PRELIMINARY STUDY ON SURVEILLANCE AND RESISTANCE STATUS OF *Aedes aegypti* AGAINST VARIOUS INSECTICIDES IN SUNDA ISLANDS, INDONESIA

AMIRAH HAZIQAH BINTI RASHID

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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Matric No: SGR 160064

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PRELIMINARY STUDY ON SURVEILLANCE AND RESISTANCE STATUS OF *Aedes aegypti* AGAINST VARIOUS INSECTICIDES IN SUNDA ISLANDS, INDONESIA

ABSTRACT

Aedes mosquitoes are well known as the vector of dengue fever (DF) and dengue hemorrhagic fever (DHF) which are a major health concern in Indonesia. Little to none study was done on the resistance status of adulticides and larvicides against the field population across Indonesia. Thus, in this study, ovitrap surveillance was conducted to determine the abundance of dengue vectors in fourteen study sites across eight provinces located in the Sunda Islands, Indonesia. High ovitrap indices up to 70% and 90% were obtained from indoor and outdoor areas, respectively. The mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.13 to 14.50 and 0.10 to 18.60, respectively. Mixed infestation (< 10%) and interchange of breeding habitat preferences of *Ae. albopictus* and *Ae. aegypti* were also observed in the present study. Field-collected and reference strains of *Ae. aegypti* larvae were tested against diagnostic dosage of 8 larvicides which belong to organophosphates and organochlorines. This study shows that *Ae. aegypti* larvae from Padang, Samarinda, Flores and Timor were susceptible to both fenitrothion and dieldrin (mortality ≥ 98%). Six out of 10 field strain of *Ae. aegypti* larvae were resistant (< 80% mortality) against fenthion, whilst Kuningan, Samarinda, Sumba and Timor exhibited some development of resistance (mortality between 80-98%). All field-collected *Ae. aegypti* larvae were resistant against diagnostic dosages of chlorpyrifos, malathion, temephos and DDT with mortality ranging from 0% to 74.67%. Field adult *Ae. aegypti* exhibited various knockdown rate, ranging from 0.00 - 100.00%, 0.00 - 44.00%, 0.00 - 6.7% and 0.00% for pyrethroids, organophosphates, carbamates and organochlorines respectively. Overall, mortality of adult *Ae. aegypti* ranges from 6.67 to 100.00% were recorded across the Indonesian populations. There were significant

correlations between the mortality rates of lambda-cyhalothrin and permethrin (r = 0.733, $p = 0.016$); lamda-cyhalothrin and deltamethrin (r = 0.83, $p = 0.003$); lambda-cyhalothrin and etofenprox (r = 0.936, $p < 0.01$); dieldrin and DDT (r = 0.701, $p = 0.024$); and DDT and deltamethrin (r = 0.69, $p = 0.027$). This showed the existence of cross resistance within the pyrethroids, organochlorines and, between pyrethroids and DDT. This study revealed that the *Ae. aegypti* were resistant to most insecticides tested. However, there are insecticides to which *Ae. aegypti* are also still susceptible and are not frequently used in Indonesia as vector control. Thus, rotating among the insecticides could be an alternative way in controlling the resistance of using a single insecticide.

Keywords: Dengue, ovitrap surveillance, insecticides, resistance, Indonesia.

KAJIAN AWAL PENINJAUAN DAN STATUS KERINTANGAN *Aedes aegypti* TERHADAP PELBAGAI INSEKTISID DI PULAU SUNDA, INDONESIA

ABSTRAK

Nyamuk *Aedes* dikenali sebagai vektor demam denggi (DF) dan demam denggi berdarah (DHF) yang menjadi kebimbangan kesihatan utama di Indonesia. Setakat ini, masih belum ada kajian yang dibuat terhadap status kerintangan nyamuk dewasa dan jejentik *Ae. aegypti* terhadap populasinya di seluruh Indonesia. Oleh itu, dalam kajian ini peninjauan ovitrap telah dilakukan untuk menentukan kelimpahan vektor denggi di empat belas tapak kajian di lapan wilayah yang terletak di Kepulauan Sunda, Indonesia. Indeks ovitrap setinggi 70% dan 90% diperolehi dari kawasan dalam dan luar rumah. Purata bilangan jejentik *Ae. aegypti* dan *Ae. albopictus* adalah antara 0.13 hingga 14.50 dan 0.10 hingga 18.60. Kajian ini juga mendapati terdapat percampuran nyamuk *Ae. aegypti* dan *Ae. albopictus* di dalam satu ovitrap (< 10%) dan pertukaran pemilihan tempat pembiakan di antara keduanya. Jejentik *Ae. aegypti* diperolehi dari lapangan dan strain rujukan telah diuji terhadap 8 racun jejentik berdos diagnostik dari kumpulan organofosfat dan organoklorin. Kajian ini menunjukkan bahawa fenitrothion dan dieldrin masih memberi kesan (kematian ≥ 98%) terhadap jejentik *Ae. aegypti* dari Padang, Samarinda, Flores dan Timor. Enam dari 10 jejentik dari lapangan telah menunjukkan kerintangan (< 80%) terhadap fenthion sementara jejentik dari Kuningan, Samarinda, Sumba dan Timor menunjukkan kerintangan yang sederhana (kematian ≥ 98%). Kesemua jejentik dari lapangan menunjukkan kerintangan terhadap dos diagnostik chlorpyrifos, malathion, temefos dan DDT dengan jumlah kematian antara 0% hingga 74.67%. Nyamuk dewasa dari lapangan menunjukkan pelbagai kadar *knockdown* iaitu 0.00 - 100%, 0.00 - 44.00%, 0.00 - 6.67% dan 0.00% untuk pyrethroids, organofosfat, carbamate dan organoklorin. Secara keseluruhnnya, kadar catatan kematian nyamuk dewasa adalah antara 6.67 - 100.00% bagi keseluruhan populasi Indonesia. Terdapat korelasi yang signifikan di antara

kadar kematian lambda-cyhalothrin dan permethrin (r = 0.733, p = 0.016); lambda-cyhalothrin dan deltamethrin (r = 0.83, p = 0.003); lambda-cyhalothrin dan etofenprox (r = 0.936, p <0.01); dieldrin dan DDT (r = 0.701, p = 0.024); dan DDT dan deltamethrin (r = 0.69, p = 0.027). Ini menunjukkan kewujudan rintangan silang di dalam kumpulan pyrethroids, dalam kumpulan organoklorin dan di antara pyrethroids dan DDT. Kajian ini menunjukkan bahawa *Ae. aegypti* rintang terhadap kebanyakan insektisid yang diuji. Akan tetapi, masih terdapat beberapa insektisid berkesan terhadap *Ae. aegypti* dan jarang digunakan di Indonesia sebagai kawalan vektor. Oleh itu, penggunaan insektisid secara bergilir dipercayai dapat mengurangkan status kerintangan nyamuk terhadap satu jenis insektisid.

Kata kunci: Denggi, peninjauan ovitrap, insektisid, kerintangan, Indonesia.

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LIST OF SYMBOLS AND ABBREVIATIONS

&	:	and
^{o}C	:	Degree Celsius
=	:	Equal
<	:	Less than
>	:	More than
≤	:	Less than or equal to
±	:	plus minus
%	:	Percent
ACh	:	Acetylcholine
AChE	:	Acetylcholinesterases
Ae.	:	*Aedes*
ANOVA	:	Analysis of variance
Bti	:	*Bacillus thuringinensis israelensis*
cm	:	Centimeter
C.L	:	confidence limit
df	:	degrees of freedom
DF	:	Dengue fever
DHF	:	Dengue hemorrhagic fever
GST	:	glutathione S-transgerases
KT_{50}	:	50% of knockdown rate
F60	:	Filial generation 60
mg/L	:	milligram per liter (concentration)
OI	:	ovitrap index
p	:	possibility value

r	:	r value
sp.	:	species (singular)
SPSS	:	statistical analysis software

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background of Study

Aedes mosquitoes are well known as the vector of dengue fever (DF) and dengue hemorrhagic fever (DHF) which are a major health concern in Indonesia (Wahyono *et al*., 2017). In 2015, Indonesia recorded a total of 126, 675 dengue cases with 1229 deaths (Ministry of Health Republic of Indonesia, 2016). The high number of dengue cases reported are due to rapid urbanization and globalization. With the aid of climate change suitable breeding sites have been created for the *Aedes* mosquitoes and as a result there is higher breeding of vectors (Kusriastuti & Sutomo, 2005).

With this increasing trend of dengue, many efforts have been done to eradicate the vectors. The easiest and most effective way to control the vector is through applying larvicide to their breeding places (Suganya *et al*., 2013). The larvicidals can be classified as synthetic or as organic insecticides base. The excessive usage of synthetic insecticides such as dichlorodiphenyltrichloroethane (DDT) and permethrin have resulted in pesticide resistance and harmful effects on non-target organisms (Rawani *et al*., 2014).

Lack of effective mosquito control plays an important role in the incidence and spread of the dengue virus (Karyanti *et al*., 2014). To reduce disease transmission, insecticides are used to control the vector *Aedes* (Ahmad *et al*., 2009). However, excessive usage of insecticides has caused the development of resistance and interferes with the control programs (Ahmad *et al*., 2009; Putra *et al*., 2016).

The most common method to monitor *Aedes* mosquitoes populations is through ovitrap surveillance (Lau *et al*., 2013). This is a simple and convenient tool for *Aedes* surveillance, ovitrap is fast, sensitive and cost-effective device to determine the presence

of egg laying females of *Aedes* mosquitoes (Norzahira *et al*., 2011; Wan-Norafikah *et al*., 2011).

1.2 Problem Statement

There are numerous molecular studies for the surveillance of dengue, but only a few focus on the vectors abundance in Indonesia (Fahri *et al*., 2013; Nusa *et al.,* 2014; Wijayanti *et al*., 2016; Martini *et al*., 2013). Molecular surveillance studies focuses on serological features of dengue which include the serotypes and genetics aspects of dengue disease.

Although there have been studies describing various insecticides (permethrin, deltamethrin, cypermethrin, temephos, and malathion) resistance from Java Island but no data are available for the major islands in the other parts of Indonesia such as Sumatra and Kalimantan. In addition, most of the previous resistance studies done targeted only on the vector mosquito larvae (Ahmad *et al*., 2009; Mulyatno *et al*., 2012; Astuti *et al*., 2014; Putra *et al*., 2016).

1.3 Significant of Study

Findings obtained from surveillance studies such as on vector density, larval habitats, distribution and the level of the insecticide susceptibility are important in enhancing the vector control strategy (Kusriastuti & Sutomo, 2005). Hence, an effective surveillance of the vector *Aedes* is crucial in determining the distribution and vector density for the implementation, planning and monitoring disease control programs (Kusriastuti & Sutomo, 2005; Basker & Ezhil, 2012).

This study will provide a baseline on the resistance status of *Ae. aegypti* against the various insecticides and the effectiveness of the insecticides vastly used on the adults and larvae mosquito.

1.4 Scope of Study

This study focuses on the distribution of *Aedes* mosquitoes throughout the Sunda Islands of Indonesia. Besides that, the resistance status of both adults and larvae of *Ae. aegypti*, the primary vector of dengue against various insecticides was evaluated in this study.

1.5 Objective of Study

The objectives of this study are;

(1) to determine the preliminary distribution and abundance of dengue vectors, *Ae. aegypti* and *Ae. albopictus* in the Sunda Islands of Indonesia.

(2) to determine the resistance status of adult *Ae. aegypti* against diagnostic dosage of adulticides

(3) to investigate the effectiveness of diagnostic dosage of larvicides against *Ae. aegypti* larvae.

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

Study 1: A preliminary survey on distribution and abundance of dengue vectors in Sunda Islands of Indonesia.

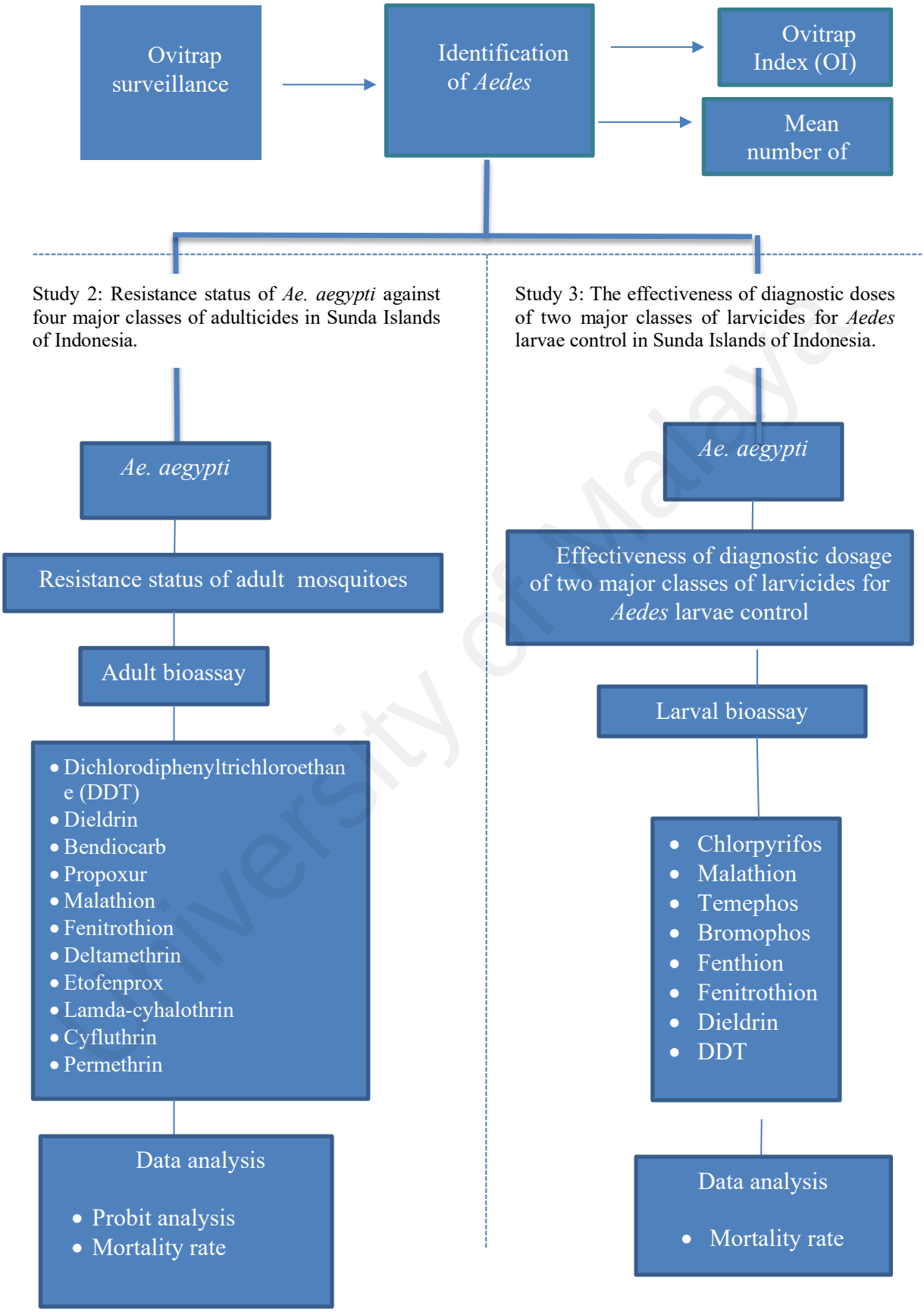


Figure 1.1: Schematic diagram of 'Preliminary study on surveillance and resistance status of *Ae. aegypti* against various insecticides in Sunda Islands, Indonesia'.

CHAPTER 2: LITERATURE REVIEW

2.1 *Aedes* Mosquitoes

The *Aedes* mosquito has four distinct life stages which are egg, larvae, pupa, and adult. The egg of *Aedes* is shiny black, and elongate-oval in shape which is often referred as boat-like shape with a flatten upper surface (Christophers, 1960; Sivanathan, 2006). The eggs are laid singly above the water line on damp surface, originally soft and white in colour when newly laid however become dark and hard as it matures (Christophers, 1960). The eggs can survive in a dry weather for several months or longer but once submerged in water hatching is triggered (Westbrook, 2010). The eggs may require repeated immersion in water due to phases in hatching and may take several days to months to hatch.

There are four larval instars in *Aedes* mosquito life cycle. Larvae stage lasts about 4 - 7 days in warm climates, and longer during shortage of food (Rozendaal, 1997). Larvae are legless, with a fully developed head and pair of antennae and a pair of compound eyes (Figure 2.1), and swim with sweeping movements. At the end posterior of the abdomen, a short barrel-shaped siphon is present that allows air breathing when coming up to the surface. According to Christophers (1960), first-instar larvae was identified based on the presence of the egg-breaker on the head. While the second and third instars larvae, they can be identified through the transverse diameter of the head approximately 0.45 mm and 0.65 mm respectively. Fully darkened head of the fourth-instar larvae are suffice to differentiate with the third-instar larvae. Also, on the shoulders of the thorax in fourth-instar larvae, elements of the future pupal respiratory trumpets can be observed. Larvae feed on debris of plant, animal origin, yeast, bacteria and small aquatic organisms (Sivanathan, 2006). They are also sensitive to vibrations and dive to bottom to escape danger or to for feeding.

Unlike most insects, the pupa of mosquito is active but does not feed. *Aedes* remains in the pupa stage for 1 - 3 days under normal condition. Pupa is comma shaped and have a plump body with breathing trumpets which are cylindrical (Christophers, 1960). The head and thorax are combined to form the cephalothorax (Service, 2012). Pupa of *Aedes* also usually remains on the surface of water, however when water is disturbed, it will move downward in a jerky motion (Sivanathan, 2006; Service, 2012). When the pupa matures, the pupal skin splits at one end and a fully developed adult mosquito emerges.

An adult mosquito usually measures about 3 – 6 mm in length. The body of a mosquito is divided into the head, thorax and abdomen (Figure 2.2). The head comprises of a conspicuous pair of kidney-shaped compound eyes and a pair of antennae. Female antennae have whorls of short hairs, while male antennae have long hairs giving them feathery appearance which can be seen by naked eyes to differentiate between the sexes (Service, 2012). Below the antennae, are the palps and arising between the palps, is the single elongated proboscis which is present in both sexes, however the piercing stylets to perforate the skin of the host are absent in males (Becker *et al*., 2003).

Since *Aedes* are container breeding mosquitoes, larvae are found in man-made containers such as tires, tin cans, bottles, flower pots and water-storage pots, or in natural container habitats such as bamboo stumps, tree holes, rock pools and leaf axils (Estrada-Franco & Craig, 1995; Service, 2012).

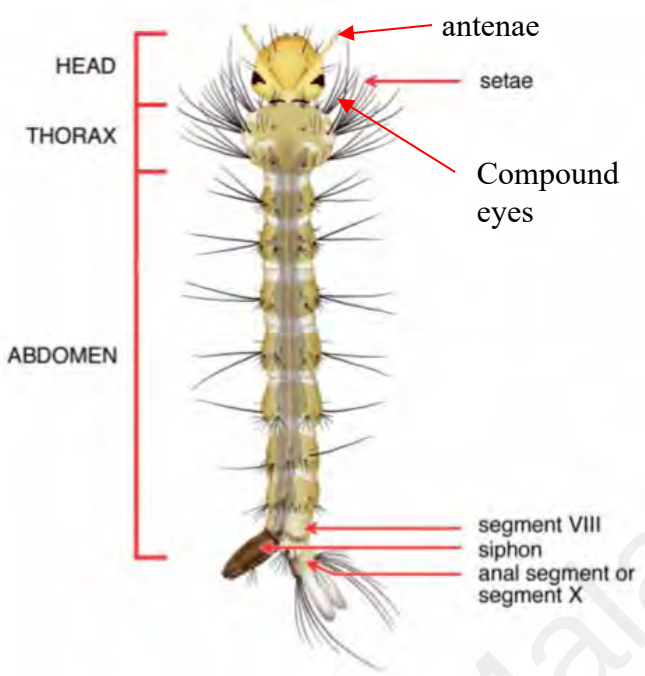


Figure 2.1: Mosquito larva. Image reproduced with permission from Rueda, 2004.

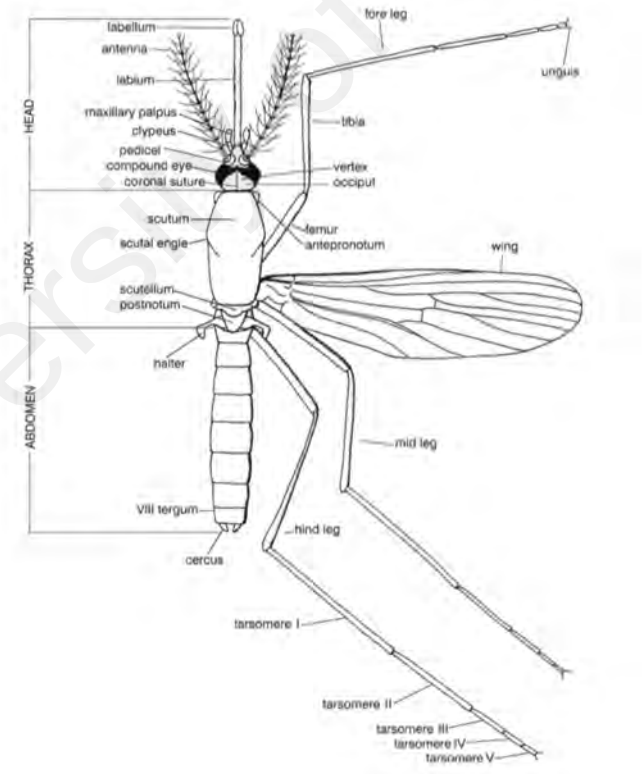


Figure 2.2: General outline of a female culicine mosquito. Image reproduced with permission from Becker *et al.,* 2003.

Just like any other mosquitoes, *Aedes* mosquitoes go through an autogenous development which allows the female mosquito bite a host for a blood-meal for necessary nutrients for the development of the eggs. Females usually mate only once but produce eggs at intervals throughout their life, whilst fully grown male adults will feed on nectar from flowers and other naturally occurring sugary secretions (Harker, 2013). However, sugar feeding is not restricted to males only, female also feed on sugary substance to obtain energy for flight and dispersal (Service, 2012). In the tropics, the digestion of a blood-meal and the simultaneous development of eggs takes 2-3 days but longer in temperate regions. The three distinct behaviour features of an adult mosquitoes are, feeding, mating and oviposition. *Aedes* prefers to bite mainly in the morning or evening and most species bite and rest outdoors but there are species that prefer to breed, feed and rest in and around houses. Mating of adult mosquitoes takes place in flight and usually occurs within one to three feet of the ground and usually lasts from 5 to 15 seconds. Females are not inseminated until they are 30 to 36 hours old and it is reported that one copulation is sufficient to fertilize one batch of eggs (Ho *et al.*, 2014).

In addition, female *Aedes* also prefer to lay eggs in solid and dark colored containers (Burke, 2009). Thus, the sampling and trapping of mosquitoes are performed based on these characteristics. Oviposition trap, a device used to sample mosquito, is usually a dark colored cup and placed outdoor to attract female *Aedes* to oviposit (Sivanathan, 2006). Ovitraps are usually used for surveillance and often used to obtain a large number of larvae and adults for studies which requires a substantial amount of eggs (Sivagnaname & Gunasekaran, 2012).

2.2 *Ae. aegypti*

Ae. aegypti is thought to be originated from Africa and introduced into Asia (Mattingly, 1957). *Ae. aegypti* is widely distributed throughout tropical, subtropical region and temperate region. Since *Ae. aegypti* cannot survive during cold winter months, preventing the establishment of permanent population in cold winter countries (Becker *et al.*, 2003).

They are likely found in rapidly developing areas with less vegetation (Ishak *et al.*, 2015). *Ae. aegypti* prefer to rest indoors such as in bed rooms, kitchens and living rooms and feed exclusively on humans during daylight hours (Yasuno & Tonn, 1970; Chadee, 2013). *Ae. aegypti* have different breeding preferences according to the season with high preference to wet season compared to the dry season (Troyo, 2008). Based on Center for Disease Control and Prevention (CDC) (2016), adult of *Ae. aegypti* depend greatly on water storage containers to lay their eggs and are typically found in areas lacking piped water systems, while the larvae prefer artificial or natural water containers such as discarded tires, flower pots and cemetery vases. Larvae can also be found in underground collections of water, such as storm drains, water meters and unsealed septic tanks.

2.3 *Ae. albopictus*

According to Becker *et al*. (2003) *Ae. albopictus* is mainly distributed in the Oriental Region and Oceania and known as the 'Asian Tiger Mosquito'. Whereas, Kraemer *et al*. (2015) stated that *Ae. albopictus* originated from the tropical forest of Southeast Asia and Madagascar but recently spread to North and South America, as well as West Africa and rapidly expanded its range to Europe, the United States and Brazil during the 1980s.

They are often found in tropical and subtropical areas, but are found more in temperate climatic zones. *Aedes* mosquitoes predominantly feed on humans, but also bite other

mammals including dogs, squirrels, dogs and cows. These arthropods often feed on humans during dusk and night, but may also found biting during daytime outside houses in shaded areas. Female *Ae. albopictus* preferred to oviposit in habitats with a rough gray surface and low reflectivity rather than on a smooth black surface with high reflectivity. Naturally, they usually lay eggs in water reservoirs containing decaying vegetable matter such as tree holes, bamboo stumps, coconut shells, rock holes, flower pots, tin cans, water jars and tires (Becker *et al*., 2003).

2.4 Similarities and differences between *Ae.* (*Stegomyia*) *aegypti* and *Ae.* (*Stegomyia*) *albopictus*

Ae. aegypti and *Ae. albopictus* are differentiated through the morphology of the larva and adult (Table 2.1).

Table 2.1: Similarities and differences between *Ae.* (*Stegomyia*) *aegypti* and *Ae.* (*Stegomyia*) *albopictus* (Becker *et al.,* 2003).

Ae. aegypti	*Ae. albopictus*
Larva (Figure 2.3)	
One branch in seta on the head	Two branches in seta on the head
Long spines on mesothorax and metathorax	Plural hair groups and lack long spines on the mesothorax and metathorax
Shorter, stout and subapical spines comb scales	Bare apical spines and a row of small spicules comb scale
Adult (Figure 2.4)	
Lyre-shaped silver pattern on the scutum	Distinct longitudinal silver line on the scutum
Scutum is predominantly covered with narrow dark brown scales	Scutum is mainly covered with narrow dark scales
Scutellum has broad white scales on the lobes and a few broad dark scales at the apex of the mid lobe	Scutellum has broad white scales over all the lobes with an apical area of dark scales on the mid lobe
• Tibiae are all dark • Dark scale proboscis • Fore and mid tarsi have white basal band on tarsomeres I and II, hind tarsus has broad basal white band on tarsomeres I-IV, and tarsomeres V all white. • Wing veins are all dark scale except small spot of white scales at the base of costaces	

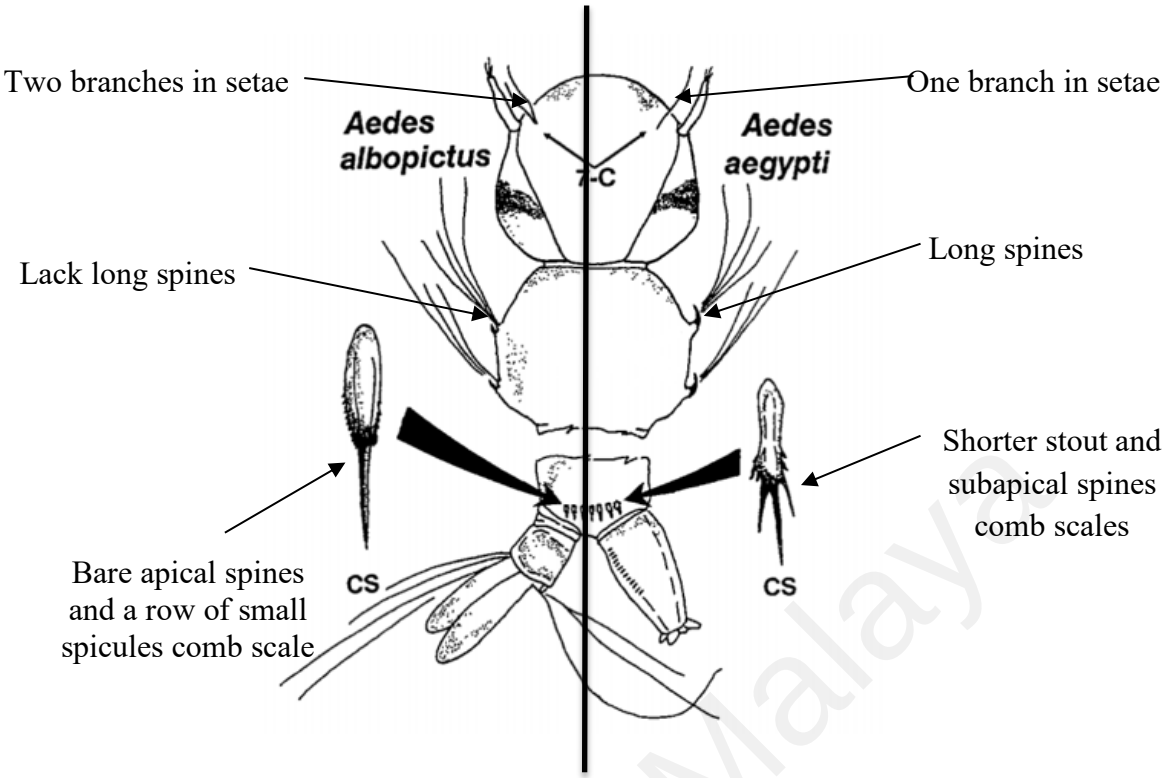


Figure 2.3: Differences between *Ae. albopictus* and *Ae. aegypti* at larval stage. Image reproduced with permission Estrada-Franco and Craig, 1995.

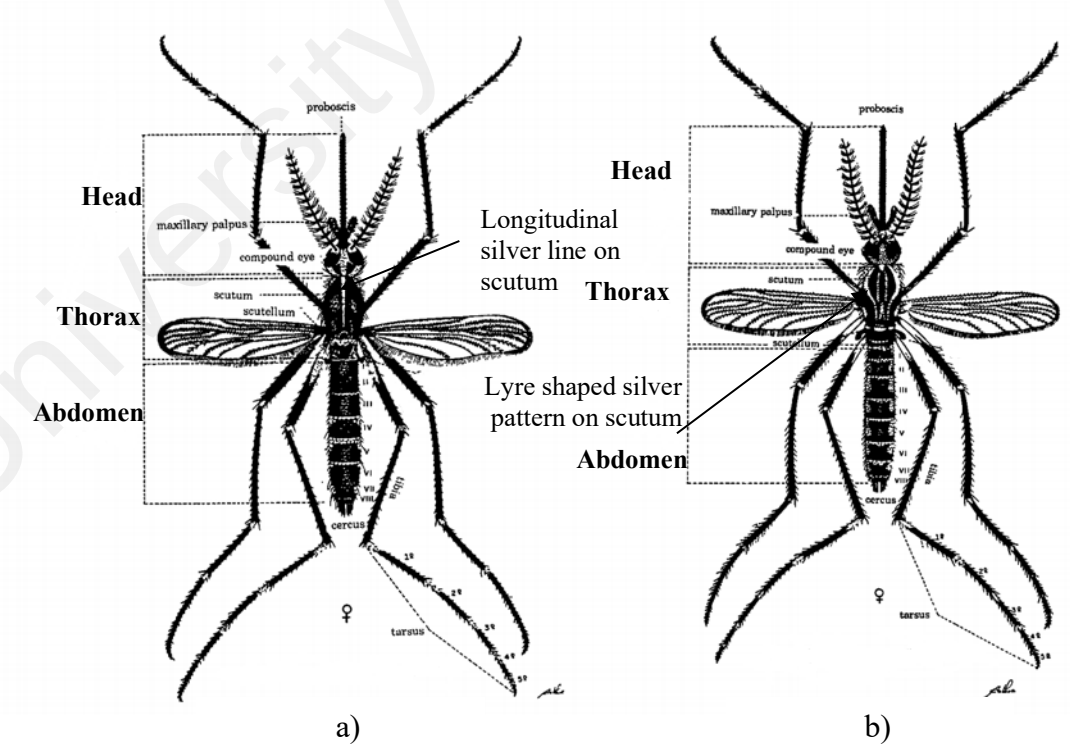


Figure 2.4: Morphological characteristics of adult a) *Ae. albopictus* and b) *Ae. aegypti* Image reproduced with permission from Estrada-Franco & Craig., 1995.

2.5 Medical Importance of *Aedes* Mosquitoes

2.5.1 Dengue fever

According to WHO (2018c), estimation of 390 million dengue cases are reported annually with 96 million manifest clinically. This could also be far from the actual condition, as dengue cases are generally underreported and many cases misclassified. However, incidence of dengue has grown dramatically around the world with an increase from 2.2 million in 2010 to 3.2 million in 2015. Back in 1970, only 9 countries experienced severe dengue epidemics, but dengue cases are now endemic in more than 100 countries including South-East Asia. Indonesia, the largest country in Southeast Asia has reported a total of 126,675 dengue cases with 1,229 deaths in 2015 (Ministry of Health Republic of Indonesia, 2016)

Up to now, dengue was believed to be caused by four antigenically distinct serotypes, Dengue Virus (DENV)-1, DENV-2, DENV-3 and DENV-4, each with a different immune response to infection. However, a case in Sarawak, Malaysia reported the existence of DENV-5 which has a phylogenetically distinct genetics sequence from the three previous forms of DENV-4 and some similarity to DENV-2. The existence of DENV-5 is believed to be caused by an antigenic shift, a combination of two different strains of the virus to form a new subtype. However, the exact reason for the emergence of DENV-5 is not clear (Mustafa *et al*., 2015). Due to different serotype resulting in different immune response, makes it difficult to treat the disease. When an individual recovers from an infection caused by one dengue serotype, temporary protection from other serotypes is approximately only two-three months after the first dengue infection. After a period, the individual can be infected with the remaining dengue serotype (Nature, 2014).

The disease is transmitted from humans through bites of the infected female vector mainly *Ae. aegypti*, but *Ae. albopictus* can also act as a vector (Rozendaal, 1997). Vector i.e. *Ae. scutellaris* group are vectors in some parts of the Western Pacific region (Service, 2012). In general, after incubation for 4 - 10 days, an infected mosquito is capable of transmitting the virus for the rest of its life (WHO, 2018c). Transovarial transmission occurs from the mother to the offspring in both species *Ae. aegypti* and *Ae. albopictus* is also reported.

Infected human can be symptomatic but majority are asymptomatic. Dengue is common among infant, young children and adult causing high fever, severe headache, nausea, vomiting, pain behind the eyes, muscle and joint pain. These symptoms usually last between 2 - 7 days. However, severe dengue can cause complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding and organ impairment leading to death.

So far, no anti-viral treatment is available for this disease, other than clinical management which is based on supportive therapy and monitoring the patient's body fluid volume. The first dengue vaccine has been available in the market since late 2015 and early 2016 (WHO, 2018c).

2.5.2 Chikungunya Virus

Chikungunya was first described during an outbreak in Tanzania in 1952. A decade after, outbreaks of Chikungunya fever was reported in Asia including in Indonesia in 1982. Since 2005, India, Maldives, Myanmar, Thailand and Indonesia have reported over 1.9 million cases (WHO, 2017a).

The two common mosquitoes involved in transmitting this virus are *Ae. aegypti* and *Ae. albopictus*. After being bitten by the infected mosquito, onset of illness occurs usually

between 4 and 8 days but can range from 2 to 12 days. Chikungunya shows the same symptom as dengue fever where there is a report of co-occurrence of both fevers. The symptom includes abrupt onset of fever frequently along with joint pain. Muscle pain, headache, nausea, fatigue and rash can also be developed by the infected patient. Serious complications are not common, but can cause death in elderly (WHO, 2017a).

To date, there is still no antiviral drug treatment for this disease. Thus, the treatment focuses on relieving the symptoms which uses antipyretics, optimal analgesics and fluids for the joint pains. Vaccine for this virus is also still not available.

2.5.3 Zika Virus

Zika virus is caused by a virus from the *Flavivirus* genus and was first isolated in 1947 from a monkey in the Zika forest, Uganda. In 1952, Zika was detected in human in Nigeria (CDC, 2016). Zika is known as an important disease in Africa, but also reported in Asian countries including Indonesia (Hayes, 2009).

Zika virus has been isolated from *Ae. aegypti*, the main vector for transmitting this disease. Other *Aedes* mosquitoes such as *Ae*. *albopictus*, *Ae. polynesiensis* and *Ae. viattus* are also known potential vectors for Zika virus. Zika can also be transmitted between humans through materno-foetal transmission, sexual transmission, through blood transfusion and organ transplantation (Musso *et al*., 2015; European Centre for Disease and Control, 2015).

Common symptoms include rash, fever, arthralgia and conjunctivitis and usually last for 2 - 7 days (WHO, 2018a). Zika virus can also cause congenital brain abnormalities, including microcephaly and trigger of Guillain-Barré syndrome if infected during pregnancy. Currently, there are no specific treatment for this disease other than treating the common symptoms and vaccine are also not available for Zika virus (WHO, 2018a).

2.5.4 Yellow fever

Yellow fever occurs mainly in Africa and tropical areas of the Americas. Approximately 200 000 cases were reported with 30 000 deaths caused by yellow fever. There have been several outbreaks in Africa started off in 2006 up to 2010, thus proving that yellow fever is recurring in Africa (Service, 2012).

Yellow fever is transmitted by *Aedes* mosquitoes namely *Ae. africanus*, *Ae. bromeliae*, *Ae. furcifer*, *Ae. opok* and *Ae. luteocephalus* mainly found in the forest and *Ae. aegypti* which can be found in urban areas (Service, 2012). There are 3 types of transmission cycles; sylvatic (jungle) yellow fever which occurs in the tropical rainforest and monkey are the primary reservoir. Thus, humans working or travelling in the forest may be infected if bitten. Intermediate yellow fever which occurs in both in the wild and around vicinity of houses. Urban yellow fever which occurs when infected people introduce the virus into a population of humans lacking immunity or unvaccinated and can be transmitted from person to person (WHO, 2018b).

The most common symptoms of yellow fever are muscle pain with prominent backache, headache, fever, loss of appetite, nausea and vomiting. Eventhough symptoms disappear after 3 to 4 days in most cases, complication involving kidney and liver can occur within the 24 hours during the toxic phase. During the toxic phase, dark urine, abdominal pain with vomiting and yellowing of the skin and eyes appear. Bleeding can also occur from the nose, mouth, eyes or stomach and can cause death within 7 to days.

Early supportive treatment is successful in treating yellow fever, and vaccine is available to prevent being infected with yellow fever (WHO, 2018b).

2.6 Mosquitoes control

The only way to control vector-borne diseases is to prevent transmission (Insecticide Resistance Action Committee, 2006). According to Foster and Walker (2002), there are three main ways to successful vector control; habitat modification, biological control and chemical control. Habitat modification is to prevent the oviposition, hatching or larval development which is also known as source reduction. Habitat modification can be done through the removing of water from containers that can enhance the oviposition. Thus, the sustainability of an integrated vector control program crucially depends on community and owners participation (Gubler, 1989).

Next is through the biological control. There are two main ways in controlling the vector biologically which are through predators and viruses. Predator such as dragonflies, birds and bats reduce the adult mosquito population. However, most biological controls using predators focus on larvae stage reduction such as mosquito fish (*Gambusia affinis),* kill fish (*Fundulus* spp.), grass carp and tilapia (Foster & Walker, 2002). Unfortunately, these biocontrol agents are ineffective in larvae control. Other than that, there are the usage of parasites and pathogen such as nematode (*Romanomermis culicivorax*), protozoan (ciliates *Lambornella*), gregarine sporozoan (*Ascogregarina*) and microsporidian (*Nosema*) (Foster & Walker, 2002). Virus is also used in the vector control such as iridescent viruses and entomopox viruses. Unfortunately, the usage of parasites and viruses are still at the experimental stage and have a limited effects. In contrast, there is a successful usage of *Bacillus thuringinensis israelensis* (Bti) which is effective against *Culex* larva (Foster & Walker, 2002).

There is also successful usage of chemicals on larvae and adults control. Adulticides are placed where the adults rest or are released into the air where the adults fly. The usage of dichlorophenyl trichloroethane (DDT) spraying, thermal fogs and ultra-low-volume

(ULV) sprays, organophosphates, carbamates and pyrethrins are the ones commonly used as adulticides. Larvicides are placed in water where larvae develop or water accumulates to control the number of larvae. Inorganic compounds that are normally used as larvicides are copper arsenate, fuel oil and organochlorine chemicals such as DDT and dieldrin. There are three main usages of larvicides which are light mineral oils, organophosphate (temephos and malathion) and insect growth regulator (methoprene) (Foster & Walker, 2002).

2.7 Insecticides

The four major classes of insecticides are organochlorines, organophosphate, carbamate and pyrethroids (Ranson *et al*., 2010). Since, Dichlorodiphenyltrichloroethane (DDT) was firstly developed during the Second World War in 1940's, it has been heavily used and depended on as an insecticide for vector control (Van de Berg *et al*., 2012). The studies of insecticides were then focused on organophosphates which originated in Germany and have been evolving since 1932. More than a decade later, carbamates were discovered in Switzerland in between 1960s and 1970s synthetic pyrethroids were developed in Japan and UK (Becker *et al*., 2003).

2.7.1 Organochlorine

Organochlorine is made up of carbon, chlorine and hydrogen and was introduced as chlorinated hydrocarbons. The similar characteristics of organochlorine subgroups are high chemical stability, moderate solubility in organic solvents, low solubility in water and low vapor pressure, but differ in structure (Hill & Waller, 1982). Organochlorine insecticides such as DDT, dieldrin, aldrin and hexachlorocyclohexane are among the widely-used pesticides in developing countries of Asia (Jayaraj *et al*., 2016).

The DDT properties were first discovered in 1939 in Switzerland, while dieldrin was first developed in 1945 in the United States (Van de Berg *et al*., 2012; Costa *et al*., 2013). The structural formula of DDT and dieldrin is shown in Figure 2.5. DDT and dieldrin are persistent and inexpensive to produce in a large scale. Both insecticides are effective in disturbing the balance of sodium and potassium ions within the sensory neurons, causing spontaneous firing and preventing normal passage of impulses. It also interferes with the stabilizing action of the calcium and causing the muscles to twitch which is followed by convulsion and death.

Due to its high stability, these insecticides have a long half-life in the soil, aquatic environment, plant and animal tissues. It cannot be easily broken down by microorganisms, enzymes, ultra violet light or heat. It also accumulates in the body systems due to its water-insoluble properties. These characteristics are hazardous to the environment and non-target organisms and presently banned worldwide. However, DDT is still being used in some rural areas or countries in malaria control (Becker *et al*., 2003).

In Indonesia, DDT was used in malaria eradication programme since 1950's but banned in the 1970s, whilst dieldrin was used in 1955 and discontinued a decade after (Asih *et al*., 2012).

2.7.2 Organophosphates

According to Becker *et al*. (2003) organophosphate, a less stable chemical is also known as organic phosphorus, phosphorus esters or phosphorus insecticides. There are two noticeable features of organophosphates, low chemical stability and more toxic than organochloride insecticides.

Organophosphates inhibit enzyme acetylcholinesterases (AChE) to phosphorylate with the acetylcholine (ACh) by binding to the enzyme. This causes the overstimulation

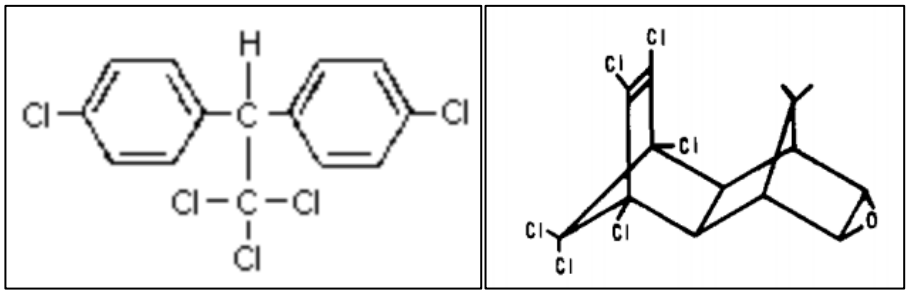
by the excess of acetylcholine in the nerve ending. Accumulation of acetylcholine, interferes with the neuromuscular junction, producing rapid twitching of muscles and final paralysis of the organism. Organophosphates includes malathion, temephos, fenitrothion, fenthion, chlorpyrifos and bromophos. Structural formula of organophosphate insecticides are shown in Figures 2.6 – 2.10.

Malathion was first introduced in 1950 for pest control, mosquito control and agriculture. Malathion is a fast action with a very low acute toxicity. Malathion is commonly used as a ULV formation in controlling the adult mosquitoes. It is majorly used in Indonesia in fogging since the early 1970's up to now (Putra *et al*., 2016).

Temephos which is marketed as Abate has been used in mosquito larviciding for a very long period of time. It has a very low mammalian toxicity and has been used in various aquatic habitats including water for human consumption. Temephos is also one of the insecticides that is toxicologically accepted to be used in potable waters. To date, Indonesia has been using temephos in mass larviciding since 1970's (Putra *et al*., 2016).

Fenitrothion or also known under a different trade name as Acothion is used in larviciding in most European countries. It is usually used as larvicide in closed water systems; sewage and waste-water containers. Fenthion is mainly used in agriculture and used in public health vector control. Fenthion are also sold under different names such as Baytex, Queletox and Lebaycid. They have been used in agriculture in most countries since 1957 to control numerous pests. Fenthion is effective in vector control by direct application and stomach action (WHO, 2003).

Chlorpyrifos is used in agriculture, forestry and mosquito control for both larvae and adults. It works as vector control on direct application, stomach and respiratory action (Becker *et al*., 2003).



A B

Figure 2.5: Structural formula of (A) DDT and (B) dieldrin. Image reproduced with permission from WHO,1979 and Henderson & Crosby, 1967.

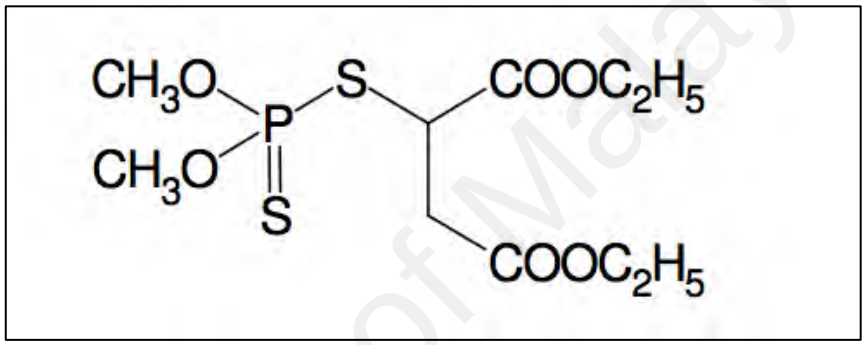


Figure 2.6: Structural formula of malathion. Image reproduced with permission from WHO, 2013.

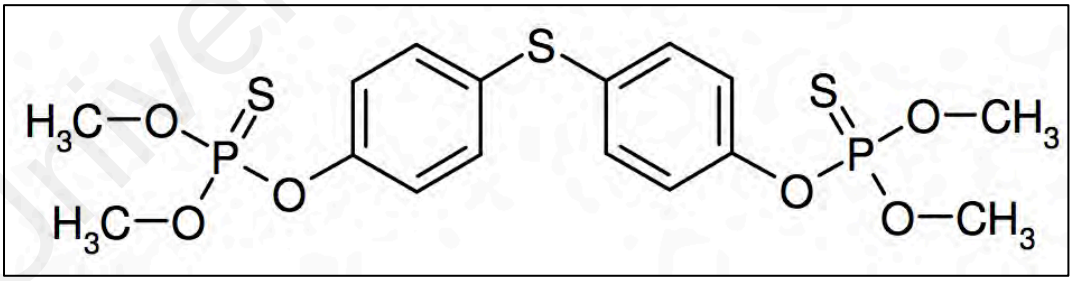


Figure 2.7: Structural formula of temephos. Image reproduced with permission from WHO, 2011.

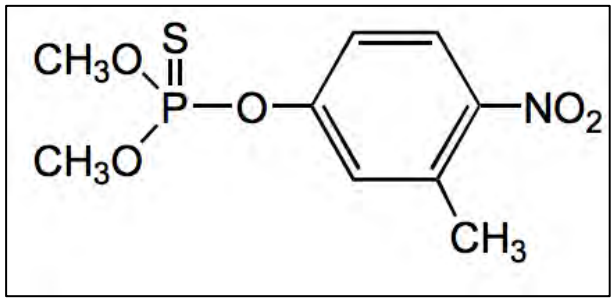


Figure 2.8: Structural formula of fenitrothion. Image reproduced with permission from WHO, 2010.

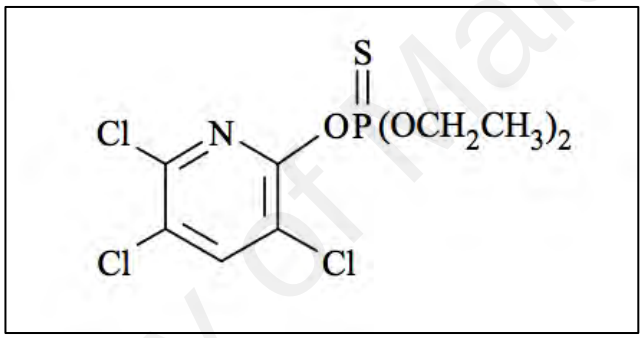


Figure 2.9: Structural formula of chlorpyrifos. Image reproduced with permission from WHO, 2009.

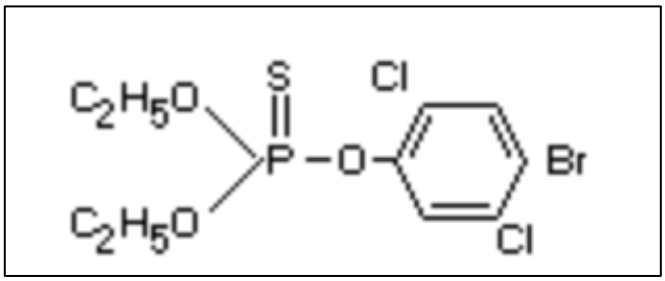


Figure 2.10: Structural formula of bromophos. Image reproduced with permission Bansch *et al*., 1974.

Bromophos has extremely low mammalian toxicity and used mainly in controlling flies and mosquitoes. It is also used in protecting crop mainly for the control of vegetable root flies and in storage pest control (Stiasni *et al*., 1967).

2.7.3 Carbamates

Carbamates were first introduced in 1951 by a company in Switzerland and developed as insecticides after the discovery of the highly active *N*-methyl carbamates.

The mode of action of carbamates are similar to organophosphates where it affects the activity of AChE, by hydrolysing the enzyme and thus overstimulation of ACh causing the prolonging action of the neurotransmitter at cholinergic synapse. This results in convulsions, paralysis and ultimately death on the insects. However, the enzyme inhibition in carbamates are more easily reserved as compared to organophosphates thus the insects can recover at lower dosages (Becker *et al*., 2003). Examples of carbamates are propoxur and bendiocarb. The structural formula of bendiocarb and propoxur are shown in Figures 2.11 and 2.12.

Bendiocarb is commonly used as residual house insecticide to control cockroaches and flies and used by public health control against adult mosquito. It has been used for both residual spray and aerial ULV applications (Becker *et al*., 2003).

Propoxur is mainly used as active ingredient in commercialized product called Baygon which is also used in Indonesia. Propoxur is mostly used in controlling the adult mosquitoes by spraying outdoor and indoor (Becker *et al*., 2003).

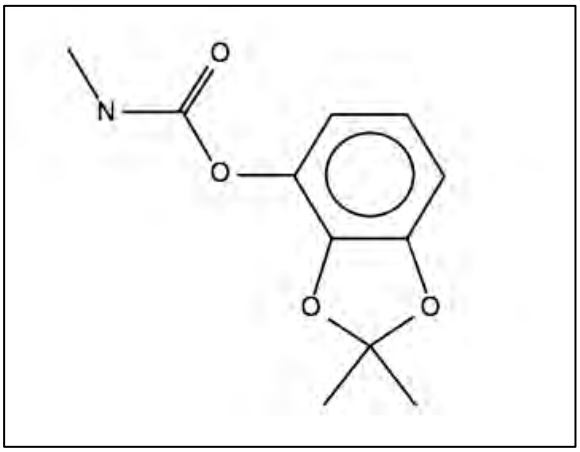


Figure 2.11: Structural formula of bendiocarb. Image reproduced with permission WHO, 2008.

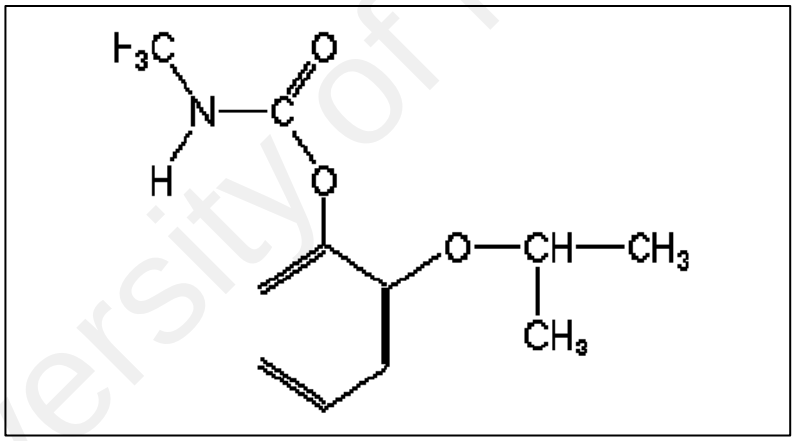


Figure 2.12: Structural formula of propoxur. Image reproduced with permission WHO, 2017b.

2.7.4 Pyrethroids

Synthetic pyrethroids are the latest developed group of insecticides and are vastly used in controlling mosquito. Natural pyrethrins, also called as pyrethrum, mixture of insecticidal esters and *Chrysanthemums* spp. flower head was widely used in 1840. In 1941, pyrethrins were used as pests control against mosquitoes, houseflies and other indoor pests in pressurized aerosol containers. However, due to its rapid degradation in the environment it cannot be used on a large scale. Fortunately, the more stable synthetic pyrethroids were successfully synthesized in late 1960's and 1970's which are more stable than pyrethrins and vastly used up to now (Van den Becken & Vijverberg., 1988; Coleman *et al*., 2017). Pyrethroids are effective since it causes knock-down effect with repellent or anti-feeding action on adult mosquitoes and can be effectively applied as mosquito larvicides. Other than that, pyrethroids have low oral toxicity to mammals but higher oral toxicity against insects (Vijverberg & Van der Becken, 1990).

Pyrethroids interferes with the nervous function system, involving the interaction with the sodium channel in nerve membranes. The balance of sodium and potassium ions within the sensory neurons are disturbed with the presence of pyrethroids insecticides causing spontaneous firing and preventing normal passage of impulses. In addition, pyrethroids also cause membrane destabilization by interfering with the calcium stabling action at the axonal surface. Then, the disruption is transmitted throughout the nervous system which cause muscles twitching, convulsions and eventually death (Wouters & Van den Bercken, 1978). Cyfluthrin, etofenprox, lambda-cyhalothrin, permethrin and deltamethrin are among the examples of pyrethroids. The structural formula of cyfluthrin, lambda-cyhalothrin, etofenprox, permethrin and deltamethrin are shown in Figure 2.13 – 2.17.

Cyfluthrin have low volatility with very low water solubility. They are mainly a contact insecticide, not systematic and does not penetrate plant tissues. They are mainly used in residual sprays, impregnation and fogging (WHO, 2003).

Lambda-cyhalothrin have low vitality and short persistence in soil. They are mainly used to control the vectors in indoor residual spraying, space spraying and treatment of mosquito nets (WHO, 2003).

Etofenprox is a non-ester pyrethroids but also acts like other pyrethroids by targeting the chloride channel of the insect nervous systems. However, etofenprox lacks the common structure of the pyrethroids classes which are the ester bond between the acid and alcoholic moiety (Clark *et al*., 2011). Etofenprox is used broadly in agriculture, forestry, horticulture and in pest control. It is mainly used in vector control by direct application or indirectly by impregnating fabrics such as mosquito nets (WHO, 2003).

Permethrin is applied in many sectors including agriculture, animal health and public health. They are used in vector control by direct application, stomach action with some repellent effects (WHO, 2003).

Deltamethrin is mainly used in agriculture sector, and also used in controlling ticks, moths and insect pests of livestock. It is used in vector control by direct application and stomach action. Stomach action acts when an insecticide is eaten by an insect where the poison will enter the stomach and being absorbed into the body (WHO, 2003).

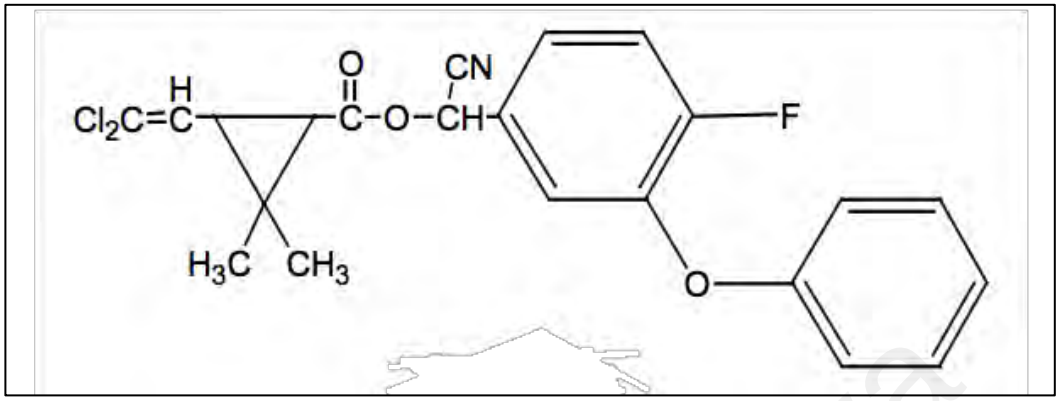


Figure 2.13: Structural formula of cyfluthrin. Image reproduced with permission Casjens, 2009.

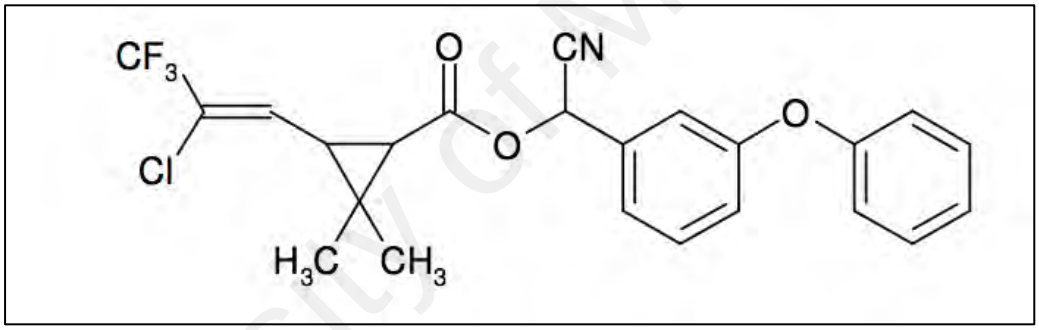


Figure 2.14: Structural formula of lambda-cyhalothrin. Image reproduced with permission WHO, 2015.

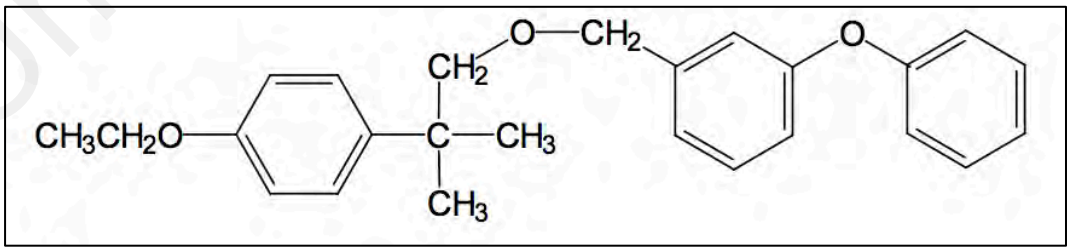


Figure 2.15: Structural formula of etofenprox. Image reproduced with permission from WHO, 2007.

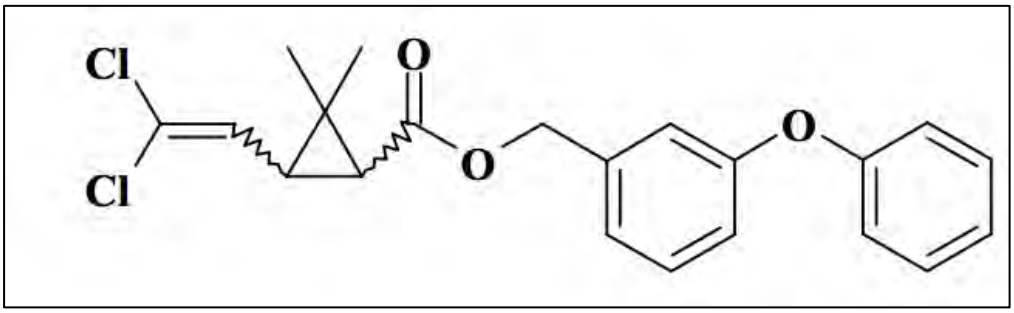


Figure 2.16: Structural formula of permethrin. Image reproduced with permission WHO, 2006.

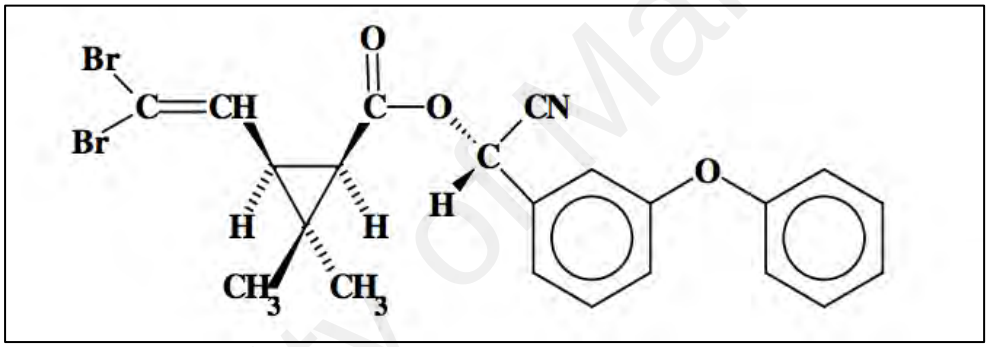


Figure 2.17: Structural formula of deltamethrin. Image reproduced with permission WHO, 2012.

2.8 Resistance status in *Aedes* Mosquitoes

Insecticide is used in vector control since the discovery of dichlorodiphenyltrichloroethane (DDT) in the 1940s. Insecticides is extensively used globally to control or eradicate the vectors for more than 2 decades. Unfortunately, the success was only temporary with vectors eventually developed resistance against the insecticide in use (Rozendaal, 1997).

Insecticide Resistance Action Committee (2006) defines resistance as 'the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended level of control when used as recommended'.

Resistance of insecticide existed since 1992 and among the insecticide-resistant vectors are anopheline and culicines mosquitoes. Resistance has been reported to occur in every chemical class of insecticide, including insect growth regulators and microbial drugs (Brogdon & McAllister, 1998).

According to Brogdon and McAllister (1998), resistance can occur when enhanced levels or modified activities of enzymes; esterases, oxidases or glutathione S-transgerases prevent the insecticide from reaching its site action and insecticide no longer binds to its target. These two types of resistance mechanisms are; detoxification mechanisms and target-site mechanisms. Esterases, oxidases, and GST are the enzymes responsible in the detoxification of toxic compounds. However, when the levels of the activities of these enzymes are modified it reduces the effectiveness of the insecticide which commonly occurs in insects with modified levels of esterases. Target site mechanisms occurs when there are alterations in the amino acids active binding site causing the insecticide to be less effective or ineffective. Other than that, Miller (1988) adds that there are also

behavioral resistant where the insects changes in behavior and no longer favors the contact where the insecticide is deposited.

Furthermore, cross resistance occurs within the same class of insecticide or with the similar mode of action. For instance, organophosphates and carbamates have the same target which are the acetylcholinesterases, whilst organochlorine and synthetic pyrethroids both target the sodium channels of the nerve sheath (Brogdon & McAllister, 1998).

2.8.1 Diagnostic Insecticide-Resistance Mosquitoes

The best and conventional way to detect the resistance is based on insecticide susceptibility through dosage-mortality (bioassay) test (Brogdon, 1989). It is being determined when mosquitoes are exposed to single dose for a period of time. It also can be used to quantify the level of insecticide resistance either by recording the mortality against series of insecticide concentrations or different exposure periods (Weetman & Donelly, 2015). Besides that, there are also advance methods to detect the resistance such as biochemical immunological, nucleic acid probe resistance detection assays and molecular diagnostic markers (Brogdon, 1989; Weetman & Donelly, 2015).

2.8.1.1 WHO Susceptibility Tests

WHO larval and adult susceptibility bioassays (2005 and 2016b) are the standard measures for early detection of insecticide resistance in mosquito populations. According to WHO (2016a), insect from a wild population that are exposed to a fixed concentration of insecticide on test papers or test solutions are designed to reliably kill susceptible insects, so that any survivors may be assumed to be resistant.

Female adult mosquito are exposed to discriminating dosage of insecticide-impregnated paper for an hour. Within the exposure period, adult mosquitoes are

observed and knockdown mosquitoes were calculated for each minute. The knockdown results are subjected to probit analysis to obtain the KT_{50} (50% knockdown time). Further, the adult mosquitoes which were provided with 10% sugar solution were left for 24 hours, to observe the mortality. After that period, dead adult mosquitoes were counted and recorded (WHO, 2016b). Similar method applies for larval bioassay, where larvae were exposed to discriminating dosage of test solutions for 24 hours and observed mortality were recorded 24 hours post-treatment (WHO, 2005).

Observed mortality from both bioassays are then interpreted to determine whether the populations are still susceptible or resistant against specific insecticides. As recommended by WHO (2016b), 98-100% mortality indicates susceptibility, 90-97% mortality is, possibility of resistance that needs to be further confirmed and less than 90% mortality is, resistance.

2.8.2 Resistance Studies on *Ae. aegypti* Mosquitoes in Indonesia

In 1996, Mardihusodo tested *Ae. aegypti* adult on the level of enzymes and relating it to the resistance status of the adult mosquito in Yogyakarta. He found that in the study sites in Yogyakarta the mosquito had high levels of enzyme and this indicated the resistance status of the adults. Hence, the resistance of the adult *Ae. aegypti* was tested against diagnostic dosage of deltamethrin and permethrin and were found to be resistant (Wuliandri *et al*., 2015).

Field strains of *Ae. aegypti* adults in Semarang and Salatiga were also found to be resistant against lambda-cyhalothrin and deltamethrin (Brengues *et al*., 2003; Sayono *et al*., 2016). *Ae. aegypti* adults in Bandung and Palembang were both resistant against the permethrin, whilst deltamethrin were effective only in Palembang (Ahmad *et al*., 2009).

However, cypermethrin was found to be susceptible against adult *Ae. aegypti* collected from Cimahi city (Astuti *et al*., 2014).

Malathion was also ineffective in the field strain of adult *Ae. aegypti* from South Sumatra, Jakarta, Semarang and Sukarta (Ambarita *et al*., 2015; Prasetyowati *et al*., 2016; Sayono *et al*., 2016). Larvae of *Ae. aegypti* were also found to be resistant in most of the divisions in Indonesia. Fuadzy and Hendri (2015) found that *Ae. aegypti* larvae collected from Kota Tasikmalaya were resistant against the diagnostic dosage of temephos. Similar finding was observed by Mulyatno *et al*. (2012) and Prasetyowati *et al*. (2016) where *Ae. aegypti* larvae from Surabaya and Jakarta were also resistant. In addition, *Ae. aegypti* larvae obtained from Semarang, Salatiga were resistant against DDT, permethrin (Brengues *et al*., 2003).

2.8.3 Resistance Studies in *Ae. aegypti* mosquitoes in other countries

Susceptibility status of *Ae. aegypti* was found to be similar in some other countries. Adult *Ae. aegypti* from Bangsar, Malaysia were resistant against diagnostic dosage of malathion (Shafie *et al*., 2012). In contrast, studies done in Thailand, the main states in Malaysia, India and Vietnam were susceptible against the diagnostic dosage of malathion (Rohani *et al*., 2001; Huong *et al*., 2004; Ponlawat *et al*., 2005; Das *et al*., 2011; Ishak *et al*., 2015).

Most pyrethroids were ineffective against the adult *Ae. aegypti*, in the main states of Malaysia. The field strain of adult *Ae. aegypti* was resistant against permethrin and deltamethrin (Rohani *et al*., 2001; Ishak *et al*., 2015). In Singapore, resistant against the permethrin, cypermethrin, deltamethrin and etofenprox was detected (Koou *et al*., 2014). Moreover, some Latin countries also reported the resistance of adult *Ae. aegypti* against deltamethrin and lambda-cyhalothrin (Rodriguez *et al*., 2002).

However adult *Ae. aegypti* obtained from certain cities in India, Viet Nam, and Thailand were found to be susceptible against the diagnostic dosage of deltamethrin, cyfluthrin, permethrin, lambda-cyhalothrin and etofenprox (Huong *et al*., 2004; Chareonvirigaphop *et al*., 2013; Das *et al*., 2011).

In general, DDT was found to be impotent in both India and Malaysia. Adult *Ae. aegypti* was resistant to DDT in Kuala Lumpur, Kelantan, Johor and Pulau Pinang, Malaysia (Rohani *et al*., 2001; Ishak *et al*., 2015). Adult *Ae. aegypti* from Delhi, Mumbai, Jodhpur, Chennai and Ranchi City, India were also resistant against both DDT and dieldrin. However, dieldrin was reported to be effective in major states of Malaysia and India for both adults and larvae (Madhukar & Pillai., 1970; Katyal *et al.,* 2001; Tikar *et al.,* 2008; Ishak *et al*., 2015).

CHAPTER 3: METHODOLOGY

3.1 Preliminary Distribution and Abundance of Dengue Vectors in The Sunda Islands of Indonesia

3.1.1 Study sites

Ovitrap surveillance was conducted throughout 14 sites across eight provinces of Indonesia. The geographical description of the study sites is given in Figure 3.1 and Table 3.1.

Table 3.1: Geographical description of study sites in Indonesia.

Sunda Island	Island	Province	Regencies	Study site	Geographical distribution	Landscape
Greater Sunda Islands	Java	West Java	Kuningan	Kuningan	S 6º13'5.260" E 106º50'15.936"	Sub-urban
	Sumatra	West Sumatra	Padang	Air Tawar Barat	S 0º53'48.260" E 100º20'45.265"	Sub-urban
		Aceh	Aceh	Banda Aceh	N 5º34'27.170" E 95º22'6.517"	Sub-urban
	Borneo	East Kalimantan	Samarinda	Sidodadi	S 0º28'41.646" E 117º08'46.441"	Sub-urban
			Paser	Long Ikis	S 1º36'15.486" E 116º10'1.292"	Rural
		West Kalimantan	Pontianak	Bangka Belitung Laut	S 0º3'31.967" E 109º21'19.322"	Sub-urban
Lesser Sunda Islands	Bali	Bali	Denpasar	Sanur	S 8º41'10.254" E 115º15'23.634"	Sub-urban
	Lombok	West Nusa Tenggara	Mataram	Ampenan	S 8º34'13.911" E 116º05'08.575"	Sub-urban
				Pagesangan	S 8º36'2.666" E 116º06'07.080"	Sub-urban
	Sumbawa		Dompu	Bada	S 8º32'20.878" E 118º27'28.799"	Sub-urban
	Flores	East Nusa Tenggara	Manggarai Barat	Labuan Bajo	S 8º29'34.269" E 119º52'40.889"	Sub-urban
	Sumba		Southwest Sumba	Tambolaka	S 9º25'50.416" E 119º14'18.636"	Sub-urban
			East Sumba	Waingapu	S 9º39'49.331" E 120º16'17.321"	Sub-urban
	Timor		South Central Timor	Soe	S 9º51'33.538" E 124º15'44.345"	Sub-urban

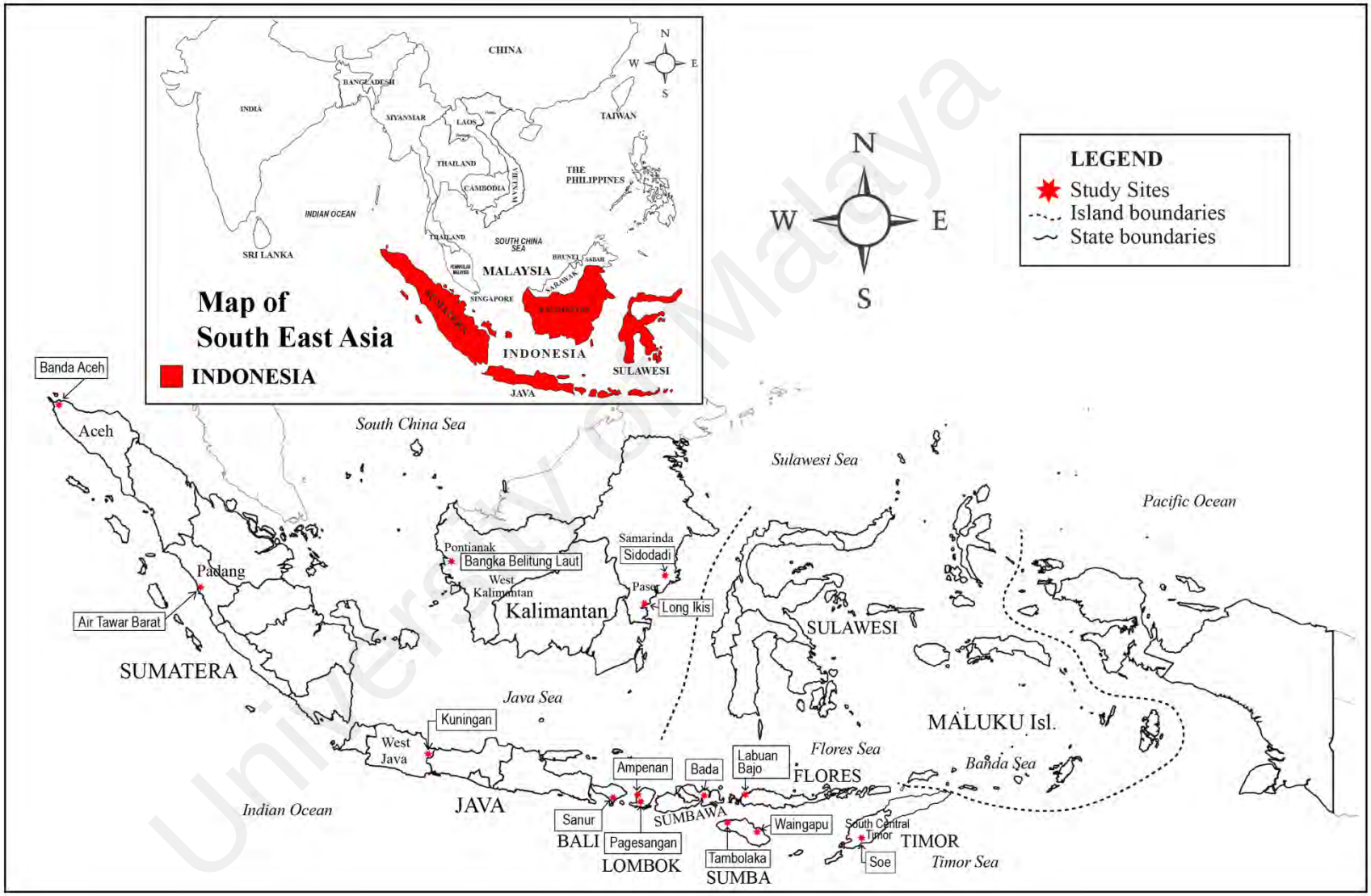


Figure 3.1: Location of study sites in Indonesia.

3.1.2 Ovitrap Surveillance

Preparation of ovitrap was performed according to Lee (1992). Ovitrap used was a 300ml plastic container with base diameter of 6.5 cm, 9.0 cm in height and 7.8 cm opening. A layer of black oil paint was sprayed and coated on the outer wall of the container (Figure 3.2A). An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) was placed diagonally into each ovitrap (Figure 3.2B). Overnight tap water was filled into each ovitrap to a level of 5.5 cm and the ovitraps were placed randomly in indoor and outdoor of selected houses. Ovitraps were placed on the ground with 25 meters apart from each other and not less than 10% in the housing area of each study sites. In this study, interior of the house is referred as "indoor", while outside of the house but confined to the immediate vicinity of the house is referred as "outdoor" i.e. car porch, corridor and under the eave (Wan-Norafikah *et al*., 2011).

3.1.3 Larvae collection and identification

Ovitraps were collected after five days of exposure and were brought back to laboratory. The contents were poured into individual plastic containers, together with the paddle. Each container was added with fresh water and the larvae were allowed to grow in the laboratory. To avoid oviposition of other mosquitoes from the vicinity, the containers were kept covered. A small amount (0.01 g) of dried and powdered beef liver was added into each container as larval food (Figure 3.3). The hatched larvae were subsequently counted and identified at 3rd instar and the numbers of larvae were recorded individually for each positive ovitrap. The identified larvae were then bred in the insectarium of Institute of Biological Sciences, University Malaya for the adult and larval bioassay (Chen *et al*., 2005a).

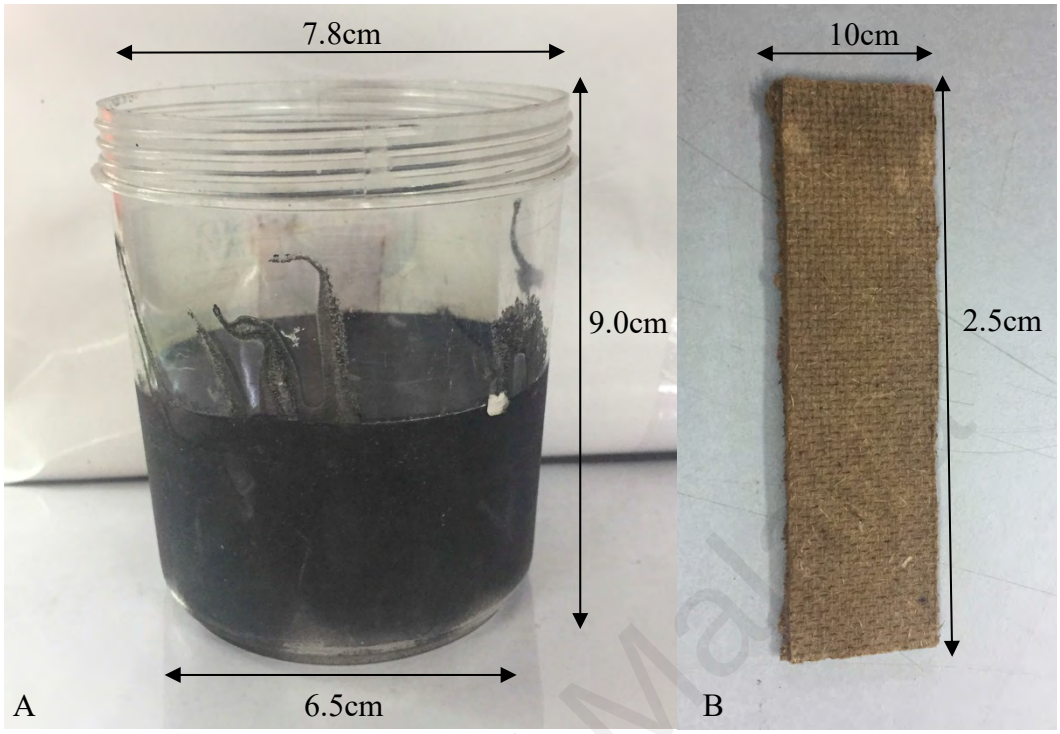


Figure 3.2: (A) Plastic container used for ovitrap and (B) oviposition paddle.

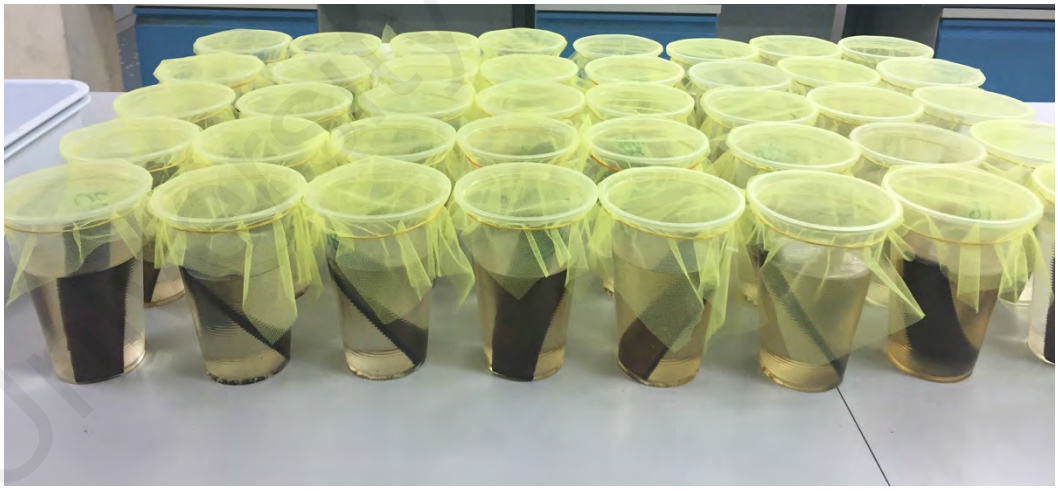


Figure 3.3: Plastic containers storing the paddle and contents of the ovitraps in the laboratory.

All 3rd instar larvae from the ovitraps were individually identified. Each larva was placed on a slide and was observed with the compound microscope. *Ae. aegypti* and *Ae. albopictus* were differentiated by observing on the 8th segment of the larvae abdomen and the spine on thorax of the larvae.

Ae. aegypti was identified with posing a single row of comb teeth with pitch-fork shaped, while *Ae. albopictus* with a single row of comb teeth with straight thorn-like shape (Figures 3.4 A and B). The spine on *Ae. aegypti* are longer and ending in a single point, while the spine is shorter or absence on *Ae. albopictus* (Figures 3.5 A and B).

3.1.4 Data analysis

The percentage of positive ovitraps to the total number of recovered ovitraps was determined for each ovitrap surveillance to obtain the Ovitrap Index (OI).

$$\text{Ovitrap index} = \frac{\text{Number of positive ovitrap}}{\text{Number of recovered ovitrap}} \times 100$$

Mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae per total number of recovered ovitrap were also determined.

$$\text{Mean numbers of larvae} = \frac{\text{Total number larvae (ovitrap 1 + …… ovitrap 20)}}{\text{Number of recovered ovitraps}}$$

All levels of statistical significance were determined at $p < 0.05$ by using statistical programme (SPSS Version 25.0, IBM, Armonk, NY).

(1) paired t-test was performed to determine the differences of the mean number of *Ae. aegypti* and *Ae. albopictus* larvae recovered per study site.

(2) ANOVA with repeated measures was performed to compare the mean number of *Ae. aegypti* and *Ae. albopictus* larvae found both indoor and outdoor.

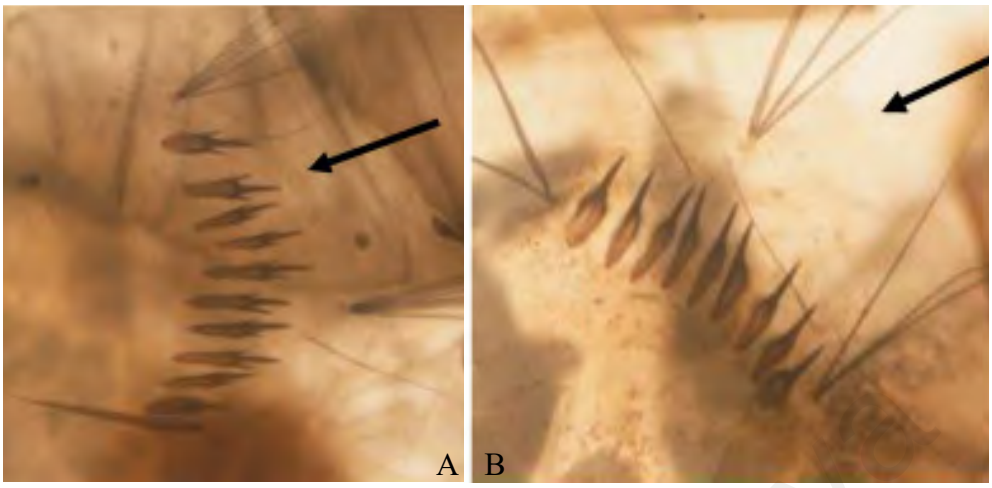


Figure 3.4: (A) *Ae. aegypti* larvae: comb scale with pitch-fork shape
(B) *Ae. albopictus*: comb scale with straight-thorn like shape. Image reproduced withpermission from Teo *et al*., 2017.

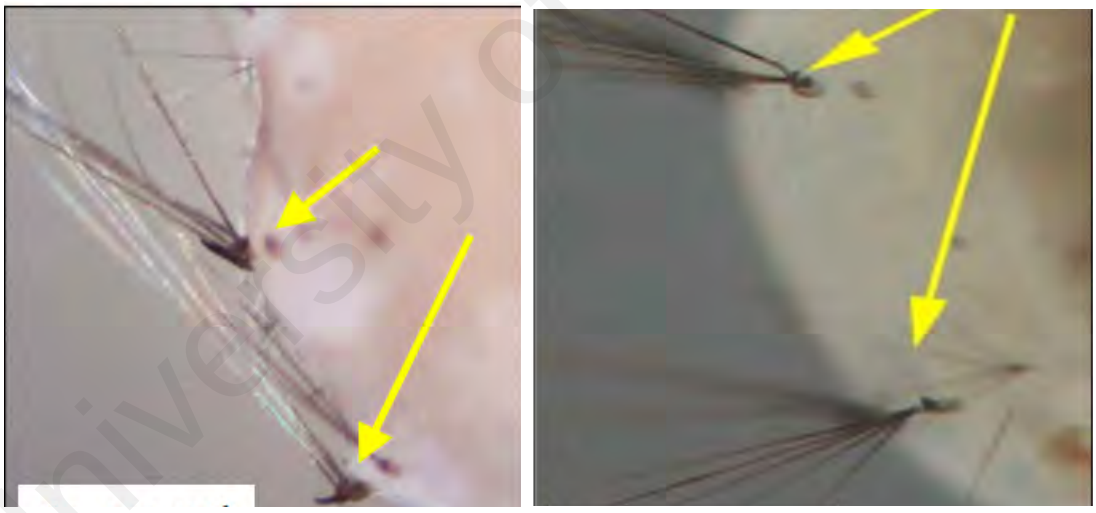


Figure 3.5: (A) *Ae. aegypti* larvae: spine longer and end in a single point
(B) *Ae. albopictus*: spine is shorter or absence. Image reproduced with permission from Yoshimizu, 2013.

3.2 Test insects

This study focuses on the *Ae. aegypti* since this species is found both indoor and outdoor in the preliminary surveillance

3.2.1 Mosquito strain

Field strains of *Ae. aegypti* obtained from the ovitrap surveillance were used in both adult and larval bioassay. While, the reference strain of *Ae. aegypti* (Bora-bora) was obtained from University Science of Malaysia (USM), was used in both adult and larval bioassay.

3.2.2 Mosquitoes Colonization

Both *Ae. aegypti* larvae collected from the field by ovitrap surveillance (labelled as F0) and reference strain of *Ae. aegypti* were colonized in the insectarium of Institute Biological Sciences, University Malaya under room temperature of 28 °C and 70% relative humidity. F1 generation of the field strain and F60 of reference strain were reared and used for WHO adult bioassay and WHO larvae bioassay. The mosquitoes were colonized by following the standard method used by Medical Entomology Division, Institute for Medical Research. Larvae were reared in rectangular plastic containers measuring 14.7 cm x 20 cm x 7.5 cm filled with overnight tap water (Figure 3.6). The larvae were fed with beef liver powder (Figure 3.7). Every 2 - 3 days, at least 50% of the water in the containers were removed and replenished with clean water to ensure the water is free of accumulated food debris. Pupae were then transferred into plastic cup and placed into mosquito cage (30 cm x 30 cm x 30 cm) for adult emergence (Figure 3.8). Adult mosquitoes were then fed with 10% sucrose solution containing 1% of vitamin B complex and four to five days old female adults were blood-fed with wire mesh confined mice for 12 hours during daytime.

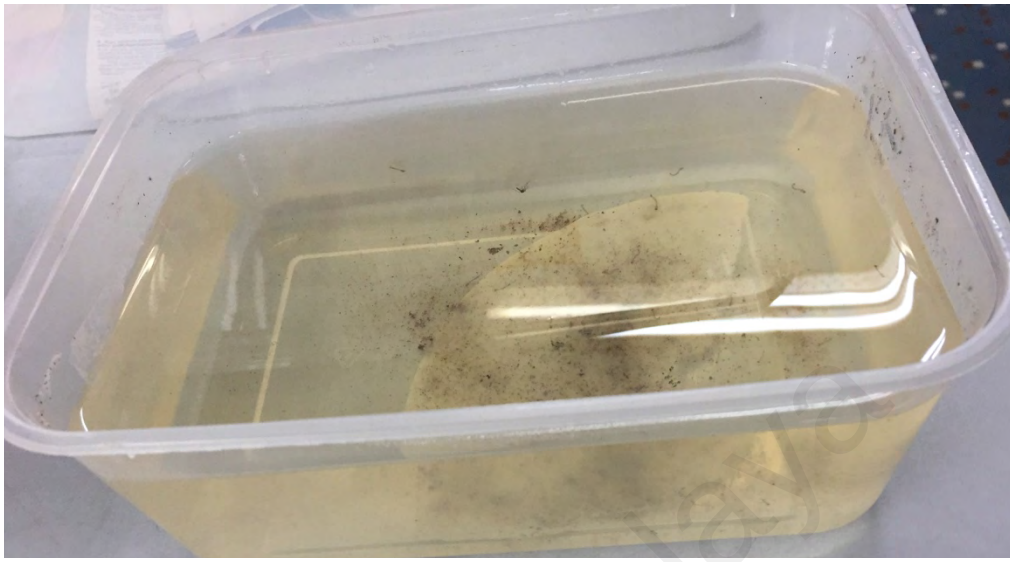


Figure 3.6: Larvae reared in rectangular plastic containers.

Figure 3.7: Beef liver powder.

Oviposition site was prepared using plastic cup (4.7 cm base diameter, 7.4 cm opening diameter and height of 7.7 cm) containing 200 ml overnight tap water and lined with filter paper. The eggs obtained were removed for air drying and the filter paper was immersed into overnight tap water to hatch the eggs (Figure 3.9).

3.2.3 Diagnostic dosage

Diagnostic dose is a fixed dose of an insecticide ingredient dissolved in a solvent that is exposed to a standard period of exposure and used to discriminate the proportions of susceptible and resistant in a sample of mosquito population (WHO, 2016b). In this study, specific diagnostic dose are applied according to the concentrations provided by WHO.

3.3 Resistance Status of *Ae. aegypti* against Four Major Classes of Adulticides in Sunda Islands of Indonesia

3.3.1 Insecticides

The insecticides used in bioassay are DDT (4%), dieldrin (0.4%), bendiocarb (0.1%), propoxur (0.1%), malathion (5%), fenitrothion (1.0%), deltamethrin (0.025%), etofenprox (0.5%), lambda-cyhalothrin (0.05%), cyfluthrin (0.15%) and permethrin (0.25%). All insecticide-impregnated paper was supplied from WHOPES Collaborating Centre in Universiti Sains Malaysia, Penang, Malaysia.

3.3.2 WHO Adult Bioassay

Insecticide susceptibility tests were performed according to WHO standard procedures (WHO, 2016b). Bioassay were carried out using 2 to 5 days old, non-blood fed adult female *Aedes* mosquitoes. For each test, 3 replicates of 15 females were exposed to insecticide-impregnated papers in the test tubes for 1 h (Figure 3.10). The number of adult mosquito's knockdown were recorded every minute up to 60 minutes. Mosquitoes were transferred into holding tubes and supplied with a 10% sugar solution and kept at 27 - 28

°C and 70 – 80% RH (Figure 3.11). Mortality of female adult mosquitoes was recorded 24 h after exposure. Control treatment was set up by exposing the mosquitoes to untreated papers (WHO, 2016b).

3.3.3 Data Analysis

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 - 97% and considered resistant if mortality rates < 98%. If the mortality in the control tubes exceeds 10%, the mortalities of all treated groups were corrected using the Abbotts's formula (WHO, 2016). The data obtained from bioassay was subjected to probit analysis to obtain the knockdown time (KT) values.

Corrected mortality (%) =

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

To investigate cross resistance among insecticides, relationships between the mortality rates of various insecticides were analysed using Spearman correlation analysis, where *p*-values ≤ 0.05 was considered statistically significant. All the data obtained were analysed using SPSS Version 25.

Figure 3.8: Mosquito cage and fed with 10% sucrose solution.

Figure 3.9: Mosquito eggs obtained.

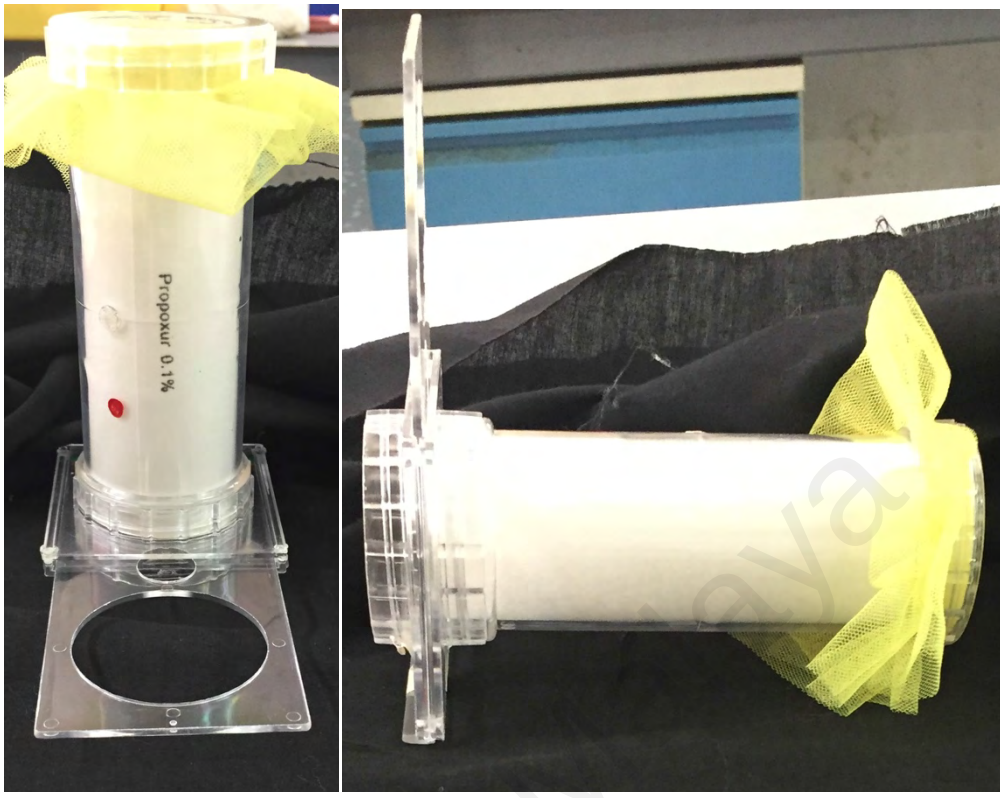


Figure 3.10: WHO Adult Bioassay.

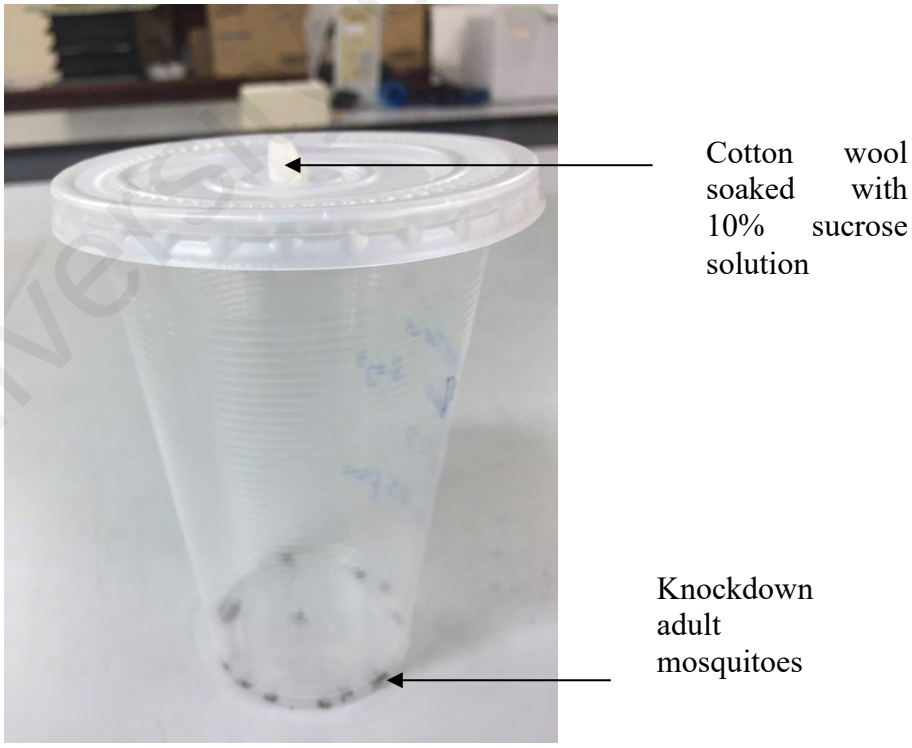


Figure 3.11: Mosquitoes transferred into holding tube.

3.4 The Effectiveness of Diagnostic Dosage of Two Major Classes of Insecticides for *Ae. aegypti* Larvae Control in The Sunda Islands of Indonesia

3.4.1 Insecticides

The larvae were tested against diagnostic dosages of bromophos (0.050 mg/l), chlorpyrifos (0.002 mg/l), fenitrothion (0.020 mg/l), fenthion (0.025 mg/l), malathion (0.125 mg/l), temephos (0.012 mg/l), dieldrin (0.025 mg/l) and DDT (0.012 mg/l). All stock solutions of larvicides were supplied from WHOPES Collaborating Centre in Universiti Sains Malaysia, Penang, Malaysia. The concentration for each larvicide was prepared by dilution from 31.25 mg/L for bromophos, fenitrothion, fenthion; 312.5 mg/L for temephos; 6.25 mg/L for chlorpyrifos; 8% for malathion; 4% for DDT and 1% for dieldrin.

3.4.2 WHO Larval Bioassay

The larvicidal activity of the insecticide were assessed according to the WHO standard procedure for larval bioassay (WHO, 2005). Twenty-five late 3rd instar larvae were introduced into 250 mL of test solution containing larvicides and ethanol in a 300 mL paper cup for 24 h (Figure 3.12). The concentrations were obtained by diluting commercial grade of larvicides stock solution with absolute ethanol. For the control, 1 mL of ethanol were added to 249 mL distilled water. The experiment were replicated three times and the mortality of the larvae were assessed after 24 h. Larvae were considered dead if they sank to the bottom of the paper cups and fail to move or float after being probed (Othman *et al*., 2010).

3.4.3 Data Analysis

The percentage mortality of the larvae was determined by dividing the number of dead larvae by the total number tested and it were corrected using Abbots formula in case

control mortality was between 5% and 20%. The larvae strain was considered susceptible if the mortality rates were greater than 98%, possible resistance if mortality is between 90 - 97% and considered resistant if mortality rates were less than 98% (WHO, 2016). Mortality were recorded 24 h after exposure.

$$\text{Percentage mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae tested}} \times 100$$

All the data obtained were analysed using SPSS Version 25.

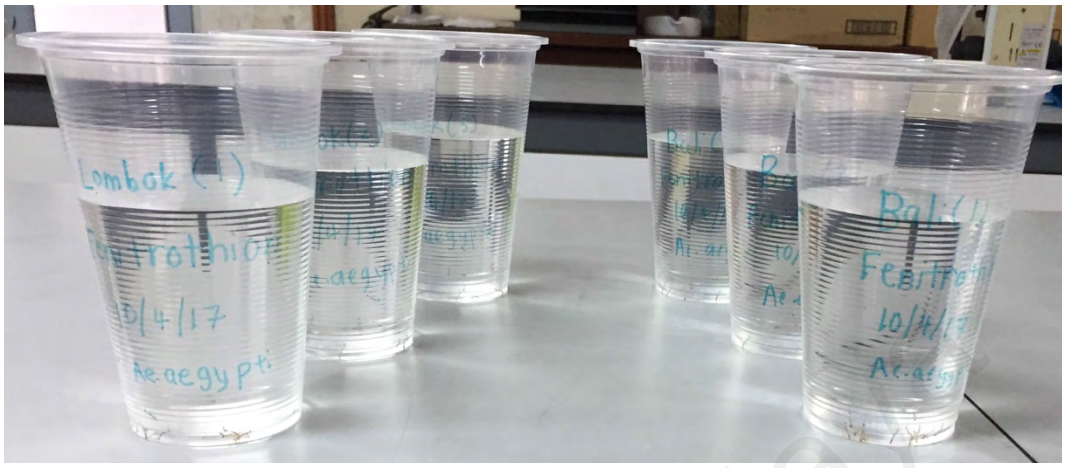


Figure 3.12: WHO larval bioassay.

CHAPTER 4: RESULTS

4.1 Preliminary Distribution and Abundance of Dengue Vectors in The Sunda Islands of Indonesia

From a total of 2431 collected larvae, 66.52% were *Ae. albopictus* and 33.48% were *Ae. aegypti*. Both species were collected from a total of 385 ovitraps placed indoor and outdoor randomly across all study sites.

The indoor OIs ranged from 0.00 to 70.00%, with the highest OI recorded in Sanur (Bali). The mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.00 to 13.35 and 0.00 to 9.00, respectively. Generally, mean numbers of *Ae. aegypti* and *Ae. albopictus* populations obtained from indoor (10 out of 13 sites) were not significantly different ($p > 0.05$). However, the populations of *Ae. aegypti* from Air Tawar Barat (West Sumatra) and Sanur (Bali) were found significantly dominant indoor compared to *Ae. albopictus* ($p < 0.05$). Conversely, a population of *Ae. albopictus* from Tambolaka (Southwest Sumba) was significantly higher than *Ae. aegypti* ($p < 0.05$). No *Aedes* breeding was found in ovitraps placed indoor in Banda Aceh (North Sumatra) and Pagesangan (West Nusa Tenggara). *Ae. albopictus* was absent indoors in eight study sites (Table 4.1).

The outdoor OIs ranged from 0.00 to 90.00%, with the highest OI recorded in Labuan Bajo (East Nusa Tenggara). Mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae ranged from 0.00 to 14.50 and 0.00 to 18.60, respectively. Although *Ae. albopictus* was more prevalent than *Ae. aegypti* in most sites (8 out of 14), only six study sites were found significantly dominant in the outdoor populations ($p < 0.05$). *Ae. aegypti* populations from Kuningan (West Java) and Sidodadi (East Kalimantan) were significantly dominant in outdoor compared to *Ae. albopictus* ($p < 0.05$). No *Aedes* breeding was found in ovitraps placed outdoor in Bada (West Nusa Tenggara) and Soe (East Nusa Tenggara) (Table 4.2).

Table 4.1: Ovitrap index (indoor), mean number of larvae and percentage of larvae density recovered at selected provinces in Indonesia.

Province	Regencies	Study sites	Number of ovitrap	Ovitrap Index (%)	Mean number (± SE)			Percentage of larvae density (%)	
					Ae. aegypti	*Ae. albopictus*	t-test	*Ae. aegypti*	*Ae. albopictus*
West Java	Kuningan	Kuningan	15	6.67	0.27 ± 0.27	0.00 ± 0.00	p= 0.334 df= 14	100	
West Sumatra	Padang	Air Tawar Barat	20	60.00	13.35 ± 4.42	0.00 ± 0.00	p= 0.007* df= 19	100	
North Sumatra	Aceh	Banda Aceh	15	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
East Kalimantan	Samarinda	Sidodadi	7	14.29	0.29 ± 0.29	0.29 ± 0.29	p= 0.356 df= 6	50	50
West Kalimantan	Pontianak	Bangka Belitung Laut	10	10.00	1.10 ± 1.10	0.00 ± 0.00	p= 0.343 df= 9	100	
Bali	Denpasar	Sanur	20	70.00	3.75 ± 1.09	0.70 ± 0.40	p= 0.015* df= 19	84.27	15.73
West Nusa Tenggara	Mataram	Ampenan	10	20.00	0.70 ± 0.70	2.10 ± 2.10	p= 0.535 df= 9	25.00	75.00
		Pagesangan	10	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
	Dompu	Bada	10	10.00	1.30 ± 1.30	0.00 ± 0.00	p= 0.343 df= 9	100	
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	20	60.00	7.15 ± 2.74	9.00 ± 2.67	p= 0.631 df= 19	44.27	55.73
	Southwest Sumba	Tambolaka	15	60.00	0.33 ± 0.33	8.00 ± 3.63	p= 0.033* df= 14	3.96	96.39
	East Sumba	Waingapu	15	13.33	3.13 ± 2.86	0.00 ± 0.00	p= 0.292 df= 14	100	
	South Central Timor	Soe	18	5.56	0.67 ± 0.67	0.00 ± 0.00	p= 0.331 df= 17	100	

N/A: Not available; *Statistically significant ($p < 0.05$); *Ae. aegypti*; *Ae. albopictus*

Table 4.2: Ovitrap index (outdoor), mean number of larvae, and percentage of larvae density recovered at selective provinces in Indonesia.

Province	Regencies	Study sites	Number of ovitrap (n)	Ovitrap Index (%)	Mean number (±SE)			Percentage of larvae density (%)	
					Ae. aegypti	*Ae. albopictus*	t-test	*Ae. aegypti*	*Ae. albopictus*
West Java	Kuningan	Kuningan	14	35.71	0.50 ± 0.19	0.00 ± 0.00	p= 0.029* df= 13	100	
West Sumatra	Padang	Air Tawar Barat	20	85.00	0.15 ± 0.11	17.95 ± 3.52	p= 0.001* df= 19	0.83	99.17
North Sumatra	Aceh	Banda Aceh	15	80.00	0.00 ± 0.00	4.93 ± 1.75	p= 0.014* df= 14		100
East Kalimantan	Samarinda	Sidodadi	8	87.50	14.50 ± 3.54	1.50 ± 1.05	p= 0.008* df= 7	90.63	9.38
	Paser	Long Ikis	15	33.33	0.00 ± 0.00	3.53 ± 1.59	p= 0.043* df= 14		100
West Kalimantan	Pontianak	Bangka Belitung Laut	10	10.00	0.00 ± 0.00	0.10 ± 0.10	p= 0.343 df= 9		100
Bali	Denpasar	Sanur	20	75.00	0.50 ± 0.35	4.40 ± 1.52	p= 0.033* df= 19	10.20	89.80
West Nusa Tenggara	Mataram	Ampenan	10	60.00	0.60 ± 0.43	12.30 ± 5.79	p= 0.074 df= 9	4.65	95.35
		Pagesangan	10	40.00	1.30 ± 0.80	0.20 ± 0.20	p= 0.213 df= 9	86.67	13.33
	Dompu	Bada	10	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	20	90.00	2.35 ± 1.00	18.60 ± 3.24	p= 0.001* df= 19	11.22	88.78
	Southwest Sumba	Tambolaka	15	80.00	0.13 ± 0.09	15.27 ± 4.08	p= 0.002* df= 14	0.84	99.16
	East Sumba	Waingapu	15	20.00	1.20 ± 0.73	0.00 ± 0.00	p= 0.123 df= 14		100
	South Central Timor	Soe	18	0.00	0.00 ± 0.00	0.00 ± 0.00	N/A	N/A	

N/A: Not available; *Statistically significant ($p <$ 0.05); *Ae. aegypti*; *Ae. albopictus*

Overall, the mean number of *Ae. aegypti* larvae per ovitrap was 3.17 ± 0.68 for indoor ovitraps and 1.19 ± 0.51 for outdoor ovitraps. The mean number of *Ae. albopictus* larvae per ovitrap was 1.19 ± 0.29 for ovitraps placed indoors as compared to 6.78 ± 0.89 ovitraps placed outdoors. The presence of both *Ae. aegypti* and *Ae. albopictus* differed significantly in indoor and outdoor ($p < 0.01$). Nonetheless, only 6 study sites i.e. Air Tawar Barat, Sanur, Banda Aceh, Sidodadi, Sanur and Labuan showed significant difference of mean larvae number of both *Ae. aegypti* and *Ae. albopictus* in indoor and outdoor ($p < 0.05$) (Table 4.3).

Mixed infestation of *Ae. aegypti* and *Ae. albopictus* was also observed in this study (Table 4.4). Only 5.4% ovitraps (10 out of 185 ovitraps) were found mixed infestation in indoor, with predomination of *Ae. aegypti* larvae in Sanur by 3.56 folds, and *Ae. albopictus* larvae in Labuan Bajo by 1.26 folds. On the other hand, co-occurrence of both *Aedes* species was also found in 8.5% ovitraps (17 out of 200 ovitraps) in outdoor across seven study sites. *Ae. albopictus* larvae were found dominating in mixed infestation ovitraps in five study sites by 7.91 - 119.67 folds, whilst *Ae. aegypti* larvae were found dominating in mixed infestation ovitraps in two study sites by 6.50 - 9.67 folds.

In addition, independent t-test also showed no significant difference between the different landscapes with OI and mean number of larvae per ovitrap of *Ae. aegypti* and *Ae. albopictus.*

Table 4.3: Composition of *Ae. albopictus* and *Ae. aegypti* larvae obtained from indoor and outdoor.

Province	Regencies	Study sites	*Ae. albopictus*	*Ae. aegypti*
West Java	Kuningan	Kuningan	N/A	*p* value= 0.384 df= (1,14)
West Sumatra	Padang	Air Tawar Barat	*p* value= 0.01* df= (1,19)	*p* value= 0.007* df= (1,19)
North Sumatra	Aceh	Banda Aceh	*p* value= 0.014* df= (1,14)	N/A
East Kalimantan	Samarinda	Sidodadi	*p* value= 0.245 df= (1,6)	*p* value= 0.014* df= (1,6)
West Kalimantan	Pontianak	Bangka Belitung Laut	*p* value= 0.343 df= (1,9)	*p* value= 0.343 df= (1,9)
Bali	Denpasar	Sanur	*p* value= 0.03* df= (1,19)	*p* value= 0.03* df= (1,19)
West Nusa Tenggara	Mataram	Ampenan	*p* value= 0.137 df= (1,19)	*p* value= 0.798 df= (1,9)
		Pagesangan	*p* value= 0.343 df= (1,9)	*p* value= 0.14 df= (1,9)
	Dompu	Bada	N/A	*p* value= 0.343 df= (1,9)
East Nusa Tenggara	Manggarai Barat	Labuan Bajo	*p* value= 0.016* df= (1,19)	*p* value= 0.076 df= (1,19)
	Southwest Sumba	Tambolaka	*p* value= 0.210 df= (1,19)	*p* value= 0.486 df= (1,19)
	East Sumba	Waingapu	N/A	*p* value= 0.445 df= (1,19)
	South Central Timor	Soe	N/A	*p* value= 0.331 df= (1,17)

*statistically significantly ($p < 0.05$)

Table 4.4: Mixed breeding of *Aedes* larvae in ovitraps obtained from the selected study sites in Indonesia.

Regencies	Study site	Number of recovered ovitraps	Total positive ovitraps	Ovitraps with mixed breeding	Percent positive ovitrap (%)			Ratio of *Ae. aegypti: Ae. albopictus* in mixed breeding
					Ae. aegypti	*Ae. albopictus*	Mixed breeding	
				Indoor				
Kuningan	Kuningan	15	1	0	100.00	0.00	0.00	N/A
Padang	Air Tawar Barat	20	12	0	100.00	0.00	0.00	N/A
Aceh	Banda Aceh	15	0	0	0.00	0.00	0.00	N/A
Samarinda	Sidodadi	7	1	0	100.00	0.00	0.00	N/A
Paser	Long Ikis				N/A			
Pontianak	Bangka Belitung Laut	10	1	0	100.00	0.00	0.00	N/A
Denpasar	Sanur	20	14	2	78.57	7.14	14.29	5.36 : 1.00
Mataram	Ampenan	10	2	0	50.00	50.00	0.00	1.00 : 3.00
	Pagesangan	10	0	0	0.00	0.00	0.00	N/A
Dompu	Bada	10	1	0	100.00	0.00	0.00	N/A
Manggarai Barat	Labuan Bajo	20	12	8	8.33	25.00	66.67	1.00 : 1.26
Southwest Sumba	Tambolaka	15	9	0	11.11	88.89	0.00	N/A
East Sumba	Waingapu	15	2	0	100.00	0.00	0.00	N/A
South Central Timor	Soe	18	1	0	100.00	0.00	0.00	N/A
				Outdoor				
Kuningan	Kuningan	14	5	0	100.00	0.00	0.00	N/A
Padang	Air Tawar Barat	20	17	2	0.00	88.24	11.76	1.00 : 119.67
Aceh	Banda Aceh	15	12	0	0.00	0.00	0.00	N/A
Samarinda	Sidodadi	8	7	2	71.43	0.00	28.57	9.67 : 1.00
Paser	Long Ikis	15	5	0	0.00	100.00	0.00	N/A
Pontianak	Bangka Belitung Laut	10	1	0	0.00	100.00	0.00	N/A
Denpasar	Sanur	20	15	2	0.00	86.67	13.33	1.00 : 8.80
Mataram	Ampenan	10	6	2	0.00	66.67	33.33	1.00 : 20.50
	Pagesangan	10	4	1	75.00	0.00	25.00	6.50 : 1.00
Dompu	Bada	10	0	0	0.00	0.00	0.00	N/A
Manggarai Barat	Labuan Bajo	20	18	6	5.56	61.11	33.33	1.00 : 7.91
Southwest Sumba	Tambolaka	15	12	2	0.00	83.33	16.67	1.00 : 18.00
East Sumba	Waingapu	15	3	0	100.00	0.00	0.00	N/A
South Central Timor	Soe	18	0	0	0.00	0.00	0.00	N/A

N/A : Not available

4.2 Resistance Status of *Ae. aegypti* against Four Major Classes of Adulticides in Sunda Islands of Indonesia

The resistance status of adult *Ae. aegypti* against 11 insecticides namely cyfluthrin, lambda-cyhalothrin, permethrin, deltamethrin, etofenprox, malathion, fenitrothion, propoxur, bendiocarb, DDT and dieldrin from four different classes of pyrethroids, organophosphates, carbamate and organochlorine were described in table 4.5, 4.6 and 4.7 respectively. Majority of the populations were not knockdown after the exposures of fenitrothion, propoxur, bendiocarb, DDT and dieldrin, thus the KT_{50} values could not be determined.

4.2.1 Susceptibility status of pyrethroids against field collected adult *Ae. aegypti*

Cyfluthrin had the lowest knockdown time, KT_{50} of 9.43 minutes and up to 57.29 minutes across all populations. Samarinda population appeared to be most resistant against all insecticides, because most of the mosquitoes were not knocked down during the 1 h exposure period (Table 4.5).

Aedes aegypti from Pontianak, Dompu and Manggarai Barat showed higher mortality rates against lambda-cyhalothrin, deltamethrin, permethrin and etofenprox which corresponded to the KT_{50} where knockdown was observed in 60 minutes exposure. Mosquitoes from Samarinda appeared to be the most resistant against all pyrethroids, because only 2.22% knockdown rate against cyfluthrin was observed, whereas no knockdown was recorded for the remaining pyrethroids. However, *Ae. aegypti* from Dompu and Manggarai Barat were most susceptible to all pyrethroids with knockdown rates ranged from 78.18 to 100% (Table 4.6).

Table 4.5: KT50 of Indonesian adult *Ae. aegypti* against four classes of insecticide.

Location	Pyrethroids				
	Cyfluthrin (0.15%)	Lambda-cyhalothrin (0.05%)	Permethrin (0.25%)	Deltamethrin (0.025%)	Etofenprox (0.5%)
	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)
Reference	13.89 (13.42-14.36)	17.98 (17.43-18.48)	29.65 (28.69-30.61)	26.55 (25.59-27.45)	50.94 (49.63-52.46)
Kuningan	57.29 (53.35-62.77)	ND (No knockdown)	ND (No knockdown)	ND	ND (No knockdown)
Padang	37.49 (36.40-38.65)	< 5% knockdown	ND (No knockdown)	233.33 (154.64-476.12)	ND (No knockdown)
Samarinda	< 5% knockdown	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Pontianak	11.08 (10.79-11.39)	23.82 (23.13-24.56)	40.97 (39.68-42.36)	24.60 (24.06-25.12)	54.21 (52.28-56.63)
Denpasar	33.01 (32.19-33.85)	ND (No knockdown)	ND	ND	ND (No knockdown)
Mataram	30.21 (29.13-31.31)	ND (No knockdown)	ND (No knockdown)	ND	ND (No knockdown)
Dompu	10.14 (9.62-10.54)	14.36 (13.98-14.74)	26.06 (25.30-26.79)	19.55 (18.98-20.10)	36.75 (35.85-37.64)
Manggarai Barat	9.43 (9.10-9.75)	27.67 (26.64-28.63)	25.78 (24.94-26.66)	14.87 (14.37-15.43)	41.38 (40.45-42.31)
East Sumba	44.60 (41.40- 50.74)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
South Central Timor	28.30 (27.48- 29.12)	ND	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)

CL, confidence limit; ND, not determined

Table 4.5, continued.

Location	Organophosphates		Carbamates		Organochlorines	
	Malathion (5%)	Fenitrothion (1%)	Propoxur (0.1%)	Bendiocarb (0.1%)	DDT (4%)	Dieldrin (0.4%)
	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)	KT50 (min) (95% CL)
Reference	69.07 (63.83-82.17)	ND	127.96 (ND)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Kuningan	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Padang	ND	ND	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Samarinda	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Pontianak	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Denpasar	ND (No knockdown)	ND (No knockdown)	ND	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Mataram	ND	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Dompu	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
Manggarai Barat	60.08 (58.54-62.62)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
East Sumba	< 5% knockdown	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)
South Central Timor	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)	ND (No knockdown)

CL, confidence limit; ND, not determine d

4.2.2 Susceptibility status of organophosphates against field collected adult *Ae. aegypti*

Only malathion can cause knockdown against adult field populations from Padang, Mataram, Manggarai Barat and East Sumba with the highest KT_{50} of 60.08 minutes, whilst fenitrothion was no longer effective because no knockdown was observed after 60 minutes. In contrast, mortality rates of the seven field strains (i.e. Padang, Pontianak, Denpasar, Mataram, Dompu, Manggarai Barat and South Central Timor) at 24h post exposure showed full susceptibility against malathion, whilst populations Kuningan, Samarinda and East Sumba were resistant (mortality < 98%). Furthermore, populations of Padang, Pontianak, Dompu and Manggarai Barat showed complete susceptibility against fenitrothion (Table 4.7).

4.2.3 Susceptibility status of carbamates against field collected adult *Ae. aegypti*

After an hour of exposures to carbamate insecticides, only Denpasar showed knockdown rate of 6.67% against propoxur, whereas no knockdown was observed in most of the populations. Likewise, mortality rates indicated that nine out of 10 populations were resistant (<98% mortality) against both propoxur and bendiocarb. Only *Ae. aegypti* from Pontianak showed possible resistant against propoxur (93.33% mortality) (Table 4.7).

4.2.4 Susceptibility status of organochlorines against field collected adult *Ae. aegypti*

Aedes aegypti from 10 study sites was resistant against both DDT and dieldrin. No knockdown was recorded after an hour of exposures. All populations displayed low mortality rates (6.67 – 93.33 % mortality) against DDT and dieldrin. All field populations were resistant to DDT with the highest mortality of 75.56.

Table 4.6: Percentage of knockdown of Indonesian adult *Ae. aegypti* against four classes of insecticides.

Location	Knockdown (%)										
	Pyrethroids				Organophosphates			Carbamates		Organochlorines	
	Cyfluthrin (0.15%)	Lambda-cyhalothrin (0.05%)	Permethrin (0.25%)	Deltamethrin (0.025%)	Etofenprox (0.5%)	Malathion (5%)	Fenitrothion (1%)	Propoxur (0.1%)	Bendiocarb (0.1%)	DDT (4%)	Dieldrin (0.4%)
Reference	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	75.56 ± 1.3	28.89 ± 0.67	6.67 ± 0.00	13.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kuningan	48.89 ± 1.86	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Padang	100.00 ± 0.00	4.44 ± 0.33	0.00 ± 0.00	26.67 ± 0.00	0.00 ± 0.00	6.67 ± 0.00	6.67 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Samarinda	2.22 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pontianak	100.00 ± 0.00	100.00 ± 0.00	84.44 ± 0.33	100.00 ± 0.00	68.89 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Denpasar	95.56 ± 0.33	0.00 ± 0.00	6.67 ± 0.00	6.67 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mataram	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 0.00	0.00 ± 0.00	24.45 ± 1.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dompu	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	91.11± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Manggarai Barat	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	78.18 ± 0.67	44.00 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
East Sumba	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.44 ± 0.67	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
South Central Timor	100.00 ± 0.00	6.67 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	p= 0.00 F= 67.87 df= 10,22	*p*= 000 F= 5501.80 df= 10,22	*p*= 0.00 F= 5183.20 df= 10,22		*p*=0.00 F= 181.436 df= 10,22			*p*= 0.00 F= 13.80 df= 10,22			

Table 4.7: Percentage of mortality of Indonesian adult *Ae. aegypti* against four classes of insecticides.

Location	Mortality (%)										
	Pyrethroids					Organophosphates		Carbamates		Organochlorines	
	Cyfluthrin (0.15%)	Lambda-cyhalothrin (0.05%)	Permethrin (0.25%)	Deltamethrin (0.025%)	Etofenprox (0.5%)	Malathion (5%)	Fenitrothion (1%)	Propoxur (0.15%)	Bendiocarb (0.1%)	DDT (4%)	Dieldrin (0.4%)
Reference	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}	97.78 ± 0.33^{P}	86.67 ± 0.58^{R}	77.78 ± 0.88^{R}	68.89 ± 0.33^{R}	77.78 ± 0.33^{R}	95.56 ± 0.33^{P}
Kuningan	53.33 ± 0.58^{R}	22.22 ± 0.67^{R}	37.78 ± 0.88^{R}	31.11 ± 0.67^{R}	8.89 ± 0.33^{R}	86.67 ± 1.00^{R}	86.67 ± 1.00^{R}	11.11 ± 0.33^{R}	20.00 ± 0.00^{R}	6.67 ± 0.00^{R}	22.22 ± 0.33^{R}
Padang	80.00 ± 0.00^{R}	26.67 ± 0.00^{R}	24.44 ± 0.33^{R}	71.11 ± 0.33^{R}	13.33 ± 0.00^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	15.56 ± 0.33^{R}	17.78 ± 0.33^{R}	75.56 ± 0.33^{R}	82.22 ± 0.33^{R}
Samarinda	82.22 ± 0.33^{R}	75.56 ± 0.33^{R}	84.44 ± 0.33^{R}	80.00 ± 0.58^{R}	46.67 ± 0.58^{R}	55.56 ± 0.33^{R}	97.78 ± 0.33^{P}	66.67 ± 0.58^{R}	77.78 ± 0.33^{R}	33.33 ± 0.58^{R}	37.78 ± 0.33^{R}
Pontianak	100.00 ± 0.00^{S}	95.56 ± 0.33^{P}	75.56 ± 0.33^{R}	100.00 ± 0.00^{S}	84.44 ± 0.33^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	93.33 ± 0.00^{P}	80.00 ± 0.58^{R}	48.89 ± 0.33^{R}	48.89 ± 0.33^{R}
Denpasar	93.33 ± 0.58^{P}	15.56 ± 0.33^{R}	24.44 ± 0.33^{R}	42.22 ± 0.33^{R}	8.89 ± 0.33^{R}	100.00 ± 0.00^{S}	80.00 ± 0.58^{R}	15.56 ± 0.33^{R}	15.56 ± 0.33^{R}	24.44 ± 0.33^{R}	68.89 ± 0.33^{R}
Mataram	100.00 ± 0.00^{S}	55.56 ± 0.33^{R}	20.00 ± 0.00^{R}	55.56 ± 0.33^{R}	28.89 ± 0.33^{R}	100.00 ± 0.00^{S}	95.56 ± 0.33^{P}	31.11 ± 0.88^{R}	24.44 ± 0.33^{R}	11.11 ± 0.67^{R}	66.67 ± 0.00^{R}
Dompu	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	88.89 ± 0.88^{R}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	37.78 ± 0.33^{R}	62.22 ± 0.33^{R}	68.89 ± 0.33^{R}	68.89 ± 0.33^{R}
Manggarai Barat	91.11 ± 0.58^{P}	100.00 ± 0.00^{S}	93.33 ± 0.58^{P}	100.00 ± 0.00^{S}	91.11 ± 0.67^{P}	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	35.56 ± 0.33^{R}	35.56 ± 0.33^{R}	66.67 ± 0.00^{R}	77.78 ± 0.33^{R}
East Sumba	73.33 ± 1.53^{R}	22.22 ± 1.20^{R}	20.00 ± 1.15^{R}	55.56 ± 0.88^{R}	17.78 ± 0.33^{R}	91.11 ± 0.88^{P}	86.67 ± 0.58^{R}	6.67 ± 0.00^{R}	26.67 ± 0.58^{R}	11.11 ± 0.33^{R}	33.33 ± 0.00^{R}
South Central Timor	95.56 ± 0.33^{P}	48.89 ± 0.33^{R}	80.00 ± 0.58^{R}	37.78 ± 0.33^{R}	13.33 ± 0.00^{R}	100.00 ± 0.00^{S}	88.89 ± 0.33^{R}	37.78 ± 0.33^{R}	13.33 ± 0.33^{R}	35.56 ± 0.33^{R}	93.33 ± 0.00^{P}
	p= 0.00 F= 16.23 df= 10,22	*p*= 0.00 F= 125.45 df= 10,22	*p*= 0.00 F= 86.13 df= 10,22	*p*= 0.00 F= 92.07 df= 10,22	*p*= 0.00 F= 167.01 df= 10,22	*p*= 0.00 F= 22.18 df= 10,22	p= 0.00 F= 5.64 df= 10,22	p= 0.00 F= 79.62 df= 10,22	*p*= 0.00 F= 107.81 df= 10,22	*p*= 0.00 F= 117. 20 df= 10,22	*p*= 0.00 F= 144.56 df= 10,22

R, resistant (mortality <90%); S, susceptible (mortality ≥ 98%), P, possible resistance (mortality 90-97%) as determined by WHO (2016

4.3 The Effectiveness of Diagnostic Dosage of Insecticides for *Aedes* Larvae Control in The Sunda Islands of Indonesia

Table 4.8 shows the percentage mortality of larvae *Ae*. *aegypti* from 10 study sites against diagnostic dosages of six organophosphate larvicides namely fenthion (0.025 mg/L), chlorpyrifos (0.002 mg/L), bromophos (0.05 mg/L), fenitrothion (0.02 mg/L), malathion (0.125 mg/L), temephos (0.012 mg/L) and 2 organochlorines namely dieldrin (0.025 mg/L) and DDT (0.012 mg/L).

4.3.1 Susceptibility status of organophosphate larvicides against field collected *Ae. aegypti* larvae

Aedes aegypti from Kuningan, Padang, Samarinda, Pontianak, Dompu, Manggarai Barat, East Sumba and Timor showed 98.67 - 100% mortality rates against fenitrothion, whilst *Ae. aegypti* from Denpasar was resistant (85% mortality). Mosquitoes from Mataram showed the possibility of resistance with mortality rates ranged from 90 - 97% (Table 4.8).

All field strains showed various percentage mortalities ranging from 0 to 74.67%, illustrating that the field strains were resistant. A wide range of mortalities were also observed in the field strain against the diagnostic doses of malathion and temephos with zero mortality up to the highest mortality of 76%, demonstrating the resistance status of both larvicides across all study sites.

On the other hand, all field collected *Ae. aegypti* showed resistance and possible resistance against fenthion, chlorpyrifos and bromophos with mortality rates ranged from 0 – 88.00%, indicating that these insecticides were no longer effective against the field populations of *Ae. aegypti* larvae (Table 4.8).

4.3.2 Susceptibility status of organochlorines larvicides against field collected *Ae. aegypti* larvae

All 10 field populations were resistant to DDT; eight of which (i.e. Kuningan, Samarinda, Pontianak, Denpasar, Mataram, Dompu, East Sumba and South Central Timor) demonstrated zero mortality after 24 hour of exposure. Five populations (Padang, Samarinda, Denpasar, Mataram and Manggarai Barat) were fully susceptible against diagnostic dose of dieldrin with 100% mortality rates. Pontianak and Dompu showed low mortality rates of 53.33% and 37.33%, respectively, against dieldrin while Kuningan, East Sumba and South Central Timor showed development to resistance with mortality rates ranged from 81.33 - 97.33% (Table 4.8).

Table 4.8: Percentage mortality of *Ae. aegypti* larvae from 10 study sites in Indonesia against diagnostic dosages of organophosphates and organochlorines larvicides for 24 hours exposure.

Location	Insecticides							
	Organophosphate						Organochlorine	
	Fenitrothion	Fenthion	Chlorpyrifos	Bromophos	Malathion	Temephos	Dieldrin	DDT
	0.02mg/L	0.025mg/L	0.002mg/L	0.05mg/L	0.125mg/L	0.012mg/L	0.025mg/L	0.012mg/L
Reference	100.00 ± 0.00^{S}	100.00 ± 0.00^{S}	92.00 ± 0.58^{P}	89.33 ± 1.67^{R}	0.00 ± 0.00^{R}	9.33 ± 0.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Kuningan	98.67 ± 0.33^{S}	88.00 ± 2.00^{R}	4.00 ± 0.00^{R}	81.33 ± 1.67^{R}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	97.33 ± 0.67^{P}	0.00 ± 0.00^{R}
Padang	100.00 ± 0.00^{S}	33.33 ± 0.88^{R}	46.67 ± 0.88^{R}	40.00 ± 0.58^{R}	0.00 ± 0.00^{R}	4.00 ± 0.00^{R}	100.00 ± 0.00^{S}	4.00 ± 0.00^{R}
Samarinda	100.00 ± 0.00^{S}	85.33 ± 0.88^{R}	1.33 ± 0.33^{R}	82.67 ± 0.33^{R}	1.33 ± 0.33^{R}	8.00 ± 0.00^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Pontianak	100.00 ± 0.00^{S}	61.33 ± 1.45^{R}	74.67 ± 1.86^{R}	38.67 ± 0.33^{R}	0.00 ± 0.00^{R}	4.00 ± 0.58^{R}	53.33 ± 1.76^{R}	0.00 ± 0.00^{R}
Denpasar	85.00 ± 0.33^{R}	11.00 ± 0.33^{R}	1.33 ± 0.33^{R}	20.00 ± 2.31^{R}	0.00 ± 0.00^{R}	25.00 ± 2.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Mataram	97.00 ± 0.33^{P}	39.00 ± 1.33^{R}	4.00 ± 0.00^{R}	9.30 ± 0.33^{R}	0.00 ± 0.00^{R}	9.30 ± 0.33^{R}	100.00 ± 0.00^{S}	0.00 ± 0.00^{R}
Dompu	100.00 ± 0.00^{S}	49.33 ± 1.67^{R}	0.00 ± 0.00^{R}	54.67 ± 0.33^{R}	1.33 ± 0.33^{R}	49.33 ± 0.88^{R}	37.33 ± 0.67^{R}	0.00 ± 0.00^{R}
Manggarai Barat	100.00 ± 0.00^{S}	35.00 ± 1.33^{R}	2.67 ± 0.33^{R}	0.00 ± 0.00^{R}	1.33 ± 0.33^{R}	76.00 ± 0.00^{R}	100.00 ± 0.00^{S}	2.67 ± 0.33^{R}
East Sumba	98.67 ± 0.33^{S}	88.00 ± 1.00^{R}	25.33 ± 0.88^{R}	81.33 ± 2.33^{R}	0.00 ± 0.00^{R}	0.00 ± 0.00^{R}	93.33 ± 0.67^{P}	0.00 ± 0.00^{R}
South Central Timor	100.00 ± 0.00^{S}	97.33 ± 0.67^{P}	9.33 ± 0.33^{R}	44.00 ± 1.00^{R}	33.33 ± 0.33^{R}	10.67 ± 0.33^{R}	81.33 ± 2.40^{R}	0.00 ± 0.00^{R}
	p= 0.00 F= 18.57 df= (10,22)	p= 0.00 F= 96.81 df= (10,22)	p= 0.00 F= 375.28 df= (10,22)	p= 0.00 F= 54.75 df= (10,22)	p= 0.00 F= 153.10 df= (10,22)	p= 0.00 F= 458.00 df= (10,22)	p= 0.00 F= 2231.76) df= (10,22)	p= 0.00 F= (11.80) df= (10,22)

R, resistant (mortality <90%); S, susceptible (mortality ≥ 98%), P, possible resistance (mortality 90-97%) as determined by WHO (2016)

CHAPTER 5: DISCUSSION

5.1 Preliminary Distribution and Abundance of Dengue Vectors in The Sunda Islands of Indonesia

Information on *Aedes* larval densities in relation to space and time determination of the breeding sources as well as predicting dengue outbreaks can be obtained through ovitrap surveillance (Tham, 2000). In this study, higher positive ovitraps were found outdoors rather than indoors in all study sites. Similar findings have also been reported by Wan-Norafikah *et al*. (2011) in Peninsular Malaysia.

Ae. aegypti is mostly found exclusively indoors and feed mostly indoor (Rudnick, 1967). In the present study, *Ae. aegypti* was found dominantly indoor in 7 out of 13 study sites – namely Kuningan, Air Tawar Barat, Bangka Belitung Laut, Sanur, Bada, Waingapu and Soe. This corresponds to the recent studies which were conducted in Perak, Selangor, Kuala Lumpur and Penang, Malaysia where *Ae. aegypti* prefers breeding indoor (Ho *et al*., 2014; Rozilawati *et al*., 2015). In contrast, *Ae. albopictus* breeds in manmade and natural containers and mostly found outdoors (Saleeza *et al*., 2013). In this study, *Ae. albopictus* was commonly found outdoors (8 out of 14 study sites) – namely Air Tawar Barat, Banda Aceh, Long Ikis, Bangka Belitung Laut, Sanur, Ampenan, Labuan Bajo and Tambolaka. This finding also concurred with the studies conducted in Selangor, Kuala Lumpur and Penang Island in Malaysia (Rozilawati *et al*., 2015).

Interestingly, interchange of breeding habitat preferences in *Ae. aegypti* and *Ae. albopictus* was observed in the three study sites – Tambolaka, Kuningan and Sidodadi. Previous studies have also reported similar phenomenon: *Ae. albopictus* breeds indoor, and in contrast *Ae. aegypti* breeds outdoor (Syarifah *et al*., 2008; Saleeza *et al*., 2013; Wan-Norafikah *et al*., 2011; Wan-Norafikah *et al*., 2012). This could be probably due to the influence of housing characteristics. Open eaves of a building allow outdoor

mosquitoes to enter the home and the usage of air conditioner to cool the home without the need of opening windows and doors (Garcia-Reion *et al*., 2008).

Ae. albopictus and *Ae. aegypti* are sympatric species which occupy similar ecological niches (Wan-Norafikah *et al*., 2012). Thus, mixed infestation of both species was also found in both indoor and outdoor areas (7 out of 14 study sites) ranged from 11.76% to 66.67%. The present results were much higher than the mixed infestation rates reported in Malaysia (5 to 45%) (Chen *et al*., 2006; Wan-Norafikah *et al*., 2011). Additionally, previous studies also showed the mixed infestation of *Aedes* with other genera of mosquitoes such as *Armigeres* spp. and *Culex quinquefasciatus* (Chen *et al*., 2006; Lau *et al*., 2017).

5.2 Susceptibility Status of *Ae. aegypti* against Four Major Classes of Adulticides in Sunda Islands of Indonesia

Within the last 15 years, the uses of pyrethroids have been escalated in Indonesia due to their rapid knockdown effect (Sayono *et al*., 2016). Hence, prolonged uses of pyrethroids may have contributed insecticide resistance in the field populations of *Ae. aegypti*. This phenomenon is shown in this study where none of the mosquito field populations were susceptible to all tested pyrethroids. Strikingly, nine out 10 field populations of adult *Ae. aegypti* were resistant against permethrin. Permethrin resistance in *Ae. aegypti* has also been reported worldwide (Brengues *et al*., 2003; Chang *et al*., 2009; Wuliandri *et al*., 2015; Amelia-Yap *et al*., 2018). Sayono (2016) reported deltamethrin resistance in adult *Ae. aegypti* in four districts of Central Java, Indonesia, while our study showed that three populations (i.e. Borneo, Sumbawa, Flores) were susceptible to deltamethrin. Although not all the tested pyrethroids are used in mosquito control programs in Indonesia, the resistance could be due to the factor of cross-resistance among the insecticides (i.e. lambda-cyhalothrin vs permethrin; deltamethrin vs

etofenprox). Overall, based on the mean mortality of mosquitoes at 24 h post treatment, toxicity levels of five pyrethroids tested decrease in the following order: cyfluthrin > deltamethrin > lambda-cyhalothrin > permethrin > etofenprox.

Malathion has been successful in reducing the density of adult mosquitoes by fogging across Indonesia in 1973 (Suroso, 1984). Surprisingly, the current study showed that most of the field populations (seven out of 10) are still susceptible to malathion despite being used for more than four decades. This result is also supported by Ahmad *et al*. (2009), where the adult field strain populations from Palembang, Surabaya, Bandung, Jakarta and Palu, Indonesia were susceptible to malathion. In contrast, Widiarti *et al*. (2011) and Dwi *et al*. (2015) reported that field strain of adult *Ae. aegypti* from Yogyakarta and Bandung were resistant against malathion, but their tested dose was much lower (0.8%) compared to the present study (5.0%).

Unlike malathion, more than half of the field populations were not fully susceptible to fenitrothion. Fenitrothion resistance in *Ae. aegypti* has also been reported in other countries (Sathanthriphop *et al*., 2006; Jirakanjanakit *et al*., 2007; Ocampo *et al*., 2011). It is possible that adult mosquitoes have developed resistance against this insecticide due to the extensive uses as an indoor residual spray since 1970's (Najera & Zaim, 2001). This study also found that both carbamates (i.e. propoxur and bendiocarb) were no longer effective to control all *Ae. aegypti* at adult stage. In Indonesia, carbamates are commonly used by professional pest control operators to control insect pests such as cockroaches (Rahayu *et al*., 2012). Widespread use of this insecticide may have indirectly contributed to resistance in *Ae. aegypti*.

Generally, among the eleven tested insecticides, adult *Ae. aegypti* populations showed high level of resistance to DDT in all study sites. It is not surprising because DDT has been heavily used for indoor residual spraying to control malaria since 1952 and

Anopheles aconitus in Central Java was first recorded to be resistant against this insecticide in 1962 (Bang, 1982). Although DDT has been banned in 1970's by Indonesian government, the result of this study indicates that DDT resistance has been prolonged. Similar observations in different mosquito species have also been reported worldwide (Cui *et al*., 2006; Das *et al*., 2011; Dia *et al*., 2011; Kamgang., 2011; Ocampo *et al*., 2011; Low *et al*., 2013; Singh *et al*., 2014; Ishak., 2015). The present study also showed cross resistance between DDT and deltamethrin which has been reported in many studies (Chadwick *et al*., 1977; Vontas *et al*., 2012; Brengues *et al*., 2013, Koou *et al*., 2014). Cross resistance between DDT and pyrethroids can occur because both classes share the same mode of action which target the sodium channels of the nerve sheath (Brogdon & McAllister, 1998).

Although dieldrin was only used in Indonesia for vector control that lasted a decade since 1956 (Asih *et al*., 2012), this study showed that nine out 10 field populations of adult mosquitoes were resistant towards dieldrin. Dissimilar results have been reported in the neighboring country Malaysia where the mosquitoes were fully susceptible against dieldrin (Ishak *et al*., 2015).

5.3 The Effectiveness of Diagnostic Dosage of Insecticides for *Ae.* Larvae Control in The Sunda Islands of Indonesia

Aedes aegypti larvae in this study showed different degrees of susceptibility towards the organophosphate and organochloride larvicides. Resistance of larvae towards a few organophosphates have been reported in Indonesia (Mulyatno *et al*., 2012; Putra *et al*., 2016) and other South-East Asian countries such as in Thailand, Malaysia and Singapore (Wesson, 1990; Chen *et al*., 2005b; Ponlawat *et al*., 2005; Chareonviriyaphap *et al*., 2013; Chen *et al*., 2013; Koou *et al*., 2014; Thongwat & Bunchu, 2015). Mass larviciding using temephos has been implemented to reduce *Ae. aegypti* populations since the early 1970's

(Putra *et al*., 2016). However, due to its prolonged uses, *Ae. aegypti* larval populations have developed resistance against the diagnostic dose of temephos. In this study, field strains of *Ae. aegypti* larvae exhibited resistance with the lowest mortality of 4% and the highest mortality of 76%. Similar findings have also been reported in *Ae. aegypti* from Surabaya, Indonesia, and Selangor, Malaysia against the diagnostic dosage of temephos (0.012 mg/L) with mortality rates ranged from 16 to 60% (Chen *et al*., 2005b; Mulyatno *et al*., 2012). Low mortality rates (0 - 1.33%) were also observed in field strain larvae that were exposed to malathion, possibly because of the prolonged use of malathion in fogging to control adult mosquitoes for the past 36 years (Putra *et al*., 2016).

Chlorpyrifos is not directly used to control the *Ae. aegypti* population in most countries including Indonesia. However, medium to high resistance in all field populations against chlorpyrifos was observed in this study. This is most probably due to the presence of cross-resistance to temephos or other organophosphates. Likewise, Rodriguez *et al*. (2002) also reported that *Ae. aegypti* larvae from Venezuela and Cuba showed similar trend of cross-reactivity against chlorpyrifos. This study also showed that all field strains of *Ae. aegypti* larvae were resistant against bromophos. Despite being used directly as a larvicide to control the mosquitoes, bromophos is known to be used as fly control and mostly used in poultry farms (Rozendaal, 1997). The pesticide residues may have contaminated the environment and thus affected other insects in the environment including mosquitoes (Thongwat & Bunchu, 2015).

Aedes aegypti larvae from Mataram and Denpasar had the lowest mortality rates against both fenthion and fenitrothion. However, the uses of fenthion and fenitrothion are not the common strategies to control *Aedes* mosquitoes in Indonesia. In contrast, fenitrothion has been commonly used in other countries such as Thailand and Cuba to control *Aedes* mosquitoes (Bisset *et al*., 2013; Thongwat & Bunchu, 2015). It is possible

that the insecticide-resistant mosquitoes have been transmitted accidentally from other countries by the tourists through planes or ships (WHO, 2017).

DDT was used for malarial control programs in Indonesia for a decade since 1950 till it was banned in 1970 (Asih *et al*., 2012). Although it has not been used for insecticide residual spray since the 20th century, larval populations in Indonesia are still resistant towards this insecticide class. Prior to the present study, the susceptibility of DDT against Indonesian *Ae. aegypti* larvae has not been reported. All tested field strain larvae were found resistant against DDT, probably due the excessive use of DDT in Indonesia in the past two decades (Dia *et al*., 2012). Nevertheless, DDT resistance has been reported in *Ae. aegypti* in Asia including Malaysia and India (Nazni *et al*., 2009; Mohsin *et al*., 2016). An increased level of enzyme glutathione S-transferases (GST) has been found to be involved in DDT resistance in insects (Tang & Tu, 1994). DDT dehydrochlorination is the major route to detoxify DDT which is the most common resistance mechanism in mosquitoes (Brown, 1986; Hemingway *et al*., 2000). In addition, DDT resistance is also associated with the mutation in target site of voltage gated sodium channel (kdr) (Hemingway *et al*., 2000; Amelia *et al*., 2018). *Aedes aegypti* from Semarang, Indonesia has been found resistance to DDT with an elevated level of GST and two kdr mutations. (Brengues *et al*., 2003).

Dieldrin was only used for a decade from 1955 to 1965 where it was banned (Asih *et al*., 2012). In the present study, half of the populations were susceptible to this insecticide. However, three populations were resistant against the diagnostic dose. Resistance to dieldrin has been linked with mutations occurring in the gamma amino-butyric acid (GABA) receptor in various insects, including *Aedes albopictus* (ffrench-Constant *et al*., 2000; Low *et al*., 2015). Du *et al*. (2005) also reported on substitution of alenine296 to

glycine has been associated with dieldrin resistance in a laboratory strain of *Anopheles gambiae*. Thus far there is no evidence of this mutation in *Ae. aegypti*.

In this study, the Bora-bora reference strain of *Ae. aegypti* also showed some levels of resistance against bromophos, temephos, malathion and DDT. Pasteur *et al*. (1995) documented resistance of *Culex pipiens quinquefasciatus* in Bora bora against temephos. Resistance is Bora-bora is expected because malathion, fenitrothion and temephos have been widely used in the vector control of *Ae. aegypti* (Failloux *et al*., 1994). Although this reference strain has been colonized in an insecticide free condition for more than 60 generations, resistance against these insecticides are still present. Similar observation (i.e. DDT resistance) has also been found in Malaysian laboratory strains of *Aedes aegypti* (Nazni *et al*., 2009) and *Culex. quinquefasciatus* (Low *et al*., 2013).

It is also of interest that there is contrasting trends between resistance of field strains adults and larvae against several insecticides such as malathion and dieldrin. Diagnostic dosage of malathion was found to be adequate in causing more than 98% mortality against the adult field population, whilst ineffective in the field larval populations. Yet, all the adult field populations were resistant against the diagnostic dosage of dieldrin, while the larval field strain were susceptible against dieldrin in more than half of the populations. Different insecticide gene expression in the larval and adult stages could play a role in this situation. As reported by Selvi *et al*. (2005) and Nazni *et al*. (2005), gene expression was more active in the larva stage compared to the adult stage. This phenomenon is supported by Strode *et al*. (2006) who reported higher gene expression in adult compared to larvae mosquitoes. Thus, resistance is not restricted to one or other different stages (Subramaniam *et al*., 2006). Additionally, similar resistance status was recorded across all study sites for both adult and larval stages against the diagnostic dosage of DDT.

Despite DDT being banned in Indonesia, resistance of DDT was still apparent. This could be due to the possibility of DDT resistance being genetically fixed (Ocampo *et al*., 2011).

CHAPTER 6: CONCLUSION

The findings from this study proved the interchange of breeding habitats between the two species of *Aedes*. Overall, *Ae. aegypti* was found both in indoor and outdoor across the Sunda Islands of Indonesia. However, *Ae. aegypti* was commonly to be dominant in indoor, whilst *Ae. albopictus* was also found to be dominant in outdoor. Nevertheless, there is an evidence showing the interchange of breeding habitats between this two species, where *Ae. aegypti* was found outdoor and *Ae. albopictus* was found indoor. Moreover, there are also mixed breeding of both species in an ovitrap suggesting the possibilities of both species breed in the same niche.

The resistant status of the field adult and larvae *Ae. aegypti* varied against the different classes of insecticides. Adult *Ae. aegypti* was found to be highly resistant against the organochlorines and carbamates, whilst showing diverse results against organophosphates and pyrethroids. Knockdown of adult mosquitoes was only seen against pyrethroids, however the mortality of the adult *Ae. aegypti* was shown to be moderately resistant and susceptible. In contrast, malathion, does not cause any knockdown effect in the adult mosquitoes but high mortality was observed post 24-hour treatment against the field strain of *Ae. aegypti.* In addition, significant correlation was found within the pyrethroids, and between the pyrethroids and DDT. This suggests that cross resistance occurred within the same group of insecticides and between groups of insecticides due to the similar mode of action. Besides that, the diagnostic dosages of temephos and malathion are no longer effective against the field strain of *Ae. aegypti* larvae. At the same time, field strain of *Ae. aegypti* larvae also showed a higher mortality against dieldrin and fenitrothion compared to the other larvicides.

The resistance status of the insecticides against both adults and larvae are worrying as the common insecticides used as the larvicides (temephos and malathion) are no longer

effective against the field strain of *Ae. aegypti*. However, there are other larvicides which are not used in the Indonesian control programme showing higher mortality against the field strain larvae. Thus, the rotation of the larvicides usage in vector control programme is advisable to avoid the resistance of an insecticide against the vector. In addition, the usage of DDT should continue to be banned as it is still causing resistance with other non-targeted organisms, such as birds, crustacean, worms and bees. Nonetheless, the increasing usage of pyrethroids and the presence of cross resistance between the pyrethroids and DDT shown in this study suggest that some pyrethroids might not be effective due to presence of the cross resistance. Thus, it is recommended to continue use the current dosage of malathion as adults of *Ae. aegypti* were still susceptible against it. Hence, a continuous monitoring on the insecticide's susceptibility status is essential to identify the efficacy of insecticides for dengue control in order to prevent the development of insecticide resistance.

Since this study only focuses on the diagnostic dosages of the insecticides which are recommended by WHO, thus redefining dosage of the insecticides is recommended to verify the resistance of the Indonesian populations of *Ae.* aegypti mosquitoes. Future study, should include the resistance status of the field strains adult and larvae by enzyme micro assay and knock down gene expression.

REFERENCES

Ahmad, I., Astari, S., Rahayu, R., & Hariani, N. (2009). Status kerentanan *Aedes aegypti* (Diptera: Culicidae) pada tahun 2006-2007 terhadap malation di Bandung, Jakarta, Surabaya, Palembang dan Palu. *Biosfera, 26*(2), 82-86.

Ambarita, L. P., Taviv, Y., Budiyanto, A., Sitorus, H., Pahlepi, R. I., & Febriyanto, F. (2015). Tingkat Kerentanan *Aedes* aegypti (Linn.) terhadap malation di Provinsi Sumatera Selatan. *Buletin Penelitian Kesehatan, 43*(2), 97-104.

Amelia-Yap, Z. H., Chen, C. D., Sofian-Azirun, M., & Low, V. L. (2018). Pyrethroid resistance in the dengue vector *Aedes aegypti* in Southeast Asia: Present situation and prospects for management. *Parasites & Vectors*, *11*(1), 332.

Asih, P. B., Syahrani, L., Rozi, I. E., Pratama, N. R., Marantina, S. S., Arsyad, D. S., & Syafruddin, D. (2012). Existence of the *rdl* mutant alleles among the *Anopheles* malaria vector in Indonesia. *Malaria Journal, 11*, 57- 61.

Astuti, E. P., Ipa, M., & Pradani, F. Y. (2014). Resistance detection of *Aedes aegypti* larvae to cypermethrin from endemic area in Cimahi City West Java. *aspirator-Journal of Vector Borne Disease Studies, 6*(1), 7-12.

Bang, Y. H., Arwati, S., & Gandahusada, S. (1982). Insecticide use for malaria control in Central Java, Indonesia: A review. *Medical Journal of Malaysia*, *37*(4), 308-315.

Bansch, R., Holtmann, H., & Knoll, H. A. (1974). Biological evaluation of bromophos for the control of storage pests. *Proceedings of The International Working Conference on Stored Product Entomology* (pp 532-541). Savanah, Georgia, United States of America.

Basker, P. & Ezhil., R. (2012). Study on the correlation of premises condition index and the presence of larvae of *Aedes* species mosquitoes in human dwellings of the Cuddalore district of Tamil Nadu, India. *Osong Public Health and Research Perspectives, 3*(1), 3-7.

Becker, N., Petrić, D., Boase, C., Lane, J., Zgomba, M., Dahl, C., & Kaiser, A. (2003). *Mosquitoes and their control*. New York, NY: Springer.

Bisset, J. A., Marín, R., Rodríguez, M. M., Severson, D. W., Ricardo, Y., French, L., & Perez, O. (2013). Insecticide resistance in two *Aedes aegypti* (Diptera: Culicidae) strains from Costa Rica. *Journal of Medical Entomology, 50*(2), 352-361.

Burke, R. L. (2009). Evaluation of *Aedes aegypti* presence and abundance in septic tanks and their impacts on dengue transmission. *Uniformed Services University of the Health Sciences Bethesda Md F Edward Hebert School of Medicine*.

Brengues, C., Hawkes, N. J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., & Hemingway, J. (2003). Pyrethroid 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. (1989). Biochemical resistance detection: An alternative to bioassay. *Parasitology Today*, *5*(2), 56-60.

Brogdon, W. G., & McAllister, J. C. (1998). Insecticide resistance and vector control. *Emerging Infectious Diseases, 4*(4), 605.

Brown, A. W. (1986). Insecticide resistance in mosquitoes: A pragmatic review. *Journal of The American Mosquito Control Association*, *2*(2), 123-140.

Casjens, H. (2009). Environmental fate of cyfluthrin. Sacramento, California: California Department of Pesticide Regulations.

Chadee, D. D. (2013). Resting behaviour of *Aedes aegypti* in Trinidad: With evidence for the re-introduction of indoor residual spraying (IRS) for dengue control. *Parasites & Vectors, 6*, 255.

Chadwick, P. R., Invest, J. F., &Bowron, M. J. (1977). An example of cross-resistance to pyrethroids in DDT-resistant *Aedes aegypti*. *Pest Management Science, 8*(6), 618-624.

Chang, C., Shen, W. K., Wang, T. T., Lin, Y. H., Hsu, E. L., & Dai, S. M. (2009). A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in *Aedes* aegypti. *Insect Biochemistry and Molecular Biology, 39*(4), 272-278.

Chareonviriyaphap, T., Bangs, M. J., Suwonkerd, W., Kongmee, M., Corbel, V., & Ngoen-Klan, R. (2013). Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasites & Vectors, 6*(1), 280.

Christophers, S. (1960). *Aedes aegypti (L.) The yellow fever mosquito: Its life history, bionomics and structure.* Cambridge, New York: Cambridge University Press.

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

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., 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 endemic areas in Kuala Lumpur and Selangor, Malaysia. *Tropical Biomedicine, 23*(2), 224-227.

Chen, C. D., Nazni, W. A., Lee, H. L., Norma-Rashid, Y., Lardizabal, M. L., & Sofian-Azirun, M. (2013). Temephos resistance in field *Aedes* (Stegomyia) *albopictus* (Skuse) from Selangor, Malaysia. *Tropical Biomedicine, 30*(2), 220-230.

Centers for Disease Control and Prevention (2016). Dengue and *Aedes aegypti* Mosquito. Retrieved 15th September 2017 from https://www.cdc.gov/dengue/resources/30jan2012/aegyptifactsheet.pdf

Clark, J. M., & Symington, S. B. (2011). Advances in the mode of action of pyrethroids. in *Pyrethroids* (pp 49-72). Berlin, Heidelberg: Springer.

Costa, L. G., Galli, C. L., & Murphy, S. D. (Eds.). (2013). Toxicology of pesticides: Experimental, clinical and regulatory perspectives. *Springer Science and Business Media, 13*, 1-2.

Coleman, M., Hemingway, J., Gleave, K. A., Wiebe, A., Gething, P. W., & Moyes, C. L. (2017). Developing global maps of insecticide resistance risk to improve vector control. *Malaria Journal*, *16*(1), 86-94.

Cui, F., Raymond, M., & Qiao, C. L. (2006). Insecticide resistance in vector mosquitoes in China. *Pest Management Science, 62*(11), 1013-1022.

Das, M. K., Singh, R. K., Lalc, R. K., & Dhimanb, R. C. (2011). Susceptibility of *Aedes aegypti* to insecticides in Ranchi city, Jharkhand state, India. *Dengue, 35*, 194.

Dia, I., Diagne, C. T., Ba, Y., Diallo, D., Konate, L., & Diallo, M. (2012). Insecticide susceptibility of *Aedes aegypti* populations from Senegal and Cape Verde Archipelago. *Parasites & Vectors, 5*(1), 238-241.

Du, W., Awolola, T. S., Howell, P., Koekemoer, L. L., Brooke, B. D., Benedict, M. Q., & Zheng, L. (2005). Independent mutations in the Rdl locus confer dieldrin resistance to *Anopheles gambiae* and *Anopheles arabiensis*. *Insect Molecular Biology*, *14*(2), 179-183.

Dwi, K., Rusmartini, T., & Purbaningsih, W. (2015). Resistensi malathion 0, 8% dan temephos 1% pada nyamuk *Aedes Aegypti* dewasa dan larva di Kecamatan Buah Batu Kota Bandung. *Prosiding Penelitian Sivitas Akademika Unisba (Kesehatan), 2*, 149-153.

European Centre for Disease and Control (2015). Vector-borne diseases. Retrieved 20th March 2017 from http://ecdc.europa.eu/en/healthtopics/climate_change/health_effects/Pages/vector_borne_diseases.aspx

Estrada-Franco, J. G., & Craig, G. B. (1995). *Biology, disease relationships, and control of Aedes albopictus*. Washington : Pan American Health Organization.

Fahri, S., Yohan, B., Trimarsanto, H., Sayono, S., Hadisaputro, S., Dharmana, E., & Sasmono, R. T. (2013). Molecular surveillance of dengue in Semarang, Indonesia revealed the circulation of an old genotype of dengue virus serotype-1. *PLoS Neglected Tropical Diseases, 7*(8), e2354.

Failloux, A. B., Ung, A., Raymond, M., & Pasteur, N. (1994). Insecticide susceptibility in mosquitoes (Diptera: Culicidae) from French Polynesia. *Journal of Medical Entomology*, *31*(5), 639-644.

ffrench-Constant, R. H., Anthony, N., Aronstein, K., Rocheleau, T., & Stilwell, G. (2000). Cyclodiene insecticide resistance: from molecular to population genetics. *Annual Review of Entomology*, *45*(1), 449-466.

Fuadzy, H., & Hendri, J. (2015). Indeks entomologi dan kerentanan larva *Aedes aegypti* terhadap temefos di Kelurahan Karsamenak Kecamatan Kawalu Kota Tasikmalaya. *Vektora: Jurnal Vektor dan Reservoir Penyakit*, *7*(2), 57-64.

Foster, W. A. & Walker, E. D. (2002). Mosquitoes (Culicidae). In Mullen, G. R., and Durden, L. A. (Eds.), *Medical and veterinary entomology* (pp. 201-262). San Diego, California: Academic Press/Elsevier.

Garcia-Rejon, J., Loroño-Pino, M. A., Farfan-Ale, J. A., Flores-Flores, L., Rosado-Paredes, E. D. P., Rivero-Cardenas, N., & Beaty, B. J. (2008). Dengue virus–infected *Aedes* Aegypti in the home environment. *The American Journal of Tropical Medicine and Hygiene*, *79*(6), 940-950.

Gubler D. J. (1989). *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: Top down or bottom up. *American Journal Tropical Medicine and Hygiene, 40*(6), 571–578.

Harker, B. W., Behura, S. K., deBruyn, B. S., Lovin, D. D., Mori, A., Romero Severson, J., & Severson, D. W. (2013). Stage-specific transcription during development of *Aedes aegypti. BMC Developmental Biology, 13*(1), 29.

Hayes, A. F. (2009). Beyond Baron and Kenny: Statistical mediation analysis in the new millennium. *Communication Monographs, 76*(4), 408-420.

Henderson, G. L., & Crosby, D. G. (1967). Photodecomposition of dieldrin and aldrin. *Journal of Agricultural and Food Chemistry*, *15*(5), 888-893.

Hemingway, J. (2000). The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochemistry and Molecular Biology*, *30*(11), 1009-1015.

Hill D. S., & Waller J. M. (1982). *Pests and diseases of tropical crops*. London, New York: Longman.

Ho, L. Y., Loh, T. S., & Yam, L. A. (2014). Surveillance and resistance status of *Aedes* population in two suburban residential areas in Kampar town, Perak, Malaysia. *Tropical Biomedicine, 31*(3), 441-448.

Huong, V. D., Ngoc, N. T. B., Hien, D. T., & Lien, N. T. B. (2004). Susceptibility of *Aedes aegypti* to insecticides in Viet Nam. *Dengue Bulletin, 28*, 179-183.

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 & Vectors, 8*(1), 181.

Insecticide Resistance Action Committee. (2006). Prevention and management of insecticide resistance in vectors and pests of public health importance. *Insecticide Resistance Action Committee*, 1, 1-49.

Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology, 9*(3-4), 90-100.

Jirakanjanakit, N., Rongnoparut, P., Saengtharatip, S., Chareonviriyaphap, T., Duchon, S., Bellec, C., & Yoksan, S. (2014). Insecticide susceptible/resistance status in *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) in Thailand during 2003–2005. *Journal of Economic Entomology*, *100*(2), 545-550.

Kamgang, B., Marcombe, S., Chandre, F., Nchoutpouen, E., Nwane, P., Etang, J., & Paupy, C. (2011). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa. *Parasites & Vectors, 4*(1), 79.

Karyanti, M. R., Uiterwaal, C. S., Kusriastuti, R., Hadinegoro, S. R., Rovers, M. M., Heesterbeek, H., & Bruijning-Verhagen, P. (2014). The changing incidence of dengue haemorrhagic fever in Indonesia: A 45-year registry-based analysis. *Bio Med Central Infectious Diseases, 14*(1), 412.

Katyal, R., Tewari, P., Rahman, S. J., Pajni, H. R., Kumar, K., & Gill, K. S. (2001). Susceptibility status of immature and adult stages of *Aedes aegypti* against conventional insecticides in Delhi, India. *Dengue Bulletin, 5*, 84-87.

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.

Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., & Hay, S. I. (2015). The Global Distribution of the Arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *eLife, 4*, e08347.

Kusriastuti, R., & Sutomo, S. (2005). Evolution of dengue prevention and control program in Indonesia. *Dengue Bulletin, 29*, 1-7.

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. *Tropical Biomedicine, 30*(1), 36-45.

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.

Low, V. L., Chen, C. D., Lee, H. L., Lim, P. E., Leong, C. S., & Sofian–Azirun, M. (2013). Current susceptibility status of Malaysian *Culex quinquefasciatus* (Diptera: Culicidae) against DDT, propoxur, malathion, and permethrin. *Journal of Medical Entomology*, *50*(1), 103-111.

Low, V. L., Vinnie-Siow, W. Y., Lim, Y. A. L., Tan, T. K., Leong, C. S., Chen, C. D., & Sofian-Azirun, M. (2015). Research note first molecular genotyping of A302S mutation in the gamma aminobutyric acid (GABA) receptor in *Aedes albopictus* from Malaysia. *Tropical Biomedicine*, *32*(3), 554-556.

Lee, H.L. (1992). *Aedes* ovitrap and larval survey in several suburban communities in Selangor, Malaysia. *Mosquito-Borne Diseases Bulletin, 9*(1), 9-15.

Madhukar, B. V. R., & Pillai, M. K. K. (1970). Development of organophosphorus resistance in Indian strains of *Aedes* aegypti (L.). *Bulletin of the World Health Organization, 43*(5), 735–742.

Mardihusodo, S. J. (1996). Application of non-specific esterase enzyme microassays to detect potential insecticide resistance of *Aedes aegypti* adults in Yogyakarta, Indonesia. *Berkala Ilmu Kedokteran, 28*, 167-171.

Martini, M., Prihatnolo, A., & Hestiningsih, R. (2013). Modification ovitrap to control of *Aedes sp*. population in Central Java. *Journal of Communication Disease, 49*(3), 52-56.

Mattingly, P. F. (1957). Genetical aspects of the *Aedes aegypti* problem. *Annals of Tropical Medicine & Parasitology, 51*(4), 392-98.

Ministry of Health Republic of Indonesia (2016). Situations of dengue in Indonesia. Retrieved 14th May 2015 from http://www.depkes.go.id/resources/download/pusdatin/infodatin/infodatin%20dbd%202016.pdf

Miller, T. A. (1988). Mechanisms of resistance to pyrethroid insecticides. *Parasitology Today, 4*(7), S8-S12.

Mohsin, M., Naz, S. I., Khan, I., Jabeen, A., Bilal, H., Ahmad, R., & IM, E. (2016). Susceptibility status of *Aedes aegypt*i and *Aedes albopictus* against insecticides at Eastern Punjab, Pakistan. *International Journal of Mosquito Research*, *3*(5), 41-46.

Mulyatno, K. C., Yamanaka, A., & Konishi, E. (2012). Resistance of *Aedes aegypti* (L.) larvae to temephos in Surabaya, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health, 43*(1), 29.

Musso, D., Roche, C., Robin, E., Nhan, T., Teissier, A., & Cao-Lormeau, V. M. (2015). Potential sexual transmission of Zika virus. *Emerging Infectious Diseases, 21*(2), 359.

Mustafa, M. S., Rasotgi, V., Jain, S., & Gupta, V. (2015). Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. *Medical Journal Armed Forces India, 71*(1), 67-70.

Najera, J. A., & Zaim M. (2011). Malaria vector control: Insecticides for residual spraying. *World Health Organization*, 1-92.

Nazni, W. A., Lee, H. L., & Azahari, A. H. (2005). Adult and larval insecticide susceptbility status of *Culex quinquefasciatus* (Say) mosquitoes in Kuala Lumpur Malaysia. *Tropical Biomedicine*, *22,* 63-68.

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

Nature (2014). Dengue Viruses. Retrieved 30th October 2017 from http://www.nature.com/scitable/topicpage/dengue-viruses-22400925.

Norzahira, R., Hidayatulfathi, O., Wong, H. M., Cheryl, A., Firdaus, R., Chew, H. S., & Lee, H. L. (2011). Ovitrap surveillance of the dengue vectors, *Aedes* (Stegomyia) *aegypti* (L.) and *Aedes* (Stegomyia) *albopictus* Skuse in selected areas in Bentong, Pahang, Malaysia. *Tropical Biomedicine, 28*(1), 48-54.

Nusa, R., Prasetyowati, H., Meutiawati, F., Yohan, B., Trimarsanto, H., Setianingsih, T. Y., & Sasmono, R. T. (2014). Molecular surveillance of dengue in Sukabumi, West Java Province, Indonesia. *The Journal of Infection in Developing Countries, 8*(6), 733-741.

Ocampo, C. B., Salazar-Terreros, M. J., Mina, N. J., McAllister, J., & Brogdon, W. (2011). Insecticide resistance status of *Aedes aegypti* in 10 localities in Colombia. *Acta Tropica, 118*(1), 37-44.

Othman, W-N., 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-Pacific Entomology, 13*(3), 175-182.

Pasteur, N., Marquine, M., Rousset, F., Failloux, A. B., Chevillon, C., & Raymond, M. (1995). The role of passive migration in the dispersal of resistance genes in *Culex pipiens quinquefasciatus* within French Polynesia. *Genetics Research*, *66*(2), 139-146.

Prasetyowati, H., Hendri, J., & Wahono, T. (2016). Status resistensi *Aedes aegypti* (Linn.) terhadap organofosfat di tiga kotamadya DKI Jakarta. *BALABA*: *Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara, 12*(1), 23-30.

Ponlawat, A., Scott, J. G., & Harrington, L. C. (2005). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *Journal of Medical Entomology, 42*(5), 821-825.

Putra, R. E., Ahmad, I., Prasetyo, D. B., Susanti, S., Rahayu, R., & Hariani, N. (2016). Detection of insecticide resistance in the larvae of some *Aedes aegypti* (Diptera: Culicidae) strains from Java, Indonesia to temephos, malathion and permethrin. *International Journal of Mosquito Research, 3*(3), 23-28.

Rahayu, R., Ahmad, I., Ratna, E. S., Tan, M. I., & Hariani, N. (2012). Present status for carbamate, pyrethroids and phenylpyrazole insecticide resistance to German cockroach, *Blattella germanica* in Indonesia. *Journal of Entomology*, *9*(6), 361-367.

Ranson, H., Burhani, J., Lumjuan, N., & Black IV, W. C. (2010). Insecticide resistance in dengue vectors. *TropIKA. Net, 1*(1), 1-12.

Rawani, A., Ghosh, A., & Chandra, G. (2014). Mosquito larvicidal potential of four common medicinal plants of India. *The Indian Journal of Medical Research, 140*(1), 102–108.

Rodríguez, M. M., Bisset, J., Ruiz, M., & Soca, A. (2002). Cross-resistance to pyrethroid and organophosphorus insecticides induced by selection with temephos in *Aedes aegypti* (Diptera: Culicidae) from Cuba. *Journal of Medical Entomology, 39*(6), 882-888.

Rohani, A., Chu, W. L., Saadiyah, 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.

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

Rozilawati, H., Tanaselvi, K., Nazni, W. A., Masri, S. M., Zairi, J., Adanan, C. R., & Lee, H. L. (2015). Surveillance of *Aedes albopictus* Skuse breeding preference in selected dengue outbreak localities, Peninsular Malaysia. *Tropical Biomedicine, 32*(1), 49-64.

Rudnick, A. (1967). *Aedes aegypti* and haemorrhagic fever. *Bulletin of the World Health Organiz*ation, *36*, 528–532.

Rueda, L. M. (2004*). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission*. New Zealand: Magnolia Press.

Saleeza, S. N. R., Norma-Rashid, Y., & Azirun, M. S. (2013). Mosquito species and outdoor breeding places in residential areas in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health, 44*(6), 963-969.

Sathantriphop, S., Paeporn, P., & Supaphathom, K. (2006). Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides. *Tropical Biomedecine, 23*(1), 97-101.

Sayono, S., Hidayati, A. P. N., Fahri, S., Sumanto, D., Dharmana, E., Hadisaputro, & S., Syafruddin, D. (2016). Distribution of voltage-gated sodium channel (*Nav*) alleles among the *Aedes aegypti* populations in Central Java Province and its association with resistance to pyrethroid insecticides. *PLoS ONE, 11*(3), e0150577.

Selvi, S., Edah, M, A., Nazni, W. A., Lee, H. L., & Azahari, A. H. (2005). Resistance development and insecticide susceptibility in *Culex quinquefasciatus* against selection pressure of malathion and permethrin and its relationship to cross-resistance towards propoxur. *Tropical Biomedicine*, *22* (2), 103-113.

Service, M. (2012). *Medical Entomology for Students*. New York: Cambridge University Press.

Shafie, F. A., Tahir, M. P. M., & Sabri, N. M. (2012). *Aedes* mosquitoes resistance in urban community setting. *Procedia-Social and Behavioral Sciences, 36*, 70-76.

Singh, R. K., Haq, S., Kumar, G., Mittal, P. K., & Dhiman, R. C. (2013). Insecticide-susceptibility status of dengue vectors *Aedes aegypti* and *Aedes albopictus* in India: a review. *Dengue, 37*, 177.

Sivanathan, M. M. (2006). The ecology and biology of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) and the resistance status of *Aedes albopictus* (field strain) against organophosphates in Penang, Malaysia (Doctoral dissertation). Retrieved 30th October 2017 from http://eprints.usm.my/9824/1/THE_ECOLOGY_AND_BIOLOGY_OF_Aedes_aegypti_(L.).pdf

Sivagnaname, N., & Gunasekaran, K. (2012). Need for an efficient adult trap for the surveillance of dengue vectors. *The Indian Journal of Medical Research, 136*(5), 739.

Syarifah, N., Rusmatini, T., Djatie, T., & Huda, F. (2008). Ovitrap ratio of *Aedes aegypti* larvae collected inside and outside houses in a community survey to prevent dengue outbreak, Bandung, Indonesia. *Proceeding Association Southeast Asian Nations Congress Tropical Medical Parasitology, 3*, 116-120.

Stiasni, M., Rehbinder, D., & Deckers, W. (1967). Absorption, distribution and metabolism of O-(4-bromo-2, 5-dichlorophenyl)-O, O-dimethylphosphorothioate (bromophos) in the rat. *Journal of Agricultural and Food Chemistry, 15*(3), 474-478.

Strode, C., Steen, K., Ortelli, F., & Ranson, H. (2006). Differential expression of the detoxification genes in the different life stages of the malaria vector *Anopheles gambiae*. *Insect Molecular Biology*, *15*(4), 523-530.

Subramaniam, S., Aris, E. M., Ahmad, N. W., Lee, H. L., & Hadi, A. A. (2006). The development of resistance and susceptibility of *Aedes aegypti* larvae and adult mosquitoes against selection pressure to malathion, permethrin, and temephos insecticides and its cross-resistance relationship against propoxur. *Malaysian Journal of Science*, *25*(1), 1-13.

Suganya, A., Murugan, K., Kovendan, K., Kumar, P. M., & Hwang, J. S. (2013). Green synthesis of silver nanoparticles using *Murraya koenigii* leaf extract against *Anopheles stephensi* and *Aedes aegypti*. *Parasitology Research, 112*(4), 1385-1397.

Suroso, D. T. (1984). Experience of the Control of *Aedes aegypti* in Indonesia. *World Health Organization, 10*, 12-19.

Tang, A. H., & Tu, C. P. (1994). Biochemical characterization of *Drosophila* glutathione S-transferases D1 and D21. *Journal of Biological Chemistry*, *269*(45), 27876-27884.

Teo, C. H., Lim, P. K. C., Voon, K., & Mak, J. W. (2017). Detection of dengue viruses and Wolbachia in *Aedes aegypti* and *Aedes albopictus* larvae from four urban localities in Kuala Lumpur, Malaysia. *Tropical Biomedicine, 34*(3), 583-597.

Tham, A.S. (2000). Surveillance of mosquitoes. In Ng F. S. P., and Yong H. S. (Eds), *Mosquitoes and mosquito-borne disease: Biology surveillance control, personal and public protection measures* (pp. 167-183). Kuala Lumpur, Malaysia: Academy Science of Malaysia.

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.

Tikar, S. N., Mendki, M. J., Chandel, K., Parashar, B. D., & Prakash, S. (2008). Susceptibility of immature stages of *Aedes* (Stegomyia) *aegypti*; vector of dengue and chikungunya to insecticides from India. *Parasitology Research*, *102*(5), 907.

Troyo, A., Calderón-arguedas, O., Fuller, D. O., Solano, M. E., Arheart, K. L., Chadee, D. D., & Beier, J. C. (2008). Seasonal profiles of *Aedes aegypti* (Diptera: Culicidae) larval habitats in an urban area of Costa Rica with a history of mosquito control. *Journal of Vector Ecology, 33*(1), 76–88.

Van den Bercken, J., & Vijverberg, H. P. (1988). Mode of action of pyrethroid insecticides. In Galli C. L., Manzo L., Spencer P. S. (Eds.), *Recent Advances in Nervous System Toxicology*. Boston : Springer.

Vijverberg, H. P., & van den Bercken, J. (1990). Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Critical Reviews in Toxicology, 21*(2), 105-126.

Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., Della Torre, A., & Ranson, H. (2012). Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticide Biochemistry and Physiology, 104*(2), 126-131.

Wahyono, T. Y. M., Nealon, J., Beucher, S., Prayitno, A., Moureau, A., Nawawi, S., & Nadjib, M. (2017). Indonesian dengue burden estimates: review of evidence by an expert panel. *Epidemiology & Infection, 145*(11), 2324-2329.

Wan-Norafikah, O., Nazni, W. A., & Noramiza, S. (2011). Ovitrap surveillance and mixed infestation of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Northern Region and Southern Region of Malaysia. *Health Environment Journal, 2*, 1-5.

Wan-Norafikah, O., Nazni, W. A., Noramiza, S., Hafa'Ar-Ko'ohar, S. S., Heah, S. K., Nor-Azlina, H., & Lee, H. L. (2012). Distribution of *Aedes* mosquitoes in three selected localities in Malaysia. *Sains Malaysiana, 41*(10), 1309-1313.

Weetman, D., & Donnelly, M. J. (2015). Evolution of insecticide resistance diagnostics in malaria vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *109*(5), 291-293.

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

Westbrook, C. J., Reiskind, M. H., Pesko, K. N., Greene, K. E., & Lounibos, L. P. (2010). Larval environmental temperature and the susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to Chikungunya virus. *Vector Borne and Zoonotic Diseases*, *10*(3), 241-247.

Widiarti, W., Heriyanto, B., Boewono, D. T., Widyastuti, U., Mujiono, M., Lasmiati, L., & Yuliadi, Y. (2011). Peta resistensi vektor demam berdarah dengue *Aedes aegypti* terhadap insektisida kelompok organofosfat, karbamat dan pyrethroid di Propinsi Jawa tengah dan Daerah Istimewa Yogyakarta. *Buletin Penelitian Kesehatan, 39*(4), 176-189.

Wijayanti, S. P., Sunaryo, S., Suprihatin, S., McFarlane, M., Rainey, S. M., Dietrich, I., & Kohl, A. (2016). Dengue in Java, Indonesia: Relevance of mosquito indices as risk predictors. *PLoS Neglected Tropical Diseases, 10*(3), e0004500.

Wuliandari, J. R., Lee, S. F., White, V. L., Tantowijoyo, W., Hoffmann, A. A., & Endersby-Harshman, N. M. (2015). Association between three mutations, F1565C, V1023G and S996P, in the voltage-sensitive sodium channel gene and knockdown resistance in *Aedes aegypti* from Yogyakarta, Indonesia. *Insects, 6*(3), 658-685.

Wouters, W., & van den Bercken, J. (1978). Action of pyrethroids. *General Pharmacology: The Vascular System, 9*(6), 387-398.

World Health Organization (1979). DDT and its derivatives. *Environmental Health Criteria.* Geneva, Switzerland: *World Health Organization*.

World Health Organization. (2003). WHO specifications and evaluations for public health pesticides. Geneva, Switzerland: *World Health Organization*.

Word Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicides. Document WHO/CDS/WHOPES/GCDPP/13. Geneva, Switzerland: World Health Organization.

World Health Organization. (2006). WHO specifications and evaluations for public health pesticides, permethrin. Geneva, Switzerland : World Health Organization

World Health Organization. (2007). WHO specifications and evaluations for public health pesticides, etofenprox. Geneva, Switzerland : World Health Organization.

World Health Organization. (2008). WHO specifications and evaluations for public health pesticides, bendiocarb. Geneva, Switzerland : World Health Organization

World Health Organization. (2009). WHO specifications and evaluations for public health pesticides, chlorpyrifos. Geneva, Switzerland : World Health Organization.

World Health Organization. (2010). WHO specifications and evaluations for public health pesticides, fenitrothion. Geneva, Switzerland : World Health Organization.

World Health Organization. (2011). WHO specifications and evaluations for public health pesticides, temephos. Geneva, Switzerland : World Health Organization.

World Health Organization. (2012). WHO specifications and evaluations for public health pesticides, deltamethrin. Geneva, Switzerland : World Health Organization.

World Health Organization. (2013). WHO specifications and evaluations for public health pesticides, malathion. Geneva, Switzerland : World Health Organization.

World Health Organization. (2015). WHO specifications and evaluations for public health pesticides, lambda-cyhalothrin. Geneva, Switzerland : World Health Organization.

World Health Organization. (2016a). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, Switzerland : World Health Organization

World Health Organization. (2016b). Monitoring and managing insecticide resistance in *Aedes* mosquito populations: Interim guidance for entomologists. Geneva, Switzerland : World Health Organization.

World Health Organization (2017a). Fact Sheet: Chikugunya. Geneva, Switzerland : World Health Organization.

World Health Organization. (2017b). WHO specifications and evaluations for public health pesticides, propoxur. Geneva, Switzerland : World Health Organization.

World Health Organization (2018a). Fact Sheet: Zika Virus. Geneva, Switzerland : World Health Organization.

World Health Organization (2018b). Fact Sheet: Yellow fever. Geneva, Switzerland : World Health Organization.

World Health Organization (2018c). Fact Sheet: Dengue and severe dengue. Geneva, Switzerland : World Health Organization.

Yasuno, M., and Tonn, R. J. (1970). A study of biting habits of *Aedes aegypti* in Bangkok, Thailand. *Bulletin of the World Health Organization, 43*(2), 319–325.

Yoshimizu M. (2013) Key Characteristics for larval *Aedes* spp. identification in CA. Retrieved 24th February 2018 from https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/*Aedes*LarvalPictureKey.pdf

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Research Article

Haziqah-Rashid, A., Chen, C.D., Lau, K.W., Low, V.L., Sofian-Azirun, M., Halim, M.R.A., … Azidah, A.A. (2019). Monitoring insecticide resistance profiles of *Aedes aegypti* (Diptera: Culicidae) in the Sunda Islands of Indonesia based on diagnostic doses of larvicides. *Journal of Medical Entomology*. https://doi.org/10.1093/jme/tjy208

Proceedings

1. **Haziqah-Rashid, A.,** Chen, C.D., Lau, K.W., Low, V.L., Azidah, A.A., Suana, I.W., Harmonis, Syahputra, E., Razak, A., Chin, A.C, Sofian-Azirun, M. (2017). Susceptibility status of dengue vectors, *Aedes aegypti* (L.) against organophosphate larvicides in major states of Indonesia. *22nd Biological Sciences Graduate Congress,* 19-21 December 2017, National University of Singapore, Singapore (Poster Presentation, Abstract: page 45).

2. **Haziqah-Rashid, A.,** Chen, C.D., Lau, K.W., Low, V.L., Azidah, A.A., Suana, I.W., Harmonis, Syahputra, E., Razak, A., Chin, A.C, Sofian-Azirun, M. (2017). Susceptibility status of dengue vector, *Aedes aegypti* (L.) against organochlorines in major states of Indonesia. *Entomology Postgraduate Symposium 2017*, 13-14 December 2017, Centre for Insect System (CIS), Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia. (Poster Presentation, Abstract: page 38).

3. **Haziqah-Rashid, A.,** Chen, C.D., Lau, K.W., Low, V.L., Azidah, A.A., Suana, I.W., Harmonis, Syahputra, E., Razak, A., Chin, A.C, Sofian-Azirun, M. (2017). 54th Revised discriminating lethal dose of temephos and malathion on *Aedes aegypti* in Indonesia. *Annual Scientific Conference of the Malaysian Society of Parasitology and Tropical Medicine (MSPTM) 2018, Tropical and Zoonotic Diseases: Stemming the Tide,* 14th-15th March, Connexion Conference and Event Centre, Bangsar South City, Kuala Lumpur, Malaysia. (E-poster presentation, Abstract: page 58).

4. **Haziqah-Rashid, A.,** Chen, C.D., Lau, K.W., Low, V.L., Azidah, A.A., Suana, I.W., Harmonis, Syahputra, E., Razak, A., Chin, A.C, Sofian-Azirun, M. (2018). Resistance status of dengue vector, *Aedes aegypti* (L.) against organophosphates in major states of Indonesia. *Malaysian Society of Parasitology and Tropical Medicine (MSPTM) Mid-year Seminar in Honour of Prof Mak Joon Wah, Relevance and Importance of Tropical Medicine*, 7th July, International Medical University Malaysia, Kuala Lumpur, Malaysia.
5. **Haziqah-Rashid, A.,** Chen, C.D., Lau, K.W., Low, V.L., Azidah, A.A., Suana, I.W., Harmonis, Syahputra, E., Razak, A., Chin, A.C, Sofian-Azirun, M. (2018). Preliminary dengue vector surveillance in the Sunda Islands, Indonesia: Interchange of breeding habitat preferences of *Aedes aegypti* and *Aedes albopictus*. *2nd Asian Simuliidae and 1st National Veterinary Parasitology Symposium, Faculty of Veterinary Medicine,Biodiversity of Vectors and Veterinary Parasitic Diseases, Bogor Agricultural University, Indonesia.* (Poster presentation, Abstract : page 21)