POPULATION PROFILE AND INSECTICIDE RESISTANCE IN DENGUE VECTOR *Aedes albopictus* Skuse IN SARAWAK, MALAYSIA

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FACULTY OF SCIENCE
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POPULATION PROFILE AND INSECTICIDE RESISTANCE IN DENGUE VECTOR *Aedes albopictus* Skuse IN SARAWAK, MALAYSIA

ABSTRACT

Dengue fever and dengue hemorrhagic fever infections have caused adverse impact on social function and economic loss in Malaysia till present day. Most studies conducted in Malaysia were focused on Peninsular Malaysia while there are insufficient published literatures related to East Malaysia. Accordingly, the populations of *Aedes albopictus* from Sarawak were studied to determine their infestation pattern, population structure, and resistance status against major insecticides used for vector control programmes along with the revelation of underlying biochemical resistance mechanism. First and foremost, ovitrap surveillance was conducted in 21 residential areas across 13 districts located in eight divisions in Sarawak State. The ovitrap index (OI) of *Aedes* larvae was found highest in urban residential area (mean OI = 90.97%), followed by suburban (69.70%), rural (65.45%) and remote (52.63%) residential areas. Interestingly, no *Aedes aegypti* was observed but two species of *Armigeres* were found co-breeding with *Ae. albopictus*. This study suggested that *Ae. albopictus* was the dominant dengue vector in Sarawak State. The emerged adults were subjected to phylogenetic study based on analyses of the mitochondrial COI gene. The analysis revealed twenty two haplotypes from 120 samples and the haplotypes were widely distributed across all the populations. Adult and larval bioassays were performed according to the WHO standard protocols to assess knockdown and mortality rate of *Ae. albopictus*. Only cyfluthrin was able to inflict complete knockdown but different susceptibility patterns were observed in other adulticides. For mortality rates, adult *Ae. albopictus* was susceptible to cyfluthrin and dieldrin. Significant correlations were found within pyrethroid and carbamates classes. This study revealed that cyfluthrin was effective against *Aedes* mosquito control in Sarawak. For larval bioassay, *Ae. albopictus* larvae were completely susceptible to

bromophos and temephos (mortality = 100%), and highly resistant to DDT, chlorpyrifos and malathion (mortality ranged from 0 – 20%). However, the larvae showed various levels of susceptibility to dieldrin, fenitrothion and fenthion. Generally, bromophos and temephos were still effective to control *Ae. albopictus* larvae in Sarawak. Enzyme assay was conducted to reveal the underlying biochemical mechanism which caused insecticide resistance. The results revealed that there were elevated α-esterases and β-esterases activities in three populations at adult and four populations in larval *Ae. albopictus* but no significant elevation of enzyme activities in mixed function oxidases (MFO) and glutathione-S-transferases (GST) among the populations. Acetylcholinsterase was insensitive to propoxur in adult *Ae. albopictus* from Sarikei and larval stage from Dalat. Moreover, association between activities of α-esterases and β-esterases, α-esterases and acetylcholinesterase, and β-esterases and acetylcholinesterase were also demonstrated. The efficacy of five insect growth regulators (IGRs) as alternative control agents against *Ae. albopictus* was also determined. Field populations of *Ae. albopictus* were susceptible to methoprene, pyriproxyfen, cyromazine and novaluron, but tolerance towards diflubenzuron. In summary, periodical monitoring of insecticide effectiveness is important to prevent development of resistance since chemical control remains as crucial approach in vector control programme. This study also suggested that IGR could possibly be an alternative selection to replace conventional insecticides.

Keywords: *Aedes albopictus*, insecticide resistance, genetic profile, Sarawak, Malaysia

PROFIL POPULASI DAN KERINTANGAN RACUN SERANGGA TERHADAP VEKTOR DEMAM DENGGI *Aedes albopictus* Skuse DARI SARAWAK, MALAYSIA

ABSTRAK

Demam denggi dan demam denggi berdarah telah membawa impak yang teruk terhadap fungsi sosial dan kerugian ekonomi di Malaysia sehingga hari ini. Kebanyakan penyelidikan yang dijalankan di Malaysia hanya memberi tumpuan di Semenanjung Malaysia mengakibatkan kekurangan hasil terbitan yang berkaitan dengan bahagian Timur Malaysia. Oleh itu, strain *Aedes albopictus* dari Sarawak telah dikaji bertujuan untuk menentukan corak pembiakan, struktur populasi dan kerintangannya terhadap racun serangga yang digunakan dalam program kawalan vektor serta mekanisme biokimia yang terlibat dalam kerintangan terhadap racun serangga. Pada mulanya, kaedah peninjauan ovitrap telah dijalankan di lapan bahagian dalam negeri Sarawak yang merangkumi 21 kawasan perumahan yang terletak di 13 daerah. Indeks ovitrap (OI) menunjukkan bahawa kawasan perumahan bandar mencatat OI yang tertinggi (min OI = 90.97%), diikuti kawasan pinggir bandar (69.70%), kawasan luar bandar (65.45%) dan kawasan terpencil (52.63%). Menariknya, tiada *Aedes aegypti* dijumpai tetapi dua spesies *Armigeres* didapati berupaya membiak bersama dengan *Ae. albopictus*. Kajian ini mencadangkan bahawa *Ae. albopictus* merupakan vektor demam denggi yang utama di negeri Sarawak. Nyamuk dewasa yang baru muncul dari pupa digunakan dalam kajian filogenetik berdasarkan analisis gen COI mitokondira. Analisis ini menunjukkan sebanyak 22 haplotip diperolehi dari 120 sampel dan semua haplotip tersebar luas merentasi semua populasi. Bioasai nyamuk dewasa dan larva dijalankan berdasarkan protokol piawai WHO untuk mengkaji kesan tumbang dan kematian *Ae. albopictus*. Hanya cyfluthrin dapat menyebabkan kesan tumbang yang sepenuhnya tetapi corak kerentanan yang berbeza diperhatikan pada racun serangga dewasa yang lain. Dari segi

kadar kematian, nyamuk dewasa *Ae. albopictus* rentan terhadap cyfluthrin dan dieldrin. Korelasi yang ketara ditemui antara racun serangga kelas piretroid dan karbamat. Kajian ini menunjukkan bahawa cyfluthrin masih berkesan untuk kawalan nyamuk *Aedes* di Sarawak. Untuk bioasai larva, *Aedes albopictus* menunjukkan kesan rentan kepada bromofos dan temefos (kadar kematian = 100%), tetapi didapati kerintangan yang tinggi terhadap DDT, chlorpyrifos dan malathion (kadar kematian antara 0 – 20%). Akan tetapi, larva *Ae. albopictus* menunjukkan pelbagai tahap kerentanan yang berbeza terhadap dieldrin, fenitrothion dan fenthion. Secara umum, bromofos dan temefos masih efektif dalam kawalan larva *Ae. albopictus* di Sarawak. Kajian asai enzim dijalankan untuk mengkaji mekanisme biokimia yang terlibat dalam kerintangan racun serangga. Kajian tersebut mengesahkan peningkatan aktiviti yang nyata bagi enzim α-esterases dan β-esterases dalam tiga populasi nyamuk dewasa dan empat populasi larva tetapi tiada peningkatan aktiviti yang nyata dalam aktiviti enzim oksidase fungsi bercampur (MFO) dan glutation-S-transferase (GST) di kalangan semua populasi. Enzim asetilkolinesterase nyamuk dewasa dari populasi Sarikei dan larva dari populasi Dalat menunjukkan ianya tidak sensitif terhadap propoksur. Tambahan lagi, keputusan kajian juga menunjukkan perhubungan yang bermakna antara α-esterases dan β-esterases, α-esterases dan asetilkolinesterase, serta β-esterases dan asetilkolinesterase. Keberkesanan lima pengawalatur pertumbuhan serangga (IGRs) sebagai agen kawalan alternatif juga dikaji. Keputusan menunjukkan populasi *Ae. albopictus* dari Sarawak rentan terhadap methoprene, pyriproxyfen, cyromazine dan novaluron tetapi toleransi terhadap diflubenzuron. Kesimpulannya, permantauan berkala terhadap keberkesanan racun serangga adalah penting untuk mengelakkan pemkembangan kerintangan racun serangga di mana bahan kimia masih merupakan langkah penting dalam program kawalan vektor. Penyelidikan ini juga mencadangkan IGRs boleh dijadikan pilihan alternatif untuk menggantikan racun serangga konvesional.

Kata kunci: *Aedes albopictus*, kerintangan racun serangga, profil genetik, Sarawak, Malaysia.

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α	:	Alpha
β	:	Beta
γ	:	Gamma
®	:	Registered trademark
™	:	Trademark
&	:	And
≈	:	Approximately
°	:	Degree
°C	:	Degree Celcius
=	:	Equal to
5^{th}	:	Fifth
4^{th}	:	Fourth
>	:	Greater than
≥	:	Greater than or equal to
<	:	Less than
≤	:	Less than or equal to
µl	:	Microliter
-	:	Minus / Negative / Absent
’	:	Minute
/	:	Per / Or
%	:	Percent
+	:	Plus
±	:	Plus-minus
”	:	Second
x	:	Times

–	:	To
3rd	:	Thrid
12L	:	Twelve hour of light (day time)
12D	:	Twelve hour of dark (night time)
AChE	:	Acetylcholinesterase
ACTHI	:	Acetylthiocholine iodine
Ae.	:	*Aedes*
An.	:	*Anopheles*
Ar.	:	*Armigeres*
ANOVA	:	Analysis of variance
BHC	:	Benzene hexachloride
Bti	:	*Bascillus thuringiensis israelensis*
BuChE	:	Buturylcholinesterase
CDNB	:	1-chloro-2, 4-dinitrobenzene
ChE	:	Cholinesterases
CL	:	Confident Limit
cm	:	Centimeter
COI	:	Cytochrome c oxidase subunit I
CSIs	:	Chitin synthesis inhibitors
Cx.	:	*Culex*
CYP	:	Plant / Mammalian cytochrome P450 oxidase
DDT	:	Dichlorodiphenyltrichloroethane
DEN	:	Dengue
DF	:	Dengue Fever
df	:	Degree of freedom
DHF	:	Dengue Haemorrhagic Fever

Div.	:	Division
DNA	:	Deoxyribonucleic acid
DSS	:	Dengue Shock Syndrome
DTNB	:	5,5-dithiobis (2-nitrobenzoic acid)
E	:	East
EC	:	Emulsifible concentrate
EI	:	Emergence inhibition
EI_{50}	:	Lethal concentration of insecticide which cause 50% emergence inhibition in bioassay
EI_{90}	:	Lethal concentration of insecticide which cause 90% emergence inhibition in bioassay
ELISA	:	Enzyme-Linked Immunosorbent Assay
EST	:	Esterases
et al.	:	Et alia / And others
F0	:	Parental generation
F1	:	First generation
FBS	:	Fast blue salt (tetrazotized o-dianisidine)
Fig.	:	Figure
F_{ST}	:	Pairwise genetic differentiation
GABA	:	Gamma-aminobutyric acid
g	:	Gram
GR	:	Granular form
GSH	:	Glutathione
GST	:	Glutathione-S-transferase
GVCR	:	Global Vector Control Response
HCH	:	hexachlorocyclohexane
H_2O_2	:	Hydrogen peroxide

H_d	:	Haplotype diversity
IBM	:	International Business Machines
i.e.	:	id est / that is
IGRs	:	Insect Growth Regulators
IMR	:	Institute for Medical Research
IPCS	:	International Programme on Chemical Safety
IRAC		Insecticide Resistance Action Commitee
ITS2	:	Internal transcribed spacer 2
IVM	:	Integrated Vector Management
JHAs	:	Juvenile hormone analogues
K	:	Average number of sequence differences
kdr	:	Knockdown resistance
KH_2PO_4	:	Potassium phosphate
KT_{50}	:	Knockdown time of insecticide which cause 50% knockdown in bioassay
L	:	Liter
LC_{50}	:	Lethal concentration of insecticide which cause 50% mortality in bioassay
LD_{50}	:	Lethal dosage of insecticide which cause 50% mortality in bioassay
LD_{90}	:	Lethal dosage of insecticide which cause 90% mortality in bioassay
M	:	Molarity
mg	:	Milligram
mm	:	Millimeter
mM	:	Millimolar
mg/L	:	Milligram per liter
MFO	:	Mixed function oxidase
N	:	North

n	:	Number
N.D.	:	Not determined
NADPH	:	Dihydronicotinamide-adenine dinucleotide phosphate
Na_2HPO_4	:	Sodium phosphate
NCBI	:	National Center for Biotechnology Information
nmoles	:	Nanomoles
*N*h	:	Haplotype number
N_m	:	Gene flow
np	:	Number of polymorphic sites
NY	:	New York
OC	:	Organochlorines
OI	:	Ovitrap Index
OP	:	Organophosphorus
P	:	Probability (statistic)
^{P}	:	Presence of resistant that need to be confirmed
PCR	:	Polymerase Chain Reaction
pH	:	Potential of hydrogen
P_i	:	Nucleotide diversity
r	:	Correlation coefficient
^{R}	:	Presence of resistance
RR	:	Resistance ratio
RR	:	Homozygous resistance
RS	:	Heterozygous
Rdl	:	Resistance to dieldrin
RFLPs	:	Restriction fragment length polymorphisms
rRNA	:	Reverse ribonucleic acid

RNA	:	Ribonucleic acid
rpm	:	Revolutions per minute
S	:	Number of segregating sites
S / ^{S}	:	Susceptible
SDS	:	Sodium dodecyl sulphate
S.E.	:	Standard Error
SIT	:	Sterile insect technique
sp.	:	Species
spp.	:	Several species
SPSS	:	Statistical Package for the Social Sciences
SS	:	Homozygous susceptible
SSLPs	:	Simple sequence length polymorphisms
SNPs	:	Single nucleotide polymorphisms
t	:	Tolerance
TMBZ	:	3,3'5,5-tetramethylbenzidine
TPA	:	Total Protected Area
Type I	:	Type one
Type II	:	Type two
ULV	:	Ultra low volume
WHO	:	World Health Organization
WP	:	Wettable powder
YF	:	Yellow Fever

CHAPTER 1: INTRODUCTION

1.1 Scope of Study

Dengue fever and dengue hemorrhagic fever infections in Malaysia are on the rise since its first report in Penang, Malaysia by Skae (1902). Due to the massive infrastructure development creating an ideal breeding environment for *Aedes* mosquitoes, both fevers had become nationwide outbreak in 1973. *Aedes aegypti* and *Aedes albopictus* have been incriminated as the principal vector of dengue (Boromisa et al., 1987; Gubler, 1988).

The first reported dengue outbreak in Sarawak was in 1982 and since then the incidence rate of dengue has been increasing from year to year (Medical Department, 1992). *Aedes* mosquitoes was first discovered in Sibu by Macdonald et al. (1965) and subsequent presence noted in Kuching, Sibu and Miri (Macdonald et al., 1967; Macdonald & Rajapaksa, 1972). Apart from previous brief mention and patchy description of presence of *Aedes* mosquitoes, a more detailed distribution and density of *Aedes* mosquitoes was reported by Chang & Jute in 1982, however, no recent information has been reported since then.

Without effective and affordable vaccine, 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 Integrated Vector Management (IVM) especially during outbreak of the disease. However, *Aedes* mosquitoes resistance against major classes of chemical insecticide has been reported and increasingly become a problem in the past decades. Weill et al. (2003) reported that mosquitoes will rapidly develop resistance to insecticide especially where the same insecticides were frequently applied.

Unfortunately, there is no information of susceptibility status of *Aedes* mosquitoes toward the insecticides available in Sarawak State.

In addition, 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 abundance and distribution of *Aedes* moquitoes in Sarawak, susceptibility status against adulticides, larvicides and IGRs as well as to promote the usage of IGRs in mosquito and other pest control programs. The objectives of the present study are:

1. To survey the *Aedes* populations in Sarawak in association with various residential areas,
2. To investigate the genetic diversity of *Ae. albopictus* in Sarawak,
3. To evaluate the susceptibility status of larvae and adult of *Ae. albopictus* against different classes of insecticides,
4. To evaluate the effectiveness and potential of insect growth regulators for *Ae. albopictus* collected in Sarawak, and
5. To elucidate the enzyme activity of non-specific esterases (α- and β- EST), mixed function oxidase (MFO), glutathione-S-transferase (GST) and acetylcholinesterase (AChE) in relation to resistance mechanisms in *Ae. albopictus*.

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

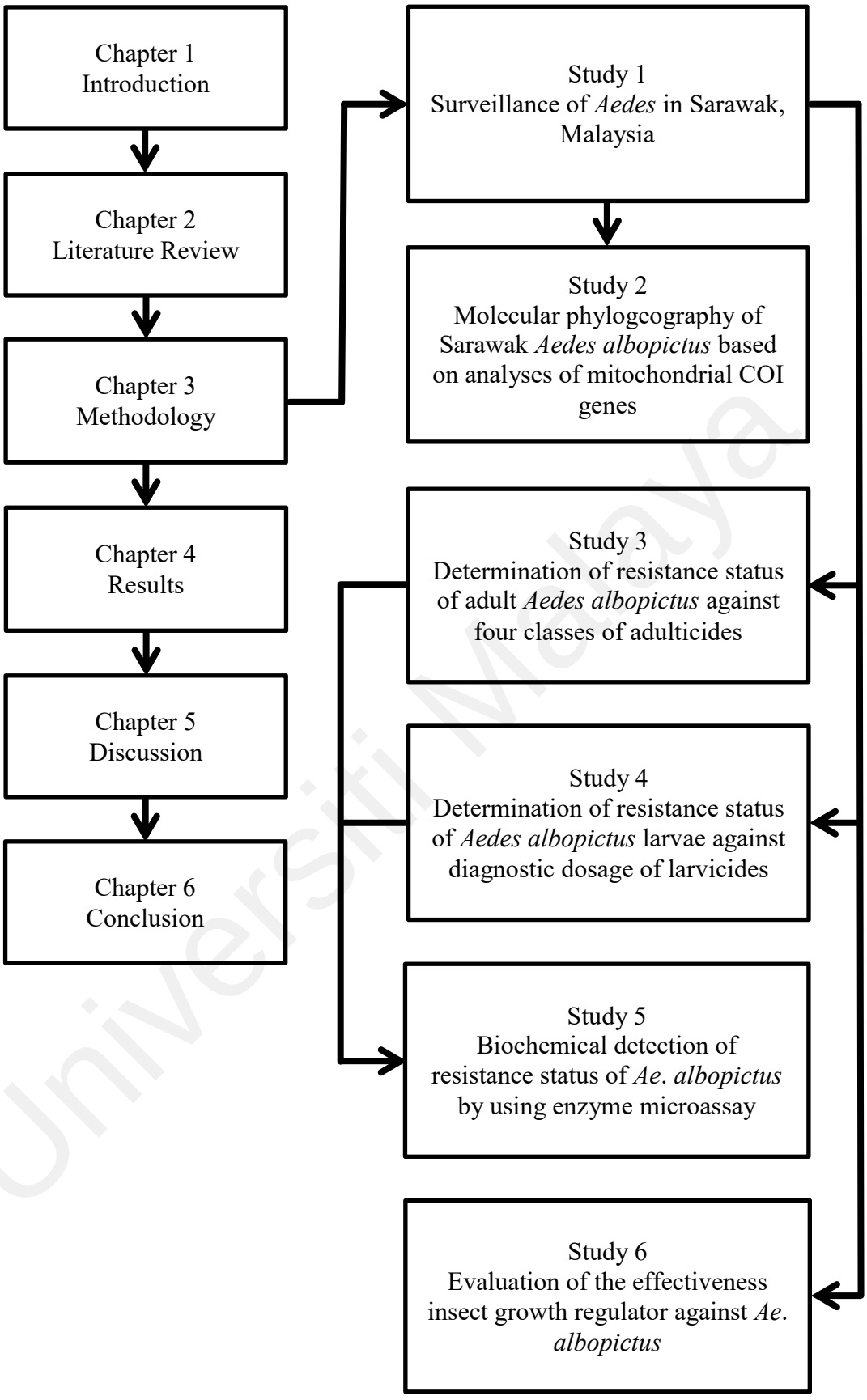

Figure 1.1 Schematic diagram of "Population profile and insecticide resistance in dengue vector *Aedes albopictus* Skuse in Sarawak, Malaysia".

CHAPTER 2: LITERATURE REVIEW

2.1 *Aedes* Mosquitoes

Mosquitoes belong to the family Culicidae, one of the families in the insect order Diptera. The best features to describe most Diptera are they possessed one pair of wings, with the hind wings are reduced to small, knobbed structures called halteres, which function as stabilizers. Mosquitoes are among the best known groups of arthropods due to their importance as pest and vector of diseases. *Aedes* mosquitoes are belong to the subfamily Culicinae, family Culicidae, Suborder Nematocera of the order Diptera. Abu Hassan & Yap (2003) reported that about 500 species of mosquitoes belonging to 20 genera in Malaysia, nevertheless, the most infamous species are *Aedes aegypti* and *Aedes albopictus*.

Both *Aedes aegypti* and *Aedes albopictus* are the vectors for dengue fever and dengue haemorrhagic fever (Nelder et al., 2010). *Aedes albopictus* has been repeatedly incriminated as a vector during dengue outbreak, particularly in Southeast Asia (Shroyer, 1986). A comparison on these two species was found that both were equally efficient in transmission of dengue-3 virus by oral route (Jumali et al., 1979). Moreover, *Aedes* species was found to be able to transmit filariasis in other regions (Rozendaal, 1997).

2.2 The Life Cycle of *Aedes* Mosquitoes

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

The mosquito eggs are small, elongate oval, seed-like bodies and intensely black 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 *Aedes* eggs can withstand desiccation for many months and hatch only when water is available. 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).

Larva hatched from egg is called first-instar and eventually followed by three successive ecdysis, leading to the second, third and fourth instar larva (Christophers, 1960). The length of the first instar is about 1.5 mm while the fourth instar is 8-10 mm. The larva possesses no legs but has well developed head and body covered with hairs. They swim by sweeping movements of the body. The larva diets are bacteria, yeast and small aquatic organisms which found around their surrounding and they ingest by using their 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). Larvae are sensitive and react rapidly to light intensity changes due to their vision is rudimentary, by 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, with fusing the head and thorax to form cephalothorax, and the abdomen hanging down form it. The *Aedes* pupal stage is mobile where they swim with progressing in a tumbling motion using their pair paddled located on the hind end of the abdomen. 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).

The adult is fully formed within the pupal cuticle when metamorphosis is complete, air is swallows by the pupa to increase internal pressure, thus splits the cuticle along the cleavage lines. The adult will slowly emerges from pupal cuticle then stands on the water surface while waiting the exoskeleton hardens and dries. The 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 of the adult are about three times as long as the head, which is hairy in the female while bushy in the male. This provides a ready means of distinguishing the sexes with the naked eye. The mouthparts in both sexes 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 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 survive by feeding 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*

The Asian tiger mosquito, *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 expanding distribution (Hawley, 1988). However, this zoophilic species progressively adapted to anthropogenic environmental changes in which provided alternative blood sources such as domestic animals and man, and water source for larval habitats (Paupy et al., 2009).

Ae. albopictus is mainly an exophagic daytime biter which prefer to bite in the early morning and late afternoon. The mosquito preferentially bites mammals; however, report showed that the female adults can feed upon most groups of vertebrates from cold to warm blooded animals, including reptiles, birds and amphibians (Scholte & Schaffner, 2007). Delatte et al. (2008) and Niebylski et al. (1994) reported that this species tend to choose human for blood source in a host choice experiment and analysis of blood meals using wild populations, respectively. Due to its opportunistic and zoophilic feeding behavior, *Ae. albopictus* not only enhances its survival but also the risk to propagate zoonotic disease from animal to animal or from animal to human (Paupy et al., 2009).

According to 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 early spread of this species to new areas mostly likely caused by the human migration towards Indo-Malayan Peninsular and the Indian Ocean islands, including Madagascar (Paupy et al., 2009). The spread was further hastened by the increase of the

intercontinental trade during 20th century. The majority of introductions are apparently due to intercontinental 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. However, *Ae. aegypti* has established a closer association with man, preferring to live inside the 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). To date, *Ae. albopictus* mainly occurs in rural and suburban areas of Asia where significant vegetation persists (Hawley, 1988).

2.4 *Aedes aegypti*

In tropical countries, *Ae. aegypti*, a closely related species to *Ae. albopcitus*, is an important vector of dengue, dengue haemorrhagic fever and other viral diseases. *Ae. aegypti* is clearly a non-indigenous species in Malaya. It probably oringinated in Africa (Mattingly, 1957) and was introduced to other part of the continent include Europe, North and South America, Asia and North Pacific Islands.

The breeding habitat of *Aedes aegypti* inevitably associated to human where natural breeding places play as subsidiary part. This species mainly breeds in the domestic environment such as water storage tanks and urns located inside or outside human dwellings, roof gutters, and temporary containers such as jars, drums, used car tyres, tin can, bottles and plant pot (Rozendaal, 1997). All these habitats typically contain relatively clean water.

According to the review paper done by MacDonald (1956), there were three tentative conclusions which can be drawn concerning the dispersal of *Ae. aegypti*. Firstly, the distribution of *Ae. aegypti* was extended by the mean of mechanical transportation of

one or other of the life-stages. Secondly, the dispersal of *Ae. aegypti* is relatively slow owing to they have difficulty in becoming established in new locality. And thirdly, the rate of spread depends on the houses and the habits of the human population once the species had established, which mean the poorer the living conditions, the more suitable is the habitat for *Ae. aegypti*. Another review paper done by Reinhold et al. (2018) reviewed that temperature of the environment can alters the mosquito population dynamics and their dispersal. In the review paper, the highest temperature threshold for *Ae. aegypti* is 34 °C and the lower limit is 16 °C, which explained this species mostly active in tropical countries.

2.5 Differences between *Aedes aegypti* and *Aedes albopictus*

The mosquitoes of this subgenus are small to medium size, black to dark in colour 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 *Aedes aegypti* and *Aedes 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 dots of white scales. ❖ Two dots of white scales on the clypeal present.	❖ Dark brown with a single longitudinal medium silvery white narrow stripe on the mesonotum. ❖ Pleurae with irregular patches of snow white scales. ❖ Scutellum with broad flat 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 The Distribution of *Aedes aegypti* and *Aedes albopictus* in Malaysia

Aedes aegypti is non-indigenous species in Malaya. It probably originated from 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). On the other hand, *Ae. albopictus* is indigenous and originated in the tropical forest of Southeast Asia. *Ae. aegypti* was probably confined to seaports and coastal area since 1850 until 1900 when present in Southeast Asia (MacDonald, 1956). 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 gradually moving further inland and becoming more common. The inference is that it was introduced to seaports by shipping, and then spread along the coast by fishing boats and local shippings towards the end of the 19th century. Lutz & Machado (1915) stated that this species can breeds in water-holding pockets in cranes, in bilge water in boats, unsealed pontoons and in drain traps. Moreover, ships especially long in tropical waters, larvae are frequently found in the containers for wash basins in cabins (Lutz & Machado, 1915). The water left in unused canoes and boats may also give rise to heavy breeding of this species (Macfie & Ingram, 1916).

The progressive invasions of both species are vital to the transmission of dengue. To date, many reports of distribution and abundance of both species have been reported by local researchers. Their appearance can be found mostly in urban and suburban area and some rural part in every state of Malaysia (Chang & Jute, 1982; Lee, 1992a; Chen et al., 2005c; Chen et al., 2013a). As *Ae. aegypti* has demonstrated equal fondness to breed in

outdoor and indoor containers, in both natural and man-made containers (Lee, 1992a), mixed breeding of both *Aedes* mosquitoes was also common in Malaysia (Chang & Jute, 1994; Chen, 2006; Rozilawati, 2007). A report also showed that *Aedes* mosquitoes can be found in high-rise apartments located in Kuala Lumpur and Selangor (Lau et al., 2013). In other words, both species had adapted well to the high-rise ecosystem in which can enhance the disease transmission.

2.7 Medical Importance of *Aedes* Mosquitoes

2.7.1 Dengue

Dengue ranks the most important mosquito borne viral disease in the world. In the past 50 years, there was incidence of significant outbreaks occurring in five of six World Health Organization (WHO) regions with 30 fold of increment. At present, dengue is endemic in 112 countries in the world (Malavige et al., 2004). Dengue has remained endemic in Malaysia since the first reported case in 1902 from Penang. 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). Dengue infections may be asymptomatic or give rise to dengue fever, dengue haemorrhagic fever or dengue shock syndrome.

Aedes mosquitoes play an important role in transmission of dengue. The primary and the most important vector is *Aedes aegypti*. Depending on geographical location, other species such as *Aedes albopictus* and *Aedes polynesiensis* may act as vectors (WHO, 1999).

Dengue fever (DF) is characterized as an acute viral with clinical symptom of a sudden onset 3 to 5 days of fever, which often is diphasic, associated with an intense headache, anorexia, abdominal discomfort and rash. According to Knudsen (1994), during the febrile phase, minor bleeding phenomena, such as petechiae and epistaxis may occur.

Dengue haemorrhagic fever (DHF) is characterized by high fever, haemorrhagic phenomena and feature of failure (Malavige et al., 2004). The clinical features of DHF are similar to 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. Symptoms such as cold blotchy skin, circumoral cyanosis and circulatory disturbances often associated with severe plasma leakage. There are some early warning signs of impending shock such as acute abdominal pain, persisting vomiting and sudden hypotension which may indicate the onset of profound shock. Metabolic acidosis is often resulting of prolonged shock which may precipitate or, on the other hand, enhance ongoing disseminated intravascular coagulation, eventually lead to massive haemorrhage. Furthermore, DSS may be associated by encephalopathy due to metabolic or electrolyte disturbances (Malavige et al., 2004).

2.7.2 Yellow Fever

Yellow fever (YF) is a zoonotic disease caused by an arbovirus which was isolated from human case in West Africa in 1927 (Gubler, 2004). There are two major cycles of endemic transmission in tropical Africa and America, i.e. the urban cycle and the sylvatic cycle which restricted to wild and rural areas. The most important vector in the urban cycle transmission is *Aedes aegypti* 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. Most of the patients suffer a short feverish illness for 3 to 4 days with headache and muscle pains and sometimes jaundice (which gives the patient a yellow color). On the other hand, starting from a brief respite, and become seriously ill with high fever associated with vomiting, severe headache and eventual death caused by gastrointestinal haemorrhage, liver or kidney failure can be seen in minority group. Death may occur within 3 days after the onset of the disease (Rozendaal, 1997; Burgess & Cowan, 1993).

WHO (2005) had recommended the immunization as the best prevention tool of yellow fever to all personal who working or visiting area where endemic with yellow fever. 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).

2.7.3 Chikungunya

Chikungunya virus belongs to genus *Alphavirus* in family *Togaviridae*. Based on serological data, Alphavirus consists of 30 species of arthropod borne viruses, which are futher subgrouped into seven serocomplexes (Khan et al., 2002). This virus was first isolated from the serum of a febrile human in Tanganyika (Tanzania) in 1953 (Powers et al., 2000). 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.

Aedes 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 & 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).

The clinical symptoms of Chikungunya virus infection often characterized by nausea, vomiting, fever, headache, myalgia, rash and arthralgia. Due to the similarity of clinical symptoms of chikungunya infection with those of dengue fever, coupled with the condition where the chikungunya virus co-circulate with dengue virus in dengue endemic area, it has been postulated that many cases of dengue virus infection are misdiagnosed. Eventually, 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, cases of

simultaneous coinfection with chikungunya and dengue viruses had also been reported (Halstead, 1966).

2.7.4 Nuisance

Outdoor activities can made impossible by swarms of biting mosquitoes (Rozendaal, 1997). They cause painful bites on human sometimes followed by localized swelling and inflammation. Irritation and itchiness may last for weeks. Thus, people are likely to be motivated to use personal protection when biting densities are high. The increased self-protection against biting may result in a reduced risk of contracting infection.

2.8 Mosquitoes Control

The impact of dengue outbreak can cause significant burden to families, communities as well as country in term of economical and society. Elder & Llyod (2007) had identified several important factors that lead to the emergence of dengue fever as follow:

- Uncontrolled urbanization and population growth increment, resulting in substandard housing, and inadequate water, sewer, and waste management systems and sanitary landfills in urban areas.
- Significant increase usage of non-biodegradable packaging, coupled with nonexistent or ineffective trash collection services.
- Large-scale global import and export of used tires.
- Constant exchange of dengue viruses and other pathogens within and between countries by frequent airplane travelling. Improved infrastructure, including roads has increased migration from rural to urban areas, as well as the general movement of people between rural and urban areas.

- Limited financial and human resources in ministries of health, leading to programs based on "crisis management" with emphasis on emergency control in response to epidemics rather than on integrated vector management to prevent epidemic transmission.

Various methods had been attempted to reduce the man-mosquito contact by controlling the mosquito populations since ancient time. At that time only several approaches were used mainly source reduction, environmental management and personal protection. However, the invention of synthetic insecticides in 1940s and 50s had changes the earlier methods and the over reliance on chemical insecticides was seen in human activities. Consequently, over application of insecticides give rise to insecticide resistance and environment problem in the 1960s and 70s which in turn force human to consider other control approaches such as insect growth regulator (IGR), biological control, a revival of the concept of environmental management and re-emphasis on personal protection as a mean of mosquito control (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.

Yap et al. (2003) suggested that source reduction and environmental management are the best approaches as they can provide long-term solutions to mosquito problem (Yap et al., 2003). Those effective measures have been reviewed by Mitchell (1996), Rozendaal (1997) and Lee (2000), 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 using biological agents such as pathogens, parasites and predators in order to reduce the number of pests. 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 often used to control mosquitoes (Yap et al., 2003).

On the other hand, chemical control is the approach involving the use of insecticide. Base on the targeted stage of mosquito, the insecticide can be categorized into two groups which are adulticide and larvicide. Adulticides are the insecticide used to control adult mosquitoes whether they are flying or resting which usually apply in fogging (air space treatment) or household application of aerosol. While larvicides are used to control the immature stages of mosquito especially the larvae and often applied in water body (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).

2.9 Insecticides

Four chemically different classes of synthetic insecticides have been available for vector control strategies to date are organochlorines (which are now banned in most countries), organophosphate, carbamates and pyrethroids (Zaim & Guillet, 2002). These insecticides have their biochemical target site in the insect central nervous system in which agonize the cholinergic nerve transmission in the insect nervous system, leading over-aggregation and death followed (Nauen, 2007).

2.9.1 Organochlorines

Organochlorines (OC) are synthetic pesticides which belong to group of chlorinated hydrocarbon derivatives (Jayaraj et al., 2016). According to Blus (2003), there are five major groups of organochlorines which are DDT, hexachlorocyclohexane (HCH), cyclodienes and similar compounds, toxaphene and the caged structures mirex and chlordecone (Kepone®) in which the grouping is based on the chemical structure of organochlorines. Organochlorines have been particularly effective in the control of pests due to chemical properties such as low water and high fat solubility, stability to photo-oxidation and low vapour pressure, in which fulfill the requirement as ideal pesticides (Kim & Smith, 2001). However, the usage of oganochlorines was banned in many countries due to its high persistency, bioaccumulation and high toxicity to environment (Jayaraj et al., 2016). The organochlorines are still detectable in present day in the environment since the discontinued usage of organochlorines for some period of time (Kim & Smith, 2001).

DDT (dichlorodiphenyltrichloroethane) is the best-known organochlorine pesticide. It was synthesized in 1874 and discovery of its insecticidal properties in 1939 by Paul Müller. The large-scale usage of DDT toward the end of World War II was a success discovery and the first chemical found to be remarkably effective in controlling insect

pest, in which subsequently led to his receiving Nobel Prize in 1948 (Blus, 2003; Zitvo, 2003). Similar to other organochlorines, one of the valued properties of DDT was its persistence in which reduced the frequency of applications and process low acute toxicity in mammals (Zitvo, 2003). In terms of economy, low frequency of applications and low cost of production had made DDT an ideal pesticide and high economic value. Soon, the insecticide euphoria ended when WHO officially reverted from malaria eradication to malaria control as the occurrence of DDT resistance in broad range of the mosquito vectors (Hemingway & Ranson, 2000). As Revised Malaria Control Strategy (1993 – 2002) was introduced and adopted (WHO, 2009b) that included restricted use of DDT, Malaysia had prohibited the application of DDT in 1998.

Aldrin and dieldrin are two closely related OCs in which they are very toxic and involved in numerous incidents of wildlife mortality (Blus, 2003). According to Blus (2003), aldrin is rapidly broken down to dieldrin when applied in the field. Dieldrin has been used in Malaysia since 1980 before being banned in 1994; though long period of discontinued usage, resistance against dieldrin has still been detected in local mosquito populations due to its high persistence (Low et al., 2015).

2.9.2 Organophosphates

Organophosphorus (OP) compound is organic compound containing phosphorus in which the term later has come include for a group of compounds that are esters of phosphoric acid, phosphorothioic acid, phosphonic acid, phosphonothioic acid and the corresponding dithioic acids. Hence, the term organophosphate is best restricted to esters of phosphoric acid (Marrs, 2001). The development of OP as insecticides only occurred in the late 1930s and early 1940s (Costa, 2006). The German chemist Gerhard Scharder is credited for the discovery of the general chemical structure of anticholinesterase OP compounds (Marrs, 2001; Costa, 2006).

Organophosphates was synthesized by reaction of alcohol and phosphoric acid, eventually on of the cage of four oxygens molecules was replaced with carbon and attaching chemical groups to the others (Marrs, 2001). In this form, they can inhibit acetylcholinesterase and cause accumulation of acetylcholine at cholinergic synapses, with over-stimulation of muscarinic and nicotinic receptors, eventually damage the central nervous system (Costa, 2006).

Organophosphate was introduced in means to replace DDT in most countries after the prohibition usage of DDT such as Africa, Bangladesh and Sri Lanka for public health insect control (N'Guessan, 2010; Rahman, 2013; Karunaratne, 2007). Example of commonly used organophosphates are bromophos, chlorpyrifos, fenitrothion, fenthion, malathion and temephos. In Malaysia, malathion, fenitrohtion, fenthion and temephos are utilized as space treatment to combat adult mosquitoes (Ong, 2016). However, malathion was replaced with pyrethroid formulations in 1996 in the vector control program in Malaysia (Ang & Singh, 2001).

2.9.3 Carbamates

Carbamate insecticides are derived from carbamic acid and induce mortality similar to organophosphate insecticides. There are more than 50 compounds classified as carbamates in which are heavily used as insecticides, fungicides, herbicides, nematicides, biocides for industrial and household products for control of household pest (Struger et al., 2016). Carbaryl was the first carbarmate insecticide introduced in 1956 and widely used throughout the world compared to other carbamates (Fishel, 2011). The feature of carbaryl's low in mammalian oral and dermal toxicity and broad spectrum has resulted in wide use in lawn and garden setting (Fishel, 2011).

Carbamate pesticides are effective insecticides by virtue of their ability to inhibit acetycholinesterase in the nervous system. They can also inhibit other esterases.

However, unlike the organophosphate compounds, the inhibitory effect on cholinesterase is brief. Some carbamates are translocated within plants, making them an effective systemic treatment. Both propoxur and bendiocarb are among the frequently used globally. One of the reason is propoxur had proposed as a replacement for DDT since the prohibition usage of DDT (WHO, 1974). However, propoxur and bendiocarb have never been applied in any vector control programme in Malaysia (Loke et al, 2012). Moreover, the use of propoxur as household aerosol was stopped by Malaysian government in 1990 (Low et al., 2013a).

2.9.4 Pyrethroids

Pyrethrins are natural insecticides produced by certain species of the chrysanthemum plant (IPCS, 1990). The pyrethrins are primarily used to control human lice, mosquitoes, cockroaches, flies and other insect pest as contact poisons which quickly penetrate the nerve system of the insect (IPCS, 1990). The positive features of pyrethrins are low mammalian toxicity but have a broad spectrum of toxic activity against insect. Unlike organocholrines, pyrethins are less persistence in environment and degradable by sunlight (Rehman et al., 2014).

Synthetic pyrethroids are insecticides derivated from *chrysanthemumic* acids with improved physical and chemical properties and greater biological activity during the modification of the chemical structures of natural pyrethrins (IPCS, 1990). Chemically, pyrethroids are ester of specific acid and alcohols which act as neuropoisons on the axons in the peripheral and central nervous system by interacting with sodium channels in insects. In other words, pyrethroids interact with the γ- amino butyric acid (GABA) receptor – ionophore complex to cause neurotoxicity (Rehman et al., 2014).

Some of the earlier synthetic pryrethroids were commercialized mainly for household insect controls. Other more recent pyrethroids have been introduced as

agricultural inseciticides because of their high insecticidal activity against wide range of insect pests at extremely low doses and low persistence in the environment. Furthermore, he pyrethroids are reapidly degraded in soil and in plants, and are hardly eluted with water once was applied (IPCS, 1990; Rehman et al., 2014).

Pyrethroids insecticides, similar to pyretrhins, are highly toxic to insects, aquatic athropods and fish but possessed low mammalian toxicity (Casida et al., 1983), but in pratical usage, no serious adverse effects have been noticed due to its low rates of application and low persistence in environment (IPCS, 1990). However, some pyrethroids (e.g. deltamethrin, fenvalerate and cypermethrin) may cause a transient itching or burning sensation in exposed human skin (IPCS, 1990). Generally, pyrethroids can be detoxified or metabolized through ester hydrolysis, oxidation and conjugation in mammals, and there is no tendency to accumulate in tissues (IPCS, 1990).

The pyrethroids constitute another group of insecticides in addition to organochlorine, organophosphorus, carbamate, and other compounds. There are two types of synthetic pyrethroids, type I and type II. Differences between type I and type II are type I pyrethroids is absence of cyano group in chemical structure and cause paralysis to insect (T Syndrome); while cyano group is present in type II at the α-carbon of the phenoxybenzylalcohol position and cause progressing to rolling convulsions to the insects (CS Syndrome) (Rehman et al., 2014). Some of the famous pyrethroids are allethrin, permethrin, deltametrhin, cypermethin and etc.

2.10 Resistance Status in *Aedes* Mosquitoes

Insecticides have renowned its important role in controlling the 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 development of resistance against every class of insecticide among the insect population had left control programs with fewer 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). In addition, it also reflected that repeated field failure of insecticides to achieve the expected level of control when the product was used without following to the label recommendations.

According to Georghiou and Lagunes-Tejeda (1991), more than 504 species of arthropods have been recorded for resistant to one or more insecticides. 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).

The aftermath of resistance could be increased pesticides application frequencies and dosage increment, decreased of yields, subsequently causing environmental damage and outbreaks of arthropod-borne human and veterinary diseases (Mullin & Scott, 1992).

The first documented case of insecticide resistance in arthropods was 1908 in Washington where the San Jose scale *Quadraspidiotus perniciosus* showed resistance 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 & Scott, 1992).

2.10.1 Resistance Studies on *Aedes* Mosquitoes in Malaysia

Since 1970s, Thomas (1970, 1976) had reported malathion-resistant *Ae. aegypti* larvae in Malaysia. In year 1978, Yan & Sudderuddin (1978) found that *Ae. aegypti* was generally more tolerant against the organophosphorus compounds (and carbaryl) showing higher CarE activity. Their toxicity tests presented the order of toxicity of larvae of *Ae. aegypti* was temephos > DDT > DDVP > malathion > lindane > carbaryl. They also found that the second-instar larvae were more susceptible than fourth-instar larvae.

Another insecticide susceptibility test on field-collected *Ae. albopictus* against DDT, permethrin, malathion and temephos were conducted by Lee et al. in 1998. The result showed that the *Ae. albopictus* larvae were highly susceptible to both temephos and malathion, while the adult mosquitoes were highly susceptible to malathion but exhibited multiple resistance to permethrin and DDT. However, they found that the non-specific esterase did not responsible for the multiple resistance in the adults of *Ae. albopictus* to tested insecticides. On the same time, Rohani et al. (1998) found that the urban strain of *Ae. albopictus* in Kuala Lumpur was exhibited multiple resistance to both permethrin and DDT.

Later on, Nazni et al. (2000) reported that *Ae. aegypti* mosquitoes are slightly tolerant to permethrin in which enzyme oxidases are involved in the resistance mechanism. They also found that resistance to malathion and temephos could be associated 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 showed resistance to DDT and permethrin.

On the other hand, field collected *Ae. albopictus* was only resistance to DDT, but strains from Selangor and Kedah were showed resistant to malathion. Rohani et al. (2001) had concluded that the effectiveness of insecticides to both adult species in descending order was malathion > permethrin > DDT, while for larvae was temephos > malathion > permethrin > DDT. From the same study, the enzyme microassay data revealed that the field strains possessed 2–5 folds elevated levels of esterases when compared to the laboratory strain in both adults and larvae stages in which explains high tolerance level to tested insecticides.

Futhermore, resistance of *Ae. aegypti* and *Ae. albopictus* against DDT, dieldrin, malathion, fenitrothion, fenthion, temephos and pyrethroid in Malaysia has also been reported by WHO (1980, 1992). To more recent year, several local researchers had discovered resistance strain in different localities. In 2013, Chen et al. (2013a) had found that *Ae. albopictus* collected from Tanjung Sepat, Selangor, showed high resistance to bendiocarb, propoxur, malathion and fenitrothion. Ishak et al. (2015) reported *Aedes* mosquitoes collected from Penang, Kuala Lumpur, Johor Bharu and Kota Bharu were showed various resistance patterns towards pyrethroids, DDT and bendiocarb.

2.10.2 Resistance Studies on *Aedes* Mosquitoes in Other Countries

In Thailand, Chareonviriyahpap et al. (1999) reported resistance of temephos, fenitrothion and malathion by *Aedes* mosquitoes. 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).

Prapanthadara et al. (2002) found that DDT resistance in two *Ae. aegypti* strains, in which R^dR^p (resistance to DDT and permethrin) and R^dS^p (resistance to DDT and

susceptible to permethrin), was cause by the increment of DDTase activity and cytochrome P^{450} content whereas permethrin resistance in R^dR^p strain probably involved a non-metabolic *kdr* mechanism.

By using biochemical assay, Paeporn et al. (2003) detected the temephos resistance in *Ae. aegypti* populations and reported that the EST detoxification was the main mechanism involved. Another enzymes biochemical assay was conducted by Paeporn et al. (2004) to detect the emergence of insecticide resistance and defined the mechanisms involved in pyrethroid resistance of *Ae. aegypti*. Their results revealed that GST was associated permethrin resistance in *Ae. aegypti* although there were significant increase of enzyme activity level in EST and MFO.

Later in 2005, Yaicharoen et al. (2005) reported low resistant to deltamethrin (resistance ratio = 8 – 17.2) and cross-resistance to DDT was found in adult *Ae. aegypti* populations collected from Bangkok and Pathum Thani provinces. Further study using biochemical analysis showed that a significant elevation of MFO and EST enzyme activity among the population. On the other hand, Sealim et al. (2005) reported that insensitive acetylcholinesterase (AChE) was not responsible for the resistance in the field-collected *Ae. aegypti* mosquitoes from Roi Et, Thailand. Both studies suggested that EST detoxification is the primary mechanism to cause resistance in the *Ae. aegypti* population.

Another study in Singapore revealed that the susceptibility (LC_{50} value) of *Ae. aegypti* to the nine insecticides in decending order 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 concluded that the Singaporean *Aedes* mosquitoes were found resistant to organochlorines and becoming

more resistant to the organophosphate compound, but were susceptible to pyrethroid and bioresmethrin (Ong et al., 1981).

In 1994, Liew et al. (1994) reported that there was a 3.5 fold increase in LD_{50} of resistance ratio for both *Ae. aegypti* and *Ae. albopictus* against temephos among the collected larval populations while the LD_{90} values had increased proportionately. According to Liew et al. (1994), the collected *Ae. albopictus* was slightly more resistant to temephos than *Ae. aegypti*. In contrast, they found that adult *Ae. aegypti* were 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.

Later in 2001, Lai et al. (2001) reported that dengue vectors were susceptible to pirimiphos-methyl, with resistance ratio for LC_{50} 1.5 and 1.4 respectively. However, *Ae. aegypti* was found resistant to permethrin (RR for LC_{50} = 12.9), which contrast to *Ae. albopictus* which was still susceptible to permethrin. They concluded that pirimiphos-methyl was still effective and promote the continuity usage in Singapore control of dengue vectors.

A review paper written by (Moyes et al., 2017) summarized the susceptibility status of *Ae. aegypti* and *Ae. albopictus* from 2008 to 2016. The emerging resistance to all 4 major insecticide classes (organochlorines, organophosphates, carbamates and pyrethroids) has been detected in the Americas, Africa and Asia. In brief, *Ae. aegypti* was found consistently reistance to deltamethrin in Brazil, French Guiana and few locations in West Africa. On the other hand, the insecticide resistance appears to be patchy in Southeast Asia. The publication also reviewed that *Ae. aegypti* populations in Asia, Africa and Latin America are consistently high resistance to organochlorines and exhibited variable resistance pattern towards carbamates. In *Ae. albopcitus* populations from Southeast Asia, evidence of resistance to all major classes of neurotoxic

insecticides has been reported as well as the resistance to the organophosphates has also been recorded in the Americas.

2.11 Insect Growth Regulators (IGRs)

According to Mulla (1995), insect growth regulators (IGRs) are a group of potent insecticides containing substances with growth inhibiting and growth retarding properties. The IGRs can be 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). JHAs disrupt the hormonal control of larval development, cause hormonal imbalance, and eventually suppress insect embryogenesis, metamorphosis and adult emergence. On the other hand, 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 (Henrick 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 et al., 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 & Mulla, 1986; Hatakoshi et al., 1987; Loh & 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 & 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, and triflumuron (Mulla, 1995; Chen et al., 2008c). 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. (2008a; 2008c) have reported studies on laboratory evaluation and field efficacy of a number of IGRs against mosquito larvae.

Cyromazine is a chemical that leads to moult disruption of targeted organism, however the mode of action of this moulting disruptor group is incompletely defined (IRAC, 2017).

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. Moreover, long

duration of residual efficacy which can reduce the frequency of application is another important feature to reduce the development of resistance. It is reasonable to assume that IGRs will be employed in mosquito and other vector control programmes.

2.12 Underlying Detoxification Mechanism in Mosquitoes

The emergence of insecticide resistance caused by extensive usage of insecticide had threatened mosquito control programmes. Strode et al. (2008) stated that to be effectively manage issue of insecticide resistance, it is crucial to understand the mechanism underlying in the phenotype. In general, insecticide resistance in insects is conferred by two major resistance mechanisms: elevated level of detoxification enzyme and alterations in the target site (Kasai et al., 2014).

2.12.1 Non-Specific Esterases (EST)

Esterase enzyme is known to play an important detoxification role in insect resistance mechanisms against organophosphate, carbamates and to a lesser extent, pyrethroids (Jackson et al., 2013; Hemingway & Ranson, 2000). This hydrolase enzyme group act by rapidly bind and hydrolyse the ester bonds, slowly detoxified the insecticide and subsequent losing insecticidal function (Jackson et al., 2013). The esterases are sequester rather than rapidly metabolize the pesticide which allow the esterases efficiently detoxify the insecticide (Hemingway & Ranson, 2000). Two common esterase loci that closely related and involve in the overproduction of esterase are *estα* and *estβ* (Karunaratne & Hemingway, 1998). Significant elevated levels of esterases activities associated with resistance developement had been found in severeal mosquito species such as *Ae. aegypti* (Chen et al., 2008b; Pethuan et al., 2007), *Ae. albopictus* (Mulyanigsih et al., 2017), *Cx. quinquefasciatus* (Low et al., 2013a), *Anopheles Culicifacies* and *Anopheles subpictus* (Karunatne, 1999).

2.12.2 Mixed Function Oxidases (MFO)

Mixed function oxidases (MFO), also known as cytochrome P450 or microsomal monooxygenases, are a superfamily of enzymes that are found in most organisms but unique to each species and no organism share identical P450 (Nelson, 2011). These enzymes are involved in the metabolism of xenobiotic and play an important role in endogenous and exogenous metabolism (Chan et al., 2014b). Mixed function oxidases metabolize insecticidal compounds by binding oxygen molecule and receive electrons from NADPH to introduce an oxygen molecule into the substrate (Hemingway & Ranson, 2000).

Elevated monooygenase activity is associated with organophosphate and pyrethroid resistance in *Ae. aegypti*, where the overtranscription of P450 genes from *CYP4*, *CYP6* and *CYP9* subfamilies were discovered (Marcombe et al., 2009). According to Ishak et al. (2016), the overtranscription of P450 gene *CYP6P12* showed high affinity to pyrethroid resistance in *Ae. albopictus* while *CYP6N3* was constantly overexpressed in DDT and carbamate resistance. The association of pyrethroid resistance with elevated of mixed function oxidases activity was also reported in *Cx. quinquefasciatus* (Kasai et al, 1998), *An. stephensi* (Brogdon et al, 1997) and *An. gambiae* (Vulule et al., 1994).

2.12.3 Glutathione-S-Transferases (GST)

Glutathione-S-transferases are dimeric multifunctional enzymes that involved in the detoxification of large range of xenobiotics (Prapanthadara et al., 1996). The enzymes catalyze the nucleophilic of reduced glutathione (GSH) to electrophilic centers of lipophilic compounds (Hemingway & Ranson, 2000). According to Che-Mendoza et al. (2009), the diversity of enzymatic functions is linked to the genetic capacity to encode different GST isoforms by most organisms. Elevated levels of GST activity have been reported to be associated to organophosphates, organochlorines and pyrethroid

resistance in mosquitoes, however, the metabolic resistance based on GST is the major mechanism of DDT resistance (Che-Mendoza et al., 2009; Hemingway and Ranson, 2000).

Glutathione-S-transferases can be divided into three main groups which are cytosolic, microsomal and mitochondrial (also known as class kappa) (Che-Mendoza et al., 2009). There are three different microsomal GST have been identified in *An. gambiae*, however, only the cytosolic GST was found responsible for insecticide resistance (Ranson et al, 2002; Ranson & Heningway, 2005). At least six classes of cytosolic proteins have been identified with similar domains to GST in insect which are delta, epsilon, omega, sigma, theta and zeta, possibly the novel GST classes (Ding et al., 2003). Among these classes, the Delta and Epsilon are specific to insects that involved in the insecticide detoxification causing metabolic resistance development (Ding et al, 2003).

There are two detoxification reactions of organochlorines by GST enzyme which are dehydrochlorination and GSH conjugation. In case of organophosphate, detoxification occurs by the conjugation of GSH via O-dealkylation or O-dearylation conjugation (Che-Mendoza et al., 2009). On the other hand, the detoxification of pyrethroid by GST mainly depends on its capacity by catalyzing lipid peroxidation products in order to reduce the peroxidative damage induced by pyrethroid.

2.12.4 Insensitive Acetylcholinesterase (AChE)

There are two major types of cholinesterases (ChE) in vertebrates: acetylcholinesterase (AChE) which found in red blood cells and nerve tissue, and butyrylcholinesterase (BuChE) which found in serum and plasma (Hemingway & Ranson, 2000). The function of AChE is hydrolyzing the neurotransmitter acetylcholine

into acetic acid and choline in order to terminate the transmission of nerve impulse in cholinergic synapses (Voet & Voet, 1995).

The AChE is the target enzyme of organophosphates and carbamates. By binding to AChE, the insecticides regulate the turnover of the neurotransmitter acetylcholine. Subsequently, the acetylcholine maintains active while the nervous influx is sustained. The over accumulated acetylcholine present in the synapse disrupt the balance state of neurotransmission and cause blockage, and eventually follow by fatality (Chevillon et al., 1999).

A study review that two AChE enzymes known as AChE1 and AChE2 differ in their substrate specificity, inhibitor sensitivity and electrophoretic migration pattern in *Cx. pipiens* (Malcolm et al., 1998). The AChE1 encoded by *ace-1* gene appears to be involved in conferring insecticide resistance while AChE2 encoded by *ace-2* gene is sex linked which is autosomal (Hemingway & Ranson, 2000). Moreover, the AChE genes have been cloned from *Ae. aegypti* and *An. stephensi*, but both genes are sex associated (Severson et al., 1997; Hemingway & Ranson, 2000).

2.13 Genetic Study

Genome is the cell in every organism that contains the essential biological information to construct and maintain that organism. The biological information contained in a genome is encoded in the nucleotide sequence of its DNA (deoxyribonucleic acid) or RNA (ribonucleic acid) molecules and is divided into discrete units called genes. Gene expression is a series of biochemical reactions that initiated by reading the information contained in a gene by proteins that attach to the genome at appropriate positions. This process generally comprising two stages, transcription and translation, where an RNA copy of the gene was produced in transcription and the second stage resulting in synthesis of a protein whose amino acid

sequence is determined, via the genetic code, by nucleotide sequence of the RNA transcript. DNA replication has to be extremely accurate in every time when a cell divides to prevent the induction of mutations into the genome copies. However, mutations do occur as error in replication or cause by the direct alteration of chemical and physical mutagens on the chemical structure of DNA. Many of these errors are corrected by DNA repair enzymes, but those escape the repair processes become permanent features of the lineage descending from the original mutated genome. Because of these events, along with genome rearrangements resulting from recombination, underlie molecular evolution, the driving force behind the evolution of living organisms (Brown, 1999).

2.13.1 DNA Barcoding

The current taxonomic follows binomial system was introduced by Linnaeus in the 1750s (Linnaeus, 1758). The identification of species following the system is mainly done on the basis of taxonomical and morphological characteristics. However, the naming system is heavily depends on specialist whose knowledge is easily lost when they retire (Tautz et al., 2003). Furthermore, identification using morphological characteristics becomes difficult when morphological features are damaged during collection (Wang et al., 2012). In addition, the morphological characteristic used to identify intact specimens may often vary between species which only can be distinguished by experienced taxonomists (Bortolus, 2008).

DNA analysis has provided a more accurate way of identifying and differentiating species after the proposal of DNA sequences as the biological classification in 2002 (Tautz et al., 2003). The combination of morphological methods and the usage of molecular data, has resolved some long-standing taxonomic questions (Wang et al., 2012).

2.13.2 Markers for DNA Barcoding

Genes are useful marker but not ideally use on genetic mapping. This is because mapping based entirely on genes is not very detailed and the gene in organisms' genome is widely spaced out with large gaps between them. Thus, other types of markers are needed. Brown (1999) stated that the ideal DNA markers are not genes that possess mapped features and must exit in at least two allelic forms. According to Lowe et al. (2004), animal mitochondrial DNA is the ideal molecular marker due to its uniparental inheritance feature, as well as lack of recombination and rapid rate of evolution. Moreover, mitochondrial DNA is the most widely used marker in animal taxa studies on the molecular ecology (Simon et al., 1994). There are three types of DNA sequence that satisfy the requirements which are restriction fragment length polymorphisms (RFLPs), simple sequence length polymorphisms (SSLPs) and single nucleotide polymorphisms (SNPs) (Brown, 1999).

RFLPs are produced by treating a DNA molecule with a restriction endonuclease in order to cuts the DNA molecule at defined sequence. On the other hand, SSLPs consists arrays of repeated sequence that display length variations and different alleles consist of different numbers of repeat units. SSLPs can be multiallelic as each SSLP can have a number of different length variants. Last but not least, SNPs are point mutations in the genome (Brown, 1999).

As time passes, the increase of available DNA markers has facilitated the accurate identification of mosquito species. For example, ITS2 (internal transcribed spacer 2) sequence was used to accurately differentiate between *Anopheles anthropophagus* and *Anopheles sinensis* than on the basis of morphology (Gao et al., 2004). Other DNA markers such as cytochrome b oxidase (Shen et al, 2013), 12S rRNA (Vences et al., 2005) and nicotinamide adenine dinucleotide dehydrogenase (Rach et al., 2008) were also used in DNA barcoding in other organism. In early of 2000s, Paul Hebert

suggested mitochondrial gene cytochrome *c* oxidase I (COI) can serve as the core for a global bioidentification system for animals (Hebert et al., 2003). Since then, COI gene-based DNA barcoding had gained increasing popularity and prove to be a useful molecular tool for identification including mosquito species (Cywinska et al., 2006; Wang et al., 2012; Chan et al., 2014a). The utility of COI sequence that can be compared universally to categorize mosquito biodiversity is also recommended by Beebe (2018).

CHAPTER 3: METHODOLOGY

3.1 Mosquito Samples

Due to the low numbers of *Ae. albopictus* from the field in this study, thus, *Ae. albopictus* populations (F0) collected during ovitrap surveillance were further bred in the insectarium to produce their offspring (F1) which were subjected to subsequent studies.

There were two strains of *Ae. albopictus* utilized in all studies, i.e. the laboratory strain and field collected strain. The *Ae. albopictus* laboratory strain (F69) was used as comparison reference strain in all studies. This laboratory strain was colonized under insecticide-free condition and maintained in the insectarium of Medical Entomology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia. Meanwhile, field populations of *Ae. albopictus* were obtained from twenty one residential areas in Sarawak via the ovitrap surveillance study. The identified field strain larvae from the ovitrap surveillance was separately reared to adulthood which known as F0. These mosquitoes were then further colonized to produce F1 progenies for subsequent studies. Only late third instar larvae and 3-5 days old sucrose-fed female mosquitioes were utilized in respective testings. One the other hand, only parental adult mosquitoes (F0) was used in the genetic study.

3.2 Mosquitoes Colonization

All populations of *Ae. albopictus* was reared according to the Standard Operating Procedure of Medical Entomology Unit, IMR (ISO/IEC 17025) which was prepared by the Medical Entomology Unit, IMR (2000). The colonies were bred in the insectarium of Institute of Biological Science, Universiti Malaya under the temperature of 27 ± 2 °C and 75 ± 10% relative humidity, and free from exposure of any insecticide.

The *Ae. albopictus* was reared and maintained separately according to populations collected from Sarawak. The adult mosquitoes were kept in cages measuring 30 cm x 30 cm x 30 cm and 10% sucrose solution was provided as food source using lint cloth. 10% sucrose solution was prepared by diluting 100g sugar in 1 L of dechlorinated water and approximately 5-10 g vitamin B complex (1%) was added. Mice were supplied as their blood meal once a week. The mice were restrained gently in a small wire modified mesh trap and left overnight inside the mosquito cages. In the next morning, the mice were removed from cages and released back to its resting cages. After blood meal, the female *Ae. albopictus* took about three to six day to oviposit.

An oviposition substrate which prepared from filter paper folded in a cone shape and put into a cup filled with dechlorinated water, was put inside the cage to allow the engorged female to lay eggs. The eggs on the filter paper were air-dried at room temperature before stored in sealed and labeled plastic bags. All *Ae. albopictus* eggs were kept until use but not exceeding six months.

For hatching purpose, the dried filter paper with eggs was soaked in plastic container measuring 14.5 cm in width x 20.0 cm length x 6.5 cm in depth filled with dechlorinated water and was added as larval food. Liver powder was prepared by mixing fine grinded of dried liver slice and yeast in ratio 4:1. The hatched larvae were allowed to grow in the plastic container until they pupated. Container was covered with mesh to avoid egg laying of other mosquitoes in the vicinity in this container. The *Ae. albopictus* pupae were then transferred into a clean container filled with dechlorinated water using pipette. The container was then put inside a new cage to allow the adult to emerge from pupal. The cage was then labeled according to their respective strains.

3.3 Ovitrap Surveillance in Sarawak

3.3.1 Geographical Description of Study Sites

An ovitrap surveillance was conducted in 21 residential areas across 13 districts located in 8 divisions in Sarawak, Malaysia. The geographical and ecological description of the study sites are given in Table 3.1. The 21 residential areas were categorized into urban, suburban, rural and remote areas according to the population density and the vegetative characteristics of the residential areas. Urban areas were high in populations and equipped with proper amenities include piped water supply and drainage system. Suburban areas were the ourskirts area situated 10 km from the urban areas but equipped with basic amenities and infrastructures. Rural areas were located ≈30 km from city with low population, equipped basic amenities but lesser infrastructures. On the other hand, remote areas were the less populated area which lack of basic amenities and infrastructures.

3.3.2 Ovitrap Surveillance

The ovitrap as described by Lee (1992b) was used in this surveillance. Each ovitrap consisted of a 300 ml black plastic container with 6.5 cm in diameter, 9.0 cm in height and the opening measuring 7.8cm in diameter. The outer wall of the container was coated with a layer of black oil paint. Fresh water was added to a level of 5.5 cm and an oviposition paddle made form hardboard (10 cm x 2.5 cm x 0.3cm) was placed diagonally with the rough surface upwards into each ovitrap.

Ovitraps were placed in not less than 10% of the houses in all residential areas. The ovitraps were placed outside the house but confined to the immediate vicinity of the house, i.e. car porch and corridor under the eave. The houses were chosen randomly.

Table 3.1: Geographical description of study sites in Sarawak, Malaysia.

Division	District	Study Sites	Coordinates	Elevation (meter a.s.l.)	Type of residential area
Kuching	Kuching	Lorong Siol Kandis	N 1º 34' 31.8"; E 110º 21' 50.6"	5	Urban
		Petra Jaya	N 1º 34' 43.0"; E 110º 20' 25.8"	7	Urban
	Bau	Kampung Atas	N 1º 28' 44.7"; E 110º 11' 11.5"	46	Remote
		Kampung Apar	N 1º 28' 30.8"; E 110º 08' 55.7"	20	Remote
Samarahan	Samarahan	Kampung Merdang Gayam	N 1º 27' 34.4"; E 110º 24' 59.5"	14	Rural
		Kampung Merdang Lumut	N 1º 27' 02.5"; E 110º 23' 52.6"	7	Rural
		Kampung Bukit Brangan	N 1º 26' 40.4"; E 110º 23' 16.7"	17	Suburban
	Serian	Kampung Melayu Tebakang	N 1º 12' 27.8"; E 110º 33' 57.2"	19	Suburban
Sibu	Sibu	Kiew Nang	N 2º 15' 47.4"; E 111º 51' 48.6"	8	Suburban
	Selangau	Pekan Selangau	N 2º 31' 24.1"; E 112º 19' 33.2"	25	Rural
Mukah	Mukah	Kampung Kuala Lama	N 2º 53' 44.4"; E 112º 05' 33.4"	4	Suburban
		Bandar Baru Mukah	N 2º 53' 34.0"; E 112º 05' 33.9"	3	Suburban
	Dalat	Pekan Dalat	N 2º 44' 20.7"; E 111º 56' 14.9"	6	Rural
Miri	Miri	Kampung Siwa Jaya	N 4º 13' 40.7"; E 113º 54' 59.3"	22	Rural
		Bandar Miri	N 4º 28' 02.4"; E 114º 00' 15.6"	6	Urban
		Lutong, Kg. Tulang	N 4º 22' 59.2"; E 113º 58' 56.9"	9	Suburban
Bintulu	Bintulu	Kemena Jaya	N 3º 10' 26.6"; E 113º 02' 53.9"	11	Suburban
		JKR Quarters	N 3º 10' 42.4"; E 113º 02' 55.3"	19	Suburban
	Tatau	Kampung Dagang Tatau	N 2º 52' 33.7"; E 112º 51' 12.6"	7	Rural
Sarikei	Sarikei	Pekan Sarikei	N 2º 07' 40.9"; E 111º 31' 7.85"	3	Suburban
Kapit	Kapit	Pekan Kapit	N 2º 01' 1.11"; E 112º 56' 14.9"	18	Rural

3.3.3 Identification of Larvae

The ovitraps were collected after 5 days and transported back to laboratory and the contents were poured individually into a labelled plastic container, together with the paddle. Dechlorinated water (tap water exposed for 24–48 hr before using) was added into the container and a small piece (10mm) of fresh beef liver was added as larval food. The hatched larvae were subsequently counted and 3rd instar-larvae were identified to species level according to the key by Mahadevan & Cheong (1974). The larval numbers were recorded individually for each positive ovitrap. The larvae from all study areas were further colonized to adulthood in insectarium to obtain their offsprings (F1) for other studies.

3.3.4 Data Analysis for Ovitrap Surveillance

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 side.

(2) Mean number of larvae per recovered ovitrap.

All levels of statistical significance were determined at $P \leq 0.05$ by using statistical programme, student t-test and one-way ANOVA (SPSS® version 21.0; IBM, Armonk, NY).

3.4 Genetic Diversity of *Aedes albopictus*

3.4.1 DNA Extraction

A total of 120 adult females of *Ae. albopictus*, comprising fifteen individuals from each of the eight divisions, were used in this study. Prior to DNA extraction, the abdomen of the mosquitoes were dissected from the mosquito sample to prevent contamination. The DNA was extracted from each specimen using the i-genomic CTB DNA Extraction Mini Kit™ (iNtRON Biotechnology, Inc., Seongnam, South Korea).

All the isolation steps were demonstrated as accordance to the manufacturer's instructions.

3.4.2 Polymerase Chain Reaction (PCR)

The amplification of extracted genomic DNA was conducted using mitochondrial primers of COI from Kumar et al. (2007) (forward primer, 5'-GGA TTT GGA AAT TGA TTA GTT CCT T-3'; reverse primer, 5'-AAA AAT TTT AAT TCC AGT TGG AAC AGC-3'). The reaction mixture for amplification of COI regions consists of 25–50 ng genomic mosquito DNA, 5 µl 10x buffer, 2.5 mM of each dNTP, 10 pmol of each forward and reverse primer, 1.5 U *Taq* polymerase (iNtRON Biotechnology, Inc.) and double-distilled water to a final volume of 50 µl. The PCR cycling was performed in a Veriti Thermal Cycler (Life Sciences, Australia) using an initial denaturation of 95°C for 5 min, followed by five cycles of 94°C for 40s (denaturation), 45°C for 1 min (annealing) and 72°C for 1 min (extension), and 35 cycles of 94°C for 40 s (denaturation), 51°C for 1 min (annealing) and 72°C for 1 min (extension), and final extension at 72°C for 10 min.

3.4.3 DNA Purification

The amplified products were electrophoresed on 2.0% agarose gel pre-stained with SYBR Safe™ (Invitrogen Corp., Carlsbad, CA, U.S.A.). The PCR products were purified with MEGAquick-spin™ PCR and Agarose Gel DNA Extraction System (iNtRON Biotechnology, Inc.). PCR products that yielded an unambiguous single band were sent to a local company for DNA sequencing.

3.4.4 DNA Sequence Alignment

Data on the nucleotide sequences of COI gene of Sarawak *Ae. albopictus* which representing unique haplotype were deposited in the National Center for Biotechnology Information (NCBI) GenBank. All obtained sequences were analysed and edited using ChromasPro 1.5® (Technelysium Pty Ltd, Brisbane, Qld, Australia) and BioEdit 7.0.9.0.® (Hall, 1999). The partial COI was preliminarily aligned using ClustalX® (Thompson et al., 1997) and subsequently aligned manually.

3.4.5 Haplotype Network Reconstruction

The genetic diversity networks of *Ae. albopictus* were analysed using TCS Networks (Clement et al, 2002) and illustrated by PopArt 1.71 (Leigh & Bryant, 2015) to calculate the minimum number of mutational steps by which the sequences could be joined with > 95% confidence. The aligned COI sequence consisted of 633 bp.

With regard to the comparison of Sarawak *Ae. albopictus* with other *Ae. albopictus* from GenBank, some downloaded sequences were trimmed in length in order to ensure equal lengths of alignment for the purposes of comparison; the final lengths of the aligned COI sequences used for analysis was 343 bp. The COI sequences deposited in GenBank that did not correspond in length or region to the sequences of Sarawak *Ae. albopictus* generated in this study were omitted. A total of 619 COI sequences (AB690835, AY748238–AY748239, AY834241, DQ424959, GQ143719, GU299768–GU299770, HF536717, HF912379, HM102286, HQ398900–HQ398902, HQ906848–HQ906851, JF810659, JN406654–JN406732, JQ388786, JQ412504–JQ412506, JQ728019–JQ728301, JX675570–JX6755572, JX679373–JX679386, KC572145–KC572496, KC920751–KC920788, KC970275–KC970276, KF042861–KF042885, KF135494–KF135495, KF657725, KJ410333, KJ410335, KP122846–KP122909, and KP211400) were retrieved from the database.

The retrieved sequences were assigned into several groups of continents following the study by Azrizal-Wahid et al. (2020) which are North America, South America, Europe, Africa, Oceania, East Asia, South Asia, West Asia, Southeast Asia, Peninsular Malaysia and East Malaysia.

3.4.6 Genetic Divergence and Haplotype Analyses

Uncorrected (*p*) pairwise genetic distances were estimated using MEGA7.0 to access the level of haplotype variation of the COI. The F_{ST} and N_m pairwise values were calculated using DNASP® (DNA Sequence Polymorphism) software (Librado & Rozas, 2009) to obtain genetic differentiation and gene flow among the *Ae. albopictus* populations in Sarawak. The levels of genetic differentiation are followed the classification criteria by Wright (1978), where $F_{ST} > 0.25$ (great differentiation), $0.15 \leq F_{ST} \leq 0.25$ (moderate differentiation) and $F_{ST} < 0.15$ (negligible differentiation). On the other hand, the levels of gene flow are determined according to criterion of Slatkin (1981) where $N_m > 1$ (high gene flow), $0.25 \leq N_m < 0.99$ (moderate gene flow) and $N_m < 0.25$ (low gene flow).

The haplotype analyses were performed using the DNASP ® software to access the number of haplotype (*N*h), haplotype diversity (H_d), nucleotide diversity (P_i), number of segregating sites (*S*), average number of sequence differences (*K*), and number of polymorphic sites (*np*) of the Sarawak *Ae. albopictus*. Moreover, the neutrality test including Tajima's *D* (Tajima, 1989) and Fu's F_s (Fu, 1997) as well as mismatch distribution analysis were also demonstrated using DNASP ® software.

3.5 Adulticide Resistance Status of *Aedes albopictus*

3.5.1 Adulticides

Eleven adulticides representing four classes of insecticides were used in adult bioassays, namely DDT (4%), dieldrin (0.4%), malathion (5%), fenitrothion (1%), bendiocarb (0.1%), propoxur (0.1%), etofenprox (0.5%), deltamethrin (0.05%), lambda-cyhalothrin (0.05%), permethrin (0.25%), and cyfluthrin (0.15%). All insecticides-impregnated papers were supplied from WHOPES Collaborating Centre, University Science of Malaysia, Penang, Malaysia.

3.5.2 WHO Adult Bioassay

The adult bioassay tests were performed according to the WHO standard procedures (WHO, 2016). A total of 25 sucrose-fed female *Ae. albopictus* aged 3–5 days were used in this study. For each test, the mosquitoes were transferred in a holding tube for an hour. After 1 hour, any knocked-down, dead or damaged mosquito was replaced with healthy one. Mosquitoes were then exposed to the insecticide impregnated papers in the test tubes for 1 hour. The knockdown numbers of mosquito were recorded every minute up to 60 minutes. A black cloth was used to cover the test tube in order to create a dark condition for mosquito to land or rest on the impregnated paper. Mosquitoes were transferred into holding cup and supplied with 10% sugar solution and mortality was recorded 24 hour after exposure. Bioassays were conducted at room temperature of 28°C ± 2°C with relative humidity of 75 ± 10%. Mosquitoes were considered knockdown when they cannot stand at normal position or fly in coordinated manner, lie on their back and immobile (WHO, 2016). Three replicates were conducted for each adulticides and control treatment was also set up by exposing 25 adult female mosquitoes to untreated paper.

3.5.3 Data Analysis for WHO Adult Bioassay

Results obtained from the bioassay were pooled and analysed using Window SPSS program (version 21.0). Kaplan-Meier survival function was used to obtain 50% knockdown time (KT_{50}). The mortality rates of the adult mosquitoes were determined by dividing the number of dead mosquitoes by the total number tested mosquitoes. The strain was considered susceptible if the mortality rates ≥ 98%, possible resistance if mortality is between 90 – 98% and considered resistant if mortality rates < 90% (WHO, 2016). If the mortality in control tubes exceeds 10%, the mortalities of all treated groups are corrected using Abbot's formula as below:

$$\text{Corrected mortality (\%)} = \frac{\text{\% mortality with treat paper} - \text{\% mortality with control}}{100 - \text{\% mortality with control}} \text{ X } 100$$

To investigate cross resistance among insecticides, associations between the mortality rates of tested adulticides were analysed using Pearson Correlation Test analysis, where *p* values ≤ 0.05 was considered statistically significant.

3.6 Lavicides Resistance Status of *Aedes albopictus*

3.6.1 Larvicides

Eight larvicides belonging to organochlorine and organophosphate groups are DDT 0,012 mg/L, dieldrin 0.050 mg/L, bromophos 0.050 mg/L, chlorpyrifos 0.012 mg/L, fenitrothion 0.020 mg/L, fenthion 0.025 mg/L, malathion 0.125 mg/L and temephos 0.012mg/L were used in this study. The stock solution of eight larvicides were supplied by the Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia, a WHOPES Collaborating Centre.

3.6.2 WHO Larval Bioassay

The larval bioassay was performed according to WHO Standard Protocol (WHO, 2016) to determine the larval susceptibility status. The diagnostic dosage of larvicide were prepared by pipetting the appropriate standard insecticide solution into 450 ml disposable cup filled with 200 ml distilled water. Twenty five of 3rd or early 4th instar larvae were introduced into the disposable cup with larvicide solution. Any abnormal larvae were discarded. The solution was then topped up to 250 ml using distilled water. The cups were held at room temperature of 28°C and 70% relative humidity. Larval mortality was recorded after 24 hours post treatment. Three replicates of each insecticide were conducted. The control (untreated) consisted of 1ml of ethanol added in distilled water was also prepared.

3.6.3 Data Analysis for WHO Larval Bioassay

The test results obtained from bioassay were pooled and analysed using analysis software (Windows SPSS program version 21.0) with a 95% confidence level. Both moribund and dead larvae were combined for data analysis. According to WHO (1970), dead larvae are those cannot be induced to move when siphon or the cervical region was probed by needle, while moribund larvae are those with characteristic diving reaction when water is disturbed, and they may show discolouration, unnatural positions, tremor, incoordination or rigour.

The percentage mortality at 24 hour post-treatment was used to determine the susceptibility status based on the WHO susceptibility criterion of mortality rate of >98%, whereas resistant represents a mortality rate of <90%; a mortality rate between 90 – 98% is a sign of tolerant/intermediate resistance (WHO, 2016). If the mortality of control was >5%, the percentage of treated was corrected by Abbott's formula:

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

With regard to cross resistance among tested larvicides, investigations of associations between the mortality rates of tested larvicides were analysed using Spearman rank-order correlation analysis, where p values ≤ 0.05 was considered statistically significant.

3.7 Biochemical Studies of Insecticide Resistance in *Aedes albopictus*

Enzyme microassays (biochemical analysis) were performed on both *Ae. albopictus* larvae and adult mosquitoes. In order to determine the possible underlying mechanism responsible for insecticide resistance, the enzyme activities of enzyme activities of non-specific esterases (α- and β-), P450-mediated monooxgenases or mixed function oxidases (MFO), glutathione-S-transferases (GST), and alteration of acetylcholinesterase (AChE) were determined using enzyme linked immunoassay (ELISA) reader (absorbance microplate reader, BIOTEK® ELx800^{TM}). For non-specific EST, MFO and GST biochemical analysis, a sample size of 24 individual from each population for each test with four replicates were conducted for adult mosquitoes and 12 individuals for larvae. For AChE biochemical analysis, a sample size of 12 individual from each strain with eight replicates were conducted.

3.7.1 Non-specific Esterases (EST) Enzyme Microassay

The Biochemical assay of non-specific esterases (EST) was conducted as described by Brogdon et al. (1988) and Lee (1990).

3.7.1.1 Preparation of Potassium Phosphate Buffer (2.0 M; pH 7.6)

The 2.0 M potassium phosphate buffer was prepared by dissolving 4.50 g of sodium phosphate dibasic (Na_2HPO_4) and 1.70 g potassium phosphate monobasic (KH_2PO_4) in 500 ml distilled water. The pH was then adjusted to 7.6 using pH meter.

3.7.1.2 Preparation of Substrate Solution

The stock solution was first prepared by dissolving 0.06 g α-naphthyl acetate in 10 ml acetone. Next, 0.5 ml of the stock solution was added into 50 ml potassium phosphate buffer to produce substrate solution.

3.7.1.3 Preparation of Indicator Solution / Coupling Reagent

The indicator solution / coupling reagent was prepared by adding 0.875 g sodium dodecyl sulphate (SDS) and 0.075 g fast blue salt (FBS) (tetrazotized o-dianisidine) into 50 ml of distilled water.

3.7.1.4 Preparation of Stopping Solution: 10% Acetic Acid

10% acetic acid which acts as stopping solution was prepared by adding 10 ml of absolute acetic acid into 90 ml of distilled water.

3.7.1.5 Procedure of Non-specific Esterases (EST) Enzyme Micorassay

Firstly, the samples were individually homogenized in 100 µl phosphate buffer in a microcentrifuge tube at 4°C using pestle and further diluted with 400 µl buffer. The homogenate was centrifuged at 15000 rpm for 10 min at 4°C. Fifty (50) µl clear homogenate was then transferred into each well of microtiter plate using micropipette. A total of four replicate aliquots of the homogenate from a single sample were obtained for this assay. Fifty (50) µl of substrate solution was then added into each well and left

to stand for 1 minute at room temperature (28°C), followed by addition of 50 µl indicator solution. The reaction was further incubated for 10 min and was stopped by addition of 50 µl 10% acetic acid. Observation of colour changes occurred where a pinkish colour appeared at first then turned to blue after incubation. This was due to the hydrolysis of α-naphthyl acetate into α-naphthol which reacted with the FBS, hence producing a change in the absorbance of the solution. The microtiter plate was incubated for 10 min at room temperature (28°C). Similar procedure was repeated for non-specific esterases (β-) with the substitution of substrates solution which made up from β-naphthyl acetate and acetone. The optical density was measured at 450 nm using absorbance microplate reader (BIOTEK® ELx800^{TM}).

3.7.2 Mixed Function Oxidases (MFO) Enzyme Microassay

Mixed function oxidases assay was carried out according to Brogdon et al. (1997) with some modifications as outlined by Nazni et al. (2000).

3.7.2.1 Preparation of Sodium Acetate Buffer (0.25 M; pH 5.0)

Exact amount of 20.51 g sodium acetate was dissolved in1000 ml distilled water to produce 0.25 M sodium acetate buffer. The buffer solution was then adjusted to pH 5.0 with acetic acid using pH meter.

3.7.2.2 Preparation of Substrate Solution: 3,3'5,5'-Tetramethylbenzidine (TMBZ) Solution

The substrate solution was freshly prepared by dissolving 0.05 g 3,3'5,5'-tetramethylbenzidine (TMBZ) in 25 ml of absolute methanol. The solution was further added with 75 ml of 0.25 M sodium acetate buffer (pH 5.0).

3.7.2.3 Preparation of Indicator Solution: 3% Hydrogen Peroxide (H_2O_2) Solution

The indicator solution was prepared by introducing 1.935 ml of 31% hydrogen peroxide into 18.065 ml of distilled water to produce 20 ml of 3% hydrogen peroxide.

3.7.2.4 Procedure of Mixed Function Oxidases (MFO) Enzyme Microassay

Each individual sample was homogenized in 100 µl sodium acetate buffer solution in a microcentrifuge tube at 4°C using pestle followed by adding 900 µl buffer solution to a total 1 ml. After 5 minutes incubation, 100 µl of each individual homogenate was then pipetted into each well of microtiter plate followed by 200 µl of 2 mM 3,3'5,5'-tetramethylbenzidine (TMBZ) and 25 µl of 3% hydrogen peroxide. Reaction of colour change took place immediately. The microtiter plate was incubated for 10 minutes before being read by absorbance microplate reader (BIOTEK® ELx800^{TM}) at a wavelength of 630 nm.

3.7.3 Glutathione-S-transferases (GST) Enzyme Microassay

Enzyme microsssay of glutathione-S-transferases (GST) was conducted according to Lee and Chong (1995).

3.7.3.1 Preparation of Potassium Phosphate Buffer (0.5 M; pH 7.4)

Solution A was prepared by dissolving exact amount of 2.724 g potassium phosphate monobasic (KH_2PO_4) in 300 ml distilled water. Meanwhile, Solution B was prepared by dissolving 9.47 g sodium phosphate dibasic (Na_2HPO_4) in 1000 ml distilled water. Next step was adding 196.0 ml of Solution A into 804.0 ml of Solution B to produce 1000 ml potassium phosphate buffer. The buffer pH was then adjusted to 7.4 using pH meter.

3.7.3.2 Preparation of Substrate Solution

The substrate solution was prepared by dissolving 0.03 g glutathione (GSH) in 50 ml potassium phosphate buffer (0.5 M; pH 7.4)

3.7.3.3 Preparation of Indicator Solution / Coupling Reagent

The coupling reagent was prepared by introducing both 0.01 g of 1-chloro-2, 4-dinitrobenzene (CDNB) and 0.5 ml acetone into 50 ml potassium phosphate buffer (0.5 M; pH 7.4).

3.7.3.4 Procedure of Glutathione-S-transferases (GST) Enzyme Microassay

Each individual sample was homogenized in 100 µl potassium phosphate buffer solution in a microcentrifuge tube at 4°C using pestle followed by another addition of 400 µl of buffer. The homogenates were then centrifuged at 14,000 rpm for 10 min at 4°C. Four replicates of homogenate with 100 µl were pipetted into each well of microtiter plate, followed by the addition of 50 µl of GSH (substrate solution) and 50 µl of CDNB (indicator solution) using a multiple eight (8) channels micropipette. Change of colour reaction took place in which yellowish colour was observed. The microtiter plate was incubated for 30 min at room temperature (28°C) before it was read by absorbance microplate reader (BIOTEK® ELx800^{TM}) at a wavelength of 410 nm.

3.7.4 Insensitive Acetylcholinesterase (AChE) Enzyme Microassay

The enzyme mircoassay for insensitive acetylcholinesterase (AChE) was conducted using a modification of Ellman's method (Brogdon et al., 1988).

3.7.4.1 Preparation of Potassium Phosphate Buffer (pH 6.8)

Potassium phosphate buffer was prepared by dissolving exact amount of 4.735 g sodium phosphate dibasic (Na_2HPO_4) and 4.540 g potassium phosphate monobasic (KH_2PO_4) in 500 ml distilled water. The pH was then adjusted to 6.8 using pH meter.

3.7.4.2 Preparation of Substrate Solution: Acetylthiocholine iodine (ACTHI)

The substrate solution was prepared by dissolving 0.075 g acetylthiocholine iodide (ACTHI) and 10 ml acetone in 90 ml potassium phosphate buffer (pH 6.8). The solution was mixed in a bottle covered with aluminium foil to prevent any exposure to light.

3.7.4.3 Preparation of Coupling Reagent: Ellman's Solution (DTNB)

The Ellmam's solution was prepared by dissolving the exact amount of 0.013 g 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) in 100 ml potassium phosphate buffer (pH 6.8). The reagent bottle was covered with aluminium foil to prevent from any exposure to light.

3.7.4.4 Preparation of Inhibitor

The 0.1% and 0.2 % propoxur solution were used as inhibitor in AChE enzyme mircoassay. Thus, 1000 mg/L and 2000 mg/L of propoxur solution was prepared as propoxur-ACTHI concentrations, respectively.

3.7.4.5 Procedure of Insensitive Acetylcholinesterase (AChE) Enzyme Microassay

Each individual sample was homogenized in 100 µl potassium phosphate buffer in a microcentrifuge tube at 4°C using pestle followed by another addition of 400 µl. The homogenate was then centrifuged at 14,000 rpm at 4°C for 10 min. In this assay, a total of 8 aliquots of homogenate from every sample were obtained, thus 8 wells of microtiter

plate were used per sample which made up two microtiter plate were used for a stain. A 50 µl of reaction mixture containing 10% acetone buffer solution of 2.6 mM acetylthiocholine iodide (ACTHI), 0.3mM of 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.1% propoxur inhibitor were added into 3 well of each samples. Another similar mixture but replaced 0.1% propoxur with 0.2% propuxur were added into another 3 well of each samples. A 50 µl of reaction mixture without propoxur inhibitor was designed as positive control. The yellowish colour or colourless solution was observed and the reaction was incubated for 30 min at room temperature (28°C) followed by the measurement of optical density at 410 nm.

3.7.5 Statistical Analysis of Enzyme Activities

The level of elevated enzyme activities obtained from each enzyme microassay for *Ae. albopictus* adult and larvae were compared with the reference strain by calculating the resistance ratio (RR) as below:

$$\text{Resistance Ratio (RR)} = \frac{\text{Mean of elevated enzyme activity of the field strain}}{\text{Mean of elevated enzyme activity of the reference strain}}$$

According to the resistance status criterion of WHO (2016), the value of RR > 10 indicated the mosquito population exhibited high resistant while RR value between 5 and 10 implying moderate resistant. The mosquito population is susceptible for RR value < 5.

Comparative measure of mean enzyme activities between different populations was performed by one-way analysis of variance (ANOVA) using SPSS (ver 21.0). Tukey's test was used to separate means in significant ANOVAs, $P \leq 0{,}05$. Independent-samples t-test was performed to indicate significant increase in mean differences for enzyme microasay of EST, MFO and GST.

With respect to insensitive acetylcholinesterase, results were interpreted as a percentage remaining activity in the propoxur inhibited fraction compared to the control (unhibited) activity. The samples were classified into three different heterogeneity categories based on their mean percent acetylsholinesterase activity in propoxur-inhibited fraction (%). Individual mosquitoes with more than 70% remaining activity are indicative of homozygous resistance (RR), 30-70% remaining activity indicative of heterozygous (RS) and less than 30% remaining activity are indicative of homozygous susceptible (SS). Because of the light absorbance of propoxur in the microplate, in certain cases, homogenates appear to show higher acetylcholinesterase activity in propoxur-inhibited fraction (>100%) and it is normal in resistant strains (WHO, 1998). The mean percent AChE activity in propoxur-inhibited fraction (%) was calculated as below:

$$\text{Mean percent acetylcholinesterase activity in propoxur-inhibited fraction (\%)} = \frac{\text{Total mean optical density of inhibited reaction (with propoxur)}}{\text{Total mean optical density of unhibited reaction (without propoxur)}} \text{ X } 100$$

Furthermore, the resistance ratio for AChE activity was calculated based on the mean present AChE activity in propoxur-inhibited fraction (%) for each larval and adult populations. Paired sample t-test was also performed to determine the differences between the mean AChE activity with the addition of propoxur and the mean AChE activity without the addition of propoxur.

Spearman rank-order correlation using SPSS was performed to determine the associations between the survival rates of larval and adult bioassays with enzyme activities, and investigate the relationships between enzyme activities.

3.8 Bioefficacy of Insect Growth Regulators (IGRs)

3.8.1 Insect Growth Regulators

Five insect growth regulators (IGRs) were used namely, methoprene 1.3% w/w GR (granules), pyriproxyfen 0.5% w/w GR (granules), diflubenzuron 25% w/w WP (wettable powder), cyromazine 75% w/w WP (wettable powder) and novaluron 10% w/w EC (emulsifiable concentrate).

Methoprene and pyriproxyfen are juvenile hormone mimics (IRAC group 7), diflubenzuron and novaluron are chitin synthesis inhibitors (IRAC group 15) and cyromazine is a moulting disruptor (IRAC group 17) (IRAC, 2017).

3.8.2 WHO Larval Bioassay for Insect Growth Regulators

The test was performed according to WHO (2005) larval susceptibility bioassay procedure for determining the susceptibility or resistance of mosquito larvae to insect growth regulators. A series of range-finding concentrations were first prepared by diluting the stock solution into 250ml water in a paper cup. Twenty-five third instar larvae were introduced into each cup and the larvae were continuously exposed to the insecticides. All cups were covered by mesh cloth and beef liver powder was provided as larvae food. Mortality and survival rates were counted daily until all individual died or emerged as adults. The test containers were held in the laboratory with room temperature (28ºC) and photoperiod of 12L:12D. Untreated control was set up in same manner without any insecticide.

3.8.3 Statistical Analysis

Results obtained from the bioassay were pooled and analysed by probit analysis (Finney, 1971) using Windows SPSS program version 21.0 with a 95% confidence level to obtain EI_{50} (50% emergence inhibition). Emergence inhibition (EI) was expressed as

the impact of the IGR. Moribund, dead larvae and pupae, as well as adult mosquitoes that had not completely separated from pupal case, were considered as "affected" and were collected for data analysis (WHO, 2005). 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 are 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:

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

RR values of 1.1 – 5.0, 5.1 – 20.0, 20.1 – 100 and > 100 were classified as tolerance, low level, moderate level and high level resistance, respectively (Wirth, 2010; Su, 2016).Values of RR less than or equal to 1 were considered as susceptible.

CHAPTER 4: RESULTS

4.1 Ovitrap Surveillance in Sarawak

Table 4.2 shows the ovitrap index (OI) and the mean number of larvae per ovitrap of *Ae. albopictus* and *Armigeres* sp. obtained from 21 residential areas across 13 districts in Sarawak. All residential areas were categorized into urban, suburban, rural and remote according to their landscapes as shown in Table 3.1. *Aedes albopictus* was present in all localities with the OI ranging from 35.00% to 100% and mean number of larvae per ovitrap ranged from 2.74 ± 1.15 to 29.41 ± 6.64.

Comparisons between OI according to landscapes show that the mean OI of the urban residential area was significantly higher than rural, suburban and remote residential area ($p < 0.05$) with mean OI at 90.97 ± 1.59%, 69.76 ± 8.34%, 65.91 ± 3.88% and 52.63 ± 15.79%, respectively. In addition, significantly highest *Ae. albopictus* mean number of larvae per ovitrap was obtained from urban residential areas (26.47 ± 1.62) compared to rural areas (14.73 ± 2.95), suburban areas (13.55 ± 2.22) and remote areas (7.06 ± 4.32) ($p < 0.05$). There difference in larval numbers was significant among all residential areas ($p \leq 0.05$).

Aedes aegypti was not detected throughout the surveillance. On the other hand, *Armigeres kesseli* and *Armigeres subalbatus* were found co-breeding with *Ae. albopictus* from 5 residential areas, namely Kampung Melayu Tebakang (District: Serian, Division: Samarahan), Kampung Merdang Lumut (Samarahan, Samarahan), Pekan Selangau (Selangau, Sibu), Kampung Atas (Bau, Kuching) and Kampung Apar (Bau, Kuching) with mean larval number per ovitrap ranging from 0.18 ± 0.18 to 1.08 ± 0.60 (Table 4.1).

Further analyses of comparisons between OI and mean larval number per ovitrap according to landscapes are shown in Table 4.2 and Table 4.3, respectively. There was

significant difference of OI between urban and other landscapes, but no significant difference of OI between suburban, rural and remote residential areas (Table 4.2). Table 4.3 reveals a significant difference between mean larval number per ovitrap obtained from urban and other landscapes; however, no significant difference between suburban, rural and remote residential areas was observed, indicating that density of the *Ae. albopictus* in urban residential areas were higher than other residential areas and distributed well with high OI observed in urban residential areas.

Table 4.4 shows mixed breeding of *Aedes albopictus* and *Armigeres* spp. larvae in residential areas in Sarawak. The percentage of mixed breeding ranged from 9.09 to 38.46%. The numbers of *Ae. albopictus* larvae were found 2.38 – 71.00 times higher than those of *Armigeres* sp. in mixed breeding ovitraps.

Table 4.1: Ovitrap index (OI) and mean number of larvae per ovitrap (mean ± S.E.) of *Aedes albopictus* larvae collected from 21 study sites across Sarawak, Malaysia.

Study Sites	Landscape	OI (%)	Mean OI	Mean *Aedes albopictus* larvae per ovitrap		Mean *Armigeres* larvae per ovitrap	
				Each study site	Type of residential area	Each study site	Type of residential area
Bandar Miri, Miri	Urban	90.91	90.97 ± 1.59	23.82 ± 3.31	26.47 ± 1.62	-	-
Lorong Siol Kandis, Kuching	Urban	88.24		29.41 ± 6.64		-	
Petra Jaya, Kuching	Urban	93.75		26.19 ± 6.11		-	
Bandar Baru Mukah, Mukah	Suburban	45.00	69.70 ± 5.10	4.25 ± 1.32	13.55 ± 2.22	-	0.11 ± 0.11
JKR Quarters, Bintulu	Suburban	60.00		15.47 ± 4.63		-	
Kampung Bukit Brangan, Samarahan	Suburban	73.68		10.26 ± 2.22		-	
Kampung Kuala Lama, Mukah	Suburban	57.89		13.05 ± 6.35		-	
Kemena Jaya, Bintulu	Suburban	72.73		18.14 ± 4.81		-	
Kiew Nang, Sibu	Suburban	78.95		12.84 ± 2.87		-	
Lutong, Kg. Tulang, Miri	Suburban	70.59		7.71 ± 2.21		-	
Pekan Sarikei, Sarikei	Suburban	68.42		12.63 ± 3.27		-	
Kampung Melayu Tebakang, Serian	Suburban	100.00		27.64 ± 2.78		0.97 ± 0.41	
Kampung Dagang Tatau, Tatau	Rural	47.37	65.46 ± 8.23	11.00 ± 4.04	14.73 ± 2.95	-	0.06 ± 0.04
Kampung Merdang Gayam, Samarahan	Rural	87.50		26.63 ± 7.24		-	
Kampung Merdang Lumut, Samarahan	Rural	80.00		17.40 ± 9.46		0.25 ± 0.16	
Kampung Siwa Jaya, Miri	Rural	63.64		16.23 ± 4.29		-	
Pekan Dalat, Dalat	Rural	35.00		4.20 ± 1.64		-	
Pekan Kapit, Kapit	Rural	92.31		20.50 ± 4.10		-	
Pekan Selangau, Selangau	Rural	52.38		7.14 ± 2.55		0.18 ± 0.18	
Kampung Atas, Bau	Remote	68.42	52.63 ± 15.79	11.37 ± 4.49	7.06 ± 4.32	1.08 ± 0.60	0.90 ± 0.19
Kampung Apar, Bau	Remote	36.84		2.74 ± 1.15		0.71 ± 0.47	
			$p = 0.043$		$p = 0.026$		$p = 0.003$

S.E. = Standard Error

Table 4.2: Comparison of mean ovitrap index (OI) between landscapes.

p value	Urban	Suburban	Rural	Remote
Urban	-	0.043	0.021	0.049
Suburban	-	-	0.654	0.212
Rural	-	-	-	0.488
Remote	-	-	-	-

Table 4.3: Comparison of mean number of larvae per ovitrap between landscapes.

p value	Urban	Suburban	Rural	Remote
Urban	-	0.010	0.039	0.015
Suburban	-	-	0.751	0.240
Rural	-	-	-	0.248
Remote	-	-	-	-

Table 4.4: Mixed breeding of *Aedes albopictus* and *Armigeres* spp. larvae in residential areas in Sarawak.

Study sites	Collected Ovitrap	Positive ovitrap		Mixed breeding ovitrap					
		n	%	n	%	Ovitrap No.	Number of Larvae		Ratio of *Ae. albopictus* : *Armigeres* spp.
							Ae. albopictus	*Armigeres* spp.	
Kampung Atas, Bau	19	13	68.42	5	38.46	1	3	1	3.00 : 1.00
						2	71	1	71.00 : 1.00
						3	53	2	26.50 : 1.00
						4	13	1	13.00 : 1.00
						5	21	8	2.63 : 1.00
Kampung Melayu Tebakang, Serian	36	36	100.00	4	11.11	1	45	7	6.43 : 1.00
						2	18	6	3.00 : 1.00
						3	49	8	6.13 : 1.00
						4	19	8	2.38 : 1.00
Kampung Merdang Lumut, Samarahan	10	8	80.00	2	25.00	1	8	1	8.00 : 1.00
						2	41	1	41.00 : 1.00
Pekan Selangau, Selangau	21	11	52.38	1	9.09	1	5	2	2.50 : 1.00
Kampung Apar, Bau	19	7	36.84	1	14.29	1	15	3	5.00 : 1.00

4.2 Genetic Diversity of *Aedes albopictus*

Based on the morphological features, all specimens were identified unambiguously, and no abnormal characters were found. The partial regions of COI were successfully sequenced from 120 individuals of *Ae. albopictus* collected from eight divisions in Sarawak with the final sequence fragment lengths aligned as 633 base pairs characters. A statistical parsimony network of 120 taxa revealed 22 haplotypes and were deposited in the GenBank under accession numbers KT211221–KT211242. From the sequence analyses, the overall value of haplotype diversity (H_d) was 0.65014 and nucleotide diversity (P_i) was 0.00176. The Kapit showed the highest diversity among divisions closely followed by Sibu.

From Table 4.5, the neutrality test of Tajima's D showed negative value for all populations except for Sibu which showed positive value. With regard to Fu's F_s test, all populations showed negative value except for Kuching and Mukah. The positive value of Fu's F_s probably suggested the occurrence of bottleneck in the populations. All the results were not significant. However, there were significant negative values for the overall result of Tajima's D (– 1.78903, $p < 0.05$) and Fu's F_s (– 20.837, $p < 0.05$), which signify an excess of low frequency of polymorphisms and suggest there was a recent population expansion in the Sarawak *Ae. albopictus*. Analysis of mismatch distribution of haplotypes in Figure 4.4 showed unimodal distribution for *Ae. albopictus*.

The overall F_{ST} was 0.18913 and Nm was 1.07, implying that the genetic differentiation was moderate and there was a high gene flow among the populations of *Ae. albopictus* in Sarawak. The value of pairwise F_{ST} and Nm between eight divisions was shown in Table 4.6. The highest F_{ST} value occurred between Mukah and Samarahan (0.46939) and the lowest was between Kuching and Miri (0.00196). However, the F_{ST} value was not significantly different in all population pairs. The

highest value of gene flow (*N*m) was found between Kuching and Miri (127.50) while the lowest was between Mukah and Samarahan.

The haplotype distribution and frequency of the haplotypes are shown in Table 4.7. The most prevalent haplotype was A1 (n = 70) followed by A15 (n = 10) and the least prevalent represent as singleton haplotypes were A2, A3, A4, A10, A11, A12, A13, A14, A16, A17, and A20. The number of haplotypes distributed in different divisions ranged from 2 to 7. The mapped haplotype network based on COI gene was a star-like shape (Fig. 4.2). There was no apparent geographical pattern observed from the network which implying lack of genetic structuring across different divisions in Sarawak. The uncorrected '*p*' genetic distances among the haplotypes of COI gene was ranged from 0.00000 – 0.0227 (Table 4.8). The highest genetic distances were observed between haplotype A2 and between A3.

Multiple aligned sequences of COI were trimmed to 343 bp to ensure the equal alignment length for global comparison. In COI analysis, a total 72 haplotypes (B1–B72) were discovered. The median joining network (Fig. 4.3) showed no clear separation of genetic structure across all continents. The composition of Sarawak *Ae. albopictus* haplotypes in the network revealed three common haplotypes were nested with sequences from Oceania (B1, B7), Southeast Asia (B1) and Peninsular Malaysia (B9) and eleven unique haplotypes (B2–B6, B8, B10–B14). Interestingly, among Malaysian *Ae. albopictus* (Peninsular Malaysia and Sarawak), only one common shared haplotype (B9) was discovered.

Table 4.5: Genetic diversity indices and neutrality test based on COI sequences of *Aedes albopictus* from eight divisions in Sarawak.

Divisions	N_h	H_d	P_i	S	K	D	F_s
Bintulu	5	0.56190	0.00156	4	0.99048	– 0.62465	– 1.548
Kapit	7	0.85714	0.00241	6	1.52381	– 0.60986	– 2.760
Kuching	3	0.25714	0.00123	5	0.78095	– 1.66013	0.414
Miri	3	0.36190	0.00081	3	0.51429	– 1.31654	– 0.379
Mukah	2	0.24762	0.00039	1	0.24762	– 0.39883	0.133
Samarahan	4	0.63810	0.00117	3	0.74286	– 0.57961	– 0.986
Sarikei	4	0.66667	0.00126	3	0.80000	– 0.39538	– 0.825
Sibu	5	0.80952	0.00286	4	1.80952	1.50974	– 0.052
Overall	22	0.65014	0.00176	17	1.11681	– 1.78903*	– 20.837*

N_h: number of haplotype; H_d: haplotype diversity; P_i: nucleotide diversity; S: number of segregating sites; K: average number of nucleotide differences; D: Tajima's D; F_s: Fu's F_s neutrality test.
*significant p value lower than 0.05 ($p < 0.05$).

Table 4.6: Pairwise genetic differentiation ($F_{ST:}$ below diagonal) and gene flow (N_m: above diagonal) among *Aedes albopictus* populations from eight divisions in Sarawak.

Division	Bintulu	Kapit	Kuching	Miri	Mukah	Samarahan	Sarikei	Sibu
Bintulu		2.01	1.94	2.15	1.35	0.41	1.38	0.75
Kapit	0.11051		2.73	5.90	2.29	1.06	2.90	1.49
Kuching	0.11429	0.08380		127.50	6.75	0.43	2.59	0.87
Miri	0.10431	0.04065	0.00196		5.00	0.36	2.81	0.78
Mukah	0.15584	0.09825	0.03571	0.04762		0.28	1.72	0.81
Samarahan	0.38095	0.19048	0.36508	0.41071	0.46939		0.61	1.24
Sarikei	0.15366	0.07948	0.08791	0.08163	0.12698	0.29155		1.01
Sibu	0.25000	0.14384	0.22286	0.24224	0.23621	0.16770	0.19789	

*significant p value lower than 0.05 ($p < 0.05$).

Table 4.7: Haplotype distribution of *Aedes albopictus* based on COI sequences.

Divisions	No. of specimens (*n*)	Haplotype COI
Bintulu	15	A1 (10), A2* (1), A3* (1), A4* (1), A5 (2)
Kapit	15	A1 (5), A6 (2), A7 (2), A8 (3), A9 (1), A10* (1) , A11* (1)
Kuching	15	A1 (13), A12* (1), A13* (1)
Miri	15	A1 (12), A6 (2), A14* (1)
Mukah	15	A1 (13), A9 (2)
Samarahan	15	A1 (5), A15 (8), A16* (1), A17* (1)
Sarikei	15	A1 (8), A6 (1), A15 (2), A18 (4)
Sibu	15	A1 (4), A19 (5), A20* (1), A21 (2), A22 (3)

*singleton haplotype

Table 4.8: Uncorrected '*p*' distance matrix among Sarawak *Aedes albopictus* haplotype based on COI gene.

Haplotype	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]
[1] A1																					
[2] A2	0.0072																				
[3] A3	0.0072	0.0072																			
[4] A4	0.0033	0.0033	0.0033																		
[5] A5	0.0033	0.0033	0.0033	0.0000																	
[6] A6	0.0033	0.0033	0.0116	0.0072	0.0072																
[7] A7	0.0033	0.0116	0.0116	0.0072	0.0072	0.0072															
[8] A8	0.0033	0.0116	0.0033	0.0072	0.0072	0.0072	0.0072														
[9] A9	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033													
[10] A10	0.0033	0.0116	0.0033	0.0072	0.0072	0.0072	0.0072	0.0000	0.0033												
[11] A11	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033	0.0000	0.0033											
[12] A12	0.0072	0.0168	0.0168	0.0116	0.0116	0.0116	0.0116	0.0116	0.0072	0.0116	0.0072										
[13] A13	0.0116	0.0227	0.0227	0.0168	0.0167	0.0168	0.0167	0.0168	0.0116	0.0168	0.0116	0.0116									
[14] A14	0.0072	0.0168	0.0072	0.0116	0.0116	0.0116	0.0116	0.0033	0.0072	0.0033	0.0072	0.0072	0.0116								
[15] A15	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033	0.0000	0.0033	0.0000	0.0072	0.0116	0.0072							
[16] A16	0.0033	0.0109	0.0109	0.0068	0.0068	0.0068	0.0068	0.0068	0.0033	0.0068	0.0033	0.0109	0.0157	0.0109	0.0033						
[17] A17	0.0033	0.0109	0.0109	0.0068	0.0068	0.0068	0.0068	0.0068	0.0033	0.0068	0.0033	0.0109	0.0157	0.0109	0.0033	0.0069					
[18] A18	0.0033	0.0116	0,0116	0.0072	0.0072	0.0072	0.0072	0.0072	0.0033	0.0071	0.0033	0.0116	0.0167	0.0116	0.0033	0.0068	0.0068				
[19] A19	0.0033	0.0116	0.0116	0.0072	0.0072	0.0072	0.0072	0.0072	0.0033	0.0071	0.0033	0.0116	0.0167	0.0116	0.0033	0.0068	0.0068	0.0072			
[20] A20	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033	0.0000	0.0033	0.0000	0.0072	0.0116	0.0072	0.0000	0.0033	0.0033	0.0033	0.0033		
[21] A21	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033	0.0000	0.0033	0.0000	0.0072	0.0116	0.0072	0.0000	0.0033	0.0033	0.0033	0.0033	0.0000	
[22] A22	0.0000	0.0072	0.0072	0.0033	0.0033	0.0033	0.0033	0.0033	0.0000	0.0033	0.0000	0.0072	0.0116	0.0072	0.0000	0.0033	0.0033	0.0033	0.0033	0.0000	0.0000

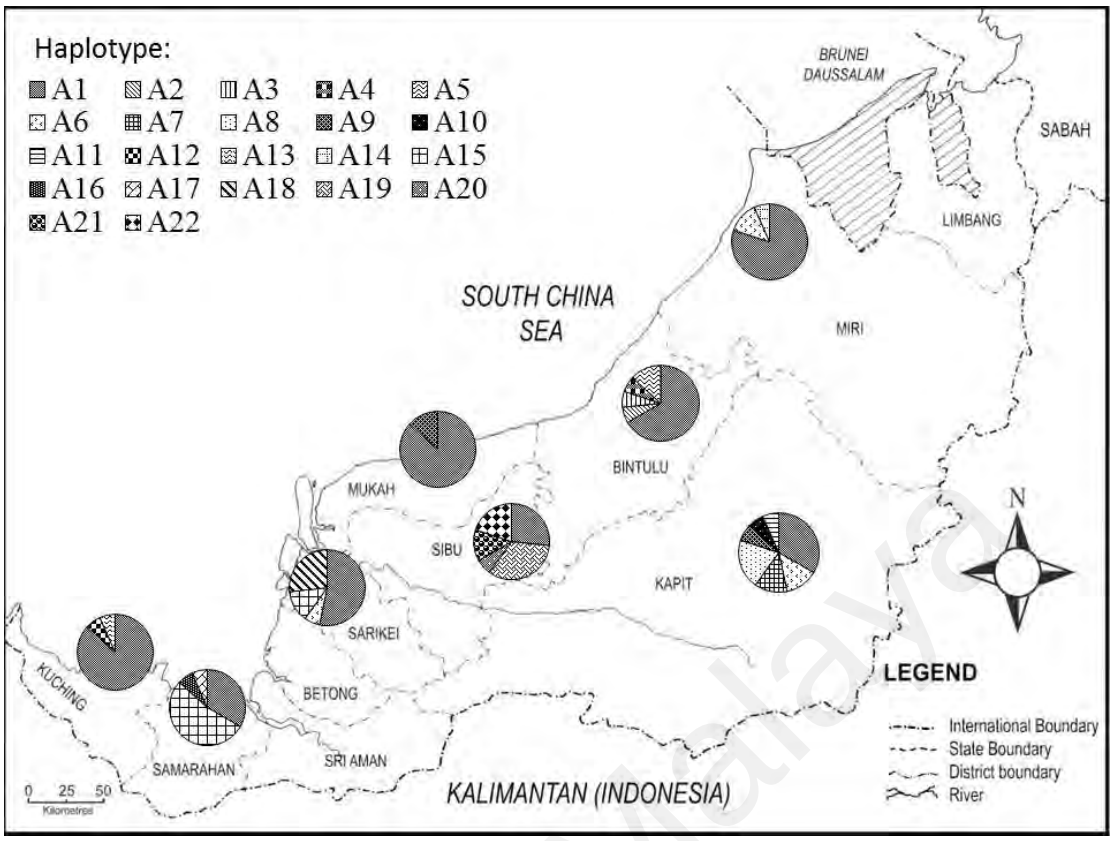

Figure 4.1: Haplotype distribution (A1–A22) base on COI sequences of *Aedes albopictus* from eight divisions in Sarawak state, Malaysia.

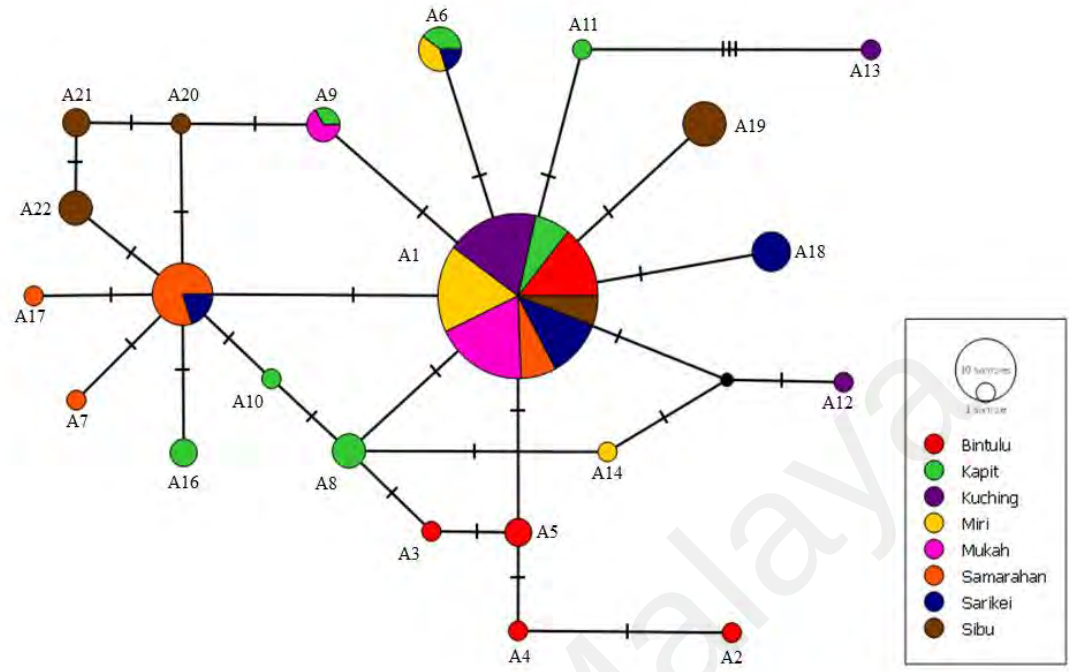

Figure 4.2: Median joining haplotype network of *Aedes albopictus* based on COI sequences isolated from eight divisions in Sarawak. Each haplotype is represented by a circle. Relative sizes of the circles indicate haplotype frequency. Circle of the same colour represent haplotypes from the same population. Small black dot connecting the haplotype represent median vector. One dash line on link connecting the haplotype indicates one mutational difference.

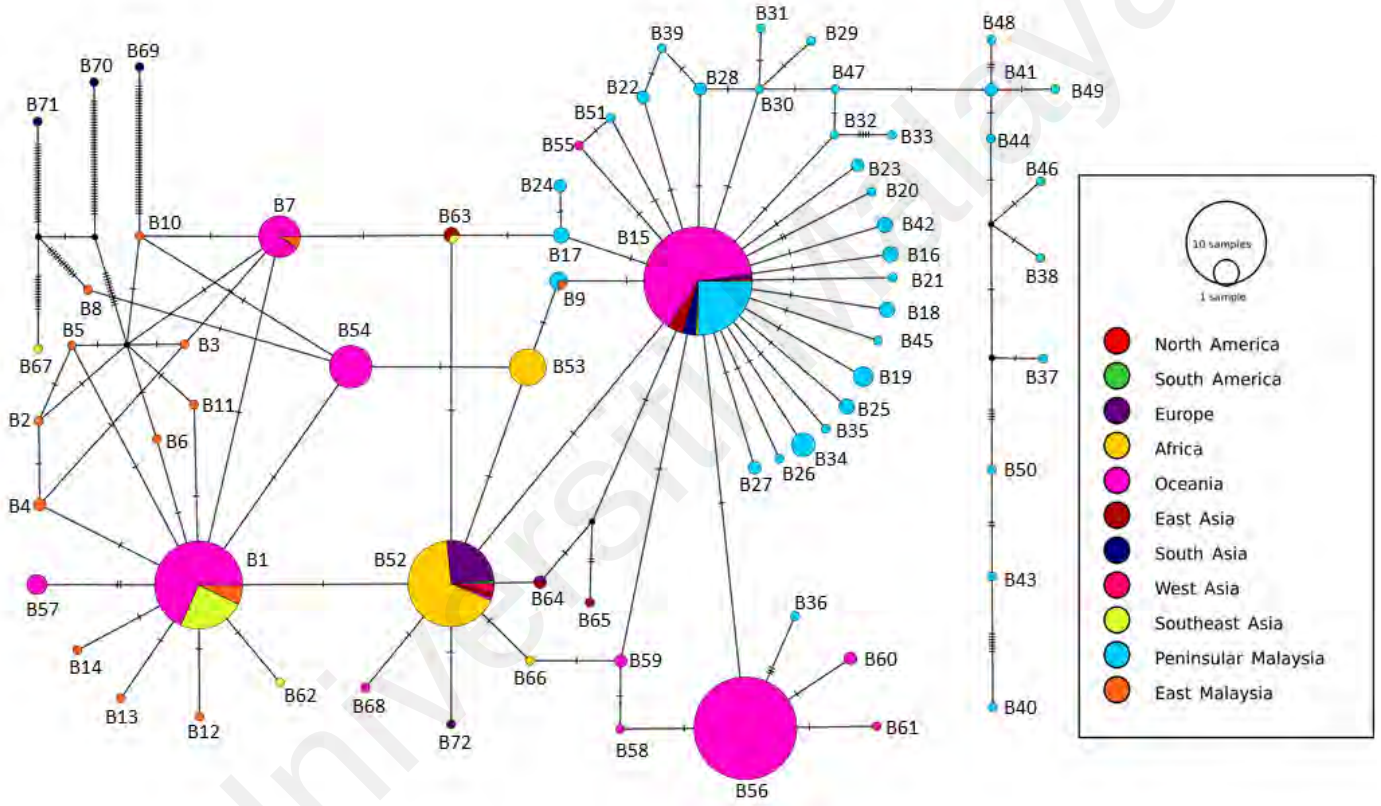

Figure 4.3: Median joining haplotype network of *Aedes albopictus* based on COI sequences isolated from eight divisions in Sarawak and those available in the GenBank. Each haplotype is represented by a circle. Relative sizes of the circles indicate haplotype frequency. Circle of the same colour represent haplotypes from the same population. Small black dot connecting the haplotype represent median vector. One dash line on link connecting the haplotype indicates one mutational difference.

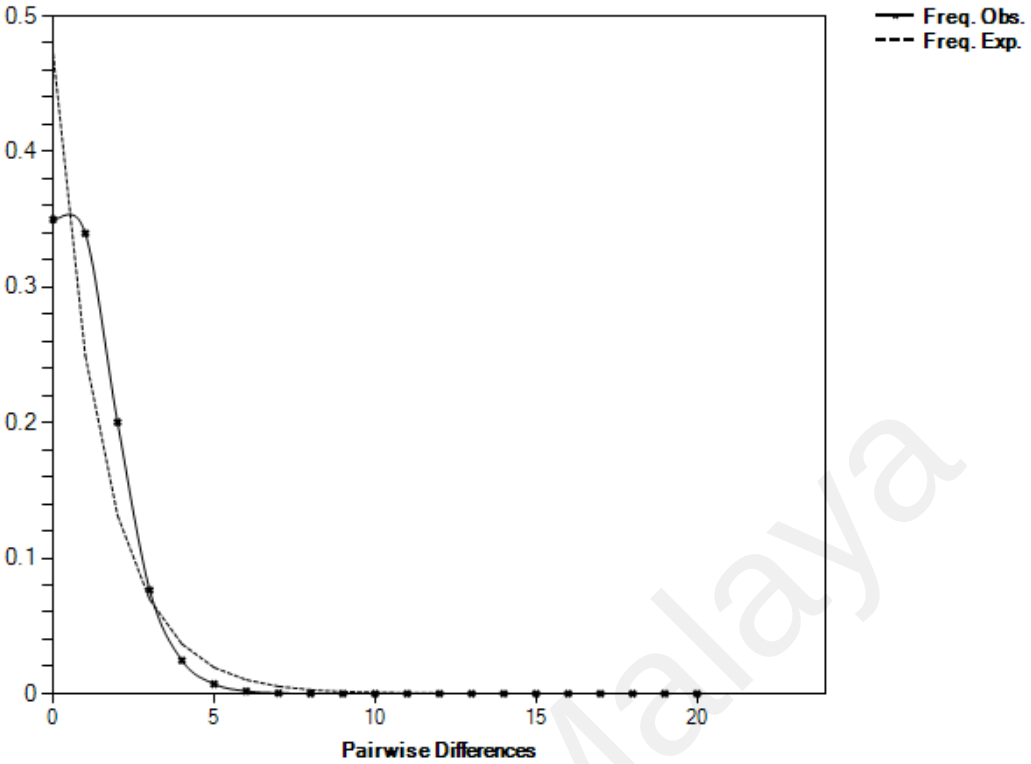

Figure 4.4: Graph of mismatch distribution analysis. Solid line (Obs) shows the empirical pairwise-difference distribution, while the dashed line (Exp) represents the equilibrium distribution with the same mean.

4.3 Adulticides Resistance Status of *Aedes albopictus*

The knockdown time of *Ae. albopictus* obtained from Sarawak against four major insecticide groups, namely organochlorine, organophosphate, carbamate and pyrethroid were shown in Table 4.9. Among the tested adulticides, cyfluthrin was able to induce rapid knockdown with KT_{50} ranging from 21.00 – 27.00 min. The KT_{50} of other tested pyrethroids ranged from 28.00 – 60.00 min. Within the tested pyrethroid, permethrin was less effective as the KT_{50} of 9 populations (69.23%) of *Ae. albopictus* was not determined due to less than 50% knockdown during the experiment. However, KT_{50} of organochlorines, organophosphates and carbamates ranged from 41.00 – 60.00, 55.00 – 59.00 and 43.00 – 60.00, respectively; with at least more than five populations of *Ae. albopictus* showed no result of KT_{50}. The descending order of knockdown effectiveness according to group was pyrethroid > carbamates > organochlorines > organophosphate. On the other hand, knockdown percentage at 1-hour post treatment summarized in Table 4.10 showed no significant difference of knockdown percentage in dieldrin, which indicated no knockdown effect caused by the chemical as well as fenitrothion. As for cyfluthrin, 100% knockdown was recorded for all populations and revealed that cyfluthrin was the most effective insecticide. Other tested insecticides showed significant difference between populations (Table 4.10), indicating knockdown effect after treatment may vary between districts. The inconsistent knockdown effect of these insecticides can increse the selection pressure of resistance population and therefore not suitable use as a universal insecticide in Sarawak.

The mortality of 24-hour post treatment which was used as an indication for susceptibility status of *Ae. albopictus* is shown in Table 4.11. Cyfluthrin was able to induce 100% mortality in all populations. However, not all populations of *Ae. albopictus* were susceptible to other four tested pyrethroids. Various susceptible levels

were found in permethrin, deltamethrin and etofenprox with 57.78 – 100.00, 75.56 – 100.00 and 80.00 – 100.00 mortalities, respectively.

All populations of *Ae. albopictus* exhibited resistance toward lambda-cyhalothrin with mortalities ranged from 68.89 to 97.78%. In addition, *Ae. albopictus* was undoubtedly resistant towards both bendiocarb and propoxur, with mortalities ranging from 53.33 to 100% and 68.89 to 95.55%, respectively. On the other hand, most populations were susceptible to fenitrothion with mortalities ranging from 95.55 to 100.00%. Conversely, 12 out of 13 populations showed resistant towards malathion with mortalities ranging from 57.78 to 100.00%. Interestingly, DDT and dieldrin of organochloride group showed contrasted results: dieldrin induced 100% mortalities to all populations while DDT exhibited various mortalities ranging from 57.78 to 100.00%.

The collected data was also subjected to Pearson's correlation analysis and summarized in Table 4.12. Significant correlations were found between insecticides within same group; namely bendiocarb and propoxur ($p = 0.036$, $r = 0.584$), etofenprox and permethrin ($p = 0.000$, $r = 0.842$), deltamethrin and lambdacyhalothrin ($p = 0.001$, $r = 0.822$), deltamethrin and permethrin ($p = 0.042$, $r = 0.570$). Moreover, insecticides belong to different group were also found significantly correlated such as malathion vs deltamethin ($p = 0.019$, $r = 0.637$), malathion vs bendiocarb ($p = 0.008$, $r = 0.698$), malathion vs propoxur ($p = 0.007$, $r = 0.708$), bendiocarb vs deltamethrin ($p = 0.031$, $r = 0.599$), signifying the presence of cross resistance.

Table 4.9: Knockdown Time (KT_{50}) of *Aedes albopictus* in Sarawak, Malaysia against 11 insecticides using Kaplan–Meier analysis.

	Insecticides										
	Organochlorides		Organophosphates		Carbamates		Pyrethroids				
District	DDT	Dieldrin	Malathion	Fenitrothion	Bendiocarb	Propoxur	Etofenprox	Deltametrhin	Lambda-cyhalothrin	Permethrin	Cyfluthrin
Laboratory	N.D.	N.D.	59.00 ± 0.00	N.D.	N.D.	N.D.	51.00 ± 2.01	34.00 ± 3.35	50.00 ± 1.10	57.00 ± 2.68	21.00 ± 1.11
Kuching	57.00 ± 1.91	N.D.	N.D.	N.D.	N.D.	N.D.	60.00 ± 0.55	57.00 ± 1.91	N.D.	N.D.	25.00 ± 0.55
Bau	N.D.	N.D.	N.D.	N.D.	48.00 ± 3.35	N.D.	51.00 ± 5.70	31.00 ± 1.10	48.00 ± 4.47	N.D.	24.00 ± 1.19
Samarahan	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	56.00 ± 1.68	N.D.	N.D.	25.00 ± 0.48
Serian	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	51.00 ± 1.66	49.00 ± 1.26	N.D.	N.D.	27.00 ± 1.34
Sibu	60.00 ± 0.00	N.D.	N.D.	N.D.	47.00 ± 2.40	50.00 ± 3.83	N.D.	45.00 ± 1.12	57.00 ± 2.67	N.D.	23.00 ± 0.72
Selangau	57.00 ± 1.26	N.D.	N.D.	N.D.	N.D.	N.D.	56.00 ± 3.35	42.00 ± 1.26	53.00 ± 3.92	N.D.	25.00 ± 1.10
Mukah	56.00 ± 1.91	N.D.	N.D.	N.D.	55.00 ± 2.10	N.D.	60.00 ± 0.00	34.00 ± 1.10	56.00 ± 4.44	N.D.	21.00 ± 0.95
Dalat	50.00 ± 2.40	N.D.	N.D.	N.D.	56.00 ± 1.50	N.D.	57.00 ± 2.23	33.00 ± 1.12	52.00 ± 4.70	N.D.	26.00 ± 1.26
Miri	42.00 ± 0.91	N.D.	N.D.	N.D.	52.00 ± 3.35	48.00 ± 0.94	48.00 ± 1.68	34.00 ± 2.87	44.00 ± 1.26	50.00 ± 1.67	24.00 ± 1.33
Bintulu	41.00 ± 4.02	N.D.	N.D.	N.D.	53.00 ± 3.35	52.00 ± 1.68	47.00 ± 1.10	36.00 ± 0.75	43.00 ± 2.39	56.00 ± 3.35	26.00 ± 1.10
Tatau	54.00 ± 1.25	N.D.	N.D.	N.D.	47.00 ± 0.96	43.00 ± 1.92	60.00 ± 0.00	37.00 ± 1.49	N.D.	N.D.	22.00 ± 0.75
Sarikei	N.D.	N.D.	55.00 ± 3.35	N.D.	56.00 ± 1.91	60.00 ± 0.00	48.00 ± 3.35	28.00 ± 1.43	42.00 ± 0.96	59.00 ± 0.67	23.00 ± 0.96
Kapit	N.D.	N.D.	N.D.	N.D.	N.D.	60.00 ± 1.33	54.00 ± 1.33	40.00 ± 2.78	53.00 ± 2.24	58.00 ± 1.34	21.00 ± 0.95

N.D. = Not determined

Table 4.10: Knockdown rates of *Aedes albopictus* after 1-hour exposure of adulticides.

	Insecticides										
	Organochlorides		Organophosphates		Carbamates		Pyrethroids				
District	DDT	Dieldrin	Malathion	Fenitrothion	Bendiocarb	Propoxur	Etofenprox	Deltamethrin	Lambda-cyhalothrin	Permethrin	Cyfluthrin
Laboratory	33.33 ± 6.67	37.78 ± 7.70	53.33 ± 6.67	0.00 ± 0.00	0.00 ± 0.00	22.22 ± 19.24	84.44 ± 7.70	93.33 ± 0.00	95.55 ± 3.85	57.78 ± 10.18	100.00 ± 0.00
Kuching	68.89 ± 5.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	42.22 ± 15.56	37.78 ± 2.22	57.78 ± 5.88	68.89 ± 8.01	40.00 ± 11.55	40.00 ± 6.67	100.00 ± 0.00
Bau	20.00 ± 3.85	2.22 ± 2.22	31.11 ± 4.44	0.00 ± 0.00	73.33 ± 3.85	48.89 ± 5.88	80.00 ± 7.70	100.00 ± 0.00	71.11 ± 8.01	42.22 ± 5.88	100.00 ± 0.00
Samarahan	15.56 ± 8.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.11 ± 4.44	2.22 ± 2.22	33.33 ± 11.55	71.11 ± 13.52	31.11 ± 5.88	13.33 ± 6.67	100.00 ± 0.00
Serian	44.44 ± 8.01	0.00 ± 0.00	4.45 ± 2.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	88.89 ± 4.44	75.56 ± 8.01	42.22 ± 13.52	15.56 ± 8.01	100.00 ± 0.00
Sibu	51.11 ± 15.56	8.89 ± 5.88	37.78 ± 5.88	0.00 ± 0.00	82.22 ± 2.22	66.67 ± 10.18	42.22 ± 8.01	93.33 ± 6.67	73.33 ± 10.19	37.78 ± 8.89	100.00 ± 0.00
Selangau	68.89 ± 27.75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	26.67 ± 13.88	64.45 ± 9.69	95.55 ± 2.22	66.67 ± 11.55	24.44 ± 8.01	100.00 ± 0.00
Mukah	60.00 ± 13.88	0.00 ± 0.00	15.56 ± 8.01	0.00 ± 0.00	62.22 ± 15.56	40.00 ± 0.00	51.11 ± 2.22	97.78 ± 2.22	64.44 ± 5.88	24.44 ± 4.44	100.00 ± 0.00
Dalat	71.11 ± 4.44	0.00 ± 0.00	22.22 ± 5.88	0.00 ± 0.00	71.11 ± 25.63	35.56 ± 18.19	60.00 ± 3.85	100.00 ± 0.00	68.89 ± 2.22	48.89 ± 11.76	100.00 ± 0.00
Miri	100.00 ± 0.00	0.00 ± 0.00	37.78 ± 4.45	0.00 ± 0.00	73.33 ± 3.85	93.33 ± 3.85	95.55 ± 2.22	100.00 ± 0.00	97.78 ± 2.22	86.67 ± 3.85	100.00 ± 0.00
Bintulu	80.00 ± 20.00	2.22 ± 2.22	22.22 ± 5.88	0.00 ± 0.00	60.00 ± 13.33	73.33 ± 16.78	93.33 ± 3.85	100.00 ± 0.00	75.56 ± 24.44	71.11 ± 8.89	100.00 ± 0.00
Tatau	88.89 ± 5.88	0.00 ± 0.00	24.44 ± 5.88	0.00 ± 0.00	91.11 ± 5.88	88.89 ± 4.44	53.33 ± 10.19	91.11 ± 8.89	46.67 ± 10.18	24.44 ± 8.01	100.00 ± 0.00
Sarikei	48.89 ± 14.57	0.00 ± 0.00	62.22 ± 8.01	0.00 ± 0.00	66.67 ± 3.85	51.11 ± 5.88	73.33 ± 10.19	95.56 ± 4.44	93.33 ± 3.85	60.00 ± 3.85	100.00 ± 0.00
Kapit	0.00 ± 0.00	0.00 ± 0.00	33.33 ± 0.00	0.00 ± 0.00	48.89 ± 2.22	55.56 ± 8.01	77.78 ± 4.45	100.00 ± 0.00	75.55 ± 9.69	57.78 ± 4.45	100.00 ± 0.00
	F = 7.223	F = 1.834	F = 14.392	F = N.D.	F = 8.565	F = 9.933	F = 7.485	F = 3.777	F = 3.491	F = 9.426	F = N.D.
	p = 0.000	p = 0.095	p = 0.000	p = N.D.	p = 0.000	p = 0.000	p = 0.000	p = 0.020	p = 0.004	p = 0.000	p = N.D.

N.D. = Not determined by one way ANOVA as the values are the same, p values ≤ 0.05 was considered statistically significant

Table 4.11: Mortality rates of *Aedes albopictus* after 24-hour post-treatments.

	Insecticides										
	Organochlorines		Organophosphates		Carbamates		Pyrethroids				
District	DDT	Dieldrin	Malathion	Fenitrothion	Bendiocarb	Propoxur	Etofenprox	Deltamethrin	Lambda-cyhalothrin	Permethrin	Cyfluthrin
Laboratory	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}
Kuching	91.11 ± 2.22^{P}	100.00 ± 0.00^{S}	73.33 ± 6.67^{R}	97.78 ± 2.22^{P}	73.33 ± 11.55^{R}	73.33 ± 3.85^{R}	97.78 ± 2.22^{P}	100.00 ± 0.00^{S}	86.67 ± 6.67^{R}	97.78 ± 2.22^{P}	100.00 ± 0.00^{S}
Bau	57.78 ± 5.88^{R}	100.00 ± 0.00^{S}	77.78 ± 2.22^{R}	95.55 ± 2.22^{P}	77.78 ± 5.88^{R}	82.22 ± 8.01^{R}	86.67 ± 0.00^{R}	91.11 ± 4.44^{P}	71.11 ± 9.69^{R}	86.67 ± 3.85^{R}	100.00 ± 0.00^{S}
Samarahan	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	57.78 ± 12.37^{R}	100.00 ± 0.00^{S}	53.33 ± 10.19^{R}	68.89 ± 2.22^{R}	77.78 ± 2.22^{R}	75.56 ± 5.88^{R}	68.89 ± 8.01^{R}	57.78 ± 9.69^{R}	100.00 ± 0.00^{S}
Serian	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	97.78 ± 2.22^{P}	100.00 ± 0.00^{S}	91.11 ± 4.44^{P}	88.89 ± 5.88^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	95.55 ± 2.22^{P}	97.78 ± 2.22^{P}	100.00 ± 0.00^{S}
Sibu	84.44 ± 12.37^{R}	100.00 ± 0.00^{S}	86.67 ± 3.85^{R}	100.00 ± 0.00^{S}	93.33 ± 3.85^{P}	77.78 ± 4.45^{R}	88.89 ± 5.88^{R}	95.55 ± 2.22^{P}	77.78 ± 5.88^{R}	75.56 ± 4.44^{R}	100.00 ± 0.00^{S}
Selangau	93.33 ± 2.22^{P}	100.00 ± 0.00^{S}	80.00 ± 11.55^{R}	97.78 ± 2.22^{P}	86.67 ± 7.70^{R}	88.89 ± 8.01^{R}	80.00 ± 3.85^{R}	95.56 ± 4.44^{P}	84.45 ± 9.69^{R}	84.44 ± 4.44^{R}	100.00 ± 0.00^{S}
Mukah	73.33 ± 3.85^{R}	100.00 ± 0.00^{S}	73.33 ± 3.85^{R}	100.00 ± 0.00^{S}	77.78 ± 5.88^{R}	71.11 ± 4.44^{R}	82.22 ± 8.01^{R}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	68.89 ± 2.22^{R}	100.00 ± 0.00^{S}
Dalat	77.78 ± 2.22^{R}	100.00 ± 0.00^{S}	75.56 ± 5.88^{R}	100.00 ± 0.00^{S}	91.11 ± 5.88^{P}	73.33 ± 3.85^{R}	86.67 ± 6.67^{R}	100.00 ± 0.00^{S}	93.33 ± 3.85^{P}	77.78 ± 2.22^{R}	100.00 ± 0.00^{S}
Miri	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	86.67 ± 7.70^{R}	100.00 ± 0.00^{S}	82.22 ± 5.88^{R}	95.55 ± 2.22^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	97.78 ± 2.22^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}
Bintulu	95.56 ± 4.44^{P}	100.00 ± 0.00^{S}	80.00 ± 11.55^{R}	97.78 ± 2.22^{P}	86.67 ± 6.67^{R}	91.11 ± 4.44^{P}	97.77 ± 2.22^{P}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}
Tatau	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	80.00 ± 10.18^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	91.11 ± 2.22^{P}	93.33 ± 3.85^{P}	91.11 ± 5.88^{P}	75.56 ± 15.55^{R}	80.00 ± 6.67^{R}	100.00 ± 0.00^{S}
Sarikei	91.11 ± 5.88^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	97.78 ± 2.22^{P}	91.11 ± 4.44^{P}	93.33 ± 3.85^{P}	80.00 ± 6.67^{R}	100.00 ± 0.00^{S}	88.89 ± 4.44^{R}	68.89 ± 8.01^{R}	100.00 ± 0.00^{S}
Kapit	68.89 ± 21.20^{R}	100.00 ± 0.00^{S}	86.67 ± 10.18^{R}	100.00 ± 0.00^{S}	80.00 ± 3.85^{R}	82.22 ± 4.45^{R}	97.78 ± 2.22^{P}	97.78 ± 2.22^{P}	82.22 ± 4.45^{R}	84.44 ± 5.88^{R}	100.00 ± 0.00^{S}
	$F = 3.735$	$F = N.D.$	$F = 1.997$	$F = 1.169$	$F = 3.174$	$F = 4.620$	$F = 4.181$	$F = 5.423$	$F = 1.932$	$F = 8.393$	$F = N.D.$
	$p = 0.002$	$p = N.D.$	$p = 0.061$	$p = 0.350$	$p = 0.005$	$p = 0.000$	$p = 0.001$	$p = 0.000$	$p = 0.070$	$p = 0.000$	$p = N.D.$

p values ≤ 0.05 was considered statistically significant; S, susceptible (mortality $\geq 98\%$); P, presence of resistant that need to be confirmed ($90\% \leq$ mortality $< 98\%$); and R, confirmed presence of resistance (mortality $< 90\%$), as determined by WHO (2016). N.D. = Not determined by one way ANOVA as the values are the same

Table 4.12: Correlation of mortality rates of *Aedes albopictus* between adulticides used in WHO bioassay.

Insecticides		Organochlorides		Organophosphates		Carbamates		Pyrethroids				
		DDT	Dieldrin	Malathion	Fenitrothion	Bendiocarb	Propoxur	Etofenprox	Deltmethrin	Lambda-cyhalothrin	Permethrin	Cyflutrhin
Organochlorides	DDT	-										
	Dieldrin	N.D.	-									
Organophosphates	Malathion	r = 0.077 *p* = 0.802	N.D.	-								
	Fenitrothion	r = 0.334 *p* = 0.265	N.D.	r = - 0.019 *p* = 0.950	-							
Carbamates	Bendiocarb	r = 0.080 *p* = 0.796	N.D.	r = 0.698 *p* = 0.008	r = 0.077 *p* = 0.802	-						
	Propoxur	r = 0.376 *p* = 0.206	N.D.	r = 0.708 *p* = 0.007	r = - 0.187 *p* = 0.541	r = 0.584 *p* = 0.036	-					
Pyrethroids	Etofenprox	r = 0.171 *p* = 0.576	N.D.	r = 0.352 *p* = 0.238	r = 0.161 *p* = 0.599	r = 0.278 *p* = 0.358	r = 0.366 *p* = 0.218	-				
	Deltamethrin	r = -0.122 *p* = 0.691	N.D.	r = 0.637 *p* = 0.019	r = - 0.043 *p* = 0.889	r = 0.599 *p* = 0.031	r = 0.355 *p* = 0.234	r = 0.471 *p* = 0.104	-			
	Lambda-cyhalothrin	r = 0.218 *p* = 0.475	N.D.	r = 0.448 *p* = 0.124	r = 0.214 *p* = 0.482	r = 0.345 *p* = 0.248	r = 0.314 *p* = 0.296	r = 0.402 *p* = 0.173	r = 0.822 *p* = 0.001	-		
	Permethrin	r = 0.149 *p* = 0.628	N.D.	r = 0.354 *p* = 0.235	r = - 0.244 *p* = 0.421	r = 0.288 *p* = 0.340	r = 0.500 *p* = 0.082	r = 0.842 *p* = 0.000	r = 0.570 *p* = 0.042	r = 0.490 *p* = 0.089	-	
	Cyfluthrin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-

r = correlation coefficients, *p* values ≤ 0.05 was considered statistically significant, N.D. = Not determined

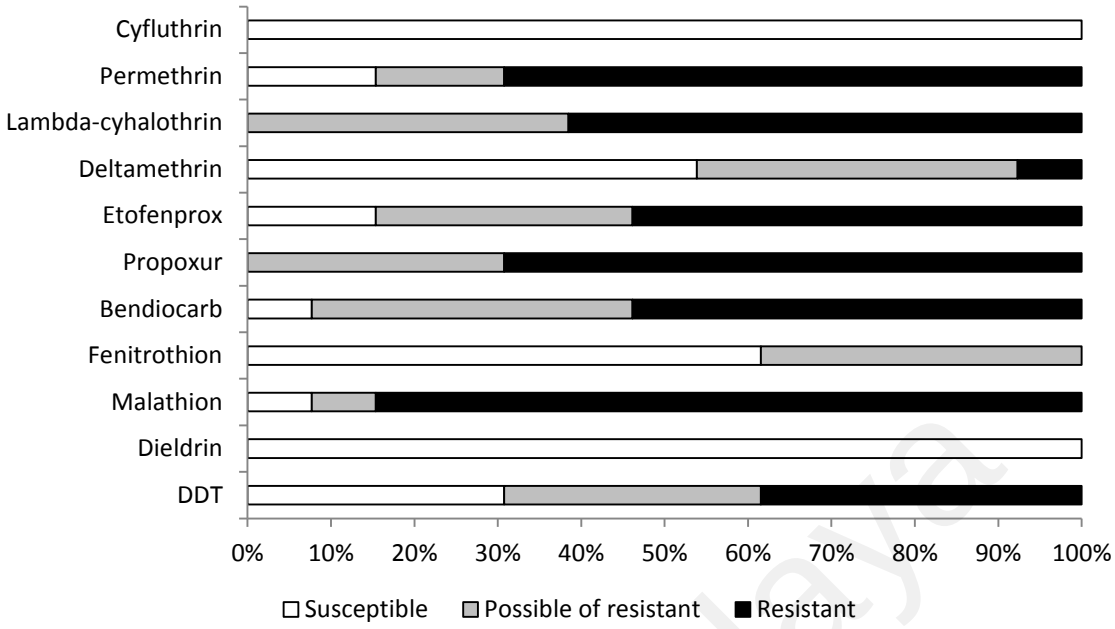

Figure 4.5: Resistance status (percentage) of *Aedes albopictus* populations against various adulticides in Sarawak.

4.4 Lavicides Resistance Status of *Aedes albopictus*

The baseline data of 24 hours post treatment mortality for each larvicides were presented in Table 4.13. The result revealed that *Ae. albopictus* collected from 13 districts were completely susceptible to bromophos and temephos (mortality = 100%), while highly resistant to DDT, chlorpyrifos and malathion (mortality ranging 0 – 20%). However, the larvae showed various level of susceptibility to fenthion, fenitrothion and dieldrin. Larval populations from Samarahan, Sibu, Mukah, Serian, Selangau, Dalat and Sarikei were susceptible to fenthion, larvae from Kuching and Bau showed intermediate resistance to fenthion, whereas larvae from Miri, Bintulu, Tatau and Kapit were resistant to fenthion. For fenitrothion, larval population from Bau, Mukah, Dalat and Sarikei were susceptible and Kuching, Samarahan, Sibu, Serian, Selangau and Miri exhibited sign of tolerant, except those from Bintulu, Tatau and Kapit, which were resistant to fenitrothion. The larval population from Kuching, Bau, Samarahan, Sibu, Mukah, Serian, Selangau, Dalat, Tatau, Sarikei and Kapit were susceptible to dieldrin, except for Miri and Bintulu, which showed sign of intermediate resistant and resistant to dieldrin, respectively.

The correlation of each tested larvicides was presented in Table 4.14. The result showed that fenitrothion was significantly correlated to fenthion ($r = 0.879$, $p = 0.000$) and dieldrin was significantly correlated to fenthion ($r = 0.495$, $p = 0.001$) and fenitrothion ($r = 0.438$, $p = 0.004$).

Table 4.13: Mortality of 24 hours post treatment of tested insecticides against field *Aedes albopictus*.

	Insecticides							
Districts	Bromophos	Chlorpyrifos	Fenthion	Fenitrothion	Temephos	Malathion	DDT	Dieldrin
Laboratory	100.00 ± 0.00^{s}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Kuching	100.00 ± 0.00^{s}	1.33 ± 1.33^{R}	93.33 ± 6.67^{P}	96.00 ± 6.67^{P}	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	10.67 ± 2.67^{R}	100.00 ± 0.00^{S}
Bau	100.00 ± 0.00^{S}	5.33 ± 5.33^{R}	96.00 ± 4.00^{P}	98.67 ± 1.33^{S}	100.00 ± 0.00^{S}	12.00 ± 8.33^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Samarahan	100.00 ± 0.00^{S}	12.00 ± 6.11^{R}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	5.33 ± 5.33^{R}	100.00 ± 0.00^{S}
Sibu	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	98.67 ± 1.33^{S}	96.00 ± 4.00^{P}	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Mukah	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Serian	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	100.00 ± 0.00^{S}	93.33 ± 6.67^{P}	100.00 ± 0.00^{S}	12.00 ± 6.11^{R}	2.67 ± 2.67^{R}	100.00 ± 0.00^{S}
Selangau	100.00 ± 0.00^{S}	2.67 ± 2.67^{R}	100.00 ± 0.00^{S}	88.00 ± 6.93^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Dalat	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}
Miri	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	68.00 ± 10.07^{R}	86.67 ± 13.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	97.33 ± 1.33^{P}
Bintulu	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	17.33 ± 5.81^{R}	36.00 ± 4.00^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	81.33 ± 3.53^{R}
Tatau	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}	5.33 ± 5.33^{R}	2.67 ± 2.67^{R}	100.00 ± 0.00^{S}
Sarikei	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	20.00 ± 11.55^{R}	0.00 ± 0.00^{R}	98.67 ± 1.33^{S}
Kapit	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}	21.33 ± 10.91^{R}	14.67 ± 10.91^{R}	100.00 ± 0.00^{S}	5.33 ± 5.33^{R}	10.67 ± 2.67^{R}	98.67 ± 1.33^{S}
	N.D	$F = 109.638$ $p = 0.000$	$F = 59.383$ $p = 0.000$	$F = 20.683$ $p = 0.000$	N.D	$F = 1.644$ $p = 0.132$	$F = 3.731$ $p = 0.002$	$F = 6.063$ $p = 0.000$

p values ≤ 0.05 was considered statistically significant; S, susceptible (mortality ≥ 98%); P, presence of resistant that need to be confirmed (90% ≤ mortality < 98%); and R, confirmed presence of resistance (mortality < 90%), as determined by WHO (2016). N.D. = Not determined by one way ANOVA as the values are the same

Table 4.14: Correlation between 24 hr mortality of tested organophosphate and organochlorine insecticides against *Aedes albopictus*.

Insecticides	Bromophos	Chlorpyrifos	Fenthion	Fenitrothion	Temephos	Malathion	DDT	Dieldrin
Bromophos	–							
Chlorpyrifos	N.D.	–						
Fenthion	N.D.	r = 0.212 *p* = 0.178	–					
Fenitrothion	N.D.	r = 0.201 *p* = 0.201	r = 0.879 *p* = 0.000	–				
Temephos	N.D.	N.D.	N.D.	N.D.	–			
Malathion	N.D.	r = -0.136 *p* = 0.389	r = 0.084 *p* = 0.595	r = 0.032 *p* = 0.842	N.D.	–		
DDT	N.D.	r = -0.095 *p* = 0.548	r = -0.241 *p* = 0.124	r = -0.223 *p* = 0.156	N.D.	r = 0.083 *p* = 0.603	–	
Dieldrin	N.D.	r = 0.129 *p* = 0.417	r = 0.495 *p* = 0.001	r = 0.438 *p* = 0.004	N.D.	r = 0.034 *p* = 0.830	r = 0.036 *p* = 0.820	–

r = correlation coefficients, *p* values ≤ 0.05 was considered statistically significant, N.D. = Not determined

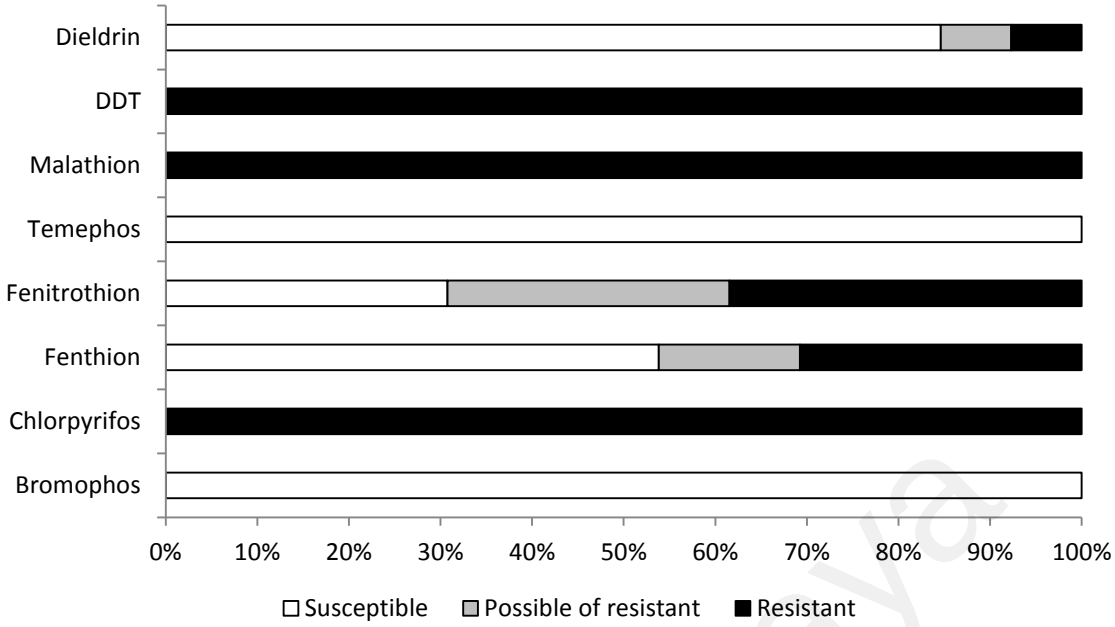

Figure 4.6: Resistance status (percentage) of *Aedes albopictus* population against various larvicides in Sarawak.

4.5 Biochemical Studies of Insecticide Resistance in *Ae. albopictus*

In order to reveal the underlying mechanism of the metabolic resistance exhibited in *Ae. albopictus* adult and larvae of Sarawak, the enzyme microassay of non-specific esterases (α- and β- EST), glutathione-S-transferases (GST), mixed function oxidases (MFO), and insensitive acetylcholinesterase (AChE) were conducted.

4.5.1 Non-specific Esterases (EST) Enzyme Microassay

One-way ANOVA revealed that there was significant difference for both elevated α-EST and β-EST activities among all *Ae. albopictus* adult populations (Table 4.15). In comparison to reference strain, significant elevated enzyme activities were observed in Miri, Bintulu and Sarikei for α-EST; and Miri, Sarikei and Kapit populations for β-EST. The resistance ratio (RR) of each strain of *Ae. albopictus* adults based on their α-EST and β-EST activities ranged from 0.825 – 1.488 folds. Similar result was observed in larval populations where there was significant difference in one-way ANOVA analysis for both α-EST and β-EST activities (Table 4.15). Larval populations from Kuching, Selangau, Sarikei and Kapit showed significant elevated enzyme activity compared to reference strain in α-EST, while elevated of β-EST activity was observed in larval populations from Samarahan, Selangau, Sarikei and Kapit. However, the RR for each larval populations ranging from 0.893 – 1.382 folds (Table 4.16).

Table 4.15: Mean (± S.E.) values of non-specific α-esterases (α-EST) and β-esterases (β-EST) activities of *Aedes albopictus* adults from 13 districts in Sarawak at absorbance 450 nm.

Strain	Mean ± S.E. (α-naphthol nmoles/min/mg protein)	Resistance Ratio (RR)	Mean ± S.E. (β-naphthol nmoles/min/mg protein)	Resistance Ratio (RR)
Reference	0.126 ± 0.004	–	0.125 ± 0.004	–
Kuching	*0.115 ± 0.003	0.913	0.117 ± 0.003	0.936
Bau	0.121 ± 0.004	0.960	0.119 ± 0.004	0.952
Samarahan	0.117 ± 0.002	0.929	0.118 ± 0.002	0.944
Serian	0.118 ± 0.002	0.937	0.121 ± 0.002	0.968
Sibu	0.120 ± 0.004	0.952	0.123 ± 0.002	0.984
Selangau	0.125 ± 0.004	0.992	0.116 ± 0.003	0.928
Mukah	*0.114 ± 0.002	0.905	*0.116 ± 0.002	0.928
Dalat	0.116 ± 0.005	0.921	0.113 ± 0.006	0.904
Miri	*0.184 ± 0.008	1.460	*0.186 ± 0.007	1.488
Bintulu	*0.145 ± 0.005	1.151	0.123 ± 0.002	0.984
Tatau	*0.104 ± 0.002	0.825	*0.103 ± 0.002	0.824
Sarikei	*0.148 ± 0.005	1.175	*0.151 ± 0.005	1.208
Kapit	0.130 ± 0.003	1.032	*0.137 ± 0.003	1.096
One Way ANOVA	F = 28.000 df = 12 *P* = 0.000		F = 37.116 df = 12 *P* = 0.000	

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean non-specific esterases of the field population / Mean non-specific esterases of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean of EST was significantly different with the reference strain ($P \leq 0.05$)(Independent samples t-tet).

Table 4.16: Mean (± S.E.) values of non-specific α-esterases (α-EST) and β-esterases (β-EST) activities of *Aedes albopictus* larvae from 13 districts in Sarawak at absorbance 450 nm.

Strain	Mean ± S.E. (α-naphthol nmoles/min/mg protein)	Resistance Ratio (RR)	Mean ± S.E. (β-naphthol nmoles/min/mg protein)	Resistance Ratio (RR)
Reference	0.131 ± 0.004	–	0.126 ± 0.003	–
Kuching	*0.155 ± 0.010	1.183	0.147 ± 0.007	1.122
Bau	0.134 ± 0.010	1.023	0.136 ± 0.009	1.038
Samarahan	0.137 ± 0.005	1.046	*0.139 ± 0.005	1.061
Serian	0.122 ± 0.006	0.931	0.117 ± 0.004	0.893
Sibu	0.120 ± 0.006	0.916	0.123 ± 0.005	0.939
Selangau	*0.169 ± 0.004	1.290	*0.168 ± 0.004	1.282
Mukah	*0.117 ± 0.004	0.893	0.122 ± 0.004	0.931
Dalat	0.133 ± 0.006	1.015	0.134 ± 0.006	1.023
Miri	0.145 ± 0.010	1.107	0.140 ± 0.008	1.069
Bintulu	0.130 ± 0.005	0.992	0.132 ± 0.003	1.007
Tatau	0.121 ± 0.004	0.924	0.122 ± 0.003	0.931
Sarikei	*0.161 ± 0.008	1.229	*0.169 ± 0.008	1.290
Kapit	*0.181 ± 0.009	1.382	*0.180 ± 0.011	1.374
One Way ANOVA	F = 8.777 df = 12 *P* = 0.000		F = 10.497 df = 12 *P* = 0.000	

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean non-specific esterases of the field population / Mean non-specific esterases of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean of EST was significantly different with the reference strain ($P \leq 0.05$) (Independent samples t-tet).

4.5.2 Mixed Function Oxidases (MFO) Enzyme Microassay

In mixed function oxidases assay (Table 4.17), one-way ANOVA showed that there was significant difference on MFO activities among all populations of adult and larvae. Significant difference in MFO activities was observed in all field populations of *Ae. albopictus* adults compared to reference strain. On the other hand, only two field populations of larval (Mukah and Bintulu) were significant difference from the reference strain. However, no significant elevated MFO activity was detected. The resistance ratios for adult and larvae ranged from 0.216 – 0.524 and 0.768 – 1.071 folds, respectively.

4.5.3 Glutathione-S-transferases (GST) Enzyme Microassay

With regard to glutathione-S-transferase assay (Table 4.18), there was a significant difference on enzyme activities among *Ae. albopictus* adult populations but no significant elevated enzyme activity was detected when compared to reference strain. On the other hand, there was no significant difference on enzyme activities among larval populations but significant elevated enzyme activity was only exhibited in larval population from Miri as compared to reference strain. The resistance ratio for *Ae. albopictus* adult populations ranged from 0.500 – 1.193 folds, while for larval populations ranged from 0.855 – 1.455 folds.

Table 4.17: Mean (± S.E.) values of mixed function oxidases (MFO) activities of *Aedes albopictus* adults and larvae from 13 districts in Sarawak at absorbance 630 nm.

	Adult		Larvae	
Strain	Mean ± S.E. (nmoles cyt c/min/mg protein)	Resistance Ratio (RR)	Mean ± S.E. (nmoles cyt c/min/mg protein)	Resistance Ratio (RR)
Reference	0.227 ± 0.016	–	0.056 ± 0.004	–
Kuching	*0.101 ± 0.007	0.445	0.055 ± 0.004	0.982
Bau	*0.089 ± 0.024	0.392	0.053 ± 0.003	0.946
Samarahan	*0.053 ± 0.004	0.233	0.054 ± 0.005	0.964
Serian	*0.102 ± 0.009	0.449	0.057 ± 0.005	1.018
Sibu	*0.066 ± 0.006	0.291	0.051 ± 0.004	0.911
Selangau	*0.055 ± 0.005	0.242	0.053 ± 0.004	0.946
Mukah	*0.119 ± 0.015	0.524	*0.044 ± 0.002	0.786
Dalat	*0.066 ± 0.006	0.291	0.049 ± 0.004	0.875
Miri	*0.089 ± 0.006	0.392	0.060 ± 0.004	1.071
Bintulu	*0.055 ± 0.004	0.242	*0.043 ± 0.001	0.768
Tatau	*0.078 ± 0.007	0.344	0.048 ± 0.002	0.857
Sarikei	*0.110 ± 0.007	0.485	0.059 ± 0.004	1.054
Kapit	*0.049 ± 0.002	0.216	0.047 ± 0.003	0.839
One Way ANOVA	F = 6.385 df = 12 *P* = 0.000		F = 2.327 df = 12 *P* = 0.009	

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean mixed function oxidases of the field population / Mean mixed function oxidases of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean of MFO was significantly different with the reference strain ($P \leq 0.05$) (Independent samples t-tet).

Table 4.18: Mean (± S.E.) values of glutathione-S-transferases (GST) activities of *Aedes albopictus* adults and larvae from 13 districts in Sarawak at absorbance 410 nm.

	Adult		Larvae	
Strain	Mean ± S.E. (nmoles CDNB/min/mg protein)	Resistance Ratio (RR)	Mean ± S.E. (nmoles CDNB/min/mg protein)	Resistance Ratio (RR)
Reference	0.088 ± 0.024	–	0.055 ± 0.003	–
Kuching	0.051 ± 0.001	0.580	0.059 ± 0.004	1.073
Bau	0.051 ± 0.002	0.580	0.048 ± 0.002	0.873
Samarahan	0.060 ± 0.002	0.682	0.052 ± 0.002	0.945
Serian	0.059 ± 0.002	0.670	0.052 ± 0.003	0.945
Sibu	0.044 ± 0.001	0.500	0.080 ± 0.031	1.455
Selangau	0.059 ± 0.002	0.670	0.055 ± 0.002	1.000
Mukah	0.045 ± 0.001	0.511	0.055 ± 0.005	1.000
Dalat	0.060 ± 0.011	0.682	0.049 ± 0.002	0.891
Miri	0.048 ± 0.001	0.545	*0.047 ± 0.001	0.855
Bintulu	0.105 ± 0.011	1.193	0.049 ± 0.003	0.891
Tatau	0.050 ± 0.002	0.568	0.059 ± 0.003	1.073
Sarikei	0.055 ± 0.001	0.625	0.056 ± 0.029	1.018
Kapit	0.053 ± 0.002	0.602	0.051 ± 0.003	0.927
One Way ANOVA	F = 19.220 df = 12 *P* = 0.000		F = 0.902 df = 12 *P* = 0.547	

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean glutathione-S-transferases of the field population / Mean glutathione-S-transferases of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean of GST was significantly different with the reference strain ($P \leq 0.05$) (Independent samples t-tet).

4.5.4 Insensitive Acetylcholinesterase (AChE) Enzyme Microassay

As for insensitive acetylcholinesterase assay on *Ae. albopictus* adult (Table 4.19), based on the Paired t-test results, lower AChE activity was observed significantly in all populations when they were treated with 0.1% and 0.2% propoxur which implied that the AChE activity of these populations was still sensitive against propoxur except for Sarikei population in both treatments. Significant mean percentage of AChE activity in propoxur inhibited fraction was also observed in all populations except populations from Bau, Bintulu, Sarikei and Sibu in both treatments. Resistance ratio of AChE activity for all adult populations were less than 5.00. Nine out of thirteen adult populations exhibited high AChE activity when tested with 0.1% propoxur and increased to twelve in 0.2% propoxur, indicating that majority of them possessed homozygous resistance (RR) against insecticide associated with AChE enzyme. Other populations were heterozygous (RS) against AChE-associated insecticides but no homozygous susceptible (SS) population was found. As for *Ae. albopictus* larvae (Table 4.20), only larval population in Dalat exhibited insensitive AChE enzyme activity as showed by non-significant difference in Paired t-test between both treatments of 0.1% and 0.2% propoxur with control without propoxur, while others tested populations showed converse results. Significant mean percentage of AChE activity in propoxur inhibited fraction was observed in all larval populations except populations from Bintulu and Serian in AChE activities in 0.1% and 0.2% propoxur. In contrast to adult populations, all larvae populations were found homozygous resistance (RR) with mean AChE activity in propoxur-inhibited fraction more than 70%.

Table 4.19: Mean (± S.E.) values of acetylcholinsterase activities in fractions with and without propoxur inhibition of *Aedes albopictus* adults from 13 districts in Sarawak at absorbance 410 nm.

Strain	Mean (± S.E.) values of AChE activities			Mean percent AChE activity in propoxur-inhibited fraction (%)	Resistance Ratio	Mean (± S.E.) values of AChE activities			Mean percent AChE activity in propoxur-inhibited fraction (%)	Resistance Ratio
	Control (without propoxur)	ACTH + 0.1% Propoxur	Paired T-test			Control (without propoxur)	ACTH + 0.2% Propoxur	Paired T-test		
Reference	0.118 ± 0.007	0.069 ± 0.001	0.000	61.23 ± 3.35	–	0.118 ± 0.007	0.070 ± 0.001	0.000	61.42 ± 3.36	–
Kuching	0.096 ± 0.003	0.074 ± 0.002	0.000	*76.61 ± 1.54	1.25	0.096 ± 0.003	0.073 ± 0.002	0.000	*75.84 ± 1.52	1.23
Bau	0.198 ± 0.042	0.069 ± 0.017	0.007	52.08 ±8.86	0.85	0.198 ± 0.042	0.071 ± 0.002	0.008	54.46 ± 9.12	0.87
Samarahan	0.094 ± 0.002	0.071 ± 0.001	0.000	*76.04 ± 1.16	1.24	0.094 ± 0.002	0.072 ± 0.001	0.000	*76.63 ± 1.04	1.25
Serian	0.093 ± 0.003	0.069 ± 0.001	0.000	*74.72 ± 1.53	1.22	0.093 ± 0.003	0.071 ± 0.001	0.000	*75.23 ± 2.13	1.22
Sibu	0.108 ± 0.008	0.071 ± 0.002	0.002	69.59 ± 4.69	1.14	0.108 ± 0.008	0.072 ± 0.001	0.002	70.40 ± 4.66	1.15
Selangau	0.089 ± 0.003	0.065 ± 0.001	0.000	*73.56 ± 1.55	1.20	0.089 ± 0.003	0.067 ± 0.001	0.000	*75.87 ± 1.75	1.24
Mukah	0.091 ± 0.002	0.068 ± 0.001	0.000	*75.17 ± 1.44	1.23	0.091 ± 0.002	0.068 ± 0.001	0.000	*75.39 ± 1.55	1.23
Dalat	0.098 ± 0.004	0.068 ± 0.001	0.000	*70.81 ± 2.41	1.16	0.098 ± 0.004	0.069 ± 0.001	0.000	*71.48 ± 2.25	1.16
Miri	0.098 ± 0.005	0.076 ± 0.007	0.018	*79.15 ± 7.70	1.29	0.098 ± 0.005	0.078 ± 0.007	0.025	*81.19 ± 7.21	1.32
Bintulu	0.103 ± 0.007	0.070 ± 0.001	0.000	69.59 ± 2.88	1.14	0.103 ± 0.007	0.070 ± 0.001	0.000	70.16 ± 2.90	1.14
Tatau	0.098 ± 0.003	0.070 ± 0.001	0.000	*72.01 ± 1.88	1.18	0.098 ± 0.003	0.070 ± 0.001	0.000	*72.39 ± 2.04	1.18
Sarikei	0.127 ± 0.026	0.073 ± 0.003	0.063	68.30 ± 5.42	1.12	0.127 ± 0.026	0.074 ± 0.003	0.072	70.01 ± 5.72	1.14
Kapit	0.098 ± 0.004	0.069 ± 0.001	0.000	*71.23 ± 2.31	1.16	0.098 ± 0.004	0.069 ± 0.001	0.000	*71.98 ± 2.30	1.17

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean percent acetylcholinesterase of the field population / Mean percent acetylcholinesterase of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean percent of AChE activity in propoxur inhibited fraction (%) was significantly different with the reference strain ($P \leq 0.05$) (Independent samples t-tet).

Table 4.20: Mean (± S.E.) values of acetylcholinsterase activities in fractions with and without propoxur inhibition of *Aedes albopictus* larvae from 13 districts in Sarawak at absorbance 410 nm.

Strain	Mean (± S.E.) values of AChE activities			Mean percent AChE activity in propoxur-inhibited fraction (%)	Resistance Ratio	Mean (± S.E.) values of AChE activities			Mean percent AChE activity in propoxur-inhibited fraction (%)	Resistance Ratio
	Control (without propoxur)	ACTH + 0.1% Propoxur	Paired T-test			Control (without propoxur)	ACTH + 0.2% Propoxur	Paired T-test		
Reference	0.087 ± 0.002	0.062 ± 0.001	0.000	71.97 ± 0.67	–	0.087 ± 0.002	0.064 ± 0.001	0.000	74.11 ± 0.62	–
Kuching	0.080 ± 0.002	0.066 ± 0.001	0.000	*82.61 ± 2.36	1.15	0.080 ± 0.002	0.067 ± 0.001	0.000	*83.35 ± 1.97	1.12
Bau	0.084 ± 0.001	0.064 ± 0.001	0.000	*76.68 ± 0.73	1.07	0.084 ± 0.001	0.065 ± 0.001	0.000	*77.95 ± 0.61	1.05
Samarahan	0.093 ± 0.001	0.065 ± 0.001	0.000	*69.15 ± 0.77	0.96	0.093 ± 0.001	0.067 ± 0.001	0.000	*71.63 ± 0.65	0.97
Serian	0.092 ± 0.001	0.065 ± 0.001	0.000	71.37 ± 0.74	0.99	0.092 ± 0.001	0.066 ± 0.002	0.000	72.46 ± 0.66	0.98
Sibu	0.089 ± 0.001	0.069 ± 0.001	0.000	*77.60 ± 0.66	1.08	0.089 ± 0.001	0.071 ± 0.001	0.000	*79.78 ± 0.73	1.08
Selangau	0.088 ± 0.001	0.068 ± 0.000	0.000	*78.15 ± 0.47	1.09	0.088 ± 0.001	0.069 ± 0.000	0.000	*78.34 ± 0.49	1.06
Mukah	0.086 ± 0.002	0.070 ± 0.001	0.000	*81.58 ± 0.68	1.13	0.086 ± 0.002	0.070 ± 0.001	0.000	*81.92 ± 0.69	1.11
Dalat	0.151 ± 0.062	0.070 ± 0.002	0.217	*78.84 ± 0.57	1.10	0.151 ± 0.062	0.071 ± 0.001	0.221	*79.79 ± 0.59	1.08
Miri	0.091 ± 0.004	0.071 ± 0.002	0.000	*78.35 ± 1.50	1.09	0.091 ± 0.004	0.071 ± 0.002	0.000	*78.30 ± 2.04	1.06
Bintulu	0.094 ± 0.002	0.066 ± 0.001	0.000	70.04 ± 0.76	0.97	0.094 ± 0.002	0.067 ± 0.001	0.000	71.23 ± 0.84	0.96
Tatau	0.089 ± 0.003	0.073 ± 0.002	0.000	*81.30 ± 0.77	1.13	0.089 ± 0.003	0.071 ± 0.001	0.000	*79.63 ± 0.96	1.07
Sarikei	0.082 ± 0.001	0.066 ± 0.001	0.000	*80.16 ± 0.55	1.11	0.082 ± 0.001	0.067 ± 0.001	0.000	*81.58 ± 0.54	1.10
Kapit	0.083 ± 0.001	0.064 ± 0.001	0.000	*77.19 ± 0.80	1.07	0.083 ± 0.001	0.064 ± 0.001	0.000	*77.69 ± 1.03	1.08

$P \leq 0.05$ indicated significant difference; S.E. = Standard Error;
Resistance Ratio (RR) = Mean percent acetylcholinesterase of the field population / Mean percent acetylcholinesterase of the reference strain
RR < 5 = susceptible, 5 ≤ RR ≤ 10 = moderate resistance, RR > 10 = high resistance as determined by WHO (2016).
* = The mean percent of AChE activity in propoxur inhibited fraction (%) was significantly different with the reference strain ($P \leq 0.05$) (Independent samples t-tet).

4.5.5 Association of the Survivability Rates of Larvae and Adults with Enzyme Activities, and Relationships between Enzyme Activities

The correlation between detoxification enzyme activities in *Ae. albopcitus* adult and larvae was determined by conducting Pearson Correlation Test in Table 4.21 and Table 4.22, respectively. The results showed that there were strong associations between α-esterases and β-esterases, and between insensitive AChE with 0.1% propoxur and 0.2% propoxur in both adults and larvae. Furthermore, correlation between α-esterases and insensitive AChE with 0.1% propoxur, α-esterases and insensitive AChE with 0.2% propoxur, β-esterases and insensitive AChE with 0.1% propoxur, and between β-esterases and insensitive AChE with 0.2% propoxur were also observed in *Ae. albopictus* adult.

The correlation of mean elevated enzyme activities for *Ae. albopictus* between larval stage and adult stage was shown in Table 4.23. There was no correlation of enzyme activities between adult and larval stage. Correlation analysis was also conducted to discover any association between the survivality of *Ae. albopictus* adults and larvae ascertained from both adult and larval mosquito bioassays and the enzyme activities, were shown in Table 4.24 and Table 4.25, repectively. There was no significant correlation in *Ae. albopictus* adult between the tested insecticides and enzyme activities. At larval stage, significant correlation was achieved only between MFO and fenitrothion with r = 0.661 and p = 0.014 while no correlation detected in others scenario.

Table 4.21: Correlation between different mean elevated enzyme activities for *Aedes albopictus* adult.

Elevated enzyme activities	α-EST	β-EST	MFO	GST	AChE (0.1% Propoxur)	AChE (0.2% Propoxur)
α-EST						
β-EST	r = 0.939 *p* = 0.000					
MFO	r = 0.035 *p* = 0.909	r = 0.165 *p* = 0.590				
GST	r = 0.188 *p* = 0.539	r = -0.115 *p* = 0.709	r = -0.406 *p* = 0.168			
AChE (0.1% Propoxur)	r = 0.569 *p* = 0.043	r = 0.659 *p* = 0.014	r = 0.322 *p* = 0.284	r = -0.147 *p* = 0.633		
AChE (0.2% Propoxur)	r = 0.678 *p* = 0.011	r = 0.768 *p* = 0.002	r = 0.310 *p* = 0.303	r = -0.191 *p* = 0.531	r = 0.941 *p* = 0.000	

$P \leq 0.05$ indicated significant difference

Table 4.22: Correlation between different mean elevated enzyme activities for *Aedes albopictus* larvae.

Elevated enzyme activities	α-EST	β-EST	MFO	GST	AChE (0.1% Propoxur)	AChE (0.2% Propoxur)
α-EST						
β-EST	r = 0.977 *p* = 0.000					
MFO	r = 0.262 *p* = 0.388	r = 0.197 *p* = 0.519				
GST	r = -0.234 *p* = 0.442	r = -0.201 *p* = 0.511	r = -0.041 *p* = 0.894			
AChE (0.1% Propoxur)	r = -0.402 *p* = 0.174	r = -0.397 *p* = 0.179	r = -0.147 *p* = 0.632	r = 0.240 *p* = 0.430		
AChE (0.2% Propoxur)	r = -0.450 *p* = 0.123	r = -0.437 *p* = 0.135	r = -0.066 *p* = 0.831	r = 0.364 *p* = 0.222	r = 0.939 *p* = 0.000	

$P \leq 0.05$ indicated significant difference

Table 4.23: Correlation of mean elevated enzyme activities for *Aedes albopictus* between larval stage and adult stage.

Elevated enzyme activities	α-EST	β-EST	MFO	GST	AChE (0.1% Propoxur)	AChE (0.2% Propoxur)
α-EST	r = 0.313 *p* = 0.297					
β-EST		r = 0.333 *p* = 0.266				
MFO			r = 0.334 *p* = 0.265			
GST				r = -0.360 *p* = 0.227		
AChE (0.1% Propoxur)					r = 0.066 *p* = 0.830	
AChE (0.2% Propoxur)						r = 0.092 *p* = 0.765

$P \leq 0.05$ indicated significant difference

Table 4.24: Correlation of survivability of *Aedes albopictus* adults with mean elevated enzyme activities.

	α-EST	β-EST	MFO	GST	AChE (0.1% Propoxur)	AChE (0.2% Propoxur)
DDT 4%	r = -0.186 *p* = 0.543	r = -0.134 *p* = 0.663	r = -0.438 *p* = 0.135	r = -0.147 *p* = 0.631	r = -0.466 *p* = 0.109	r = -0.360 p = 0.227
Dieldrin 0.4%	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Malathion 5%	r = -0.154 *p* = 0.616	r = -0.063 *p* = 0.839	r = 0.473 *p* = 0.103	r = -0.457 *p* = 0.116	r = -0.053 *p* = 0.864	r = -0.078 *p* = 0.800
Fenitrothion 1%	r = -0.185 *p* = 0.545	r = -0.197 *p* = 0.519	r = -0.511 *p* = 0.075	r = -0.096 *p* = 0.756	r = -0.245 *p* = 0.420	r = -0.148 *p* = 0.630
Bendiocarb 0.1%	r = -0.068 *p* = 0.826	r = 0.110 *p* = 0.973	r = 0.500 *p* = 0.082	r = -0.404 *p* = 0.171	r = 0.061 *p* = 0.843	r = 0.024 *p* = 0.939
Propoxur 0.1%	r = -0.161 *p* = 0.599	r = -0.096 *p* = 0.756	r = 0.214 *p* = 0.482	r = -0.074 *p* = 0.810	r = 0.019 *p* = 0.951	r = -0.040 *p* = 0.895
Etofenprox 0.5%	r = 0.115 *p* = 0.709	r = 0.213 *p* = 0.484	r = -0.129 *p* = 0.674	r = -0.284 *p* = 0.346	r = -0.253 *p* = 0.404	r = -0.171 *p* = 0.577
Deltamethrin 0.05%	r = -0.041 *p* = 0.893	r = -0.038 *p* = 0.902	r = 0.071 *p* = 0.817	r = -0.225 *p* = 0.461	r = -0.295 *p* = 0.328	r = -0.255 *p* = 0.400
Lambda-cyhalothrin 0.05%	r = -0.036 *p* = 0.906	r = 0.103 *p* = 0.738	r = 0.193 *p* = 0.528	r = -0.388 *p* = 0.190	r = 0.042 *p* = 0.892	r = 0.027 *p* = 0.931
Permethrin 0.25%	r = 0.465 *p* = 0.109	r = 0.552 *p* = 0.051	r = 0.017 *p* = 0.956	r = -0.346 *p* = 0.247	r = 0.089 *p* = 0.772	r = 0.150 *p* = 0.625
Cyfluthrin 0.15%	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = cannot be computed because at least one of the variables is constant.
$P \leq 0.05$ indicated significant difference

Table 4.25: Correlation of survivability of *Aedes albopictus* larvae with mean elevated enzyme activities.

	α-EST	β-EST	MFO	GST	AChE (0.1% Propoxur)	AChE (0.2% Propoxur)
Bromophos	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chlorpyrifos	r = 0.443 *p* = 0.129	r = 0.411 *p* = 0.163	r = 0.200 *p* = 0.512	r = -0.294 *p* = 0.329	r = -0.422 *p* = 0.151	r = -0.511 *p* = 0.074
Fenthion	r = 0.236 *p* = 0.438	r = 0.272 *p* = 0.368	r = 0.221 *p* = 0.469	r = -0.075 *p* = 0.807	r = -0.225 *p* = 0.459	r = -0.095 *p* = 0.757
Fenitrothion	r = 0.254 *p* = 0.402	r = 0.259 *p* = 0.392	r = 0.661 *p* = 0.014	r = -0.255 *p* = 0.401	r = 0.112 *p* = 0.716	r = 0.166 *p* = 0.589
Temephos	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Malathion	r = -0.160 *p* = 0.601	r = -0.156 *p* = 0.611	r = 0.095 *p* = 0.758	r = 0.144 *p* = 0.638	r = 0.090 *p* = 0.770	r = 0.061 *p* = 0.842
DDT	r = 0.238 *p* = 0.434	r = 0.209 *p* = 0.493	r = 0.132 *p* = 0.668	r = 0.096 *p* = 0.756	r = 0.176 *p* = 0.565	r = 0.127 *p* = 0.680
Dieldrin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = cannot be computed because at least one of the variables is constant.
$P \leq 0.05$ indicated significant difference

4.6 Bioefficacy of Insect Growth Regulators (IGRs)

The EI and RR of *Aedes albopictus* against juvenile hormones, chitin synthesis inhibitors and moulting disruptor are summarized in Table 4.26, Table 4.27 and Table 4.28 respectively. *Aedes albopictus* collected from 13 districts in Sarawak were susceptible to all tested juvenile hormones, with RRs for methoprene and pyriproxyfen ranging from 0.19 – 0.38 and 0.05 – 0.14, respectively (Table 4.26).

For the chitin synthesis inhibitors, *Ae. albopictus* was susceptible to novaluron with RRs ranging from 0.75 – 1.00, but exhibited tolerance towards diflubenzuron with RRs ranging from 0.33 – 1.33. *Aedes albopictus* was susceptible to cyromazine, a moulting distruptor, with RRs ranging from 0.50 – 0.95.

Among the tested populations, only *Ae. albopictus* from Bintulu district showed tolerance towards diflubenzuron ($1.00 < RR < 5.00$). In general, the mean RR of five IGRs was less than 1.00 which implied that *Ae. albopictus* from Sarawak were susceptible to all tested IGRs.

Table 4.26: Emergence inhibition (50% and 90%) and resistance ratio of *Aedes albopictus* from Sarawak, Malaysia against juvenile hormone mimics.

Methoprene				
District	EI_{50} (mg/liter; 95% CL)	EI_{90} (mg/liter; 95% CL)	Regression Line	RR
Laboratory	0.005 (0.004 – 0.007)	0.027 (0.019 – 0.044)	y = 1.82x + 4.14	–
Kuching	0.001 (0.000 – 0.002)	0.025 (0.013 – 0.096)	y = 0.80x + 2.57	0.19[s]
Bau	0.002 (0.001 – 0.003)	0.048 (0.025 – 0.165)	y = 0.93x + 2.50	0.38[s]
Samarahan	0.001 (0.000 – 0.002)	0.030 (0.017 – 0.093)	y = 0.94x + 2.71	0.19[s]
Serian	0.001 (0.000 – 0.002)	0.031 (0.015 – 0.142)	y = 0.77x + 2.45	0.19[s]
Sibu	0.001 (0.000 – 0.002)	0.016 (0.010 – 0.038)	y = 1.04x + 3.16	0.19[s]
Selangau	0.001 (0.000 – 0.002)	0.012 (0.008 – 0.028)	y = 1.07x + 3.32	0.19[s]
Mukah	0.002 (0.001 – 0.003)	0.041 (0.022 – 0.123)	y = 0.99x + 2.65	0.38[s]
Dalat	0.002 (0.001 – 0.003)	0.042 (0.022 – 0.146)	y = 0.90x + 2.53	0.38[s]
Miri	0.001 (0.000 – 0.002)	0.034 (0.016 – 0.141)	y = 0.68x + 2.29	0.19[s]
Bintulu	0.002 (0.001 – 0.003)	0.055 (0.029 – 0.182)	y = 0.95x + 2.48	0.38[s]
Tatau	0.001 (0.000 – 0.001)	0.012 (0.007 – 0.030)	y = 0.98x + 3.15	0.19[s]
Sarikei	0.001 (0.000 – 0.002)	0.034 (0.017 – 0.170)	y = 0.75x + 2.39	0.19[s]
Kapit	0.001 (0.000 – 0.001)	0.010 (0.006 – 0.023)	y = 0.95x + 3.20	0.19[s]
Mean ± S.E.	0.001 ± 0.001	0.030 ± 0.004		0.25 ± 0.25[s]
Pyriproxyfen				
District	EI_{50} (mg/liter; 95% CL)	EI_{90} (mg/liter; 95% CL)	Regression Line	RR
Laboratory	0.044 (0.030 – 0.073)	1.086 (0.414 – 6.561)	y = 0.92x + 1.25	–
Kuching	0.002 (0.000 – 0.005)	0.764 (0.172 – 221.27)	y = 0.48x + 1.34	0.05[s]
Bau	0.006 (0.003 – 0.008)	0.103 (0.060 – 0.259)	y = 1.01x + 2.28	0.14[s]
Samarahan	0.004 (0.002 – 0.006)	0.060 (0.037 – 0.133)	y = 1.04x + 2.56	0.09[s]
Serian	0.002 (0.000 – 0.003)	0.035 (0.022 – 0.077)	y = 0.97x + 2.70	0.05[s]
Sibu	0.006 (0.003 – 0.009)	0.079 (0.054 – 0.127)	y = 1.15x + 2.55	0.14[s]
Selangau	0.002 (0.000 – 0.004)	0.052 (0.030 – 0.146)	y = 0.86x + 2.38	0.05[s]
Mukah	0.003 (0.001 – 0.006)	0.084 (0.048 – 0.237)	y = 0.91x + 2.26	0.07[s]
Dalat	0.003 (0.001 – 0.006)	0.077 (0.045 – 0.202)	y = 0.95x + 2.34	0.07[s]
Miri	0.003 (0.000 – 0.009)	0.032 (0.012 – 0.182)	y = 1.31x + 3.24	0.07[s]
Bintulu	0.005 (0.002 – 0.008)	0.133 (0.072 – 0.416)	y = 0.91x + 2.08	0.11[s]
Tatau	0.006 (0.005 – 0.008)	0.085 (0.051 – 0.096)	y = 1.11x + 2.47	0.14[s]
Sarikei	0.004 (0.003 – 0.006)	0.284 (0.125 – 0.347)	y = 0.69x + 1.66	0.09[s]
Kapit	0.002 (0.001 – 0.003)	0.054 (0.029 -0.063)	y = 0.90x + 2.41	0.05[s]
Mean ± S.E.	0.0036 ± 0.0004	0.142 ± 0.055		0.09 ± 0.01[s]

CL = Confidence Limit, s = susceptible, t = tolerance

Table 4.27: Emergence inhibition (50% and 90%) and resistance ratio of *Aedes albopictus* from Sarawak, Malaysia against chitin synthesis inhibitors.

Diflubenzuron				
District	EI_{50} (mg/liter; 95% CL)	EI_{90} (mg/liter; 95% CL)	Regression Line	RR
Laboratory	0.00006 (0.00003 – 0.00010)	0.0018 (0.0009 – 0.0067)	y = 0.87x + 3.66	–
Kuching	0.00003 (0.00001 – 0.00005)	0.0010 (0.0004 – 0.0020)	y = 0.98x + 4.40	0.50[s]
Bau	0.00006 (0.00003 – 0.00008)	0.0010 (0.0010 – 0.0020)	y = 1.08x + 4.54	1.00[s]
Samarahan	0.00002 (0.0000 – 0.00004)	0.0005 (0.0003 – 0.0010)	y = 0.99x + 4.61	0.33[s]
Serian	0.00002 (0.00001 – 0.00004)	0.0010 (0.0003 – 0.0010)	y = 0.88x + 4.19	0.33[s]
Sibu	0.00004 (0.00002 – 0.00007)	0.0010 (0.0005 – 0.0020)	y = 1.01x + 4.43	0.67[s]
Selangau	0.00002 (0.00001 – 0.00002)	0.0004 (0.0002 -0.0005)	y = 0.93x + 4.42	0.33[s]
Mukah	0.00003 (0.00002 – 0.00005)	0.0010 (0.0004 – 0.0010)	y = 0.92x + 4.11	0.50[s]
Dalat	0.00005 (0.00002 – 0.00008)	0.0010 (0.0010 – 0.0030)	y = 0.92x + 4.00	0.83[s]
Miri	0.00005 (0.00002 -0.00007)	0.0010 (0.0010 – 0.0040)	y = 0.90x + 3.92	0.83[s]
Bintulu	0.00008 (0.00004 – 0.00011)	0.0020 (0.0010 – 0.0050)	y = 0.97x + 4.00	1.33[t]
Tatau	0.00003 (0.00001 – 0.00004)	0.0010 (0.0003 – 0.0010)	y = 1.01x + 4.61	0.50[s]
Sarikei	0.00003 (0.00001 – 0.00005)	0.0010 (0.0004 – 0.0020)	y = 0.88x + 4.06	0.50[s]
Kapit	0.00002 (0.00001 – 0.00004)	0.0003 (0.0002 – 0.0010)	y = 1.12x + 5.17	0.33[s]
Mean ± S.E.	0.00004 ± 0.000005	0.00094 ± 0.00011		0.61 ± 0.09[s]
Novaluron				
District	EI_{50} (mg/liter; 95% CL)	EI_{90} (mg/liter; 95% CL)	Regression Line	RR
Laboratory	0.00004 (0.00003 – 0.00004)	0.00009 (0.00008 – 0.00013)	y = 3.49x + 15.38	–
Kuching	0.00003 (0.00003 – 0.00005)	0.00015 (0.00011 – 0.00016)	y = 2.00x + 8.94	0.75[s]
Bau	0.00004 (0.00003 – 0.00004)	0.00009 (0.00008 – 0.00011)	y = 3.44x + 15.22	1.00[s]
Samarahan	0.00003 (0.00002 – 0.00004)	0.00009 (0.00008 – 0.00013)	y = 2.57x + 11.66	0.75[s]
Serian	0.00003 (0.00002 – 0.00003)	0.00008 (0.00006 – 0.00010)	y = 2.88x + 13.17	0.75[s]
Sibu	0.00004 (0.00003 – 0.00005)	0.00014 (0.00010 – 0.00014)	y = 2.51x + 10.99	1.00[s]
Selangau	0.00003 (0.00003 – 0.00004)	0.00009 (0.00007 – 0.00011)	y = 3.09x + 13.81	0.75[s]
Mukah	0.00003 (0.00003 – 0.00004)	0.00008 (0.00007 – 0.00010)	y = 3.24x + 14.55	0.75[s]
Dalat	0.00004 (0.00003 – 0.00005)	0.00012 (0.00010 – 0.00018)	y = 2.60x + 11.45	1.00[s]
Miri	0.00004 (0.00002 – 0.00004)	0.00020 (0.00013 – 0.00057)	y = 1.68x + 7.47	1.00[s]
Bintulu	0.00004 (0.00003 – 0.00005)	0.00019 (0.00013 – 0.00043)	y = 1.83x + 8.10	1.00[s]
Tatau	0.00003 (0.00002 – 0.00004)	0.00010 (0.00008 – 0.00014)	y = 2.41x + 10.94	0.75[s]
Sarikei	0.00004 (0.00003 – 0.00004)	0.00014 (0.00011 – 0.00023)	y = 2.24x + 9.90	1.00[s]
Kapit	0.00003 (0.00002 – 0.00003)	0.00009 (0.00007 – 0.00010)	y = 2.47x + 11.29	0.75[s]
Mean ± S.E.	0.000035 ± 0.000001	0.00012 ± 0.00001		0.87 ± 0.04[s]

CL = Confidence Limit, s = susceptible, t = tolerance

Table 4.28: Emergence inhibition (50% and 90%) and resistance ratio of *Aedes albopictus* from Sarawak, Malaysia against moulting disruptor.

Cyromazine				
District	EI_{50} (mg/liter; 95% CL)	EI_{90} (mg/liter; 95% CL)	Regression Line	RR
Laboratory	0.107 (0.099 – 0.116)	0.201 (0.179 – 0.235)	y = 4.71x + 4.57	–
Kuching	0.102 (0.090 – 0.115)	0.329 (0.252 – 0.509)	y = 2.52x + 2.50	0.95[s]
Bau	0.095 (0.085 – 0.105)	0.244 (0.203 – 0.321)	y = 3.14x + 3.20	0.88[s]
Samarahan	0.089 (0.080 – 0.099)	0.213 (0.179 – 0.277)	y = 3.07x + 3.34	0.83[s]
Serian	0.059 (0.023 – 0.080)	0.190 (0.080 – 0.185)	y = 2.50x + 3.08	0.55[s]
Sibu	0.075 (0.064 – 0.085)	0.233 (0.189 – 0.327)	y = 2.61x + 2.93	0.70[s]
Selangau	0.060 (0.048 – 0.070)	0.196 (0.161 – 0.272)	y = 2.49x + 3.04	0.56[s]
Mukah	0.079 (0.069 – 0.087)	0.201 (0.171 – 0.257)	y = 3.15x + 3.47	0.74[s]
Dalat	0.054 (0.044 – 0.063)	0.149 (0.128 – 0.188)	y = 2.94x + 3.71	0.50[s]
Miri	0.088 (0.071 – 0.105)	0.497 (0.316 – 1.284)	y = 1.71x + 1.80	0.82[s]
Bintulu	0.066 (0.058 – 0.073)	0.143 (0.127 – 0.170)	y = 3.80x + 4.49	0.61[s]
Tatau	0.059 (0.041 – 0.072)	0.299 (0.215 – 0.580)	y = 1.82x + 2.23	0.55[s]
Sarikei	0.069 (0.058 – 0.077)	0.191 (0.160 – 0.249)	y = 2.88x + 3.35	0.64[s]
Kapit	0.059 (0.048 – 0.069)	0.186 (0.154 – 0.252)	y = 2.59x + 3.17	0.55[s]
Mean ± S.E.	0.073 ± 0.004	0.236 ± 0.026		0.68 ± 0.04[s]

CL = Confidence Limit, s = susceptible, t = tolerance

CHAPTER 5: DISCUSSION

Among the mosquitoes, both *Ae. aegypti* and *Ae. albopictus* are the vectors for dengue fever and dengue haemorrhagic fever (Nelder et al, 2010). In Malaysia, the container-breeding *Ae. aegypti* is the primary dengue vector while *Ae. albopictus* is the secondary vector (Lee, 2000). However, a study conducted in Sarawak revealed that *Ae. albopictus* was the main vector that circulating dengue virus with absent of *Ae. aegypti* (Harvie et al., 2020). In addition, previous report showed that *Ae. albopictus* is the predominant species in Sarawak (Lau et al., 2017). Being the largest state in Malaysia, Sarawak contains large tracts of tropical rainforest with diverse flora and fauna which are largely found in Total Protected Area (TPA) (Tisen, 2008).

In the meantime of developing effective vaccine to counter dengue fever, many studies on *Ae. albopictus* were conducted on their ecological distribution, chemical resistance status as well as molecular studies (Chen et al., 2006; Elia-Amira et al., 2019; Low et al., 2015). In Malaysia, several population genetics studies of *Ae. albopictus* were conducted in various localities in Peninsular Malaysia, while the information on the *Ae. albopictus* in Sarawak is underreported.

5.1 Ovitrap Surveillance in Sarawak

Our results indicate that *Ae. albopictus* was more abundant in urban residential area and the density was significantly higher in urban area than those of other categories of residential areas, with mean ovitrap index (OI) 90.97 ± 1.59 % and mean number of larvae per ovitrap 26.47 ± 1.62.

The differences in OI and mean number of larvae per ovitrap of *Ae. albopictus* between landscapes can possibly be the results of geo-physical and socio-environmental set up of the residential area with reference to location and basic amenities (Chang &

Jute, 1982). According to Chang & Jute (1982), the density of *Ae. albopictus* was higher in coastal and rural areas and comparatively low in urban and suburban areas due to the absence of basic amenities and the consequential water storage activities in coastal and rural area which in turn become breeding grounds for *Ae. albopictus*. The condition of recent rural areas is different from those reported by Chang & Jute (1982) 34 years ago. Road, communication, water supply and garbage disposal system have been improved, and an effective vector control programme is now actively implemented by local authorities, thus reducing water storage activities and the number of breeding grounds for *Ae. albopictus*. However, still in several rural and remote areas, the lack of basic amenities has led to indiscriminate disposal of garbage and many water holding containers were still used widely, similarly as reported by Chang & Jute (1982).

Aedes albopictus is well known as a semidomestic breeder in urban areas where it feeds on humans and domestic animals and oviposits in natural and artificial water containers near human dwellings (Hawley, 1988). Heavy vegetation was observed around the urban areas and a variety of man-made breeding grounds for *Ae. albopictus*, such as plastic rubbish and water ditches yielded by urban activities was also observed.

Aedes aegypti was previously reported in Sibu (Macdonald et al., 1965), Kuching (Macdonald et al., 1967; Surtees, 1970) and Miri (Macdonald & Rajapaksa, 1972). A survey done by Chang & Jute (1982) in 1980 found that *Ae. aegypti* was present in 37 localities out of 73. Interestingly, no *Ae. aegypti* was recovered from this study. Chang & Jute (1982) also reported that *Ae. aegypti* had been eliminated in 5 urban localities after 3 years of vector control programme since 1978. Chan et al. (1971) reported that *Ae. aegypti* breeds predominately inside houses while *Ae. albopictus* breeds mainly outside houses. Most of the control programme targets indoor areas due to intensive malaria vector control in the past 3 decades (Tee, 2000), whereas the outdoor breeding behavior of *Ae. albopcitus* might have increased their survival when they were hidden

in the inner deep of heavy vegetation where control application hardly reached. In the long run, the population of *Ae. aegypti* was lowered and thus *Ae. albopictus* become dominant in urban area due to the absence of interspecies competition in outdoor breeding sites. The lack of proper means of transportation from urban to other areas could also affect the dispersal of *Ae. aegypti* in the past 3 decades (Chang & Jute, 1982) and this might be the reason why the populations of *Ae. aegypti* were unable to spread while suppressed by the control programme. Barrera (1992) reported that *Ae. albopictus* could withstand starvation longer than *Ae. aegypti* when reared on oak leaves, in other word, the heavy coverage of vegetation around the residential area favors the *Ae. albopictus* populations. With all the factors may explain why *Ae. albopictus* become a dominant species in urban residential areas. In suburban and rural areas, the distribution is somewhat similar although ovitrap index in urban area was higher. Both residential areas share the similarity of geo-physical and socio-environment factors such as water supply, shop lots and residential. The human population and activities which provide more food source and favorable habitats for *Ae. albopictus* contribute to higher OI in suburban residential area than rural and remote residential area.

Larvae of *Armigeres* spp. were also found co-breeding with *Ae. albopcitus* in 5 residential areas. *Armigeres kesseli* and *Armigeres subalbatus* are commonly found close to human dwellings and may adapt to breeding habitats similar to *Aedes* mosquitoes such as artificial containers, coconut shells, hollow bamboos and mostly polluted water (Pandian & Chandrashekaran, 1980; Rajavel, 1992, Nurin-Zulkifli et al., 2015). The larvae of *Armigeres* spp. are voracious biter that had been reported to be predacious (Buddle, 1928) as well as cannibalistic (Rajavel, 1992). The presence of *Armigeres* sp. increases the interspecies competition as well as the predation on the *Ae. albopictus* larvae.

The factors contributing to the failure of establishment of *Ae. aegypti* in all residential areas when compared to data reported by Chang & Jute (1982) are not fully understood. The absence of *Ae. aegypti* was also previously reported in an university campus and an island of peninsular Malaysia (Wan-Norafikah et al., 2009; Chen et al., 2009; Lau et al., 2013; Noor-Afizah et al, 2015). This phenomenon was probably due to lack of favorable breeding foci of *Ae. aegypti* (Wan-Norafikah et al., 2009; Chen et al., 2009; Lau et al., 2013). Noor-Afizah et al. (2015) suggested that other *Aedes* species was prevented from establishing themselves because the population of *Ae. albopictus* was so dominant, as the establishment of *Ae. albopictus* was associated with reduction in the abundance and range of *Ae. aegypti*.

5.2 Genetic Diversity of *Aedes albopictus*

Analysis revealed that haplotype A1 was the most widespread haplotype of *Ae. albopictus* as a result of its dispersion in Sarawak (Fig. 4.1 and Table 4.7). *Aedes albopictus* from Kapit exhibited higher divergence with the identification of seven haplotypes. In contrast, the Mukah was the least diverse in genetic with only two haplotypes detected. In this study, haplotype A1 was suggested as the common ancestor due to its wide dispersion across Sarawak. This haplotype probably had experienced evolution over time into various haplotypes (A2–A22) in order to adapt the habitat and demographical changes and eventually became distributed across Sarawak by high gene flow activities. Moreover, the haplotypes were shared across different populations indicating recent gene flow between populations (Koopman et al., 2007).

Numbers of haplotypes discovered in this study were considered low with only twenty two haplotypes and presence of several singleton haplotype that restricted to particular areas. Previous study conducted in Penang and in Subang Jaya, revealed 37 and 21 haplotypes, respectively (Zawani et al., 2014; Ismail et al., 2015). Sarawak is the

largest state in Malaysia with diverse of flora and fauna. In comparison to Penang and Subang Jaya, Sarawak is expected to harbor more diversified gene pool. However, low number of haplotypes may indicate low degree of COI gene polymorphisms in *Ae. albopictus* in Sarawak. A possible reason would be attributed to the demographic of the sampling site. Although Sarawak is flourished with biodiversity, the infrastructures was improved and equipped with well-developed road access in order to promote eco-tourism (Tisen, 2008). The lack of geographical barriers that restrict human activities is more likely to increase the dispersal movement of *Ae. albopictus* in which the distribution of this species was found mediated by human activity (Manni et al., 2015). Indirectly, this will increase the possibility of genetic transferring within and between populations.

In regard to the comparison of *Ae. albopictus* in Sarawak with global database, several unique haplotypes discovered in this study have not been recorded in any other countries, suggesting that they may represent either new mutations that have yet to disperse or haplotypes that have not been surveyed. In haplotype network analysis, two haplotypes (B1 and B7) were identical or nearly identical to sequences from Southeast Asian and Oceania and one shared haplotype (B9) with sequence from Peninsular Malaysia. This indicates a close genetic affinity of *Ae. albopictus* populations and restricted only in Southeast Asian and Oceania regions. Upon the shared haplotypes, *Ae. albopictus* in Sarawak also showed genetic proximity with the haplotypes from Oceania, Europe, North and South America, East and West Asia, Africa by one or two mutational steps, however, many mutational steps were discovered when compared to sequences from South Asia. According to Gratz (2004), the mosquito *Ae. albopictus* was originally from Southeast Asia, islands of the Western Pacific and Indian Ocean. This relation could be resulted from human migration towards Indo-Malayan Peninsular and the Indian Ocean islands, including Madagascar in early spread of the species (Paupy *et al*.,

2009). The spread was further hastened by the increase of the intercontinental trade during 20th century. The majority of introductions were apparently due to intercontinental transportation of dormant eggs in tyres. Several study also suggested that this invasive species originated from Asia and spread to western countries, for instance, population in Cameroon was closer to tropical populations such as Cambodia, Thailand and Vietnam (Kamgang et al., 2011), American populations originated from China (Zhang et al., 2018) and North American populations from Japan (Battaglia et al., 2016). Moreover, evidence suggested that China is the one of the ancestral and being the oldest among the East Asian *Ae. albopictus* populations (Manni et al., 2017; Zhang et al., 2018). Interestingly, only one shared haplotype was recorded with haplotypes discovered in Penang. Oceanographic could be the factor for intra-specific genetic discontinuities (Castella et al., 2000). Borneo including Sarawak is isolated from the Southeast Asia mainland (including Peninsular Malaysia) by the South China Sea. This likely to be a barrier to gene flow between *Ae. albopictus* in both localities, leading to discrepancy in genetic diversification. Similar scenarios were also reported for some fly species such as *Simulium nobile* and *Haematobia exigua* in the mainland and islands of Southeast Asia (Low et al., 2016; Low et al., 2017).

A study on global genetic character of *Ae. albopictus* revealed high values of H_d in most studied populations which ranged from 0.059 (Netherlands) to 0.946 (China) (Zhang et al., 2018). In our study, the genetic diversity was considered low (H_d = 0.65014, P_i = 0.00176) when compared to previous study. It was expected that the Asisan *Ae. albopictus* would harbour higher genetic diversity in its native area compared to invaded areas (Zhang et al. 2018). This hypothesis was further supported by our results when compared to the non-native areas such as Cameroon (H_d=0.24) and Croatia (H_d = 0.282) (Kamgang et al, 2011; Žitko et al., 2011). Coupled with the low values of genetic distance obtained (Table 4.8), this phenomenon of low genetic

diversity in *Ae. albopictus* might be a result of high gene flow and effectual dispersal throughout and between the populations. Similar result was found in *Ae. albopictus* in Manaus in which the low genetic variation was resulted from insufficient time for accumulating genetic differences among populations (Maia et al., 2009). On the other hand, dispersal of the species also contributes to the gene flow. The normal flight range of this species is limited and have not been observed fly in strong winds (Novak, 1992). However, another study revealed that the flight range might increase in the event of female unsuccessfully in search for suitable oviposition sites with the aid of wind (Delatte et al., 2013). In Sarawak, the presence of mountain range and geographical patches were not the significant barriers among *Ae. albopictus* populations because of *Ae. albopictus* is well known as a semidomestic breeder where can oviposit in natural and artificial water containers (Hawley, 1988). Hence, the human activities play an important role that could increase the rate of genetic transfer among the population. For instant, transportation of used and waste tires as well as water-holding container that harboured the *Ae. albopictus* eggs (Novak, 1992).

Another plausible factor contributed to low genetic diversity is the genetic variation losses that result from genetic drift by repeating bottleneck effect on the population of *Ae. albopictus* (Nei et al., 1975). *Aedes albopictus* populations in Sarawak may have experienced bottleneck effect cause by the vector-borne disease control activities such as source reduction and insecticide application. Previous study conducted by Yan et al. (1998) had revealed that the reduced in genetic variation was associated with hitchhiking effect that take place in other genome regions around a putative organophosphate target site. In Malaysia, insecticide application such as larviciding with temephos and space spraying treatment with insecticide were used in integrated vector management (IVM) programme to combat this notorious dengue vector (Ong, 2016). However, intensive and repeated used of insecticide have contributed to the

development of insecticide resistance among the populations. This may suggest that the development of insecticide resistance is associated with hitchhiking effect that was contributed by the reduced level of genetic variation in the populations. Similar observation was also reported in Malaysian *Culex quinquefasciatus* (Low et al., 2013c)

The neutrality test result showed that the *Ae. albopcitus* populations in Sarawak may have experienced population expansion history resulting in significant negative value in both Tajima's D and Fu's F_s test. This phenomenon was further corroborated by the mismatch distribution analysis. The unimodal distribution (Fig. 4.4) obtained for *Ae. albopictus* provide further evidence for a recent population expansion for this species. In this study, heavy vegetation include ornamental plants was observed around the study sites and variety of man-made breeding grounds such as presence of rubbish. This provided favourable breeding habitats for *Ae. albopictus* contribute to their expansion in populations (Lau et al., 2017). Similar finding also suggested that environment factors such as the availability of breeding sites, habitat preferences and food source, may influence the distribution of *Ae. albopictus* which eventually affect the expansion of the populations (Kamgang et al., 2013). The recent expansion of *Ae. albopictus* may explain the high gene flow values and low genetic variation obtained from this study. Moreover, this expansion may also explain the *Ae. albopictus* in Sarawak, coupled with the advantage of favourable habitats, had successfully displace and compete with *Ae. aegypti* for further dispersion. This finding further support previous study that *Ae. albopictus* as the dominant dengue vector in Sarawak (Chang & Jute, 1982; Lau et al., 2017).

With the available result from this study, the Sarawak *Ae. albopictus* has possibly experienced recent bottlenecks and population expansions. Similar result was observed in China (Zhang et al., 2018), the application of insecticide for the control of dengue epidemics might have reduced but not eliminated *Ae. albopictus* populations and thus

isolating them into small and fragmented gene pools. The remaining populations were then expanded rapidly after the reduction of chemical usage in IVM (Zhang et al., 2018). This indirectly produced several singleton haplotype in particular area in Sarawak. Knowledge of genetics on medically insect species is required element in order to further understand the disease epidemiology, vector transmission as well as disease control (Tabachnick & Black, 1995). By understanding the genetic diversity and identification of the dispersal routes include demographic origin of *Ae. albopictus* would facilitate the control and prevention of epidemic outbreaks (Manni et al., 2015). Hence, in order to fully understand the ecology and epidemiology of *Ae. albopictus*, further research on wider geographical areas in Malaysia especially East coast of Peninsular Malaysia and Sabah, in addition of incorporation of more variable markers, will be beneficial in revealing the presence of additional haplotypes.

5.3 Adulticides Resistance Status of *Aedes albopictus*

The present study revealed that *Ae. albopictus* collected from Sarawak, Malaysia was susceptible to cyfluthrin and dieldrin but exhibited different degrees of susceptibilities to other tested insecticides. Among the tested insecticides, cyfluthrin was the most effective pyrethroid accounted for the shortest KT_{50} and induced complete mortality to all populations.

Over the past few decades, the efficiency of cyfluthrin was proven to be effective against *Aedes* mosquitoes with fast knockdown effect and high mortality (Vythilingam et al., 1992; Sulaiman et al., 2002; Hidayati et al., 2011, Chen et al., 2013a). On the other hand, permethrin resistance was reported in Malaysia due to its widely uses in dengue control program (Wan-Norafikah et al., 2010; Chan & Jaal, 2103; Ishak et al., 2015). Pyrethroids are one of the most widely used insecticides to control mosquitoes in government control operation and household insecticides in the forms of aerosol, coils

and vaporizer (Yap et al., 2000). Over-use of the pyrethroid-based insecticides might confer pyrethroid resistance to the populations.

A mixed resistance pattern was observed against both bendiocarb and propoxur. The results were in line to past studies in Malaysia where widespread of resistance toward both tested insecticides were reported (Ishak et al., 2015; Elia-Amira et al., 2019). Carbamates have been heavily used in agriculture as insecticides, fungicides and herbicides for more than half century (Struger et al., 2016). The exophilic behaviour of *Ae. albopictus* may increase chance of exposure to carbamate in agriculture area. Indirect exposure may cause the resistance development in the populations because both bendiocarb and propoxur were not commonly used in dengue vector control in Malaysia.

Malathion has been used in the control of mosquito populations in Malaysia for more than 20 years by fogging practice and proven to be efficient to control the dengue vector in early 2000 (VBCDP, 1999; Rohani et al., 2001). However, recent studies showed that various resistance patterns were discovered from several localities in Malaysia which similar to our findings (Chen et al., 2013a; Ishak et al., 2015). On the other hand, most of the tested populations were susceptible to fenitrothion. Both organophosphates have been routinely used as active ingredients in space treatment to control dengue vectors in Malaysia, resistance towards these insecticides may become intensive because both share the same mode of actions (Ong, 2016). Moreover, both insecticides were incapable to induce high knockdown effect on adults, especially fenitrothion, as observed in Chen et al. (2013a).

The use of DDT as control agent was dated back to 1960s in Malaysia but it was banned in 1998 on account of their persistent and carcinogenic nature. Residual spraying of DDT was extensively used in the malaria eradication programme since 1967 in Malaysia. Over reliance on the DDT had led to resistance development among the anopheline mosquitoes (Rohani et al., 2014). Similar situation has occurred in *Aedes*

mosquitoes, despite the removal of DDT from public health uses for more than 20 years, DDT resistance in *Aedes* mosquitoes has been reported by many researchers till present day (Chen et al., 2013a; Ishak et al., 2015; Rahim et al., 2017). Our results showed that *Ae. albopictus* was susceptible towards dieldrin but exhibited a wide range of DDT susceptibilities. Similar observation was also reported in Ishak et al. (2015) and Elia-Amira et al. (2019).

Cross-resistance occurs when resistance to one insecticide confers resistance to another insecticide within or between insecticide classes with same mode of mechanism. Unsurprisingly, among the tested pyrethroids, the insecticides exhibited cross resistance within the group owing to the same mechanism to cause lethal effect by attacking the voltage-gated sodium channels and interferes neuron flow of the mosquitoes (Coats, 1990). Additionally, elevated expression of detoxification enzymes such as MFO (Mixed Function Oxidase), GST (Glutathione S-Transferase), and EST (Non-Specific Esterases), have also been associated with pyrethroid resistance in *Aedes* mosquitoes (Wan-Norafikah et al., 2008; Amelia-Yap et al., 2019). On the other hand, carbamate insecticides bendiocarb and propoxur were found cross-resistant with each other. Similar findings were also reported in *Culex quinquefasciatus* from Southern California (Georghiou et al., 1966). Furthermore, this study also identified cross-resistance between organophosphate and carbamate. Both insecticides share the same mechanism basis by attacking the acetylcholinesterase of insects. When overexposure to either class of insecticide may eventually lead to the development of cross-resistance due to the insensitivity of acetylcholinesterase target site (Hemingway et al., 2004).

Overall, among 11 tested adulticides, only cyfluthrin was effective to control *Ae. Albopictus* in Sarawak. The susceptibility status of 11 tested insecticides decreases in the following order: cyfluthrin, dieldrin > fenitrothion > deltametthin > DDT > etfenrpox > permetrhin > bendiocarb > malathion > lambda-cyhalotrhin > propoxur.

Other new generation pyrethroid should be considered as a routine insecticide in order to prevent resistance development to cyfluthrin in future.

5.4 Lavicides Resistance Status of *Aedes albopictus*

The *Ae. albopictus* larvae showed different degrees of susceptibility status towards the organochlorine and organophosphate larvicides. The organophosphate was alternative replacement since the prohibition of organochlorine usage in pest management control due to its deteriorative features to the environment. Among the organophosphate larvicides, temephos is the approved larvicide that is widely used to control container-inhabiting *Aedes* mosquitoes since 1973 (Rahim et al., 2016). In this study, *Ae. albopictus* from Sarawak were completely susceptible to temephos. However, *Aedes* mosquitoes had been reported for resistant toward temephos in Peninsular Malaysia such as Selangor and Penang (Lee, 1991; Chen et al., 2005b; Loke et al., 2010; Ishak et al., 2015) as temephos was extensively used in dengue vector control program. The *Aedes* larvae were also susceptible to bromophos in which this insecticide is commonly used to control stored grain infestation and crop pest in agriculture industry (Eichler, 1972) but not in dengue control program in Malaysia. Because of the less exposure of the mosquito to bromophos, the possibility of resistance development towards the insecticide was reduced.

Unlike bromophos, despite of fenthion and fenitrothion were commonly used in agriculture and horticulture industry (WHO, 1971; 2004), both insecticides were also used as insecticide for space treatment in dengue control program in Malaysia (Ong, 2016). The development of resistance in certain districts could be caused by continuous selection pressure from vector control activities (Lee et al., 1998). Space treatment fogging was mainly conducted by trained government health practitioners but insecticide application in agricultural industry was conducted by private or local entities.

Thus, irregular and inappropriate application of dosage may lead to resistance development toward the conventional insecticide (Lee, 2005; Ong, 2016). Similarly, chlorpyrifos was used to control agriculture pest, cockroach and termite in Malaysia but rarely in larviciding programme (Lee, 2002; Ngan et al., 2005). The present study exhibited all field populations were resistance against chlorpyrifos. The resistance development could be caused by indirectly long period exposure of the insecticide residues used in other control programme (Thongwat & Bunchu, 2015). According to Margaret & Chai (2007), chlorpyrifos residue was found exceeding the maximum residue limit in vegetables sample collected in Sarawak. Although there is no report of chlorpyrifos resistance mosquito reported in Malaysia, cases of chlorpyrifos resistance development in *Ae. albopictus* were reported in Thailand and USA (Wesson, 1990; Liu et al., 2004). Therefore, it is advisably to restrain the usage of chlorpyrifos as alternative chemical in dengue vector control progamme in future.

Moreover, our present study showed that all populations of mosquitoes resistant to malathion and DDT. The resistance of malathion by *Aedes* larvae has been reported worldwide, for instance Thailand and Malaysia (Ali et al.,1995; Chen et al., 2005b; Ponlawat et al., 2005; Wesson, 1990). Similar to fenitrothion and fenthion, malathion is used in fogging operation to control adult mosquito in Malaysia (Ong, 2016). The incipient resistance can be cause by the insecticide droplet dropped into the larvae habitat or an inherited character that transferred to progenies (Hemingway et al., 2004). On the other hand, DDT was used in malaria control since 1952 in Sarawak with the beginning of Sarawak Malaria Pilot Project (Zulueta & LaChance, 1956).

In Malaysia, DDT was widely used in malaria control programs since the initiation of MEP (Malaria Eradication Programme) in 1950s (Lee & Chong, 1995). However, the usage of organochlorines are prohibited include DDT and dieldrin and both insecticides had been discontinued after 1998 (Zuriati et al., 2003). Although the discontinuation

usage of DDT, the mosquito populations of Sarawak were found resistant against DDT. Similar situation has also been observed in *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus* in Malaysia (Nazni et al., 2009; Low et al., 2013a; Ishak et al., 2015). The resistance of DDT often is related to the mutation in target site of voltage gated sodium channel (*kdr*) (Hemingway et al., 2000). Elevated levels of glutathione-S-transferase (GST) enzyme also play an important role in DDT resistance (Grant et al., 1991).

On the other hand, resistance to dieldrin has been linked with mutations occurring in gamma amina-butyric acid (GABA) receptor. Hemingway et al. (2004) reported that the substitution of alanine to serine (A302S) accord to resistance to dieldrin (known as *Rdl* gene). Although dieldrin was found effective in 12 populations in our study, *Rdl* gene has been discovered at a relatively high frequency in *Ae. albopictus* from Peninsular Malaysia (Low et al., 2015).

Cross resistance can occurred in the *Aedes* mosquito population since *Ae. albopictus* of Sarawak showed resistance to chlopyrifos, malathion, fenitrothion and fenthion, of which these insecticides are belonging to the organophosphate group of insecticides. Cross-resistance between different insecticide classes in conjunction with the target-site mechanism are often discussed between pyrethroid and DDT (Amin & Hemingway, 1989; Brengues et al., 2003). Our study showed that dieldrin was significantly correlated to fenthion and fenitrothion indicating cross-resistance may have occured. However, a more detail investigation using enzyme bioassay should be implemented.

In short, the Sarawak *Ae. albopictus* larvae were susceptible to bromophos and temephos. However, there is possibility of larvae population may develop resistance to bromophos and temephos in future if uncontrolled application of insecticides continues. Thus, regular resistance monitoring should be conducted to confirm the efficacy of insecticides for dengue control.

5.5 Biochemical Studies of Insecticide Resistance in *Aedes albopictus*

Generally, different activity levels of each enzyme were expressed at both larvae and adult stages of *Ae. albopictus* populations. Although, none of the tested enzymes are responsible for the resistance mechanism among the *Ae. albopictus* populations, but a detoxification enzyme activity was noticeable between fenitrothion survivability rate in larvae bioassay and mixed function oxidases (MFO). This phenomenon suggested that the development of fenitrothion resistance in these larval populations was due to MFO enzyme activities. Mixed function oxidases was reported mostly associated with the pyrethroid resistance (Brooke et al., 2001), but also showed relation to organophosphates and carbamates resistance (Alvarez et al., 2013; Ishak et al., 2016; Marcombe et al., 2009). A similar case reported by Alvarez et al. (2013) that MFO appeared to be an incipiently altered mechanism for *Ae. aegypti* in Western Venezuela. In other insects, MFO was also involved in fenitrothion resistance of *Daphnia magna* in Spain (Damásio et al., 2007). However, monooxygenases play only a minor role in organophosphate resistance (Nauen, 2007). In Malaysia, elevated oxidases level was frequently reported on pyrethroid resistance in *Ae. albopictus* (Nazni et al., 2000; Wan-Norafikah et al., 2008) and *Ae. aegypti* (Wan-Norafikah et al., 2010).

In the present study, the significant reduction of acetylcholinesterase upon addition of propoxur indicating this insecticide was still effective against tested *Ae. albopictus* except populations from Sarikei and Dalat. However, no correlation was found between AChE activity and tested insecticides, indicating that insensitive AChE did not play a clear role in any insecticide resistance. Altered AChE plays an important role in resistance mechanism to organophosphate for many insects. For instance, elevated level of AChE had involved in organophosphate resistance in *Culex quinquefasciatus* (Liu et al., 2005; Low *et al*., 2013b), *Ae. albopictus* (Lee et al., 2014), *Ae, aegypti* (Rosilawati

et al., 2018) and *Culex pipiens* (Cui et al., 2006). In spite of that, AChE enzyme does not always cause insecticide resistance in *Ae. aegypti* by several researchers (Polson et al., 2011; Koou et al., 2014; Leong et al., 2019).

The absence of correlations of enzyme activities of non-specific esterases (EST) and glutathione-S-transferases (GST) with other insecticidal bioassay survivability rates, implied that both enzyme did not contribute to the insecticide resistance development among the Sarawak populations. Similar results were reported by Chen et al. (2013b) where there was no correlation with temephos resistance of *Ae. albopictus* with esterases in Selangor and Kuala Lumpur strains. However, previous studies reported that elevated levels of esterases activity were correlated with organophosphates resistance in Malaysian *Ae. aegypti* (Chen et al., 2008b) and *Cx. quinquefasciatus* (Low et al., 2013b). Moreover, elevated esterases activites also played a predominant role in conferring organophosphate resistance in *Ae. aegypti* in Thailand (Pethuan et al., 2007). On the other hand, GST was reported to be involved in organophosphate and pyrethroids resistance, but primarily associated with DDT resistance (Hollingworth & Dong, 2008). Earliar study has attempted to demonstrate the relation between elevated GST activities in *Ae. aegypti*, *Cx. quinquefasciatus* and *Anopheles maculatus*, but no clear correlation was discovered (Lee & Chong, 1995). In Malaysia, the usage of DDT was discontinued in 1998 due to persistent and carcinogenic nature, but the resistance phenotype still remains in Malaysian mosquito populations (Low et al., 2013b). Previous study had suggested that the evolution of knockdown resistance could be a plausible explanation for the lack of correlation between GST activity and DDT resistance (Low et al., 2013b). The occurrence of knockdown resistance (*kdr*) had been reported for *Ae. aegypti* worldwide in which F1534C and V1016G mutations associated with DDT and pyrethroids resistance (Ishak et al., 2015; Kawada et al., 2014). Additionally, the F1534C *kdr* mutation also detected in Singapore strain *Ae. albopictus*

(Kasai *et al*., 2011). In contrast, no *kdr* gene mutation was detected in any field *Ae. albopictus* in Malaysia to date. Another suspected mechanism is the *Rdl* gene mutation in the GABA-gated chloride channel. In Malaysia, the first discovery of *Rdl* gene mutation in *Ae. albopictus* was A302S mutation (Low et al., 2015). The joint action of gene mutation and detoxification mechanisms might be the reason for development of resistance.

A study conducted by Bisset et al. (2014) showed that, although there were increased of enzyme level in both adult and larvae stage, the larvae displayed resistant to chlorpyrifos and deltamethin but not in the adult stage. This is because the adult mosquito do no possesses similar detoxification mechanisms as do larvae. This may be a pausible factor cause the phenomena that adult *Ae. albopictus* complete susceptible to dieldrin but larvae showed various susceptible status to dieldrin in our findings.

The discovery of multidisciplinary of detoxification mechanism involved in insecticide resistance among mosquitoes is not a new phenomenon. Previous study had documented an association between α-esterases and β-esterases in Malaysian *Cx. quinquefasciatus* (Low et al., 2013b) similar to our findings. Hemingway & Ranson (2000) had suggested that the occurrence of this correlation was due to co-amplification of two enzymes ($est\alpha^1$ and $est\beta^1$) which is commonly found in organophosphate-resistance *Cx. quinquefasicatus*.

Metabolic resistance is important in the absence of *kdr* mutations (Ngoagouni et al., 2016). However, the elevated enzyme activities do not necessarily correlate to the toxicological changes since the metabolic pathways could involve different forms of particular enzyme (Siegfried & Scott, 1992). In our study, the lack of correlation between enzyme activity and insecticide resistance level does not strengthen the hypothesis that enzyme activities respond to the survivability rate of Sarawak strain *Ae. albopictus*. Although elevated enzyme activities could play an important role in the

insecticide resistance, it is clear that from our present study that enzyme activity cannot account for the insecticide resistance observed. Therefore, further investigation on target site insensitivity mechanism in *Ae. albopictus* populations should be performed in order to understand the underlying causes contributed to the insecticide resistance.

The results provide the basic information on the mechanisms of α-esterases, β-esterases, mixed function oxidases, glutathione-S-transferase and acetylcholinesterase towards the survivability rate of *Ae. albopictus* in Sarawak. Evidence of mixed function oxidases is responsible to fenitrothion resistance in larval stage was discovered.

5.6 Bioefficacy of Insect Growth Regulators (IGRs)

Insect growth regulators are shown to offer good control and management of insect population since 1956 (Mian & Mulla, 1982). However, the lack of available information in Sarawak has confined the use of IGRs in the local dengue control programme. In the present study, three groups of IGRs were selected based on mode of action. Methoprene and pyriproxyfen are juvenile hormone mimics or juvenoids (JHs), which are chemically related to the natural juvenile hormones of insects, that disrupt the hormonal development and eventually inhibit pupation (Slama et al., 1974). On the other hand, diflubenzuron and novaluron belong to chitin synthesis inhibitors (CSIs), which inhibit cuticle formation and hence treated insects fail to moult or have soft weak cuticle that cannot protect them, and soon die after ecdysis (Itoh, 1981; Mulla, 1995). Cyromazine is a chemical that leads to moult disruption of targeted organism, however the mode of action of is not defined (IRAC, 2017). Most IGRs are known to have no apparent ill effect on non-target organisms and good margin of safety to fish and wildlife (Vythilingam, 2005).

The development of resistance toward insecticides caused by frequent and excessive use of similar chemical in vector control have been reported in different countries

including Malaysia (Georghiou, 1987; Chen et al., 2005b). Resistance towards conventional larvicide such as temephos is frequently reported in Malaysia which has led to other alternatives to chemical agents (Chen, et al., 2005b; Nazni et al., 2000).The present study documents excellent larvicidal efficacy of five IGRs against field collected *Ae. albopictus* larvae from Sarawak. All tested populations of *Ae. albopictus* larvae collected from 13 districts were susceptible to methoprene, pyriproxyfen, novaluron and cyromazine. Many researchers have documented that IGRs are effective chemical agents to control mosquito larvae, for instant, pyriproxyfen was effectively known to induce emergence inhibition (EI) towards mosquito larvae (Unlu et al., 2017; Schaefer & Mulligan, 1991; Seccacini et al., 2008). Similarly, methopene, diflubenzuron and novaluron also proved to be effective insecticides against mosquito larvae (Seccacini et al., 2008; Baruah & Das, 1996; Mulla et al., 2003; Ali et al., 1999).

Good residual efficacy of chemical agents can prolong the duration of effectiveness of the control programme and reduce the frequency of application, which in return reduces costs for man power and operation. Pyriproxyfen has been reported to induce complete inhibition over 4 months up to 43 weeks under indoor experiment and simulated field trial (Lau et al., 2015; Seccacini et al., 2008; Vythilingam et al., 2005; Marcombe et al., 2011). Diflubenzuron has also been reported to show complete inhibition for more than 4 weeks up to 4 months (Chen et al., 2008c; Lam, 1990; Seccacini et al., 2008). Additionally, researchers also showed that EC10 of novaluron (0.05 – 1 mg/L) exhibited 86 – 96% of IE for about 190 days (≈27 weeks), while 0.001 – 0.02 mg/L achieved 80 – 100% of IE for 2 months (≈8 weeks) (Mulla et al., 2003).

Among the tested populations, larvae from Bintulu showed tolerance to diflubenzuron. Because of the scarcity of the available information on the use of IGRs in these study sites, the cause of tolerance is unknown. However, IGRs have also been widely used for other pest control such as house flies, fleas, tsetse flies and cockroaches

(Cetin et al., 2009; Palma & Meola, 1990; Langley et al., 1990; Chow & Yang, 1990), hence, we cannot exclude the possibility of the uncontrolled use of IGRs on other pests which may indirectly exhibit selection pressure on our surveyed *Aedes* populations.

The horizontal effect is another significant feature of IGR. Chism & Apperson (2003) conducted a study using pyriproxyfen against *Ae. albopictus* and showed that with sufficient pyriproxyfen carried by gravid females can transfer effective larvicidal doses to larval microcosms. Itoh et al. (1994) showed that pyriproxyfen treated *Aedes aegypti* which allowed to lay eggs in water containing fourth instars can cause subsequent mortality to those immatures. Similar scenario was showed by Sihuincha et al. (2005) that pyriproxyfen could be transferred and caused subsequent mortality to larvae in new treatment free oviposition site by treated female mosquitoes. Moreover, Kamal & Khater (2010) reported a reduction of egg hatchability in mosquito adults that emerged from the treatments of pyriproxyfen and diflubenzuron. In short, the horizontal efficacy of IGR has shed some light on the extended larvicidal effect in mosquito populations.

In terms of user preference, direct application of IGR is simple and easy in areas such as drains, ponds and in places where long-term control is desired. Moreover, pyriproxyfen, diflubenzuron and novaluron have been accepted by WHO for application in drinking water and which do not emit smell or produce turbidity in treated water like temephos (WHO, 2008a,b,c; WHO, 2009a). However, IGRs do not induce instant mortality to larvae after treatment. The late mortality that occurs would imply ineffectiveness compared to other larvicides, and this may discourage the use of IGRs as alternative option. Certainly, users and local authorities should be imformed on the use of IGRs in which a significant mortality can be observed after 72 hr post-treatment of IGR (Su & Cheng 2014). Weekly surveillance after treatment would be ideal period to assess the efficacy of IGRs because the mode of action of IGR affecting the moulting

and metamorphosis processes on the mosquito life cycle may take place within the 7 days (Chen et al., 2005a; Lee, 1992b).

However, there are potential effects of IGRs on non-target organisms. A review paper written by Tunaz &d Uygun (2004) reviewed that crustaceans and other aquatic arthropods are sensitive to IGRs. Since aquatic arthopods share similar molting process and hormones as mosquito, the application of IGRs would bring adverse effect to the non-target organism if irrational planning is implemented.

This study concluded that the tested IGRs showed promising result and can be used as alternative chemical agents especially methoprene, pyriproxyfen and cyromazine. This study clearly provides baseline referral information for local authorities and IVM operator for future counter resistance programme.

5.7 Mosquitoes Studies in Sarawak: Challenges and Limitations

In order to obtain in depth understanding on the dengue vector, the first step to be considered is to determine the distribution and abundance. Previous study had proved that the ovitrap surveillance is a reliable and sensitive tool for detecting presence of dengue vectors (Lau et al., 2013). The ovitrap surveillance in this study was able to prove that Sarawak *Ae. albopictus* was able to establish better than *Ae. aegypti* in all residential areas. In other words, *Ae. albopictus* is the dominant vector and incriminated for the transmission of dengue fever. Moreover, all the surveyed residential areas are in high risk of dengue transmission where OI was more than 10% (Tham, 2000). Ovitrap surveillance is a key component of any local integrated vector management to quantify human risk to dengue fever. Thus, regular survey ovitrap surveillance should implement frequently to act as an early warning and prevent dengue outbreak.

Sarawak is the largest state in Malaysia with large tracts of tropical rainforest with diverse flora and fauna in Total Protected Area (TPA) (Tisen, 2008). Suprisingly, *Ae.*

aegypti was absent although commonly found in Peninsular Malaysia. *Ae. aegypti* was previously reported in Sarawak in between 1965 and 1982 (Macdonald et al., 1965; Macdonald et al., 1967; Macdonald & Rajapaksa, 1972; Chang & Jute, 1982), but this species was absent in recent studies (Lau et al., 2017; Harvie et al., 2020). Though from the genetic study showed the Sarawak *Ae. albopictus* populations experienced expansion which may indicating suppressed or halted the establishment of *Ae. aegypti*. Another plausible explanation is the frequency of study sampling sites was not high enough to detect *Ae. aegypti*. Ovitrap surveillance should continue in other regions in Sarawak.

Despite of World Health Organization suggested several approaches for integrated vector management, chemical control using insecticides remains the most preferred strategy in many countries including Malaysia. Nevertheless, the uncontrolled and persistent usage of insecticides has led to development of insecticide resistance among the mosquito populations. Unable escape from the similar fate, the Sarawak *Ae. albopictus* was also found to be resistance to several common insecticides in our studies. Apart from the fact of over reliance on the insecticides usage, the lack of standardisation and professional knowledge among the operational personel could be another contributing factor. From preparation stage to insecticides application, staff should be aware to the changes during operation such as overdose in chemical and early observation of efficacy insecticides application. Thus, proper training should be implemented to inculcate correct operating procedure in order to reduce error during insecticide application operation.

Moreover, our findings provide the basic information on the mechanisms of α-esterases, β-esterases, mixed function oxidases, glutathione-S-transferase and acetylcholinesterase towards the survivability rate of *Ae. albopictus* in Sarawak state. Evidence of mixed function oxidases is responsible to fenitrothion resistance in larval

stage was discovered. Although other tested enzymes did not play a role in insecticide resistance in the survival rate of Sarawak *Ae. albopictus*, significant elevated enzymes activities were observed. This indicates that the enzymes could become a contributing factor in the development of insecticide resistance in future if appropriate approaches are not adopted. Fortunately, the tested IGRs showed promising result and can be used as alternative chemical agents especially methoprene, pyriproxyfen and cyromazine. The study clearly provides baseline referral information for local authorities and IVM operator for future counter resistance programme. However, there will be a similar scenario if the use of IGRs is not planned properly.

In nut shell, although dengue vector control promulgated by the government is intended to be same in all parts of Malaysia, insecticide susceptibility patterns exhibited by mosquito were not homogeneous across geographic regions of Sarawak. Thus, regular surveillance and monitoring on the distribution and susceptibility status is essential for early detection on the insecticide resistance development, and to ensure the efficacy of the insecticides in vector control programme which allows appropriate solution can be implemented when insecticide resistance is identified.

5.8 The Way Towards Integrated Vector Managemant (IVM)

Apart from previously stated suggestions for regular surveillance and monitoring on mosquitoes distribution and susceptibility status, source reduction remains the ideal approach in vector control strategies. Pocquet et al. (2014) noted that the elimination of breeding habitats was the crucial tool for mosquito control in Mayotte while insecticide application was rarely performed. However, source reduction is a labour-intensive and time consuming approach. Hence, by raising public awareness and encouraging the use of personal protection measures can reduce the exposure to mosquito bites. Moreover, cooperation between local authorities and community can also be further established. A

success scenario was presented by Professor Dina Fonseca in which engaging community members to perform mosquito control in their yards (Corbel et al., 2019). By the interactions betweem residents and scientist through exchange of data and results can provide immediate results at the end of the season. Subsequently, this can help to maintain interest and enthusiasm in reducing the mosquito populations amongst residents. There was a significant decrease of female *Ae. albopictus* in the community from their result and proved that community-based approach can play an important role in IVM.

According to WHO (2017), WHO Global Vector Control Response (GVCR) strategies need to consist 5 key areas in order to reduce the threat of vector borne diseases. The key components are (i) engaging communities and build resilience against future disease outbreaks, (ii) enhancing the surveillance for early warning response, (iii) aligning actions across sectors, such as ministries of health and other relevant ministries and city planner, (iv) scaling-up vector control tools in combination to optimum its impact on disease, and (v) monitoring and evaluating the implemented control program. In order to facilitate more effective IVM, information sharing among the responsible local authorities would ease the investigation of resistance development and prediction of disease outbreak. Malaria control has been implemented since 1952 in Sarawak with the commencement of Sarawak Malaria Pilot Project (Zulueta & LaChance, 1956), in conjunction with dengue outbreak. The dosage and types of insecticide applied in both IVM would be difference and may eventually lead to the development of cross-resistance. With better communication between concurrent IVM, cross-resistance of similar type insecticide can be avoid and indirectly reduce the cost burden to the country economy. One example from our study was the resistance of chlopyrifos by *Ae. albopictus* larvae. As mentioned before that chlopyrifos was not applied in local IVM but in agricultural sector since the chemical residue was found in vegetable. Both

Ministry of Health and Ministry of Agriculture and Food Industries should align in actions to review the application of current insecticides in order to prevent further resistance development in either targeted pest as we are running out of control choices.

Integrated management in conjunction of new vector tools such as *Wolbachia* infected mosquitoes, sterile insect technique (SIT), biological control agent (e.g. *Bacillus thuringiensis* var, *israelensis*, Bti) and other non-chemical approaches should be considered to maximize the effectiveness of the control programs. For instance, introduction of *Wolbachia* infected mosquitoes into wild population is now a replacement strategy with trials now conducted in 11 countries (Corbel et al., 2019). A pilot studies conducted in Townsville and Cairns, Australia showed promising results with this method (O'Neil et al., 2018). Another positive result was obtained in Singapore in which 50% suppression of the urban *Ae. aegypti* mosquito population achieved after the deployment of *Wolbachia*-infected *Ae. aegypti* (Corbel et al., 2019). To ensure the effectiveness and achievement of vector control, application of insecticide should not be the sole approach in managing dengue vector owing to the side effect of resistance development and contamination to environment.

CHAPTER 6: CONCLUSION

In order to obtain optimum results from an integrated vector management (IVM) programme, it is important to understand the information about the targeted vector. Thus, the population profile and resistance status of the Sarawak *Ae. albopictus* can provide a baseline information for local authorities. From the study, the *Ae. albopictus* was the predominant dengue vector in residential areas in Sarawak. Unlike in Peninsular Malaysia, *Ae. aegypti* was absent in ovitrap surveillance conducted in Sarawak. Mixed breeding of *Ae. albopictus* and *Armigeres* species was found in several residential areas in Sarawak. Moreover, the genetic diversity of *Ae. albopictus* populations in Sarawak was low due to high gene flow. Another factor was the populations had experienced a history of expansion and bottleneck effect. A total of 22 haplotypes was discovered in the Sarawak populations.

Chemical control continues to be the preferred approach in Malaysia. However, unplanned and extensive usage of insecticide can induce the development of insecticide resistance. Thus, understanding of current resistance status of *Ae. albopictus* can help to formulate a better IVM. Among 11 tested adulticides in adult bioassay, only cyfluthrin was effective to control *Ae. albopictus* in Sarawak. The susceptibility status of 11 tested adulticides decreases in the following order: cyfluthrin, dieldrin > fenitrothion > deltametthin > DDT > etfenrpox > permetrhin > bendiocarb > malathion > lambda-cyhalotrhin > propoxur. Cross resistances in adult *Ae. albopictus* were found within pyrethroid (i.e., deltamethrin and lambda-cyhalothrin, deltamethrin and permethrin, permethrin and etofenprox) and carbamates (i.e., bendiocarb and propoxur) classes. Furthermore, Cross resistances were also detected between different classes of adulticides in adult *Ae. albopictus* (i.e., malathion and deltamethin, malathion and bendiocarb, malathion and propoxur, bendiocarb and deltamethrin). On the other hand,

the larval bioassay revealed that *Ae. albopictus* larvae from Sarawak was completely susceptible to bromophos and temephos, but highly resistant to DDT, chlorpyrifos and malathion (mortality < 20%), indicating bromophos and temephos are still effective for *Ae. albopictus* larval control in Sarawak. Cross resistances were also found in *Ae. albopictus* larvae (i.e. fenitrothion vs fenthion, fenitrothion vs dieldrin, and fenthion vs dieldrin). Regarding to development of insecticide resistance, enzymes are playing an important role in the detoxification mechanism of *Ae. albopictus*. From the results, acetylcholinsterase was insensitive to propoxur in adult stage of Sarikei population and larval stage of Dalat population. Mixed function oxidases (MFO) was involved in fenitrothion resistant among the *Ae. albopictus* larvae populations in Sarawak. However, from the study, MFO, non-specific esterases (α- and β-) and glutathione-S-transferases (GST) were not responsible for insecticide resistant among the *Ae. albopictus* populations in Sarawak.

To prevent further development of insecticide resistance, alternative approaches or chemical choices should be made. With regard to bioefficacy of IGRs, Sarawak populations of *Ae. albopictus* were susceptible to methoprene, pyriproxyfen, cyromazine and novaluron, except diflubenzuron Sarawak populations of *Ae. albopictus* were susceptible to methoprene, pyriproxyfen, cyromazine and novaluron, except diflubenzuron. Although IGRs can induce negative effect on the non-targeted aquatic arthropod, IGRs exhibited promising results. With proper planned control program integrated with IGRs, the chemicals can be used as alternative control agents against field populations of *Ae. albopictus* in Sarawak, Malaysia. Regular resistance surveillance needs to be conducted since an early detection on the insecticide resistance development allows appropriate solution to be implemented in a timely manner.

REFERENCES

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

Ali, A., Chowdhury, M. A., Hossain, M. I., Mahmud-Ul-Ameen, Habiba, D. B., & Aslam, A. F. (1999). Laboratory evaluation of selected larvicides and insect growth regulators against field collected *Culex quenqufasciatus* larvae from urban Dhaka, Bangladesh. *Journal of the American Mosquito Control Association*, *15*, 43–47.

Ali, A., Nayar, J. K., & Xue, R. D. (1995). Comparative toxicity of selected larvicides and insect growth regulators to a Florida laboratory population of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, *11*, 72–76.

Alvarez, L. C., Ponce, G., Oviedo, M., Lopez, B., & Flores, A.E. (2013). Resistance to malathion and deltamethrin in *Ae. aegypti* (Diptera: Culicidae) from Western Venezuela. *Journal of Medical Entomology*, *50*(5), 1031–1039.

Amelia-Yap, Z. H., Sofian-Azirun, M., Chen, C. D., Suana, I. W., Lau, K. W., Elia-Amira, N. M. R., Haziqah-Rashid, A., Tan, T. K., Lim, Y. A. L., & Low, V. L. (2019). Pyrethroids use: threats on metabolic-mediated resistance mechanisms in the primary dengue vector *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, *56*, 811–816.

Amin, A. M., & Hemingway, J. (1989). Preliminary investigation of the mechanisms of DDT and pyrethroid resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae) from Saudi Arabia. *Bulletin of Entomological Research*, *79*, 361–366.

Ang, K. T., & Singh, S. (2001). Epidemiology and new initiatives in the preventive and control of dengue in Malaysia. *Dengue Bulletin*, *25*, 7–14.

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

Azrizal-Wahid, N., Sofian-Azirun, M., & Low, V. L. New insights into the haloptype diversity of the cosmopolitan cat flea *Ctenocephalides felis* (Siphonaptera: Pulicidae). *Veterinary Parasitology*, *281*, Article#109102. DOI:10.1016/j.vetpar.2020.109102

Barrera, R. (1996). Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecology of Entomology*, *21*, 112–127.

Baruah, I., & Das, S. C. (1996). Evaluation of Methoprene (Altosid) and Diflubenzuron (Dimilin) for control of mosquito breeding in Tezpur (Assam). *Indian Journal of Malariology*, *33*, 61–66.

Battaglia, V., Gabrieli, P., Brandini, S., Capodiferro, M. R., Javier, P. A., Chen, X. G., Achilli, A., Semino, O., Gomulski, L. M., Malacrida, A. R., Gasperi, G., Torroni, A., & Olivieri, A. (2016). The worldwide spread of the tiger mosquito as revealed by Mitogenome Haplogroup diversity. *Frontier in Genetics*, *23*,7(e2361) DOI: 10.3389/fgene.2016.00208.

Beebe, N. W. (2018). DNA barcoding mosquitoes: advice for potential prospectors. *Parasitology*, *145*, 622–633.

Bisset, J. A., Rodríguez, M. M., French, L., Severson, D. W., Gutiérrez, G., Hurtado, D., & Fuentes, I. (2014). Insecticide resistance and metabolic mechanisms involved in larval and adult stages of *Aedes aegypti* insecticide-resistant reference strains from Cuba. *Journal of American Mosquito Control Association*, *30*(4), 298–304.

Blus, L. J. (2003). Chapter 13 Organochlorine Pesticides. In: Hoffman, D. J., Rattner, B. A., Burton, G. A. & Carins, J. (Eds.), *Handbook of Ecotoxicology* (2nd ed.) (pp. 314–329). Lewis publishers, CRC press company.

Boromisa, R. D., Rai, K. S., & 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.

Bortolus, A. (2008). Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in Ecology. *AMBIO: A Journal of The Human Environment*, *37*(2), 114–118.

Brengues, C., Hawkes, N. J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., Manguin, S., Morgan, J. C., & Hemingway, J. (2003). Pyrethriond and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Medical and Veterinary Entomology*, *17*(1), 87–94.

Brogdon, W. G., Hobbs, J. H., St Jean, Y., Jacques, J. R., & Charles, L. B. (1988). Microplate assay analysis of reduced fenitrothion susceptibility in Haitian *Anopheles albimanus*. *Journal of the American Mosquito Control Association*, *4*(2), 152–158.

Brogdon, W. G., McAllister, J. G., & Vulule, J. (1997). Heme peroxide activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *Journal of the American Mosquito Control Association*, *13*, 233–237.

Brooke, B. D., Kloke, G., Hunt, R. H., Koekemoer, L. L., Temu, E. A., Taylor, M. E., Small, G., Hemingway, J., & Coetzee, M. (2001). Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). *Bulletin of Entomological Research*, *91*(4), 265–272.

Brown, T. A. (1999). Chapter 1 What is a genome. In: *Genomes* (pp. 2–12). John Wiley & Sons (Asia) Pte. Ltd.

Buddle, R. (1928). Entomological notes on the Canton Delta. *Journal of the Royal Naval Medical Service*, *14*, 190–200.

Burgess, N. R. H. & 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.

Casida, J. E., Gammon, D. W., Glickman, A. H., & Lawrence, L. J. (1983). Mechanisms of selective action of pyrethroid insecticides. *Annual Review Phormacology and Toxicology*, *23*, 413–438.

Castella, V., Ruedi, M., Excoffier, L., Ibáñez, C., Arlettaz, R., & Hausser, J. (2000). Is the Gibraltar Strait a barrier to gene flow fo the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? *Molecular Ecology*, *9*, 1761–1772.

Cetin, H., Erler, F., & 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.

Chan, A., Chiang, L., Hapuarachchi, H. C., Tan, C., Pang, S., Lee, R., Lee, K., Ng, L., & Lam-Phua, S. (2014a). DNA barcoding: complementing morphological identification of mosquito species in Singapore. *Parasites & Vectors*, *7*, Article#569.

Chan, H. H., & Jaal, Z. (2013). Permethrin resistance in *Aedes albopictus* (Diptera: Culicidae) and associated fitness costs. *Journal of Medical Entomology*, *50*(2), 362–370.

Chan, H. H., Wajidi, M. F. F., & Jaal, Z. (2014b). Molecular cloning and xenobiotic induction of seven novel cytochrome P450 monooxygenases in *Aedes albopictus*. *Journal of Insect Science*, *14*, Article#163.

Chan, K. L., Chan, Y. C., & Ho, B.C. (1971). *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 4. Competition between species. *Bulletin of World Health Organizatoin*, *44*, 643–649.

Chang, M. S., & Jute, N. (1982). Distribution and density of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Sarawak. *Medical Journal of Malaysia*, *37*(3), 205–210.

Chang, M. S., & Jute, N. (1994). Breeding of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in urban housing of Sibu town, Sarawak. *Southeast Asian Journal of Tropical Medicine and Public Health*, *25*(3), 543–548.

Chang, M. S., Rubins, P., Jute, M., & Lim, T. W. (1981). Entomological aspects of endemic dengue fever in Sarawak 1973–1980. *Medical Journal of Malaysia*, *36*, 79–82.

Chareonviriyaphap, T., Aum-aung, B., & Ratanatham, S. (1999). Current insecticide resistance pattern in mosquito vectors in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *30*, 184–194.

Che-Mendoza, A., Penilla, R. P., & Rodriguez, D. A. (2009). Insecticide resistance and glutathione S-transferases in mosquitoes: A review. *African Journal of Biotechnology*, *8*(8), 1386–1397.

Chen, C. D., Andy-Tan, W. A., Loke, S. R., Lee, H. L., Yasmin, A. R., & Sofian-Azirun, M (2008a). Effectiveness of pyriproxyfen-controlled release block against larvae of *Aedes* (Stegomyia) *aegypti* in Kuala Lumpur, Malaysia. *Dengue Bulletin*, *32*, 119–206.

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

Chen, C. D., Low, V. L., Lau, K. W., Lee, H. L., Nazni, W. A., Heo, C. C., Azidah, A. A., & Sofian-Azirun, M. (2013a). First report on adulticide susceptibility status of *Aedes albopictus*, *Culex quinquefasciatus*, and *Culex vishnui* from a pig farm in Tanjung Sepat, Selangor, Malaysia. *Journal of the America Mosquito Control Association*, *29*, 243–250.

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., & 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., Norma-Rashid, Y., Lardizabal, M. L., & Sofian-Azirun, M. (2013b). Temephos resistance in field *Aedes (Stegomyia) albopictus* (Skuse) from Selangor, Malaysia. *Tropical Biomedicine*, *30*(2), 220–230.

Chen, C. D., Nazni, W. A., Lee, H. L., Seleena, B., & Sofian-Azirun, M. (2008b). Biochemical detection of temephos resistance in *Aedes (Stegomyia) aegypti* (Linnaeus) from dengue-endemic areas of Selangor State, Malaysia. *Proceeding of the 3rd ASEAN Congress of Tropical Medicine and Parasitology 2008*, pp. 6–20.

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., & Lee, H. L. (2008c). 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.

Chevillon, C., Raymond, M., Guillemaud, T., Lenormand, T., & Pasteur, N. (1999). Population genetics of insecticide resistance in mosquito *Culex pipiens*. *Biological Journal of the Linnean Society*, *68*, 147–157.

Chism, B. D., & Apperson, C. S. (2003). Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Medical and Veterinary Entomology*, *17*, 211–220.

Chow, Y. S., & 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.

Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: estimating gene genealogies. *Proceeding of 16th International Parallel and Distributed Processing Symposium*, *2*, 184.

Coats, J. R. (1990). Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. *Environmental Health Perspectives*, *87*, 255–262.

Corbel. V., Durot, C., Achee, N. L., Chandre, F., Coulibaly, M. B., David, J. P., Devine, G. J., Dusfour, I., Fonseca, D. M., Griego, J., Jumtarajumnong, W., Lenhart, A., Kasai, S., Martins, A., Moyes, C., Ng, L. C., Pinto, J., Pompon, J. F., Raghavendra, K., Roiz, D., Vatandoost, H., Vontas, J., & Weetman, D. (2019). Second WIN International Conference on "Integrated approaches and innovative tools for combating insecticide resistance in vectors of arboviruses", October 2018, Singapore. *Parasites & Vectors*, *12*, 331.

Costa, L. G. (2006). Current issues in organophosphate toxicology. *Clinica Chimica Acta, 366*, 1–13. DOI: 10.1016/j.cca.2005.10.008

Cui, F. Raymond, M., Berthomieu, A., Alout, H., Weill, M., & Qiao, C. L. (2006). Recent emergence of insensitive acetylcholinesterase in Chinese populations of *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, *43*, 879–883.

Cywinska, A., Hunter, F. F., & Hebert, P. D. (2006). Identifying Canadian mosquito species through DNA barcodes. *Medical and Veterinary Entomology*, *20*(4), 413–424.

Damásio, J., Guilhermino, L., Soares, A. M. V. M., Riva, C. M., & Barata, C. (2007). Biochemical Mechanisms of Resistance *Daphnia Magna* exposed to the insecticide fenitrothion. *Chemosphere*, *70*(1), 74–82.

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

Delatte, H., Desvars, A., Bouétard, S. Bord, Gimonneau, G., Vourc'h, G., & Fontenille, D. (2008). Blood-feeding behaviour of *Aedes albopictus*, vector of chikungunya on La Réunion. *Vector Born Zoonotic Diseases*, *8*, 25–34.

Delatte, H., Toty, C., Boyer, S., Bouetard, A., Bastien, F., & Fontenille, D. (2013). Evidence of habitat structuring *Aedes albopictus* populations in Reunion Island. *PLoS Neglected Tropical Diseases*, *7*, Article#e2111.

Ding, Y., Ortelli, F., Rossiter, L. C., Hemingway, J., & Ranson, H. (2003). The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics*, *4*, Article#35.

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). pp. 1–10.

Eichler, D. (1972). Bromophos and bromophos ethyl residues. *Residue Reviews*, *41*, 65–112.

Elder, J. P., & Lloyd, L. (2007). Achieving behavior change for dengue control: methods, scaling-up and sustainability. In: *Report of the Scientific Working Group on Dengue 2006* (pp.140–149). WHO, Geneva.

Elia-Amira, N. M. R., Chen, C. D., Low, V. L., Lau, K. W., Haziqah-Rashid, A., Amelia-Yap, Z. H., Lee, H. L., & Sofian-Azirun, M. (2019). Adulticide resistance status of *Aedes albopictus* (Diptera: Culicidae) in Sabah, Malaysia: A statewide assessment. *Journal of Medical Entomology*, *56*(6), 1715–1725.

Estrada, J. G., & 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: Beaty, B. J. and Marquardt, W. C. (Eds.), *The biology of disease vectors* (pp. 512–529). University Press of Colorado, Niwot, Colorado.

Finney, D. J. (1971). Probit analysis. Cambrige University Press, Cambridge, United Kingdom.

Fishel, F. M. (2011). Pesticide toxicity profile: carbamate pesticides. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville. pp 1–51.

Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiling and background selection. *Genetics*, *147*, 915–925.

Gao, Q., Beebe, N., & Cooper, R. (2004). Molecular identification of the malaria vectors *Anopheles anthropophagus* and *Anopheles sinensis* (Diptera: Culicidae) in central China using polymerase chain reaction and appraisal of their position within the *Hyrcanus Group*. *Journal of Medical Entomology*, *41*(1), 5–11.

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

Georghiou, G. P., Metcalf, R. L., & Gidden, F. E. (1966). Carbamate resistance in mosquitos. Selection of *Culex pipiens fatigans* Wiedemann (=*C. quinquefasciatus* Say) for resistance to Baygon. *Bulletin of World Health Organization*, *35*, 691–708.

Georghiou, G. P., Wirth, M., Saume, F., &d 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.

Grant, D. F., Dietze, E. C., & Hammock, B. D. (1991). Glutathione S-transferase isozymes in *Aedes aegypti*: purification, characterization, and isozyme-specific regulation. *Insect Biochemistry*, *21*, 421–433.

Gratz, N. G. (2004). Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, *18*(3), 215–227.

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

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.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*: 95–98.

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., & 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., & 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.

Harvie, S., Nor-Aliza, A. R., Lela, S., & Razitasham, S. (2020). Detection of dengue virus serotype 2 (DENV-2) in population of *Aedes* mosquitoes from Sibu and Miri divisions of Sarawak using reserve transcription polymerase chain reaction (RT-PCR) and semi-nested PCR. *Tropical Biomedicine*, *37*(2), 258–272.

Hatakoshi, M., Kawada, H., Nishida, S., Kisida, H., & 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*, *1*, 1–39.

Hebert, P., Cywinska, A., Ball, S., & deWaard, J. (2003). Biological identifications through DNA barcodes. *Proceedings of The Royal Society B*, *270*(1512), 313–321.

Hemingway, J., & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, *45*, 371–391.

Hemingway, J., Hawkes, N. J., McCarroll, L., & Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology*, *34*, 653–665.

Hendrick, C. A., Staal, G. B., & 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.

Hidayati, H., Nazni, W. A., Lee, H. L., & Sofian-Azirun, M. (2011). Insecticide resistance development in *Aedes aegypti* upon selection pressure with malathion. *Tropical Biomedice*, *28*, 425–437.

Hii, J. (1977). A re-survey of potential vectors of dengue fever and dengue haemaorrhagic fever in Sabah. *Medical Journal of Malaysia*, *32*, 193–196.

Hollingworth, R. M., & Dong, K. (2008). The biochemical and molecular genetic basis of resistance to pesticides in arthropods. In: Whalon M. E., Mota-Sanchez, Hollingworth, R. M., (Eds), *Global pesticide resistance in arthropods* (pp. 68). USA: CAB International. pp68.

IPCS (International Programme on Chemical Safety). (1990). Environmental Health Criteria 97, Deltamethrin. World Health Organization, Geneva.

(IRAC) Insecticide Resistance Action Committee. 2017. IRAC mode of action classification scheme, version 8.3 ed. IRAC.

Ishak, I. H., Jaal, Z., Ranson, H., & Wondji, C. S. (2015). Contrasting patterns of insecticide resistance and knockdown resistance (*kdr*) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasites and Vectors*, *8*, 181. DOI: 10.1186/s13071-015-0797-2.

Ishak, I. H, Riveron, J. M., Ibrahim, S. S., Stott, R., Longbottom, J., Irving, H., & Wondji, C.S.. (2016). The cytochrome P450 gene *CYP6P12* confers pyrethroid resistance in *kdr*-free Malaysian populations of the dengue vector *Aedes albopictus*. *Scientific Reports*, *6*, Article#24707.

Ismail, N., Dom, N. C., Ismail, R., Ahmad, A. H., Zaki, A., & Camalxaman, S. N. (2015). Mitochondrial cytochrome oxidase I gene sequence analysis of *Aedes albopictus* in Malaysia. *Journal of the American Mosquito Control Association*, *31*(4), 305–312.

Itoh, T. (1981). Field application of biologically active substances of insects, a juvenile hormone analogue and a chitin synthesis inhibitor against mosquito larvae. *Tropical Medicine*, *21*, 73–84.

Itoh, T., Kawada, H., Abe, A., Eshita, Y., Rongsriyam, Y., & Igarashi, A. (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.

Jackson, C. J., Liu, J. W., Carr, P. D., Younus, F., Coppin, C., Meirelles, T., Lethier, M., Pandey, G., Ollis, D. L., Russell, R. J., Weik, M., & Oakeshott, J. G. (2013). Stucture and function of an insect α-carboxylesterase (α*Esterase7*) associated with insecticide resistance. *Proceedings of the National Academy of Sciences*, *110*(25), 10177–10182.

Jayarai, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology*, *9*, 90–100.

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

Kamal, H. A., & Khater, E. I. (2010). The biological effects of the insect growth regulators; pyriproxyfen and diflubenzuron on the mosquito *Aedes aegypti*. *Journal of the Egyptian Society of Parasitology*, *40*, 565–574.

Kamgang, B., Brengues, C., Fontenille, D., Njiokou, F., Simard, F., & Paupy, C. (2011). Genetic structure of the tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). *PLoS One*, *6*, Article#e20257. DOI:10.1371/journal.pone.0020257.

Kamgang, B., Ngoagouni, C., Manirakiza, A., Nakouné, E., Paupy, C., & Kazanji, M. (2013). Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. *PLoS Neglected Tropical Diseases*, *7*, Article#e2590. DOI: 10.1371/journal.pntd.0002590.

Karunaratne, S. H. (1999). Insecticide cross-resistance spectra and underlying resistance mechanisms of Sri Lankan anopheline vectors of malaria. *Southeast Asian Journal of Tropical Medicine and Public Health*, *30*(3), 460–469.

Karunaratne, S. H. P. P., Damayanthi, B. T., Fareena, M. H. J., Imbuldeniya, V., & Hemingway, J. (2007). Insecticide resistance in the tropical bedbug *Cimex hemipterus*. *Pesticide Biochemistry and Physiology, 88*, 102–107.

Karunaratne, S. H. P. P., & Hemingway, J. (1998). Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical Veterinary and Entomology*, *12*, 1–12.

Kasai, S., Komagata, O., Itokawa, K., Shono, T., Ng, L. C., Kobayashi, M., & Tomita, T. (2014). Mechanisms of pyrethroid resistance in dengue mosquito vector, *Aedes aegypti*: Target site insensitivity, penetration and metabolism. *PLoS Neglected Tropical Diseases*, *8*(6), Article#e2948.

Kasai, S., Ng, L. C., Lam-Phua, S. G., & Tang, C. S. (2011). First detection of a pupative knockdown resistance gene in major mosquito vector, *Aedes albopictus*. *Japanese Journal of Infectious Diseases*, *64*, 217–221.

Kasai, S., Weerasinghe, I. S., & Shono, T. (1998). P450 Monooxygenases are an important mechanism of permethrin resistance in *Culex quinquefasciatus* say larvae. *Archives of Insect Biochemistry and Physiology*, *37*(1), 47–56.

Kawada, H., Oo, S. Z. M., Thaung, S., Kawashima, E., Maung, Y. N. M., Thu, H. M., Thant, K. Z., & Minakawa, N. (2014). Co-occurrence of point mutations in the voltage-gated sodium channel of pyrethroid-resistant *Aedes aegypti* populations in Myanmar. *PLoS Neglected Tropcial Diseases*, *8*(7), Article#e3032.

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

Kim, J. H., & Smith, A. (2001). Distribution of organochlorine pesticides in soils from South Korea. *Chemosphere, 43*(2), 137–140.

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.

Koopman, W. J. M., Li, Y-H., Coart, E., Van De Weg, W. E., Vosman, B., Roldan-Ruiz, I., & Smulders, M. J. M. (2007). Linked vs. unlinked markers: multilocus microsatellite haplotype-sharing as a tool to estimate gene flow and introgression. *Molecular Ecology*, *16*(2), 243–256.

Koou, S. Y., Chong, C. S., Vythilingam, I., Ng, L. C., & Lee, C. Y. (2014). Pyrethroid resistance in *Aedes aegypti* larvae (Diptera: Culicidae) from Singapore. *Journal of Medical Entomology*, *51*(1), 170–181.

Kumar, N. P., Rajavel, A. R., Natarajan, R., & Jambulingam, P. (2007). DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, *44*, 1–7.

Lai, T. P., Rosinah, Y., & 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 the 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., & Sampson, I.A. (2001). Chikungunya infection – an emerging disease in Malaysia. *Southeast Asian Journal of Tropical Medisine 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., & Oouchi, H. (1990). Formulation of pyriproxyfen, a juvenile hormone mimic, for tsetse control. *Medical and Veterinary Enotomology*, *4*, 127–133.

Lau, K. W., Chen. C. D., Lee, H. L., Izzul, A. A., Asri-Isa, M., Zulfadli, M., & Sofian-Azirun, M. (2013). Vertical distribution of *Aedes* mosquitoes in multiple storey buildings in Selangor and Kuala Lumpur, Malaysia. *Tropcial Biomedicine*, *30*(1), 36–45.

Lau, K. W., Chen, C. D., Lee, H. L., & Sofian-Azirun, M. (2015). Evaluation of insect growth regulators, temephos and *Bacillus thuringiensis israelensis* against *Aedes aegypti* (*L.*) in plastic containers. *Tropical Biomedicine*, *32*, 1–9.

Lau, K. W., Chen, C. D., Lee, H. L., Low, V. L., Moh, H. H., & Sofian-Azirun, M. (2017). Ovitrap surveillance in Sarawak, Malaysia: A comprehensive study. *Tropical Biomedicine*, *34*(4), 795–803.

Lee, C. Y. (2002). Subterranean termite pests and their control in the urban environment in Malaysia. *Sociobiology*, *40*(1), 3–9.

Lee, H. L. (1990). A rapid and simple biochemical method for the detection of insecticide resistance due to elevated esterase activity in mosquito larvae of *Culex quinquefasciatus*. *Tropical Biomedicine*, *7*, 21–28.

Lee, H. L. (1991). A nationwide resurvey of the factors affecting the breeding of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) in urban towns of peninsular Malaysia 1988 – 1989. *Tropical Biomedicine*, *8*, 185–189.

Lee, H. L. (1992a). *Aedes* ovitrap and larval survey in several suburban communities is Selangor, Malaysia. *Mosquito-Borne Diseases Bulletin*, *9*, 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. (2000). *Aedes*: mosquitoes that spread dengue fever. In: F.S.P. Ng & Yong H.S. (Eds.) (pp. 45–61), *Mosquitoes and mosquito-borne diseases*. Malaysia: Academy of Sciences Malaysia.

Lee, H. L. (2005). Germ warfare against mosquitoes. What now? In: Proceedings of the Fifth International Conference on Urban Pests.

Lee, H. L., Asikin, N., Nazni, W.A., & 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., & Chong, W.L. (1995). Glutathion S-Transferase activity and DDT-susceptiblity of Malaysian mosquitos. *Southeast Asian Journal of Tropical Medicine and Public Health*, *26*(1), 164–167.

Lee, H. L., & Hishamudin, M. (1990). Nationwide *Aedes* larvae survey in urban towns of peninsular Malaysia (1988–89). *Tropical Biomedicine*, *7*, 185–188.

Lee, R. M. L., Choong, C. T. H., Goh, B. P. L., Ng, L.C., & Lam-Phua, S. G. (2014). Bioassay and biochemical studies of the status of pirimiphos-methyl and cypermethrin resistance in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Singapore. *Tropical Biomedicine*, *31*(4), 670–679.

Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116.

Leong, C. S., Vythilingam, I., Liew, J. W. K., Wong, M. L., Wan Sulaiman, W. Y., & Lau, Y. L. (2019). Enzymatic and molecular characterization of insecticide resistance mechanisms in field populations of *Aedes aegypti* from Selangor, Malaysia. *Parasites and Vectors*, *12*, 236.

Librado, P., & Rozas, J. (2009). DNASP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452.

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

Linnaeus, C. (1758). Systema Nature, Editio decimal. *Laur. Salvius*.

Liu, H, Cupp, E. W., Guo, A., & Liu, N. (2004). Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *Journal of Medical Entomology*, *41*(5), 946–952.

Liu, H., Xu, Q., Zhang, L., & Liu, N. (2005). Chlorpyrifos resistance in mosquito *Culex quinquefasicatus*. *Journal of Medical Entomology*, *42*(5), 815–820.

Loh, P. Y., & 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.

Loke, S. R., Andy-Tan, W. A., Benjamin, S., Lee, H. L., & Sofian-Azirun, M. (2010). Susceptibility of field-collected *Aedes aegypti* (L.) (Diptera: Culicidae) to *Bacillius thuringiensis israelensis* and temephos. *Tropical Biomedicine*, *27*(3), 493–503.

Loke, S. R., Andy-Tan, W. A., Nazni, W. A., Lee, H. L., & Sofian-Azirun, M. (2012). Insecticide susceptibility status of field-collected *Aedes (Stegomyia) aegypti* (L.) at a dengue endemic site in Shah Alam, Selangor, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health, 43*(1), 34–47.

Low, V. L., Chen, C.D., Lee, H.L., Lim, P.E., Leong, C.S. & Sofian-Azirun, M. (2013a). Current susceptibility status of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae) against DDT, propoxur, malathion and permethrin. *Journal of Medical Entomology*, *50*, 103–111.

Low, V. L., Chen, C. D., Lee, H. L., Tan, T. K., Chen, C. F., Leong, C. S., Lim, Y. A. L., Lim, P. E., Norma-Rashid, Y., & Sofian-Azirun, M. (2013b). Enzymatic characterization of insecticide resistance mechanisms in field populations of Malaysian *Culex quinquefasciatus* Say (Diptera: Culicidae). *PLoS ONE*, *8*(11), Article#e79928.

Low, V. L., Lim, P. E., Chen, C. D., Lim, Y. A. L., Tan, K. T., Norma-Rashid, Y., Lee, H. L., & Sofian-Azirun, M. (2013c). Mitochondrial DNA analyses reveal low genetic diversity in *Culex quinquefasciatus* from residential areas in Malaysia. *Medical and Veterinary Entomology*. DOI: 10.1111/mve.12022.

Low, V. L., Takaoka, H., Pramual, P., Adler, P. H., Ya'cob, Z., Chen, C. D., Yotopranoto, S., Zaid, A., Hadi, U. K., Lardizabal, M. L., Nasruddin-Roshidi, A., & Sofian-Azirun, M. (2016). Three taxa in one: Cryptic diversity in the black fly *Simulium nobile* (Diptera: Simuliidae) in Southeast Asia. *Journal of Medical Entomology*, *53*(4), 972–976.

Low, V. L., Tan, T. K., Prakash, B. K., Vinnie-Siow, W. Y., Tay, S. T., Masmeatathip, R., Hadi, K. U., Lim, Y. A. L., Chen, C. D., Norma-Rashid, Y., & Sofian-Azirun, M. (2017). Contrasting evolutionary patterns between two haplogroups of *Haematobia exiya* (Diptera: Muscidae) from the mainland and islands of Southeast Asia. *Scientific Reports*, *7*, 5871. DOI: 10.1038/s41598-017-05921-w

Low, V. L., Vinnie-Siow, W. Y., Lim, Y. A. L., Tan, T. K., Leong, C. S., Chen, C. D., Azidah, A. A., & Sofian-Azirun, M. (2015). First molecular genotyping of A302S mutation in the gamma aminobutyric acid (GABA) receptor in *Aedes albopictus* from Malaysia. *Tropical Biomedicine*, *32*, 554–556.

Lowe, A., Harris, S., Harris, S. E., & Ashton, P. (2004). *Ecological genetics design, analysis, and application.* Oxford, United Kingdom: Blackwell Science Ltd.

Lutz, A., & Machado, A. (1915). A journey on the S. Francisco River and on some of its tributaries between Pirapora and Joazeiro. *Memorias do Instituto Oswaldo Cruz, 7*, 5–50.

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

Macdonald, W. W., Smith, C. E. G., Dawson, P. S., Ganapathipillai, A., and Mahadevan, S. (1967). Arbovirus infections in Sarawak: Further observations on mosquitoes. *Journal of Medical Entomology*, *4*, 146–157.

Macdonald, W. W., Smith, C. E. G., & Webb, H. E. (1965). Arbovirus infections in Sarawak: Observations on the mosquitoes. *Journal of Medical Entomology*, *1*, 335–347.

Macdonald, W. W., & Rajapaksa, N. (1972). A survey of the distribution and relative prevalence of *Aedes aegypti* in Sabah, Brunei and Sarawak. *Bulletin of World Health Organization*, *46*, 203–209.

Macfie, J. W. S., & Ingram, A. (1916). The domestic mosquitoes of Accra. *Bulletin of Entomological Research, 7*, 162.

Mahadevan, S., & Cheong, W. H. (1974). Chart to the identification of *Aedes* (*Stegomiya*) group and pictorial key to *Mansonia* (Mansonidae). Kuala Lumpur: Institute for Medical Research.

Maia, R. T., Scarpassa, V. M., Maciel-Litaiff, L. H., & Tadei, W. P. (2009). Reduced levels of genetic variation in *Aedes albopictus* (Diptera: Culicidae) from Manaus, Amazonas State, Brazial, based on analysis of the mitochondrial DNA *ND5* gene. *Genetics and Molecular Research*, *8*(3), 998–1007.

Malavige, G. N., Fernando, S., Fernando, D. J., & Seneviratne, S. L. (2004). Dengue viral infections. *Postgraduate Medical Journal*, *80*, 588–601.

Malcolm, C. A., Bourguet, D., Ascolillo, A., Rooker, S. J., Garvey, C. F., Hall, L. M. C., Pasteur, N., & Raymond, M. (1998). A sex-linked *Ace* gene, not linked to insensitive acetylcholinesterase-mediated insecticide resistance in *Culex pipiens*. *Insect Molecular Biology*, *7*(2), 107–120.

Manni, M., Gomulski, L. M., Aketatawong, N., Tait, G., Scolari, F., Somboon, P., Guglielmino, C. R., Malacrida, A. R., & Gasperi, G. (2015). Molecular markers for analyses of intraspecific genetic diversity in the Asian Tiger mosquito, *Aedes albopictus*. *Parasites & Vectors*, *8*, 188.

Manni, M., Guglielmino, C. R., Scolari, F., Vega-Rúa, A., Failloux, A. B., Somboon, P., Lisa, A., Savini, G., Bonizzoni, M., Gomulski, L. M., Malacrida, A. R., & Gasperi, G. (2017). Genetic evidence for a worldwide chaotic dispersion pattern of the arbovirus vector, *Aedes albopictus*. *PLoS Neglected Tropical Diseases*, *11*(1), Article#e0005332. DOI: 10.1371/journal.pntd.0005332.

Marcombe, S., Poupardin, R., Darriet, F., Reynaud, S., Bonnet, J., Strode, C., Brengues, C., Yébakima, A., Ranson, H., Corbel, V., & David J.P. (2009). Exploring the molecular basis of insecticide resistance in the dengue vector *Aedes aegypti*: A case study in Martinique Island (French West Indies). *BMC Genomics*, *10*, Article#494.

Marcombe, S., Darriet, F., Agnew, P., Etienne, M., Yp-Tcha, M. M., Yebakima, A., & Corbel, V. (2011). Field efficacy of new larvicide products for control of multi-resistant *Aedes aegypti* populaitons in Martinique (French West Indies). *American Journal of Tropical Medicine and Hygiene*, *84*, 118–126.

Margaret, A., & Chai, L. K. (2007). Pesticide residues in local vegetables in Sarawak is under control: Trend analysis from 2000 to 2006. Proceeding of Senior Officers' Conference. 11 – 14 Dec 2007, Kuching. Department of Agriculture, Sarawak. pp. 398–407.

Marrs, T. C. (2001). Chapter 1 Organophosphates: History, Chemistry, Pharmocology. In: Karalliedde, L., Feldman, S., Henry, J., & Marrs, T. (Eds.), *Organophosphates and Heath* (pp. 1–36). United Kingdom: Imperial College Press, London.

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

Medical Department. (1992). Annual Report, Sarawak.

Mian, L. S., & Mulla, M. S. (1982). Biological and environmental dynamics of insect growth regulators (IGRs) as used against Diptera of public health importance. *Residue Reviews*, *84*, 28–35.

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

Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., Raghavendra, K., Pinto, J., Corbel, V., David, J. P., & Weetman, D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLOS Neglected Tropical Diseases*, *11*(7), Article#e0005625. DOI: https://doi.org/10.1371/journal.pntd.0005625

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., Thavara, U., Tawatsin, A., Chompoosri, J., Zaim, M., & 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., & Scott, J. G. (1992). Biomolecular basis for insecticide resistance. In: Mullin, C.A. and Scott, J.G. (eds.), *Molecular mechanisms of insecticide resistiance: Diversity among insects* (pp. 1–13). USA: American Chemical Society, Washington.

Mulyaningsih, B., Umniyati, S. R., & Hadianto, T. (2017). Detection of nonspecific esterase activity in organophosphate resistance strain of *Aedes albopictus* Skuse (Diptera: Culicidae) larvae in Yogyakarta, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, *48*(3), 552–560.

Nauen, R. (2007). Insecticide resistance in disease vectors of public health importance. *Pest Management Science*, *63*(7), 628–633.

Nayar, J. K., Ali, A., & 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 condtions. *Journal of American Mosquito Control Association*, *3*, 196–201.

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

Nazni, W. A., Selvi, S., Lee, H. L., Sadiyah, I., Azahari, H., Derric, N., & Vasan, S. (2009). Susceptibility status of transgenic *Aedes aegypti* (L.) against insecticides. *Dengue Bulletin*, *33*, 124–129.

Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, *29*, 1–10.

Nelder, M., Kesavaraju, B., Farajollahi, A., Healy, S., Unlu, I., Crepeau, T., Ragavendran, A., Fonseca, D., & Gaugler, R. (2010). Suppressing *Aedes albopictus*, an emerging vector of dengue and chikungunya viruses, by novel combination of a monomolecular film and an insect-growth regulator. *The American Journal of Tropical Medicine and Hyginene*, *82*(5), 831–837.

Nelson, D. R. (2011). Progress in tracing the evolutionary paths of cytochrome P450. *Biochimica et Biophysica Acta*, *1814*, 14–18.

Ngan, C. K., Cheah, U. B., Abdullah, W. Y. W, Lim, K. P., & Ismail, B. S. (2005). Fate of chlorothalanil, chlopyrifos and profenofos in a vegetable farm in Cameron Highlands, Malaysia. *Water, Air, and Soil Pollution: Focus*, *5*, 125–136.

Ngoagouni, C., Kamgang, B., Brengues, C., Yahouedo, G., Paupy, C., Nakoune, E., Kazanji, M., & Chandre, F. (2016). Suscepbility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and Deltamethrin in the Central African Republic. *Parasites and Vectors*, *9*, Article#599.

N'Guessan, R., Boko, P., Odjo, A., Chabi, J., Akogbeto, M., & Rowland, M. (2010). Control of pyrethroid and DDT-resistance *Anopheles gambiae* by application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate inseciticide, chlorpyrifos-methyl. *Malaria Journal*, *9*, 44.

Niebylski, M. L., Savage, H. M., Nasci, R. S., & Craig Jr., G. B. (1994). Blood hosts of *Aedes albopictus* in the United State. *Journal of American Mosquito Control Association*, *10*, 447–450.

Noor-Afizah, A., Mahirah, M. N., Azahari, A. H., Khairul-Asuad, M., Nazni, W. A., & Lee, H. L. (2015). Absence of *Aedes aegypti* (L.) on and ecological island: competitive exclusion? *Southeast Asian Journal of Tropical Medicine and Public Health*, *46*(5), 850–856.

Novak, R. (1992). The Asian Tiger Mosquito, *Aedes albopictus*. *Wing Beats*, *3*, 5.

Nurin-Zulkifli, I. M., Chen, C. D., Wan-Norafikah, O., Lee, H. L., Faezah, K., Izzul, A. A., Abdullah, A. G., Lau, K. W., Norma-Rashid, Y., & Sofian-Azirun, M. (2015). Temporal changes of *Aedes* and *Armigeres* populations in suburban and forested areas in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, *46*(4), 574–585.

O'Neill, S. L., Ryan, P. A., Turley, A. P., Wilson, G., Retzki, K., Iturbe-Ormaetxe, I. et al. (2018). Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. *Gates Open Research*, *2*, 36. DOI: 10.12688/gatesopenres.12844.3

Ong, K. H., Chew, L. M., & 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.

Ong, S. Q. (2016). Dengue vector control in Malaysia: a review for current and alternative strategies. *Sains Malaysiana*, *45*, 777–785.

Paeporn, P., Komalamisra, N., Deesin, V., Rongsriyam, Y., Eshita, Y., & 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., & 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., & Meola, R. W. (1990). Field evaluation of Nylar for control of cat fleas in home yards. *Journal of Mecical Entomology*, *27*, 1045–1049.

Pandian, R. S., & Chandrashekaran, M. K. (1980). Rhythms in the biting behaviour of a mosquito *Armigeres subalbatus*. *Oecologia*, *47*, 89–95.

Paupy, C., Delatte, H., Bagny, L., Corbel, V., & Fontenille, D. (2009). *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection*, *11*, 1177–1185.

Pethuan, S., Jirakanjanakit, N., Saengtharatip, S., Chareonviriyaphap, T., Kaewpa, D., and Rongnoparut, P. (2007). Biochemical studies of insecticide resistance in *Aedes (Stegomyia aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand. *Tropical Biomedicine*, *24*(1): 7–15.

Pocquet, N., Darriet, F., Zumbo, B., Milesi, P., Thiria, J., Bernard, V., Toty, C., Labbé, P., & Chandre, F. (2014). Insecticide resistance in disease vectors from Mayotte: an opportunity for integrated vector management. *Parasites & Vectors*, *7*, Article#299.

Polson, K. A., Brogdon, W. G., Rawlins, S. C., & Chadee, D. D. (2011). Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. *Acta Tropica*, *117*(1), 31–38.

Ponlawat, A., Scott, J. G., & Harrington, L. C. (2005). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *Journal of Medical Entomology*, *42*, 821–825.

Powers, A. M., Brault, A. C., Tesh, R. B., & 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., Koottathep, S., Promtet, N., Hemingway, J., & Ketterman, A. J. (1996). Purification and characterization of a major glutathione S-transferase from the mosquito *Anopheles dirus* (Species B). *Insect Biochemistry and Molecular Biology*, *26*(3), 277–285.

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

Rach, J., Desalle, R., Sarkar, I. N., Schierwater, B., & Hadrys, H. (2008). Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. *Proceeding of The Royal Society B*, *275*(1632), 237–247.

Rahim, J., Ahmad, A. H., Ahmad, H., Ishak, I. H., Rus, A. C., & Naimusa, H. A. (2017). Adulticidal susceptibility evaluation of *Aedes albopictus* using new diagnostic doses in Penang island, Malaysia. *Journal of the American Mosquito Control Association*, *33*(3), 200–208.

Rahim, J., Ahmad, A. H., Kassim, N. F. A., Ahmad, H., Ishak, I. H., Rus, A. C., & Maimusa, H.A. (2016). Revised discrimination lethal doses for resistance monitoring program on *Aedes albopictus* against temephos and malathion in Penang Island, Malaysia. *Journal of the American Mosquito Control Association*, *32*(3), 210–216.

Rahman, M. M. (2013). Insecticide substitutes for DDT to control mosquitoes may be causes of several diseases. *Environmental Science and Pollution Research*, *20*, 2064–2069.

Rajavel, A. R. (1992). Cannibalistic behaviour in *Armigeres subalbatus* (Diptera: Culicidae). *Southeast Asean Journal of Tropical Medicine and Public Health*, *23*(3), 453–457.

Ranson, H., Claudianos, C., Ortelli, F., Abgrall, C., Hemingway, J., Sharakhova, M. V., Unger, M. F., Collins, F. H., & Feyereisen, R. (2002). Evolution of supergene families associated with insecticide resistance. *Science*, *298*, 179–181.

Ranson, H., & Hemingway, J. (2005). Mosquito glutathione transferases Review. *Methods in Enzymology*, *401*, 226–241.

Reinhold, J. M., Lazzari, C. R., & Lahondère, C. (2018). Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: A review. *Insects*, *9*(4), 158.

Rehman, H., Aziz, A. T., Saggu, S., Abbas, Z. K., Mohan, A., & Ansari, A. A. (2014). Systematic review on pyrethriod toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies*, *2*(5), 1–6.

Rohani, A., Aziz, I., Zurainee, M. N., Rohana, S. H., Zamree, I., & Lee, H. L. (2014). Current insecticide susceptibility status of Malaysian *Anopheles maculatus* Theobald to malathion, permethrin, DDT and deltamethrin. *Tropcial Biomedicine*, *31*(1), 159–165.

Rohani, A., Chu, W. L., Sa'diyah, I., Lee, H. L., & 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., & Lee, H. L. (1998). Evaluation of susceptibility of urban and rural *Aedes albopictus* to commonly used insecticides. *Vector Journal*, *4*, 15–27.

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

Rosilawati, R., Lee, H. L., Nazni, W. A., Fikri, S. F. F., Ali, R., Muhamed, K. A., Azahaari, A. H., Liu, Q. Y., & Feng, X. M. (2018). Susceptibility status and resistance mechanisms in permethrin-selected, laboratory susceptible and field-collected *Aedes aegypti* from Malaysia. *Insects*, *9*(2), 43.

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.

Saelim, V., Brogdon, W. G., Rojanapremsuk, J., Suvannadabba, S., Pandii, W., Jones, J.W., & 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), 417–425.

Schaefer, C. H., & Mullingan, F. S. (1991). Potential for resistance to pyriproxyfen: A promising new mosquito larvicide. *Journal of the American Mosquito Control Association*, *7*, 409–411.

Scholte, E. J., & Schaffner, F. (2007). Waiting the tiger: establishment and spread of the *Aedes albopictus* mosquito in Europe. In: Takken, W. & Knols, B. G. J. (Eds.), *Emerging Pests and Vector-Borne Diseases in Europe* (pp. 241–260). Netherlands: Wageningen Academic Publishers, Wageningen.

Seccacini, E., Lucia, A., Harburguer, L., Zerba, E., Licastro, S., & 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.

Severson, D. W., Anthony, N. M., Andreev, O., & ffrench-Constant, R. H. (1997). Molecular mapping of insecticide resistance genes in the yellow fever mosquito (*Aedes aegypti*). *Journal of Heredity*, *88*(6), 520–524.

Shen, Y-Y., Chen, X., & Murphy, R. W. (2013). Assessing DNA barcoding as a tool for species identification and data quality control. *PLoS ONE*, *8*, Article#e57125. DOI: 10.1371/journal.pone.0057125

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

Siegfried, B. D., & Scott, J. G. (1992). Biochemical characterization of hydrolytic and oxidative enzymes in insecticide resistant and susceptible strains of the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology*, *85*(4), 1092–1098.

Sihuincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J.D., López-Sifuentes, V., Vidal-Oré, C., & 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.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, *87*, 651–701.

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

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

Slatkin, M. (1981). Estimating levels of gene flow in natural populations. *Genetics*, *99*, 323–335.

Smith, C. E. G. (1956). The history of dengue in tropical Asia and its probable relation to the mosquito *Aedes aegypti*. *Journal of Tropical Medicene and Hygiene*, *59*(10), 243–251.

Somboon, P., Prapanthadara, L., & 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.

Strode, C., Wondji, C. S., David, J. P., Hawkes, N. J., Lumjuan, N., Nelson, D. R., Drane, D. R., Karunaratne, S. H. P. P., Hemingway, J., Black IV, W. C., & Ranson, H. (2008). Genomic analysis of detoxification genes in the mosquito *Aedes aegypti*. *Insect Biochemistry and Molecular Biology, 38*(1), 113–123.

Struger, J., Grabuski, J., Cagampan, S., Sverko, E., & Marvin, C. (2016). Occurrence and distribution of carbamate pesticides and Metalaxyl in Southern Ontario surface waters 2007 – 2010. *Bulletin of Environmental Contamination and Toxicology*, *96*, 423–431.

Su, T. Y. (2016). Resistance and its management to microbial and insect growth regulator larvicides in mosquitoes. *Insecticides Resistance*. DOI:10.5772/61658.

Su, T., & Cheng, M. L. (2014). Cross resistance in spinosad-resistant *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology*, *51*, 428–435.

Sulaiman, S., Pawanchee, Z. A., Othman, H. F., Shaari, N., Yahaya, S., Wahab, A., & Ismail, S. (2002). Field evaluation of cypermerthrin and cyfluthrin against dengue vectors in a housing estate in Malaysia. *Journal of Vector Ecology*, *27*(2), 230–234.

Surtees, G. (1970). Mosquito breeding in the Kuching area, Sarawak, with special reference on the epidemiology of dengue fever. *Journal of Medical Entomology*, *8*, 713–716.

Tabachnick, W. J., & Black, W. C. (1995). Making a case for molecular population genetic studies of arthropod vectors. *Parasitology Today*, *11*, 27–30.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585–595.

Tautz, D., & Actander, P. (2003). A plea for DNA taxonomy. *Trends in Ecology & Evolution*, *18*(2), 70–74.

Tee, A. S. (2000). Malaria control in Malaysia. In: *Mosquitoes and mosquito-borne disease* (ed. F.S.P. Ng & H.S. Yong) pp. 191–196.

Tham, A.S. (2000). Surveillance of mosquitoes. In: Ng, F. S. P., & Yong, H. S. (eds.), *Mosuqitoes and mosquito-borne diseases* (pp. 167–183). Malaysia: Academy of Sciences Malaysia, Kuala Lumpur.

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.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *24*, 4876–4882.

Thongwat, D., & Bunchu, N. (2015) Susceptibility to temephos, permethrin and deltamethrin of *Aedes aegypti* (Diptera: Culicidae) from Muang district, Phitsanulok Province, Thailand. *Asian Pacific Journal of Tropical Medicine*, *8*(1), 14–18.

Tisen, O. B. (2008). Biodiversity in Recreation: Sarawak's National Parks and Nature Reserves. In: Biodiversity and National Developmetn: Achievement, Opportunities and Chagllenges. *Proceeding of Conference on Biodiversity and National Development*, 28 May 2008. pp. 138–160.

Tunaz, H., & Uygun, N. (2004). Insect growth regulators for insect pest control. *Turkish Journal of Agriculture and Forestry*, *28*, 377–387.

Unlu, I., Suman, D. S., Wang, Y., Klingler, K., Faraji, A., & Gaugler, R. (2017). Effectiveness of autodissemination stations containing pyriproxyfen in reducing immature *Aedes albopictus* populations. *Parasites & Vector*, *10*, Article#139 DOI: 10.1186/s13071-017-2034-7.

VBDCP. (1999). Annual Report Vector Borne Disease Control Programme, Ministry of Health. pp. 171.

Vences, M., Thomas, M., van der Meijden, A., Chiari, Y., & Vieites, D. R. (2005). Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, *2*, Article#5 DOI: 10.1186/1742-9994-2-5.

Voet, D., & Voet, J. G. (1995). *Biochemistry*. 2nd Edition. John Wiley and Sons: New York.

Vulule, J. M., Beach, R. F., Atieli, F. K., Roberts, J. M., Mount, D. L., & Mwangi, R. W. (1994). Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Medical and Veterinary Entomology*, *8*, 71–75.

Vythilingam, I., Chiang, G. L., & Amatachaya, C. (1992). Adulticidal effect of cyfluthrin against mosquitos of public health importance in Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 23(1), 111–115.

Vythllingam, I., Luz, B. M., Hanni, R., Tan, S. B., & 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., Lee, H. L., Chen, C. D., Wan-Norjuliana, W. M., Azahari, A. H., & Sofian-Azirun, M. (2008). Detection of permethrin resistance in *Aedes albopictus* Skuse, collected from Titiwangsa Zone, Kuala Lumpur, Malaysia. *Proceeding of ASEAN Congress of Tropical Medicine and Parasitology*, *3*, 69–77.

Wan-Norafikah, O., Nazni, W. A., Lee, H. L., Zainol-Ariffin, P., & Sofian-Azirun, M. (2010). Permethrin resistance in *Aedes aegypti* (Linnaeus) collected from Kuala Lumpur, Malaysia. *Journal of Asia Pasific Entomology*, *13*, 175–182.

Wang, G., Li, C., Guo, X., Xing, D., Dong, Y., Wang, Z., Zhang, Y., Liu, M., Zheng, Z., Zhang, H., Zhu, X., Wu, Z., & Zhao, T. (2012). Identifying the main mosquito species in China based on DNA barcoding. *PLoS ONE*, *7*(10), Article#e47051. DOI: 10.1371/journal.pone.0047051.

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

Wesson, D. M. (1990). Susceptibility to organophosphate insecticides in larval *Aedes albopictus*. *Journal of the American Mosquito Control Association*, *6*(2), 258–264.

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

World Health Organization. (1971). Evaluations of some pesticide residues in food. *WHO Pesticide Residues Series*, No.1.

World Health Organization (1974). Propoxur. Evaluation of Some Pesticides Residue in Food. pp.330–378. *WHO Pesticide Residue Series No. 3, 1973*. World Health Organization, Geneva.

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

World Health Organization. (1992). Vector resistance to pesticides. *WHO Technical Report Series*, *818*, 2–34.

World Health Organization. (1998). Techniques to detect insecticide resistance mechanisms (field and laboratory manual). World Health Organization, Geneva, Switzerland.

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

World Health Organization. (2003). Yellow fever vaccine. *Weekly epidemiological record. 78*(40), 349–360.

World Health Organization. (2004). Fenitrothion in drinking-water. Background document for development of WHO *Guidelines for drinkin-water quality*. WHO/SDE/WSH/03.04/95.

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

World Health Organization. (2008a). 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.

World Health Organization. (2008b). 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.

World Health Organization. (2008c). 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.

World Health Organization. (2009a). Temephos in drinking water: Use for the vector control in drinking-water sources and containers. Background document for development of WHO Guidelines for drinking-water quality. Geneva, Switzerland, WHO/HSE/WSH/09.01/1.

World Health Organization. (2009b). World Malaria Report. Geneva, Switzwerland.

World Health Organization. (2016). Monitoring and managing insecticide resistance in *Aedes* mosquito populations: Interim guidance for entomologists. https://apps.who.int/iris/bitstream/10665/204588/2/WHO_ZIKV_VC_16.1_eng.pdf (accessed 25 November 2018).

World Health Organization. (2017) Global vector control response 2017 – 2030. Geneva: World Health Organization.

Wright, S. (1978). Evolution and Genetics of Populations. University of Chicago, Chicago.

Wright, M. C. (2010). Mosquito resistance to bacterial larvicidal toxins. *Open Toxinology Journal*, *3*, 101–115.

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

Yan, G., Chadee, D. D., & Severson, D. W. (1998). Evidence for genetic hitchhiking effect associated with insecticide resistance in *Aedes aegypti*. *Genetics*, *148*(2), 793–800.

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

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

Zaim, M., & Guillet, P. (2002). Alternative insecticides: An urgent need. *Trends of Parasitology*, *18*, 161–163.

Zawani, M. K. N., Abu, H. A., Sazaly, A. B., Zary, S. Y., & Darlina, M. N. (2014). Population genetic structure of *Aedes albopictus* in Penang, Malaysia. *Genetics and Molecular Research*, *13*(4), 8184–8196.

Zhang, R., Liu, T., Dezhen, M., & Zhong, Z. (2018). Genetic characters of the globally spread tiger mosquito, *Aedes albopictus* (Diptera, Culicidae): implicatons from mitochondrial gene COI. *Journal of Vector Ecology*, *43*(1), 89–97.

Žitko, T., Kovačić, A., Desdevises, Y., & Puizina, J. (2011). Genetic variation in East-Adriatic populations of the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), inferred from *NADH5* and *COI* sequence variability. *European Journal of Entomology*, *108*, 501–508.

Zitvo, V. (2003). Chlorinated Pesticides: Aldrin, DDT, Endrin, Dieldrin, Mirex. In: Fiedler, H. (eds), *Persistent Organic Pollutants*. *The Handbook of Environmetnal Chemistry (Vol. 3 Series: Anthropogenic Compounds*) Vol. 30. Springer, Berlin, Heidelberg.

Zulueta, J., & LaChance, F. (1956). A malaria-control experiment in the interior of Borneo. *Bulletin of the World Health Organization*, *15*, 673–693.

Zuriati, Z., Lee, Y. H., Pauzi, A., Rosita, O., & Laily, D. (2003). The environmental contamination by organochlorine insecticides of some agricultural areas in Malaysia. *Malaysian Journal of Chemistry*, *5*(1), 78–85.