action. Brown *et al*. (1978) reported that methoprene-resistance developed in *Culex pipiens* after 40 generations of selection pressure and extended in cross-resistance to 5 other JH mimics but not to diflubenzuron. This is in line with our study. Bloomcamp *et al.* (1987) reported that no cross resistance to methoprene (JHA) was found in the cyromazine (CSI) resistant *Musca domestica*. Our result was similar to Bloomcamp *et al.* (1987), in which JH and CSI was negatively correlated, indicating the absence of cross-resistance between these two groups of IGRs.

Although resistance was observed from methoprene and diflubenzuron, the EI_{50} value obtained in this study was lower than those reported by other researchers. Our study concluded that the tested IGRs especially cyromazine and novaluron provided promising results and can be used to control field populations of *Ae. aegypti* and *Ae. albopictus*. The use of IGR should be considered as an alternative when larvae develop resistance to conventional insecticides. Cerf and Georghiou (1972) reported that there has been cross-resistance between organophosphorus and some juvenile hormone analogue in house flies. The development of resistance and high EI_{50} value of pyriproxyfen might cause a cross-resistance, as pyriproxyfen is a juvenile hormone analogue. In this case, there is a need to conduct regular surveys to monitor the susceptibility status of *Aedes* mosquitoes towards conventional insecticides as well as IGRs in order to prevent resistance development. Regular surveys will provide information for early detection and establishment of more effective control programs.

CHAPTER 5

EVALUATION OF INSECT GROWTH REGULATORS (IGRS), TEMEPHOS AND *Bacillus thuringiensis israelensis* (Bti) AGAINST *Aedes aegypti* (Linnaeus) IN PLASTIC CONTAINERS

5.1 INTRODUCTION

Currently, dengue is considered as the most important arboviral disease of human in term of its public health importance in tropical, subtropical and temperate regions of the world (Gubler, 1989; Gubler *et al.*, 1998). Dengue has remained endemic in Malaysia since the first documented case in 1902, while the first major national dengue outbreak occurred in 1973 (Skae, 1902; Lee, 1994). *Aedes aegypti* (Linnaeus) has been incriminated as the primary vector in the transmission of dengue fever (DF) and dengue haemorrhagic fever (DHF) (Chen *et al*., 2005).

Without an effective dengue vaccine and specific treatment, the use of chemical agents is one of the most important methods of controlling dengue vector. The control approaches used by the Vector Borne Disease Control Program (VBDCP) in Malaysia are fogging with chemical insecticides and source reductions in affected areas (Lee *et al*, 2008). Larviciding using temephos is recommended by WHO since early 1970 for the control of container-breeding *Aedes* mosquitoes (WHO, 1985). In Malaysia, temephos (Abate® 1% sand granules) was widely used by the public to control the immature of *Ae. aegypti* for the last 3 decades. However, several studies in Malaysia had shown that

the susceptibility of *Aedes* larvae to temephos is decreasing due to the development of resistance (Lee & Lime, 1989; Chen *et al*., 2005). Another larvicide, *Bacillus thuringiensis* var. *israelensis* (Bti) is a microbial control agent known for the efficacy and selectivity against mosquito larvae. Although *Bacillus thuringiensis* var. *israelensis* (Bti) can be used as an alternative control agent, the bacteria cannot self-replicate and thus the residual efficacy is reduced (Vythilingam *at al*., 2005).

Insect growth regulators (IGRs) are group of insecticides containing substances that possess growth retarding and growth inhibiting properties selective for immature of insects including mosquito larvae and have no apparent ill effect on non-target organisms including mammals (Mulla *et al.*, 1986; Mulla, 1995). Insect growth regulators are now increasingly used to control *Aedes* and other mosquito larvae. Most IGRs are being developed to satisfy all the factors that enable larviciding more desirable when dealing with problem of pest/disease outbreaks. Through hormonal imbalance and inhibition of chitin formation caused by IGRs, these chemicals suppress insect embryogenesis, metamorphosis, and adult emergence (Mulla, 1995). In past decade, Lam (1990), Mulla (1995), Seccacini *et al*. (2008) and Chen *et al*. (2008) have reported studies on laboratory evaluation and field efficacy of a number of IGRs against mosquito larvae.

This study was designed to evaluate the residual effectiveness of five IGRs, namely pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron in comparison to temephos and Bti.

5.2 MATERIALS AND METHODS

5.2.1 Test container

Plastic containers with an opening of 22.0 cm in diameter, base diameter of 19.5cm and 21.7cm in height were used in this study. Five replicates were used for each chemical. Before initiating the study, all containers were washed with tap water and tested for the presence of any larvicidal contaminant by introducing 30 lab-bred early 3rd instar *Ae. aegypti* larvae. The larvae were observed daily until complete emergence as adults.

5.2.2 Test insecticides

Five insect growth regulators (IGRs) used in this study were pyriproxyfen 0.5% w/w GR (granules), methoprene 1.3% w/w GR (granules), diflubenzuron 25% w/w WP (wettable powder), cyromzine 75% w/w WP (wettable powder) and novaluron 10% w/w EC (emulsifiable concentrate). Bti wettable granule (VectorBac WG, recommended dosage = 8g / 1000 L) and temephos sand granule (Abate 1.1G recommended dosage = 1 mg/L) were also tested in this study.

5.2.3 Test insect

Laboratory-bred 3rd instar *Ae. aegypti* were used in the test. The colony was maintained in the laboratory for more than 30 years and not exposed to any control agents.

Table 5.1 Concentration of EI_{90} of each IGRs against laboratory strain of *Ae. aegypti* and test concentration (10 x EI_{90}) used in this study.

Insect growth regulator	EI_{90} (mg/L) against *Aedes aegypti* (95% C.L.*)	10 X EI_{90} (mg/L) used in this study
Pyriproxyfen	0.076 (0.051–0.144)	0.761
Methoprene	0.025 (0.011–0.129)	0.245
Diflubenzuron	0.011 (0.004–0.103)	0.111
Cyromazine	0.664 (0.458–1.139)	6.636
Novaluron	0.003 (0.001–0.014)	0.033

*C.L. = Confidence Limit

5.2.4 Trail Procedure

The trial procedure was modified according to the protocol used by Chen *et al*. (2008). The applied concentration of IGR was 10 times of 90% emergence inhibition (EI_{90}) (Table 5.1). The EI_{90} of each IGR against laboratory strain of *Ae. aegypti* was obtained by using standard larval bioassay procedures recommended by WHO (1981). Bti (VectorBac WG) and temephos (Abate®) were also tested for comparison purpose. Five containers holding 5 litres of water were set up in indoor (laboratory condition) and outdoor (simulated field condition) under the eave for each chemical. Five containers without chemicals served as untreated control. In each arm of study, 30 laboratory-bred 3^{rd} instar larvae were introduced into each plastic container and mortality of larvae, pupae and adults were monitored daily. A small piece of liver was added to each container as larvae food. In both experiments, the containers were covered with net to prevent oviposition of wild mosquitoes and to prevent emerged adults from escaping from the containers. After 7 days of exposure, the live larvae and pupae were collected, recorded and transferred into paper cups covered with net for observation until all individuals died or emerged as adults. A 50% of the total volume of water (2.5 litres) was removed and replenished weekly. The same procedure was repeated by adding fresh batch of larvae (30 larvae) into each container weekly.

5.2.5 Statistical analysis

Statistical software (SPSS v11.5) was used to analysis the data. The indicators of effectiveness of tested chemicals for these studies were:

i. duration of effectiveness of tested chemical, and

ii. percentage of emergence inhibition (EI) =

$$\frac{\textbf{Number of larvae introduced – Number of adult emerged}}{\textbf{Number of larvae introduced}} \textbf{ X 100\%}$$

A cut-off point of emergence inhibition (EI) or mortality ≥ 50% was considered effective. If percentage of untreated EI was > 5% the percentage of treated EI was corrected by Abbott's formula:

$$\frac{\text{\% treated EI – \% control EI}}{\text{100 - \% control EI}} \text{ X 100\%}$$

5.3 RESULTS

Figure 5.1 showed the weekly percentage of emergence inhibition (EI) of *Ae. aegypti* in indoor plastic containers treated with 5 IGRs, temephos and Bti . Complete emergence inhibition/mortality of *Ae. aegypti* larvae was found in pyriproxyfen treated containers for 28 weeks, followed by temephos (22 weeks), novaluron (15 weeks), methoprene (12 weeks), Bti (8 weeks), cyromazine (7 weeks) and diflubenzuron (6 weeks). By using 50% emergence inhibition as the indicator of residual efficacy, pyriproxyfen exhibited longest residual effect lasted for 43 weeks before declining to 50% EI and lower on week 44. The residual activity of larvicides against *Ae. aegypti* in container placed indoor in descending order was: pyriproxyfen> temephos> novaluron> methoprene> Bti> cyromazine> dlifubenzuron with 50% EI at 43 weeks, 26 weeks, 23 weeks, 21 weeks, 14 weeks, 12 weeks, and 11 weeks, respectively.

Figure 5.2 shows the weekly percentage of emergence inhibition (EI) of *Ae. aegypti* in plastic container treated with 5 IGRs, temephos and Bti under outdoor condition. The plastic containers placed outdoor treated with pyriproxyfen induced complete inhibition for 15 weeks, followed by temephos (12 weeks). Both novaluron and methoprene showed complete inhibition for 9 weeks, while cyromazine, diflubenzurona and Bti showed complete inhibition for 6 weeks, 4 weeks and 1 week, respectively. The residual activity of pyriproxyfen against *Ae. aegypti* under outdoor condition exhibited up to 26 weeks with emergence inhibition more than 50% (Table 5.2). The residual efficacy of container treated with pyriproxyfen was the longest while the shortest was treated by Bti with 2 weeks of residue effect. The residual activity of larvicides against *Ae. aegypti* in container placed outdoor in descending order was: pyriproxyfen> temephos> methoprene> novaluron> cyromazine> diflubenzuron> Bti with 50% EI at 26 weeks, 16 weeks, 15 weeks, 13 weeks, 8 weeks, 6 weeks and 2 weeks, respectively. In all untreated containers, all the pupae emerged successfully.

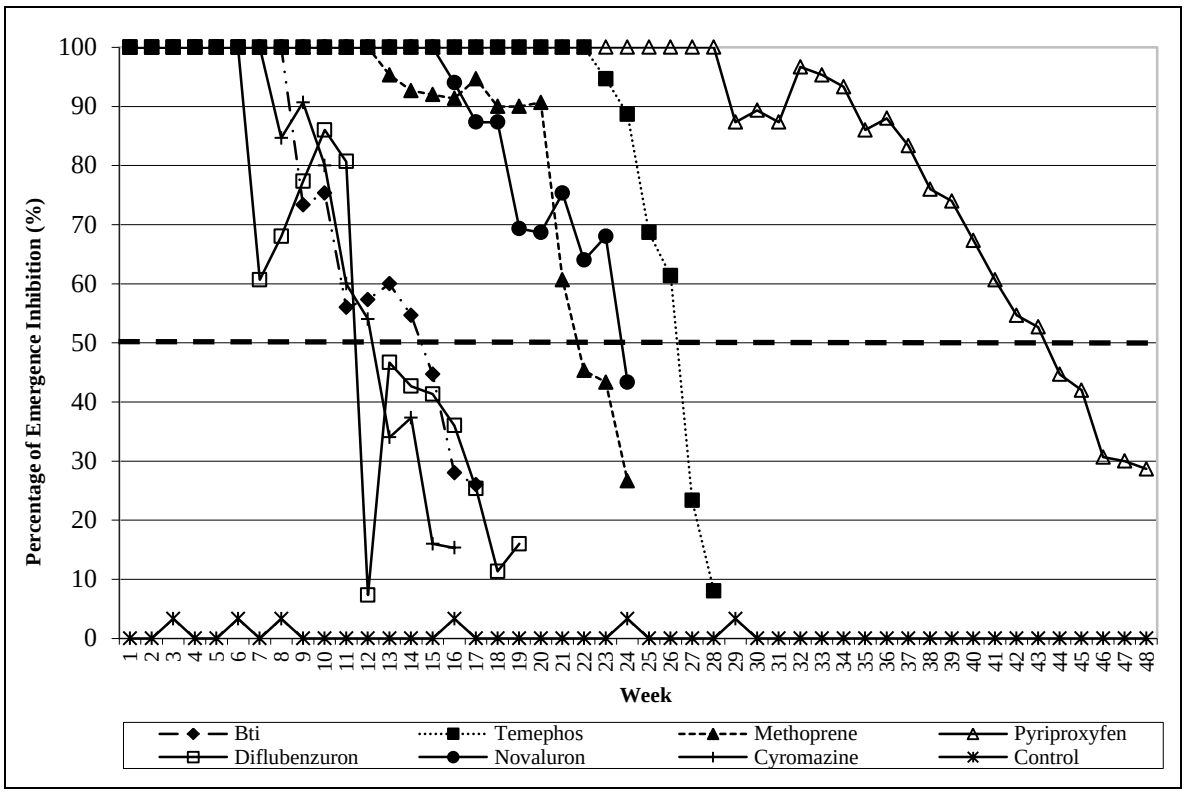

Figure 5.1 Bioefficacy of insect growth regulators, temephos and Bti against *Ae. aegypti* in plastic containers under indoor condition. Dotted line indicated the residual efficacy at cut-off point at ≥50% EI.

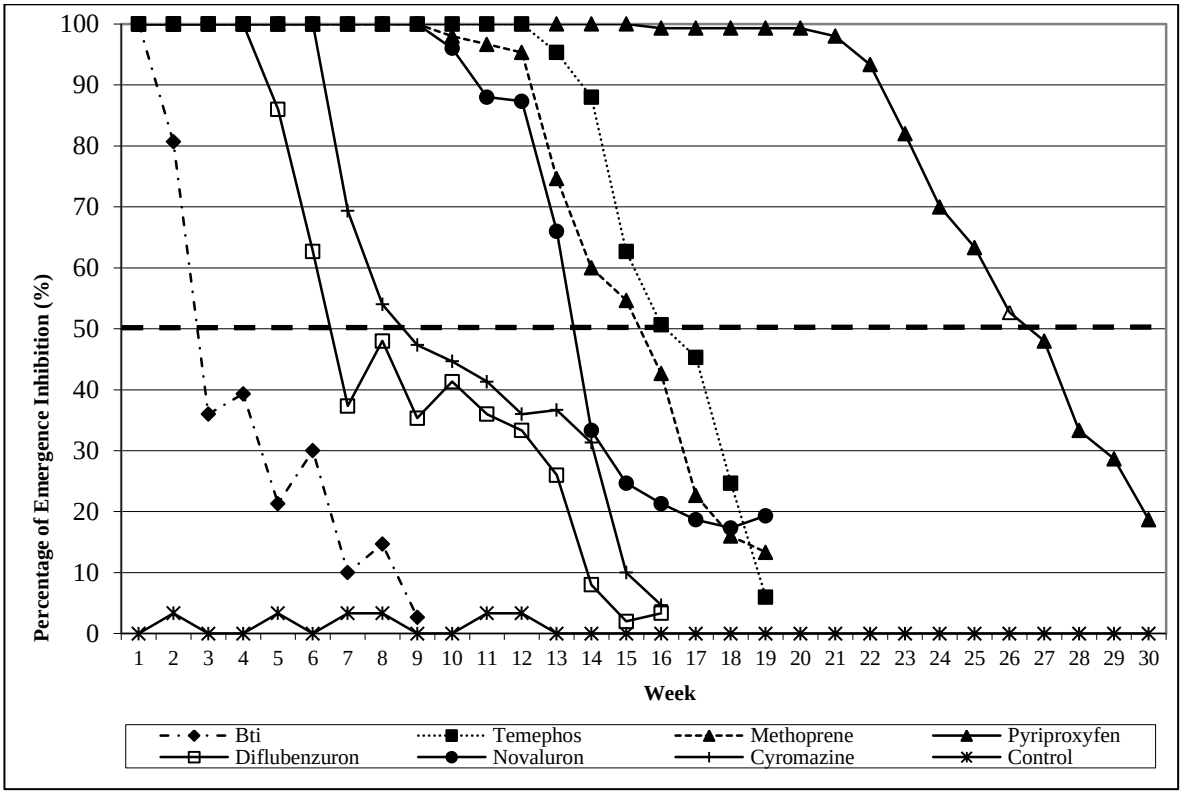

Figure 5.2 Bioefficacy of insect growth regulators, temephos and Bti against *Ae. aegypti* in plastic containers under outdoor condition. Dotted line indicated the residual efficacy cut-off point at ≥50% EI.

Table 5.2 Residual activity of 5 IGRs, temephos and Bti against *Ae. aegypti* larvae in plastic containers placed in indoor and outdoor.

Insecticides	Number of Week			
	Indoor		Outdoor	
	100% EI	≥ 50% EI	100% EI	≥ 50% EI
Diflubenzuron	6	11	4	6
Cyromazine	7	12	6	8
Novaluron	15	23	9	13
Pyriproxyfen	28	43	15	26
Methoprene	12	21	9	15
Temephos	22	26	12	16
Bti	8	14	1	2

EI = Emergence Inhibition
Bti = *Bacillus thuringiensis israelensis*

5.4 DISCUSSION

Our results showed that pyriproxyfen was the most effective IGR in terms of duration with complete inhibition and residual activity throughout the experiment under indoor and outdoor conditions. In indoor conditions, treatment with pyriproxyfen showed 28 weeks of complete inhibition and residual activity up to 43 weeks. Vythilingam *at al*. (2005) reported that 0.01 and 0.02 mg/L pyriproxyfen were highly effective against *Ae. aegypti* for 16 weeks with replacement of water in simulated field trial. Seccacini *et al*. (2008) also reported that the 0.1 mg/L granular sand formulations of pyriproxyfen remained active for over 4 months (>16 weeks). Studies by WHO (2001) and Nayar *et al*. (2002) also reported complete EI against *Ae. aegypti* for 6 weeks in plastic tubes placed outdoor.

The outdoor containers treated with diflubenzuron showed complete inhibition for 4 weeks, similar to that reported by Chen *et al.* (2008). Lam (1990) reported that the duration of effectiveness after application of wettable formulation of diflubenzuron (Dimilin® WP-25) in septic tanks to control *Ae. albopictus* breedings was up to 8 weeks. Seccacini *et al*. (2008) reported that in a simulated field study, the 0.1mg/L granular formulation of diflubenzuron was able to control *Ae. aegypti* up to 4 months (≈16

weeks). Unlike our results, Thavara *et al*. (2007) reported that the efficacy of the 0.02 mg/L of tablet and granular formulations lasted for 21 and 22 weeks post-treatment, respectively. Under the conditions where half of the water in treated jar was removed and refilled, tablet and granular formulation achieved 96–100% EI up to 21 weeks post-treatment (Thavara *et al*., 2007). Cetin *et al.* (2006) conducted a study on diflubenzuron (25% wettable powder and 4% granular formulation) against *Culex pipens*. Their results indicated that both formulations tested at 0.01, 0.02 and 0.03 mg a.i./L were able to achieve 100% adult inhibition up to 4 weeks post treatment.

The residual efficacy of methoprene, novaluron and cyromazine was shorter than pyriproxyfen and temephos but longer than diflubenzuron and Bti. Nayar *et al*. (2002) reported that the residual activity of 0.02 and 0.05 mg/L of methorpene was less effective compared to same concentration of pyriproxyfen with EI 22.3 – 93.7% during 6 weeks of observation. An experiment conducted by Mulla *et al*. (2003) in Thailand under field condition showed that EC10 of novaluron (0.05 – 1 mg/L) exhibited 86 – 96% of EI for about 190 days (≈27 weeks), while 0.001 – 0.02 mg/L achieved 80 – 100% of EI for 2 months (≈8 weeks). Because of the scarcity of data on residual activity of cyromazine against *Ae. aegypti*, the result obtained in this study was useful for consideration in future field evaluation.

Temephos in our result showed second longest residual activity in both indoor and outdoor conditions. Temephos is an organophosphorus compound with very low mammalian toxicity and has been used for the control of *Aedes* larvae in potable water since the early 1970s (Chen and Lee, 2006). Chen and Lee (2006) reported that the residual effect of 1 mg a.i./L. temephos in earthen jar lasted 15 weeks under laboratory condition. Mulla *et al*. (2004) reported that glazed clay water storage jars treated with temephos sand granules (1%) and temephos zeolite granules (1%) yielded almost 100% mortality for more than 6 months (≈24 weeks). Thavara *et al*. (2004) also reported that a

single application of temephos zeolite granules at 1 mg a.i./L provided high and satisfactory control period of at least 3 months (≈12 weeks) in water storage containers in field under normal water use practices.

Plastic containers treated with Bti exhibited 14 weeks of residual larvicidal activity in indoor but only 2 weeks in outdoor. Lee and Zairi (2005) reported that more than 80% reduction of mosquitoes were recorded in earthen jars treated with Bti up to 40 days, while Lima *et al*. (2005) reported larval mortality of 70% or more attained for 2 – 5 weeks in containers treated with Bti. The field efficacy of Bti reported by Lee and Cheong (1987) was up to 6 weeks. Chen *et al*. (2009) also reported that 80% larvae mortality was obtained in earthen jars without plants up to 10 weeks while earthen jars with aquatic plants achieved more than 50% mortality up to 7 weeks. According to Becker *et al*. (2010), although $Cobalt^{60}$ source is well suited for Bti product sterilization without significantly reducing their toxicity, exposure of strong sunlight appears to reduce the larvicidal effect of Bti. Becker *et al*. (1992) also reported that the LC_{90} value at sunny sites (LC_{90}= 0.235 ± 0.036 ppm) was 4 times higher than in shaded conditions (LC_{90} = 0.054 ± 0.008 ppm) in which the third-instar larvae of *Culex pipiens* were treated with Bti powder at the same time and under identical conditions with temperature of 25 ± 1 ℃.

In general, the residual activity in outdoor conditions had reduced compared to indoor, probably the insecticides in outdoor containers were degraded by sunlight and heat as the stability of insecticides are affected by direct sunlight and temperature. Robertson and Pope (2005) and Ogg *et al*. (2007) reported that freezing and excess heat can shorten the shelf life of insecticides and direct sunlight will also degrade the insecticides. Ho *et al*. (1990) conducted an experiment by exposing IGRs to ultraviolet irradiation or heat management (45 ℃ – 60 ℃) and showed that diflubenzuron and flufenoxuron were very stable but the other tested IGRs such as methoprene was not

included in the study. However, study of the degradation rate of the insecticides by sunlight and heat was not conducted in this trial.

In addition to possessing effectiveness, formulation is another factor affecting the residual activity. Seccacini *et al*. (2008) reported that the emulsifiable concentrate formulations (EC) of diflubenzuron diminished the concentration of the compound ingested by larvae due to instability in water and low aqueous solubility. On the other hand, the EC pyriproxyfen was 5 times more effective than the technical grade. Emulsifiable concentrates (EC) are liquid formulations in which the active ingredient has been dissolved in oil or solvents that can be mixed with water or oil for spraying purpose. Wettable powders (WP) are dry powdered pesticides formulations containing wetting and dispersing agents, which are suitable for some active ingredients which cannot be formulated into EC. Chen *et al.* (2008) reported that the diflubenzuron WP mixed well in water and did not produce turbidity which was similar to our observation. In this study, sand granule (GR) formulation of insecticides was more effective than the EC and wettable powder (WP). The sand granule formulation was designed to sink to the bottom of the water body to release the active ingredient slowly so that the concentration was maintained in treated water body. Thavara *et al*. (2007) showed that residue efficacy of the granular formulation of diflubenzuron was up to 22 weeks post-treatment, indicating that this formulation provides significantly long residual activity.

In terms of user preference, direct application method is simple and can be easily applied in areas such as drains and ponds and in places where long-term control is desired. The IGRs do not smell or produce turbidity in treated water like temephos. Moreover, pyriproxyfen, diflubenzuron and novaluron have been accepted by WHO for application in drinking water (WHO, 2008). The IGRs induce late mortality after treatment and this is a desirable feature of a control agent since mosquito larvae and other vectors are important food source for aquatic animals (Mulla, 1995). However, the

treated larvae will still be present and alive until late mortality occurs due to the mode of action of IGR, and this may discourage the use of these insecticides in some countries. In countries like Malaysia, the presence of *Aedes* larvae is ground for the enforcement officers to take legal action against the house-owners in spite of the application of IGR. Thus, the user and the enforcer should be educated on the use of IGR.

In conclusion, pyriproxyfen has shown long-term effectiveness against immature stages of *Ae. aegypti* compared with other IGRs and larvicides. It appears to be one of the best alternatives to conventional chemical insecticides such as temephos where *Aedes* larvae had been shown to develop resistance.

CHAPTER 6

GENERAL DISCUSSION

In tropical countries, *Ae. aegypti* and *Ae. albopictus* are important vectors of dengue, dengue hemorrhagic fever, yellow fever, chikungunya and other viral diseases. (Lee *et al*., 1997).

The first step to be considered in Integrated Vector Management (IVM) for dengue control is to determine the distribution and abundance of the dengue vectors. Ovitrap surveillance is a method to obtain spatial-temporal information on *Aedes* larval densities to determine the major breeding sources as well as early forecast of impending outbreaks of dengue, so that early remedial action can be taken to suppress the outbreak (Tham, 2000).

In Study 1 (Chapter 3), ovitrap surveillance conducted in 4 high-rise apartments, namely Kg. Baiduri (KB), Student Hostel of University of Malaya (UM), Kg. Kerinchi (KK) and Hang Tuah (HT) located in Selangor and Kuala Lumpur. Among the 4 high-rise apartments, HT showed significantly higher Ovitrap Index (OI) than other apartments. However, the mean number of larvae in each ovitraps were less than 10. This phenomenon may be due to avoidance of "superoviposion" by female as reported by Chadee *et al*. (1990). In other word, the female mosquitoes preferred to lay eggs in ovitraps having a small number of pre-existing eggs to ensure the survival of their progeny (William *et al*., 2008).

Aedes aegypti was dominant in Kg. Baiduri, Kg. Kerinchi and Hang Tuah and similar observations were reported by Lee (1992a) and Chen *et al.* (2006) in *Aedes* surveillance conducted in Selangor state. However, *Aedes albopictus* predominated in UM, similarly reported by Wan-Norafikah (2009) and Chen *et al.* (2009). Rudnick *et al*. (1986) reported that *Ae. albopictus* has a preference for forest-fringe habitats and well-vegetated habitats with trees. In this study the 12th Residential College was surrounded by trees and other vegetation, thus supporting the breeding of *Ae. albopictus* as reported by Rudnick *et al*. (1986). The absence of *Ae. aegypti* in UM may be due to the lack of preferred breeding condition.

Our study revealed that *Ae. aegypti* and *Ae. albopictus* can be found from ground floor to highest floor in all study sites. The tallest building is Hang Tuah with 16 floors in height (45.1 – 48.0 m). The results were similar to those reported by Liew & Curtis (2004) and Chadee (2004). This suggests that the high-rise apartment creates a complete ecosystem and provides an ecological niche with biotic and abiotic components suitable for *Aedes* breeding. Biotic components comprised humans, plants and pet animals in houses, while abiotic factors are temperature, humidity, containers and house structure. Collectively, all the components provide blood meals, water for aquatic stage in house with aquatic plant or unclean rubbish and resting place for adults at various elevations in high-rise apartments. Chadee (2004) reported that the adaptive nature of *Ae. aegypti* to house design had improved from ground floor to higher elevation apartment buildings. Tinker (1974) also suggested that the movement of *Ae. aegypti* above the ground level may result from the insecticide pressure on breeding sites at ground level.

In addition, the waste management and sanitation status of high-rise apartment play an important role. According to Knudsen & Slooff (1992), garbage collection services and surface-water drainage system combined to create a favourable habitat for

vectors and may lead to vector-borne disease outbreak. Ho *et al*. (2004) also stated that unhygienic environment not only created nuisance to occupants, but was also conducive to pest problem and growth of micro-organisms, which led to infectious diseases outbreak. Our observations in some apartments showed that improper disposal habit and poor drainage system led to higher ovitrap index. This was supported by Chen *et al.* (2005), who reported that drainage system with stagnant water served as a good artificial breeding site for *Aedes* mosquitoes. To prevent breeding of *Aedes*, operations and maintenance are crucial. Operation refers to standards of cleaning, pest control and refuse handling conditions, whereas maintenance refers to the inspection and maintenance of various building service such as water supply and drainage system.

Among the approaches on controlling the *Aedes* mosquito populations, chemical control is a common component in IVM. In Malaysia, insect growth regulators (IGRs) are seldom used in control strategies and information on these insecticides are scarce. In Study 2 (Chapter 4), the resistance status of the *Ae. aegypti* and *Ae. albopictus* against IGRs was determined. Ovitraps were set up in 12 states in Malaysia to obtain representative strain of *Aedes* mosquitoes. Larvae bioassay was conducted with field collected *Ae. aegypti* and *Ae. albopictus* to measure the level of resistance to 5 insect growth regulators, namely pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron.

Insect growth regulator, in general, is highly active and selective against larvae of mosquitoes and others insect, and has a good margin of safety to most non-target biota including invertebrates, fish, birds and human. The common characteristic of these chemicals is that they do not induce instant mortality in the treated larvae. The treated larvae survive but die in the pupal or adult stage.

The result obtained from Study 2 showed that field populations of *Ae. aegypti* showed moderate levels of resistance toward methoprene and low resistance toward

pyriproxyfen by 12.65-folds and 1.35-folds, respectively, but susceptible to diflubenzuron, cyromazine and novaluron. On the other hand, field populations of *Ae. albopcitus* only exhibited low resistance toward diflubenzuron by 2.08-folds. Resistance built up by field populations of *Aedes* mosquitoes may be due to increased insecticide application frequencies and increased dosage. There are significant correlations within juvenile hormone analogue (JHA) group and within chitin synthesis inhibitor (CSI) group, indicating that cross resistance between insecticides within the same group. Our result was similar to Bloomcamp *et al*. (1987), in which JHA and CSI was negatively correlated and indicated absence of cross-resistance between these two groups. Although IGR is less popular in mosquito control, some IGRs have been widely used to control agricultural pest since last decade, such as teflubenzuron, buprofezin and lufenuron in Malaysia (Furlong *et al*., 1994). Cross resistance may occur on the survived larvae breeding in the same contaminated environment together with the agriculture pests without the knowledge of the user. Thus, regular monitoring is important for early resistance detection.

The residue efficacy of pyriproxyfen, methoprene, diflubenzuron, cyromazine and novaluron was determined and compared with operational dosage of temephos (1 mg/L) and *Bacillus thuringiensis israelensis* (Bti) (0.008mg/L). Among the tested IGRs, pyriproxyfen was the most effective in terms of duration with complete inhibition and residual activity throughout the experiment under indoor and outdoor conditions. Temephos exhibited second longest residual activity in our study in both indoor and outdoor. In general, the residual activity of tested larvicides in descending order was pyriproxyfen > temephos > novaluron > methoprene > Bti > cyromazine > diflubenzuron under indoor condition; and pyriproxyfen > temephos > methoprene > novaluron > cyromazine > diflubenzuron > Bti under outdoor condition.

Hence this study confirmed that pyriproxyfen is the best alternative control agent applied to temephos-resistant *Aedes* larvae in term of residual efficacy. However, *Aedes* larvae resistance against pyriproxyfen, methoprene and diflubenzuron were detected in Study 2. Hence, these IGRs cannot be recommended as alternative insecticides to control *Aedes* larvae. Novaluron and cyromazine would be the best alternative choice of insecticide used in control strategy since resistance was not detected.

Our study has also addressed some of the problems related to the distribution of the *Aedes* mosquitoes. The vertical distribution of *Aedes* population was shown to be determined by the availability of resting habitats, blood source and the presence of oviposition sites. The invasion of *Aedes* mosquitoes could increase the transmission of dengue. Although field collected *Aedes* larvae were susceptible to novaluron and cyromazine, resistance to pyriproxyfen, methoprene and diflubenzuron was detected. These indicated the presence of resistance gene in the field population of *Ae. aegypti* and *Ae. albopictus*. It is also likely that *Ae. aegypti* and *Ae. albopictus* may be resistant to other IGR due to cross-resistance found in field populations.

It is obvious that there is a need to improve present dengue control methods. Integrated Vector Management (IVM) comprising surveillance, source reduction, education and public awareness, biological control, chemical control as well as personal protection should be implemented to suppress the *Aedes* populations and prevent the disease outbreak. The public should be educated on the importance of creating a clean and hygienic environment in high-rise apartments to prevent further invasion of dengue vectors .

Chemical control method such as fogging should be carried out floor to floor in order to eliminate the vector. In Trinidad, West Indies, Chadee (1988) reported that for security reasons, many apartments are closed for most parts of the day and vector

control is difficult to execute. This phenomenon also can be seen in Malaysia. Thus, the IVM should be developed to educate households on the potential breeding sites around the high-rise apartments as well as suitable vector control measures in order to prevent future threats of dengue transmission.

For effective control, there is a need for continuous monitoring of insecticide susceptibility of *Aedes* vector as chemical control is still the main approach used in control strategies. Biological control can be carried out in situation where the chemicals fail to kill the larvae. Biological control is the control of pest using biological agents such as pathogens, parasites and predators. Mermithid nematodes as parasites, *Romanomermis culicivorax* and *Romanomermis iyengari* are effectively used to control mosquitoes in open fields (Platzer, 1981). For predators, indigenous fish species such as *Poecilia reticulate* and *Aplocheilus* species have been used to control mosquito larvae (Chandra *et al*., 2008). Another successful biological agent, *Bacillus thuringiensis israelensis* H-14 (Bti), also has been used to control mosquitoes (Yap *et al*., 2003).

Moreover, research on surveillance and effective control should be implemented to improve the effectiveness of current methods. New and innovative methods such as electronic mosquito detection device is useful for early detection of the presence of mosquito. Other techniques such as detection of infection protein in dengue-infected mosquitoes and detection of transovarial dengue virus can be used as an indicator for outbreak prediction. Other innovations such as transgenic *Aedes* mosquitoes, residual spraying, barrier spraying of insecticide and personal protection device would provide alternative control strategy.

Dengue will continue to be a growing problem globally. Future strategies to control dengue vector will have to be internationally collaborative, because the range of mosquitoes is not limited by geographical boundaries. In order to provide economical and effective mean of control, both chemical and biological insecticides will have to be

judiciously used. This means that there will be a need for continuous basic and applied research to be carried out not only limited to laboratories but also in the fields. Development of new model of control and novel concept of strategies will be the goal for future public health pest control.

CHAPTER 7

CONCLUSION

1. The results implied that *Aedes* mosquitoes could be found from ground floor to highest floor of multiple storey buildings but no significant difference in abundance was found.

2. The study suggests that the invasion of *Aedes* mosquitoes in high-rise apartments could enhance the transmission of dengue virus, and approach on vector control in this type of residential areas should be developed.

3. Field population of *Aedes aegypti* exhibited moderate resistance toward methoprene (Resistance Ratio, RR = 12.65) and low resistance toward pyriproxyfen (RR = 1.35).

4. Field populations of *Ae. albopictus* exhibited low resistance against diflubenzuron (RR = 2.08).

5. Cyromazine and novaluron still provide promising effect toward field populations of *Ae. aegypti* and *Ae. albopictus*.

6. Pyriproxyfen was shown to be the longest residual activity in both indoor (43 weeks) and outdoor (26 weeks) conditions, followed by temephos (26 weeks in indoor and 16 weeks in outdoor).

7. There are significant correlations within juvenile hormone analogue (JHA) group and within chitin synthesis inhibitor (CSI) group indicating there were cross resistance between IGRs within the same group could occur.

8. This study indicated that IGRs can be an alternative long-term control measure in stagnant water body.

9. Regular surveys will provide information for early detection and establishment of more effective control programs that cover all aspect of the resistance problem can be overcame.

REFERENCES

Abu Hassan, A. & Yap, H.H. (2003). Chapter 4 Mosquitoes. *In: Urban pest control a Malaysian perspective.* 2nd edition. Lee, C.Y., Zairi, J., Yap, H.H. & Chong, N.L. (eds.). Vector Control Research Unit, School of Biological Science, Universiti Sains Malaysia, p. 27 – 42.

Apandi, Y., Lau, S.K., Norfaezah, A., Nur Izmawati, A.R., Liyana, A.Z. Khairul, I.H. and Zainah, S. (2011). Epidemiology and molecular characterization of chikungunya virus involved in the 2008 to 2009 outbreak in Malaysia. *Journal of General and Molecular Virology*. **3**(2): 35–42.

Apandi, Y., Nazni, W.A., Noor Azleen, Z.A., Vythilingam, I., Noorazian, M.Y., Azahari, A.H., Zainah, S. and Lee, H.L. (2009). The first isolation of chikungunya virus from non-human primates in Malaysia. *Journal of General and Molecular Virology*. **1**(3): 35–39.

Asmad, M. and Satwant, S. (2000). Chikungunya: Its epidemiology and foresights for future prevention and control in Malaysia. *Vector Journal*. **6** (1): 21 – 26.

Becker, N., Petric, D., Zgomba, M., Boase, Clive, Madon, M., Dahl, C. and Kaiser, A. (2010). Chapter 16 Biological Control. In: Mosquitoes and Their Control (2nd eds). *Springer*. page 405–432.

Becker, N., Zgomba, M., Ludwig, M., Petric, D. and Rettich, F. (1992). Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. *Journal of the American Mosquito Control Association*. **8**(3): 285–289.

Bisset, J.A., Rodriguez, M., Soca, A., Pasteur, N. and Raymond, M. (1997). Cross resistance to pyrethroid and organophosphorus insecticides in the southern house mosquito (Diptera:Culicidae) from Cuba. *Journal of Medical Entomology*. **34**: 244–246.

Bloomcamp, C.L., Patterson, R.S. and Koehler, P.G. (1987). Cyromazine resistance in the house fly (Diptera, Muscidae). *Journal of Economic Entomology*. **80**: 352–357.

Boromisa, R.D., Rai, K.S. and Gramstd, P. (1987). Variation in the vector competence of geographical stain *Aedes albopictus* for dengue 1 virus. *Journal of American Mosquito Control Association*. **3**: 378–386.

Brown, T.M., Devrives, D.M. and Brown, A.W.A. (1978). Induction of resistance to insect growth regulators. *Journal of Economic Entomology*. **71**(2): 223–229.

Burgess, N.R.H. and Cowan, G.O. (1993). *The color atlas of medical entomology.* London: Chapman & Hall Medical.

Busvine, J.R. (1980). *Insect and hygiene*. U.S.A.: Chapman and Hall.

Cerf, D.C. and Georghiou, G.P. (1972). Evidence of cross-resistance to a juvenile hormone analogue in some insecticide-resistant house flies. *Nature*. **239**: 401–402.

Cetin, H., Erler, F. and Yanikoglu, A. (2009). Survey of insect growth regulator (IGR) resistance in house flies (*Musca domestica* L.) from southwestern Turkey. *Journal of Vector Ecology*. **34**(2): 329–337.

Cetin, H., Yaniloglu, A. and Cilek, J.E. (2006). Efficacy of diflubenzuron, a chitin synthesis inhibitor, against *Culex pipens* larvae in sptic tank water. *Journal of American Mosquito Control Association*. **22**: 343–345.

Chadee, D.D. (1988). Effects of "closed" houses on the *Aedes aegypti* eradication programme in Trinidad. *Medical and Veterinary Entomology.* **2**: 193–198.

Chadee, D.D. (2004). Obsevations on the seasonal prevalence and vertical distribution patterns of oviposition by *Aedes aegypti (L.)* (Diptera: Culicidae) in urban high-rise apartments in Trinidad, West Indies. *Journal of Vector Ecology.* **29**(2): 323–330.

Chandra, G., Bhattacharjee, I., Chatterjee, S.N. and Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian Journal of Medical Research*. **127**: 13–27.

Chareonviriyaphpap, T., Aum-aung, B., Ratanatham, S. (1990) Current insecticide resistance patterns in mosquito vectors in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. **30**: 184–194.

Chen, C.D. (2006). The surveillance and resistance status of the dengue vectors, *Aedes (Stegomyia) aegypit* (L.) and *Aedes (Stegomyia) albopictus* Skuse against temephos in Kuala Lumpur and Selangor, Malaysia. *M. Sc. Thesis of University of Malaya, Malaysia*.

Chen, C.D. and Lee, H.L. (2006). Labortory bioefficacy of CREEK 1.0G (temephos) against *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) larvae. *Tropical Biomedicine*. **23**(2): 220–223.

Chen, C.D., Lee, H.L., Nazni, W.A., Benjamin, S., Lau, K.W., Daliza, A.R., Ella, S.S. and Sofian-Azirun, M. (2009). Field effectiveness of *Bacillus thuringiensis israelensis* (Bti) against *Aedes (Stegomyia) aegypti* (Linnaeus) in ornamental ceramic containers with common aquatic plants. *Tropical Biomedicine*. **26**(1): 100–105.

Chen, C.D., Nazni, W.A., Lee, H.L. & Sofian-Azirun, M. (2005a). Weekly variation on susceptibility status of *Aedes* mosquitoes against temephos in Selangor, Malaysia. *Tropical Biomedicine.* **22**(2): 195–206.

Chen, C.D., Nazni, W.A., Lee, H.L. and Sofian-Azirun, M. (2005b). Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia. *Tropical Biomedicine*. **22**(2): 207–216.

Chen, C.D., Nazni, W.A., Lee, H.L., Seleena, B., Mohd Masri, S., Chiang, Y.F. & Sofian-Azirun, M. (2006). Mixed breeding of *Aedes aegypti* (L.) and *Aedes albopictus* Skuse in four dengue endermic areas in Kuala Lumpur and Selangor, Malaysia. *Tropical Biomedicine.* **23**(2): 224–227.

Chen, C.D., Seleena, B., Chiang, Y.F. and Lee, H.L. (2008). Field evaluation of the bioefficacy of diflubenzuron (Dimilin®) against container-breeding *Aedes* sp. mosquitoes. *Tropical Biomedicine*. **25**: 80–86.

Chen, C.D., Seleena, B., Mohd Masri, S., Chiang, Y.F., Lee, H.L., Nazni, W.A. & Sofian-Azirun, M. (2005c). Dengue vector surveillance in urban residential and settlement areas in Selangor, Malaysia. *Tropical Biomedicine.* **22**(1): 39–43.

Chen, CD., Lee, H.L., Stella-Wong, S.P., Lau, K.W. & Sofian-Azirun, M. (2009). Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia. *Dengue Bulletin.* **33**: 187–193.

Cheng, M.L., Ho, B.C., Bartnett, R.E. & Goodwin, N. (1982). Role of a modified ovitrap in the control of *Aedes aegypti* in Houston, Texas, USA. *Bulletin WHO* **60**: 291–296.

Chow, Y.S. and Yang, H.T. (1990). The application of sex pheromones and juvenile hormone analogs on cockroaches (In Chinese), Kaohsiung. *Journal of Medical Science.* **6**: 389–401.

Christophers, S.R. (1960). *Aedes aegypti* (L.): *The yellow fever mosquito*. Cambridge University Press.

Dhadialla, G.R. and Carlson, D.P. Le. (1998). New insecticides with ecdysteroidal and juvenile hormone activity. *Annual Reveal of Entomology*. **43**: 545–569.

Div. of Medical Entomology, Institute for Medical Research. (2000). Simple key to common genera of mosquitoes larvae and adult. In: *Medical Entomology, Volume III* (Unpublished). 1 – 10.

Estrada, J.G. and Mulla, M.S. (1986). Evaluation of 2 new insect growth-regulators against mosquitoes in the laboratory. *Journal of the American Mosquito Control Association*. **2**: 57–60.

Ferrari, J.A. (1996). Insecticide resistance. *In:* The biology of disease vectors. Beaty, B.J. and Marquardt, W.C. (eds.). University Press of Colorado, Niwot, Colorado. pp. 512 – 529.

Foo, L.C., Lim, T.W., L, H.L. & Fang, R. (1985). Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health.* **16**(4): 560–568.

Furlong, M.J., Verkerk, R.H. and Wright, D.J. (1994). Differential effects of the acylurea insect growth regulator teflubenzruon on the adults of two endolarval parasitoids of *Plutella xylostella*, *Cotesia plutellae* and *Diadegma semiclausum*. *Pesticide Science*. **41**(4): 359–364.

Georghiou, G.P. and Legunes, S. (1991). The occurrence of resistance to pesticides in arthropods. Food and Agriculture Organization of the United Nations, Rome.

Georghiou, G.P., Wirth, M., Saume, F. and Knudsen, A.B. (1987). Potential for organophosphate resistance in *Aedes aegypti* (Diptera:Culicidae) in the Caribbean area and neighbouring countries. *Journal of Economic Entomology*. **24**: 290–294.

Gratz, N.G. (2004). Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*. **18** (3): 215 – 27.

Gubbler, D.J. (1988). Dengue. *In*: The arboviruses. Vol II. epidemiology and ecology. Monach, T. (eds). CRC Press, Florida. pp. 223–260.

Gubler, D.J. (1989). *Aedes aegypti* and *Aedes aegypti* borne disease control in the 1990s. Top down or bottom up. *American Journal of Tropical Medicine and Hygiene*. **40**: 571–578.

Gubler, D.J. (2004). The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle. *Comparative Immunology, Microbiology & Infections Diseases.* **27**: 319 – 330.

Gubler, D.J., Mount, G.A., Scanlon, J.E., Ford, H.R. and Sullivan, M.F. (1998). Dengue and dengue haemorrhagic fever. *Clinical Microbiology Review*. **11**: 480–496.

Halstead, S.B. (1966). Mosquito-borne haemorrhagic fevers of South and Southeast Asia. *Bulletin of the World Health Organization.* **35**: 3 – 15.

Halstead, S.B. (1990). Global epidemiology of dengue haemorrhagic fever. *Southeast Asian Journal of Tropical Medicine and Public Health.* **21**(4): 636 – 641.

Halstead, S.B., Scanlon, J.E., Umpaivit, P. and Udomsakdi, S. (1969a). Dengue and Chikungunya virus infection in man in Thailand, 1962 – 1964. IV. Epidemiologic studies in the Bangkok metropolitan area. *American Journal of Tropical Medicine and Hygiene.* **18**: 997 – 1021.

Halstead, S.B., Udomsakdi, S., Scanlon, J.E. and Rohitayodhin, S. (1969b). Dengue and Chikungunya virus infection in man in Thailand, 1962 – 1964. V. Epidemiologic observations outside Bangkok. *American Journal of Tropical Medicine and Hygiene.* **18**: 1021 – 1033.

Hasanuddin, I., Miyagi, I., Toma, T. & Kamimura, K. (1997). Breeding habitats of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in villages of Barru, South Sulawesi, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health.* **28** (4): 844–850.

Hatakoshi, M., Kawada, H., Nishida, S., Kisida, H. and Nakayama, I. (1987). Laboratory evaluation of 2-[1-methyl-2-(4-phenoxyphenoxy)-ethoxy] pyridine against larvae of mosquitoes and housefly. *Japanese Journal of Sanitary Zoology*. **38**: 271–274.

Hawley, W.A. (1988). The biology of *Aedes albopictus*. *Journal of American Mosquito Control Association*. **4**: 1 – 39.

Hendrick, C.A., Staal, G.B. and Siddal, J.B. (1973). Alkyl 3,7,11-trimethyl-1,4-dodecadenoates, a new class of potent insect growth regulator with juvenile hormone activity. *Journal of Agricultural and Food Chemistry*. **21**(3): 354–359.

Ho, C.M., Wu, S.H. and Wu, C.C. (1990). Evaluation of the control of mosquitoes with insect growth regulators. *The Kaohsiung Journal of Medical Sciences*. **6**(7): 366–374.

Ho, D.C.W., Leung, H.F., Wong, S.K., Cheung, A.K.C., Lau, S.S.Y., Wong, W.S., Lung, D.P.Y. & Chau, K.W. (2004). Assessing the health and hygiene performance of apartment buildings. *Facilities.* **22**(3): 58–69.

Iseki, A. and Georghiou, G.P. (1986). Toxicity of cyromazine to strains of the house fly (Diptera, Muscidae) variously resistant to insecticides. *Journal of Economic Entomology*. **79**: 1192–1195.

Itoh, T. (1994). Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator, pyriproxyfen to larval habitats. *Tropical Medicine*. **36**: 243–248.

James, P.J., Cramp, A.P. and Hook, S.E. (2008). Resistance to insect growth regulator insecticides in populations of sheep lice as assessed by a moulting disruption assay. *Medical and Veterinary Entomology*. **22**(4): 326–330.

Jumali, S., Gubler, D.J., Nalim, S., Eram, S., Sulianti, Saroso. J. (1979). Epidemic dengue haemorrhagic fever in rural Indonesia. III. Entomological studies. *American Journal of Tropical Medicine and Hygiene.* **28**:717 – 724.

Karimzadeh, R., Hejazi, M.J., Rahimzadeh-Khoei, F., and Moghaddam, M. (2007). Laboratory evaluation of five chitin synthesis inhibitors against the Colorado potato beetle, *Leptinotarsa decemlineata*. *Journal of Insect Science*. **7**: 1–6.

Khan, A.H., Morita, K., Parguet, M.C., Hasebe, F., Mathenge, E.G.M. and Igarashi, A. (2002). Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. *Journal of General Virology*. **83**: 3075 – 3084.

Kim, Y.J., Lee, H.S., Lee. S.W. and Ahn, Y.J. (2004). Fenproximate resistance in *Tetranychus urticae* (Acari: Tetranychidae): cross resistance and biochemical resistance mechanisms. *Pest Management Science*. **60**: 1001–1006.

Knudsen, A.B. & Slooff, R. (1992). Vector-borne disease problems in rapid urbanization: new approaches to vector control. *Bulletin of the World Health Organization.* **70**(1): 1–6.

Knudsen, A.B. (1994). A global strategy for the prevention and control of dengue and dengue haemorrhagic fever. In: *First International Congress of the Parasitology and Tropical Medicine*. pp. 39 – 45.

Kristensen, M, Spencer, A.G. and Jespersen, J.B. (2003). Larvicide resistance in *Musca domestica* (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. *Journal of Economic Entomology*. **96**: 1300–1306.

Lai, T.P., Rosinah, Y. and Lam-Phua, S.G. (2001). Susceptibility of adult field strains of *Aedes aegypti* and *Aedes albopictus* in Singapore to pirimiphos-methyl and permethrin. *Journal of American Mosquito Control Association.* **17** (2): 144 – 146.

Lam, S.K. (1993). Two decades of dengue in Malaysia. *Tropical Biomedicine* **10**: 195–200.

Lam, S.K., Chua, K.B., Hooi, P.S., Rahimah, M.A., Kumari, S., Tharmaratnam, M., Chuah, S.K., Smith, D.W. and Sampson, I.A. (2001). Chikungunya infection – an emerging disease in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health.* **32** (3): 447 – 451.

Lam, W.K. (1990). A field trial to evaluate Dimilin WP-25, an insect growth regulator, as a larvicide for controlling *Aedes albopictus* (Skuse) breeding in septic tanks in Kuala Kangsar, Perak. *Tropical Biomedicine*. **7**: 83–89.

Langley, P.A., Felton, T., Stafford, K. and Oouchi, H. (1990). Formulation of pyriproxyfen, a juvenile hormone mimic, for tsetse control. *Medical and Veterinary Enotomology*. **4**: 127–133.

Lee, H.L. (1992a). *Aedes* ovitrap and larval survey in several suburban community in Selangor, Malaysia. *Mosuqito Borne Disease Bulletin.* **9**(1): 9–15.

Lee, H.L. (1992b). Sequential sampling: Its application in ovitrap surveillance of *Aedes* (Diptera: Culicidae) in Selangor, Malaysia. *Tropical Biomedicine.* **9**: 29–34.

Lee, H.L. (1994). Research on dengue vectors: An overview. In: *First International Congress of Parasitology and Tropical Medicine 1994*. pp. 48–55.

Lee, H.L. (2000a). Aedes: mosquitoes that spread dengue fever. *In*: *Mosquitoes and mosquito-borne diseases*. Ng, F.S.P. and Yong, H.S. (eds.). Academy of Sciences Malaysia, Kuala Lumpur, Malaysia. pp. 45 – 61.

Lee, H.L. (2000b). Environmental friendly approaches to mosquito control. *In*: *Mosquitoes and mosquito-borne diseases*. Ng, F.S.P. and Yong, H.S. (eds.). Academy of Sciences Malaysia, Kuala Lumpur, Malaysia. pp. 223 – 233.

Lee, H.L. and Cheong, W.H. (1987). A preliminary *Aedes aegypti* larval survey in the suburbans of Kuala Lumpur city. *Tropical Biomedicine*. **4**: 111–118.

Lee, H.L. and Chong W.L. (1995). Glutathion S-transferase activity and DDT-susceptibility of Malaysian mosquitoes. *Southeast Asian Journal of Tropical Medicine and Public Health.* **26** (1): 164–167.

Lee, H.L. and Lime, W. (1989). A re-evaluation of the susceptibility of field collected *Aedes (Stegomyia) aegypti* (Linnaeus) larvae to temephos in Malaysia. *Mosquito Borne Disease Bulletin*. **4**: 91–95.

Lee, H.L., Asikin, N., Nazni, W.A. and Sallehuddin, S. (1998). Temporal variations of insecticide susceptibility status of field collected *Aedes albopictus* (Skuse) in Malaysia. *Tropical Biomedicine*. **15**(2): 43–50.

Lee, H.L., Chen, C.D., Mohd-Masif, S., Chiang, Y.F., Chooi, K.H. and Benjamin, S. (2008). Impact of larviciding with a *Bacillus thuringiensis israelensis* formulation, VectorBac WG®, on dengue mosquito vectors in a dengue endemic site in Selangor state, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*. **39**(4): 601–609.

Lee, H.L., Kasemsri, T. and Cheong, W.H. (1987). Laboratory evaluation of the resistance status of field-collected *Aedes* (*Stegomyia*) *aegypti* (L.) to malathion in Kuala Lumpur. *Tropical Biomedicine.* **4**: 192 – 195.

Lee, H.L., Mustafakamal, I. and Rohani, A. (1997). Does transovarian transmission of dengue virus occur in Malaysia *Aedes aegypti* and *Aedes albopictus*? *Southeast Asian Journal of Tropical Medicine and Public Health*. **28**: 230–232.

Lee, Y.W., and Zairi, J. (2006). Field evaluation of *Bacillus thuringiensis* H-14 against *Aedes* mosquitoes. *Tropical Biomedicine.* **23**(1): 37–44.

Lewis, J.L. and Forschler, B.T. (2010). Impact of five commercial baits containing chitin synthesis inhibitors on the protest community in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Environmental Entomology*. **39**(1): 98–104.

Liew, C. & Curtis, C.F. (2004). Horizontal and vertical dispersal of dengue vector mosquitoes, *Aedes aegypti* and *Aedes albopictus*, in Singapore. *Medical and Veterinary Entomology* **18**: 351–360.

Liew, C., Lam-Phua, S.G. and Curtis, C.F. (1994). The susceptibility status of Singapore *Aedes* vectors to temephos and pirimiphos-methyl. In: *First International Congress of the Parasitology and Tropical Medicine*. 68 – 77.

Lima, J.B., de Melo, N.V. and Valle, D. (2005). Residual effect of two *Bacillus thuringiensis* var. *israelensis* products assayed against *Aedes aegypti* (Diptera: Culicidae) in laboratory and outdoors at Rio de Janeiro, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*. **47**(3): 125–130.

Loh, P.Y. and Yap, H.H. (1989). Laboratory studies on the efficacy and sublethal effects of an insect growth regulator, pyriproxyfen (S-31183) against *Aedes aegypti* (Linnaeus). *Tropical Biomedicine*. **6**: 7–12.

Macdonald, W.W. (1956). *Aedes aegypti* in Malaya, I. Distribution and dispersal. *Annals of Tropical Medicine and Parasitology*. **50**: 385 – 398.

Malavige, G.N., Fernando, S., Fernando, D.J. and Seneviratne, S.L. (2004). Dengue viral infections. *Postgrad. Med. J.* **80**: 588 – 601.

Mascari, T.M., Mitchell, M.A., Rowton, E.D. and Foil, L.D. (2007). Laboratory evaluation of diflubenzuron as a feed-through for control of immature sand flies (Diptera: Psychodidae). *Journal of Medical Entomology*. **44**(2): 171–174.

Mattingly, P.F. (1957). Genetical aspects of the *Ae. aegypti* problem I. Taxonomy and bionomics. *Annals of Tropical Medicine and Parasitology*. **51**: 392 – 408.

McCarry, M.J. (1996). Efficacy and presistance of Altosid pellets against *Culex* species in catch basins in Michigan. *Journal of the American Mosquito Control Association*. **12**: 144–146.

Ministry of Health Malaysia. (2011). *Situasi semasa deman denggi di Malaysia bagi minggu 52/2011.* Kenyataan Akhbar Ketua Pengarah Kesihatan Malaysia. pp. 1-4.

Mitchell, C.J. (1996). Environmental management for vector control. *In*: *The biology of disease vectors*. Beaty, B.J. and Marquardt, W.C. (eds.). University Press of Colorado, Niwot, Colorado. pp. 292 – 293.

Mulla, M.S. (1995). The future of insect growth regulators in vector control. *Journal of the American Mosquito Control Association*. **2**: 269–273.

Mulla, M.S., Darwaazeh, H.A., Kennedy, B. and Dawson, D.M. (1986). Evaluation of new insect growth regulator against mosquitoes with notes on nontarget organisms. *Journal of American Mosquito Control Association*. **2**: 314–320.

Mulla, M.S., Thavara, U., Tawatsin, A. and Chompoosri, J. (2004). Procedures for evaluation of field efficacy of slow release formulations of larvicides against *Aedes aegypti* in water storage containers. *Journal of the American Mosquito Control Association*. **20**: 64–73.

Mulla, M.S., Thavara, U., Tawatsin, A., Chompoosri, J., Zaim, M. and Su. T. (2003). Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator, against *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology*. **28**(2): 241–254.

Mullin, C.A. and Scott, J.G. (1992). Biomolecular basis for insecticide resistance. *In*: Molecular mechanisms of insecticide resistance: Diversity among insects. Mullin, C.A. and Scott, J.G. (eds.). American Chemical Society, Washington. pp. 1–13.

Nayar, J.K., Ali, A. and Zaim, M. (2002). Effectiveness and residual activity comparison of granular formulation of insect growth regulator pyriproxyfen and S-methoprene against Florida mosquitoes in laboratory and outdoor conditions. *Journal of American Mosquito Control Association*. **3**: 196–201.

Nazni, W.A., Kamaludin, M.Y., Lee, H.L., T Rogayah, T.A.R. and Sa'diyah, I. (2000). Oxidase activity in relation to insecticide resistance in vectors of public health importance. *Tropical Biomedicine*. **17** (2): 69 – 79.

Ogg, C.L., Schultz, L.D. and Kamble, S.T. (2007). *SAFE: Transport, Storage and Disposal of Pesticides*. Extension Bulletin EC 2507, University of Nebraska, Lincoln.

Ong, K.H., Chew, L.M. and Chan, K.L. (1981). Current insecticidal susceptibility status of mosquitoes in Singapore. *Southeast Asian Journal of Tropical Medicine and Public Health.* **12** (2): 222 – 227.

Paeporn, P., Komalamisra, N., Deesin, V., Rongsriyam, Y., Eshita, Y. and Thongrungkiat, S. (2003). Temephos resistance in two forms of *Aedes aegypti* and its significance for the resistance mechanism. *Southeast Asian Journal of Tropical Medicine and Public Health.* **34** (4): 786 – 792.

Paeporn, P., Supaphathom, K., Srisawat, R., Komalamisra, N., Deesin, V., Ya-umphan, P. and Sawat, S.L. (2004). Biochemical detection of pyrethroid resistance mechanism in *Aedes aegypti* in Ratchaburi, province, Thailand. *Tropical Biomedicine.* **21** (2): 145 – 151.

Palma, K.G. and Meola, R.W. (1990). Field evaluation of Nylar for control of cat fleas in home yards. *Journal of Mecical Entomology*. **27**: 1045–1049.

Platzer, E.G. (1981). Biological control of mosquitoes with mermithids. *Journal of Nematology*. **13**(3): 257–262.

Powers, A.M., Brault, A.C., Tesh, R.B. and Weaver, S.C. (2000). Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *Journal of General Virology.* **81**: 471 – 479.

Prapanthadara, L., Promtet, N., Koottathep, S., Somboon, P., Suwonkerd, W., McCarroll, L. and Hemingway, J. (2002). Mechanisms of DDT and permethrin resistance in *Aedes aegypti* from Chiang Mai, Thailand. *Dengue Bulletin*. **26**: 185 – 189.

Robertson, A. and Pope, R. (2005). *Storing fungicides safety*. Iowa State Integrated Crop Management, USA.

Rohani, A., Chu, W.L., Sa'diyah, I., Lee, H.L. and Phang, S.M. (2001). Insecticide resistance status of *Aedes albopictus* and *Aedes aegypti* collected from urban and rural areas in major towns of Malaysia. *Tropical Biomedicine.* **18** (1): 29 – 39.

Rohani, A., Nazni, W.A., Bugor, H. and Lee, H.L. (1998). Evaluation of susceptibility of urban and rural *Aedes albopictus* to commonly used insecticides. *Vector Journal.* **4**: 15 – 27.

Rohani, A., Yulfi, H. Zamree, I. and Lee, H.L. (2005). Rapid detection of chikungunya virus in laboratory infected *Aedes aegppti* by Reverse-Transcriptase-Polymerase Chain Reaction (RT-PCR). *Tropical Biomedicine*. **22**(2): 149–154.

Rozendaal, J.A. (1997). Vector control: Methods for use by individuals and communities. *World Health Organization*: Geneva, Switzerland. 1 – 17.

Rozilawati, H., Zairi, J. & Adanan, C.R. (2007). Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia. *Tropical Biomedicine.* **24**(1): 83–94.

Rudnick, A., Marchette, R., Garcia, R. & MacVean, D.W. (1986). Dengue virus ecology in Malaysia – the general survey, 1965 – 1969. In: Rudnick, A. and Lim, T.W. (Eds.), *Dengue fever studies in Malaysia* (59 – 75). Institute for Medical Research, Malaysia.

Saelim, V., Brogdon, W.G., Rojanapremsuk, J., Suvannadabba, S., Pandii, W., Jones, J.W. and Sithiprasasna, R. (2005). Bottle and biochemical assays on temephos resistance in *Aedes aegypti* in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health.* **36** (2): 417 – 25.

Schoeppner, R.F. (1978). The effectiveness of Altosid briquettes in controlling *Culex pipiens* in catch basins. *California Mosquito Vector Control Associations*. **46**: 115–117.

Seccacini, E., Lucia, A., Harburguer, L., Zerba, E., Licastro, S. and Masuh, H. (2008). Effectiveness of pyriproxyfen and diflubenzuron formualtions as larvicide against *Aedes aegypti*. *Journal of the American Mosquito Control Association*. **24**(3): 398–403.

Service, M.W. (1992). Importance of ecology in *Aedes aegypti* control. *Southeast Asian Journal of Tropical Medicine and Public Health.* **23**: 681–688.

Shroyer, D.A. (1986). *Aedes albopictus* and arboviruses: A concise review of the literature. *Journal of American Mosquito Control Association*. **2**: 424 – 428.

Sihuincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J.D., López-Sifuentes, V., Vidal-Oré, C. and Devine, G.J. (2005). Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Perú. *Journal of Medical Entomology*. **42**(4): 620–630.

Silva, J.J. and Mendes, J. (2007). Susceptibility of *Aedes aegypti* (L.) to the insect growth regulators diflubenzuron & methoprene in Uberlândia, State of Minas Gerais. *Brazilian Society of Tropical Medicine*. **40**(6): 612–616.

Skae, F.M. (1902). Dengue fever in Penang. *British Medical Journal*. **2**: 1581–1582.

Sláma, K., Romaňuk, M. and Šorm, F. (1974). Insect Hormones and Bioanalogues. Spinger, Wien, New York. pp. 477.

Smith, C.E.G. (1956). The history of dengue in tropical Asia and its probable relation to the mosquito *Aedes aegypti*. *Journal of Tropical Medicine and Hygiene*. 3 – 11.

Somboon, P., Prapanthadara, L. and Suwonkerd, W. (2003). Insecticide susceptibility tests of *Anopheles minimus*, *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* in Northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health.* **34** (1): 87 – 93.

Stará, J. and Kocourek, F. (2007). Insecticidal resistance and cross-resistance in populations of *Cydia pomonella* (Lepidoptera: Tortricidae) in central Europe. *Journal of Economic Entomology*. **100**(5): 1587–1595.

Thaikruea, L., Charearnsook, O., Reanphumkarnkit, S., Dissomboon, P., Phonjan, R., Ratchbud, S., Kounsang, Y. and Buranapiyawong, D. (1997). Chickungunya in Thailand: A re-emerging disease? *Southeast Asian Journal of Tropical Medicine and Public Health*. **28**(2): 359–364.

Tham, A.S. (2000). Surveillance of mosquitoes. In: *Mosquitoes and mosquito-borne disease* (ed. F.S.P. Ng and H.S. Yong). pp. 167–183.

Thavara, U., Tawatsin A., Chansang, C., Asavasachanukorn, P., Zaim, M. and Mulla, M.S. (2007). Simulated and field evaluation of the efficacy of two formulations of diflubenzuron, a chitin synthesis inhibitor against larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) in water-storage containers. *Southeast Asian Journal of Tropical Medicine and Public Health*. **38**(2): 269–275.

Thavara, U., Tawatsin A., Kong-Ngamsuk, W. and Mulla, M.S. (2004). Efficacy and longevity of a new formulation of Temephos larvicide tested in village-scale trails against *Aedes aegypti* larvae in water storage containers. *Journal of American Mosquito Control Association*. **20**: 176–182.

Thomas, V. (1970). Present status of resistance and susceptibility of four species of West Malaysia culicine mosquito larvae to insecticides. *Medical Journal of Malaya.* **25**: 142 – 148.

Thomas, V. (1976). Resistance of insect vectors of diseases to insecticides in Malaysia and other Southeast Asian countries. *Medical Journal of Malaya.* **31**: 77 – 80.

Tinker, M.E. (1974). *Aedes aegypti* larva habitats in Surinam. *Bulletin of the Pan American Health Organization.* **8**(4): 293–301.

Vythllingam, I., Luz, B.M., Hanni, R., Tan, S.B and Tan, C.H. (2005). Laboratory and field evaluation of the insect growth regulator pyriproxyfen (SUMILARV 0.5G) against dengue vectors. *Journal of the American Mosquito Control Association*. **21**(3): 296–300.

Wan-Norafikah, O., Chen, C.D., Soh, H.N., Lee, H.L., Nazni, W.A. & Sofian-Azirun, M. (2009). Surveillance of *Aedes* mosquitoes in a university campus in Kuala Lumpur, Malaysia. *Tropical Biomedicine* **26**(2): 206–215.

Wan-Norafikah, O., Nazni, W.A., Noramiza, S., Shafa'ar-Ko'ohar, S., Azirol-Hisham, A., Nor-Hafizah, R., Sumarni, M.G., Mohd-Hasrul, H., SofianAzirun, M. & Lee, H.L. (2010). Vertical dispersal of *Aedes (Stegomyia)* spp. In high-rise apartments in Putrajaya, Malaysia. *Tropical Biomedicine.* **27**(3): 662–667.

Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C., Pasteur, N., Philips, A., Fort, P. and Raymond, M. (2003). Insecticide resistance in mosquito vectors. *Nature*. **423**: 136–137.

William, C.R., Leach, K.J., Wilson, N.J. and Swart, V.R. (2008). The Allee effect in site choice behavior of egg-laying dengue vector mosquitoes. *Tropical Biomedicine*. **25**(2): 140–144.

WHO. (1970). Insecticide resistance and vector control: Seventeenth report of the WHO Expert Committee on insecticides. *WHO Technical Report Series*. **443**: 73–79.

WHO. (1980). Resistance of vectors of disease to pesticides. *WHO Tech. Rpt. Series.* **655**.

WHO. (1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insect development inhibitors. *WHO/VBC/81.812.* pp. 1 – 6.

WHO. (1985). Safe use of pesticides: Ninth report of the WHO Expert Committee on Vector Biology and Control. *WHO Technical Report Series*, 813.

WHO. (1992). Vector resistance to pesticides. *WHO Technical Report Series.* **818**: 2–34.

WHO. (1997). Dengue haemorrhagic fever. *Diagnosis, treatment, prevention and control. 2nd ed.* Geneva, Switzerland: World Health Organization.

WHO. (1999). Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. *WHO Regional Publication*. **29**.

WHO. (2001). Review of insect growth regulator pyriproxyfen GR. In: Report fo the fourth WHOPES working group meeting. 2000 December 4–5. Geneva, Switzweland: WHO/CDS, WHOPES/2001.2. pp 50–67.

WHO. (2003). Yellow fever vaccine. *Weekly epidemiological record.* 78(40): 349 – 360.

WHO. (2005). *International travel and health.* World Health Organization, Geneva, Switzerland.

WHO. (2008). Diflubenzuron in drinking water: Use for vector control in drinking water sources and containers. *Background document for development of WHO Guidelines for drinking-water quality*. Geneva, Switzerland: WHO/HSE/AMR/08.03/6.

WHO. (2008). Methoprene in drinking water: Use for vector control in drinking water sources and containers. *Background document for development of WHO Guidelines for drinking-water quality*. Geneva, Switzerland: WHO/HSE/AMR/08.03/14.

WHO. (2008). Novaluron in drinking water: Use for vector control in drinking water sources and containers. *Background document for development of WHO Guidelines for drinking-water quality*. Geneva, Switzerland: WHO/HSE/AMR/08.03/11.

WHO. (2008). Pyriproxyfen in drinking water: Use for vector control in drinking water sources and containers. *Background document for development of WHO Guidelines for drinking-water quality*. Geneva, Switzerland: WHO/HSE/AMR/08.03/9.

WHO. (2007). Dengue haemorrhagic fever. *Diagnosis, treatment, prevention and control. 2nd ed*.

Yaicharoen, R., Kiatfuengfoo, R., Chareonviritaphap, T. and Rongnoparut, P. (2005). Characterization of deltamethrin resistance in field populations of *Aedes aegypti* in Thailand. *Journal of Vector Ecology*. **30** (1): 144 – 50.

Yan, P.C. and Sudderuddin, K.I. (1978). Toxicology studies of insecticides on *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.). *Southeast Asian Journal of Tropical Medicine and Public Health*. **9** (3): 378 – 383.

Yap, H.H., Lee, Y.W. and Zairi, J. (2000). Chemical control of mosquitoes. *In*: *Mosuqitoes and mosquito-borne diseases*. Ng, F.S.P. and Yong, H.S. (eds.). Academy of Sciences Malaysia, Kuala Lumpur, Malaysia. pp. 233 – 243.

Yap, H.H., Lee, Y.W. and Zairi, J. (2003). Chapter 2: insecticide Usage in the Urban Environment. *In*: *Urban pest control a Malaysian perspective*. 2nd edition. Lee, C.Y., Zairi, J., Yap, H.H. and Chong, N.L. (eds). Vector Control Research Unit, School of Biological Science, Universiti Sains Malaysia, p. 9 – 15.

Zhu, K.Y., Heise, S., Zhang, J., Anderson, T.D. and Starkey, S.R. (2007). Comparative study on effects of three chitin synthesis inhibitors on common malaria mosquito (Diptera: Culicidae). *Journal of Medical Entomology*. **44**(6): 1047–1053.

PUBLICATIONS FROM THIS WORK

Lau, K.W., Chen, C.D., Lee, H.L., Izzul, A.A., Ari-Isa, M., Zulfadli, M. and Sofian-Azirun, M. Vertical distribution of *Aedes* mosquitoes in multiple storey buildings in Selangor and Kuala Lumpur, Malaysia. *Tropical Biomedicine*. (Accepted for publication).

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2012). Vertical distribution of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in high rise apartments in Selangor and Kuala Lumpur, Malaysia. 64th Annual Meeting of the Japan Society of Medical Entomology and Zoology, Shinshu University, Ueda, Nagano, Japan. Volume 63. pp 48.

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2012). Residual bioefficacy of diflubenzuron (Dimilin®) against larvae of dengue vector, *Aedes aegypti* (Linnaeus) in Kuala Lumpur, Malaysia. *2nd International Conference on Chemical, Biological and Environmental Engineering (ICBEE 2010)*. pp. 311–313.

PRESENTATIONS FROM THIS WORK

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2012). Vertical distribution of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in high rise apartment in Selangor and Kuala Lumpur, Malaysia. 64th Annual Meeting of the Japan Society of Medical Entomology and Zoology, 29 – 31 March 2012, Shinshu University, Ueda, Nagano, Japan. (Abstract: page 48).

Lau, K.W., Chen, C.D., Lee, H.L., Izzul, A.A., Asri-Isa, M., Zulfadli, M. and Sofian-Azirun, M. (2011). Vertical distribution of *Aedes* mosquitoes in high-rise apartment in Kuala Lumpur, Malaysia. Taxonomist and Ecologist Conference 2011, 19 – 20 April 2011, Universiti Malaysia Sarawak, Sarawak, Malaysia. (Abstract: page 33).

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2010). Field evaluation of residue efficacy of insect growth regulators (IGRs) against *Aedes aegypti* (Linnaeus). IXth European Congress of Entomology (ECE 2010), 22 – 27 August 2010, Budapest, Hungary. (Abstract: page 76).

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2010). Evaluation of insect growth regulators (IGRs) against field collected *Aedes aegypti* (Linnaeus) from Kuala Lumpur and Selangor, Malaysia. 46th MSPTM Annual Scientific Conference, 24 – 25 March 2010, Grand Seasons Hotel, Kuala Lumpur, Malaysia. (Abstract: page 92).

Lau, K.W., Chen, C.D., Lee, H.L. and Sofian-Azirun, M. (2009). Evaluation of insect growth regulator (IGRs), diflubenzuron, pyriproxyfen, novaluron, cyromazine and methoprene against *Aedes aegypti* (Linnaeus). The 14th Biological Siences Graduate Congress, 10 – 12 December 2009, Chulalongkorn University, Bangkok, Thailand. (Abstract: page 191).