PUBLIC PERCEPTION ON *Aedes albopictus* MOSQUITOES, FITNESS COST TO INSECTICIDES EXPOSURE AND ITS ACTIVITY PATTERN IN PENANG RESIDENTIAL AREAS

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LIST OF ABBREVIATIONS

AchE	Acetycholinesterase
CHIKV	Chikungunya virus
CNS	Center Nervous System
DDT	Dichlorodiphenyltricholoroethane
DENV-1	Dengue Virus serotype 1
DENV-2	Dengue Virus serotype 2
DENV-3	Dengue Virus serotype 3
DENV-4	Dengue Virus serotype 4
DHF	Dengue hemorrhagic fever
INTS	Insecticide impregnated net
IRS	Insecticide residual surface
MBD	Mosquito-borne disease
OC	Organochlorine
OP	Organophosphate
VCRU	Vector Control Research Unit
WHO	World Health Organization
WPRO	Western Pacific Region Office

PERSEPSI ORANG AWAM TERHADAP NYAMUK *Aedes Albopictus*, KOS KECERGASAN KEPADA PENDEDAHAN INSEKTISID DAN POLA AKTIVITINYA DI KAWASAN KEDIAMAN DI PULAU PINANG

ABSTRAK

Satu kajian telah dijalankan ke atas nyamuk *Aedes albopictus* di kawasan kediaman dan kos kecergasan sebagai tindak balas terhadap pendedahan insektisid. Kajian untuk menilai tahap pengetahuan semasa mengenai nyamuk *Aedes* dan denggi di kalangan penduduk Penang di bandar Sg. Dua dan pinggir bandar Batu Maung melibatkan 202 responden, terutamanya mengenai pemerhatian terhadap tingkah laku umum nyamuk *Aedes* dan langkah pencegahannya, dan gaya hidup (ke atas penggunaan cahaya buatan) dalam mengubah tingkahlaku nyamuk *Aedes*. Terdapat hubungan kait antara pengetahuan kekerapan gigitan nyamuk *Aedes* dengan gaya hidup manusia pada penggunaan cahaya. Walau bagaimanapun, tahap pengetahuan pada *Aedes* dan denggi untuk kedua-dua responden bandar dan pinggir bandar adalah rendah. Berdasarkan kajian soal selidik, siasatan kesan insektisid malathion dan permethrin kepada sejarah hidup nyamuk *Ae. albopictus* telah dipilih untuk kajian seterusnya. Nyamuk betina didedahkan dengan kepekatan submaut 1.5% malathion dan 0.2% permethrin menggunakan ujian WHO bioesei dewasa dan nyamuk betina yang terselamat dinilai untuk parameter kecergasan. Kami mendapati insektisid 0.2% pemethrin mempunyai kesan lebih ketara ke atas kos kecergasan *Ae. albopictus,* walaupun telah membina daya rintangan terhadap insektisid permethrin. Permethrin mengurangkan fecunditi, memendekkan tempoh pembesaran pada setiap peringkat larva dan mempengaruhi nisbah jantina dengan lebih banyak kemunculan

nyamuk jantan *Ae. albopictus*. Berdasarkan keprihatinan mengenai aktiviti semasa nyamuk *Ae. albopictus* dan *Cx. quinquefasciatus* yang semakin meningkat di kawasan perumahan, tangkapan 24 jam telah dijalankan di kawasan kediaman bandar, Sg. Dua dan pinggir bandar, Batu Maung dengan menggunakan dua perangkap yang berbeza; Perangkap BG-Sentinel dan Perangkap cahaya CDC Fay-Prince. Perangkap cahaya CDC Fay-Prince lebih berkesan dalam memerangkap nyamuk *Cx. quinquefasciatus*. Manakala, kedua-dua perangkap tersebut tidak berkesan untuk memerangkap dan memantau aktiviti nyamuk *Ae. albopictus*. Oleh itu pensampelan perangkap telur adalah kaedah yang paling berkesan untuk menangkap *Ae. albopictus* di kawasan kediaman. Waktu kemuncak aktiviti *Ae. albopictus* tidak mengikut aktiviti 24 jam pencarian-perumah di mana spesies ini aktif pada siang (9:00 am dan 11:00 am), waktu malam (10:00 pm dan 12:00 am) dan waktu hampir subuh (5:00 am), manakala kebanyakkannya nyamuk *Cx. quinquefasctus* telah ditangkap pada waktu nokturnal. Parameter meteorologi yang dicatatkan dalam kajian tidak mempunyai hubungkait dengan kelimpahan kedua-dua spesies nyamuk. Walau bagaimanapun, keamatan cahaya sangat ketara mempengaruhi kelimpahan nyamuk *Cx. quinquefasciatus* di kedua-dua kawasan kediaman.

PUBLIC PERCEPTION ON *Aedes Albopictus* MOSQUITOES, FITNESS COST TO INSECTICIDES EXPOSURE AND ITS ACTIVITY PATTERN IN PENANG RESIDENTIAL AREAS

ABSTRACT

A study was carried on *Aedes albopictus* mosquito in residential areas and its fitness cost in response to the insecticide exposure. The study to evaluate the current knowledge level on *Aedes* mosquitoes and dengue among Penang residence in urban Sg. Dua and sub-urban Batu Maung involved 202 respondents mainly on the observation of general *Aedes'* mosquito behaviour and its preventive measure, and lifestyle (on usage of artificial light) in changing the behaviour of *Aedes* mosquitoes. There is a direct link between the knowledge of the *Aedes* biting frequency with human lifestyle on the light use. However, the level of knowledge is poor on *Aedes* mosquitoes and dengue in both urban and sub-urban respondents. Following the survey, to investigate the effects of malathion and permethrin insecticides on the life history of *Ae. albopictus* mosquitoes were chosen for subsequent studies. The female mosquitoes were exposed to sub-lethal concentration of 1.5% malathion and 0.2% permethrin using WHO adult bioassay and female survivors were evaluated for their fitness parameters. We discovered 0.2% permethrin have significantly more effects on the fitness cost of *Ae. albopictus*, even though had developed resistance towards permethrin. Permethrin has decrease the fecundity, shorten the development periods on each larval stages and influence the sex ratio with more emerge male of *Ae. albopictus*. Regarding the increasing concern on the current activity of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes in the residential areas, 24 hour trapping was conducted at urban Sg. Dua and sub-urban Batu Maung residence area using two different traps; BG- Sentinel Trap and CDC Fay-Prince Light Trap. CDC Fay-Prince light traps was most effective in trapping *Cx. quinquefasciatus.* While, neither of both trap was effective to trap and monitoring the activities of *Ae. albopictus.* Therefore ovitrap sampling is the most effective method to capturing *Ae. albopictus* in residence area. The peak activity of *Ae. albopictus* is not following the normal diel host-seeking activity which this species active at daylight (9:00 am and 11:00 am), night (10:00 pm and 12:00 am) and nearly dawn (5:00 am), while *Cx. quinquefasciatus* was captured mostly during the nocturnal time. Meteorological parameter recorded didn't correlate with the abundance of both mosquito species. However, light intensity significantly affected *Cx. quinquefasciatus* mosquitoes abundance in both residence areas.

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

Aedes albopictus has been known as main dengue fever vectors in Southeast Asia (Knudsen, 1995). In addition, this species also potential act as vector of several additional arboviruses such as chikungunya virus that considerable medical importance to human-being. (Gómez et al., 2011).

Aedes albopictus has become cosmopolitan due for its introduction by human worldwide (Knudsen, 1995). This species has been described as fundamental a rural and sub-urban mosquitoes which is normally encountered in cities and vegetation (Hawley, 1988). *Aedes albopictus* is an important dengue vector in rural and sub-urban areas in Southeast Asia (Phillips, 2008). A study by Petrić et al., (2014), showed that over recent decade, the frequent contact between human and mosquitoes happened as peri-urban and sub-urbans expanded into previously undisturbed natural area. Therefore, it providing a huge number and variety of mosquitoes breeding places than inner-city areas. Urbanized areas are facing an invasion of *Aedes albopictus* known as container-breeding mosquitoes which is an aggressive biter during the day, where females seeking for bloodmeals (Petrić et al., 2014).

This study is aimed to identify, whether did *Aedes albopictus* biting cycle and resting activities remain as bimodal pattern with morning and evening twilight peak even although the human lifestyle such as 24 hour light source may affect the mosquitoes activities.

Aedes albopictus has been reported to develop of container-breeding almost every year (Petrić et al., 2014) .The resting and biting behaviour of *Ae. albopictus* is crucial as it act as importance vector in transmitting dengue viruses and they rapidly expanding in global range (Gratz, 2004; Nelder et al., 2010). Most studies have found *Ae. albopictus* as resting and feeding outdoor (exophilic and exophagic) (Paupy et al., 2009). However, study by Valerio et al. (2010) reported of new variations in *Ae. albopictus* behaviour in gravid female which they was captured indoor in Rome, Italy. *Aedes albopictus* showed a bimodal pattern in their biting cycle and resting activites with morning (0730 hour) and evening (1730 hour) twilight peak (Hassan et al., 1996).

Observations made by Moore and Mitchell (1997) on early dispersal of *Ae. albopictus* are consistent with the hypothesis of dispersal by human lifestyles which probably movement of old tires through the interstate highway system. We able to conclude the changing of biology of *Ae. albopictus* may affect by human lifestyles which also influence the transmission of dengue viruses to humans. To encounter this problem, human started to using chemical control such as insecticides.

In Malaysia, government started to use fogging operation with malathion in early 1970s and followed with permethrin in early 1996 against *Aedes* species for dengue control. However, the use of chemical insecticides had developed resistance toward certain strain of *Ae. albopictus* mosquitoes in some localities (Nazni et al., 1998). Insecticide gave sub-lethal effects such as changes in body size or lengthens development time which can influence pathogen transmission especially dengue viruses (Muturi et al., 2010).

By understanding mosquitoes' strategy to maximizing their fitness cost in transmitting dengue viruses after insecticide exposure, make us capable to supports the development of effective control programme to encounter dengue and the evaluation of their impact to human (Petrić et al., 2014).

Our early observation noted that there are changes in *Ae. albopictus* behaviours in seeking bloodmeal and resting behaviour due to modern human lifestyle and which most of Malaysian spend their time outdoor. This may increase the chances of getting contact with dengue vector. Due to this reason, *Ae. albopictus* start to alter their behaviour in feeding, resting and oviposition strategies which will caused more establishment of their population in environment. We proposed that the feeding and resting behaviour of mosquitoes may have evolve in relation to the changing modern human life style.

The objectives of this study are:

1. To evaluate the current knowledge level and information on *Aedes* mosquitoes and dengue among Penang residence in the urban (Sg. Dua) and sub- urban areas (Batu Maung).
2. To determine the fitness cost of *Ae. albopictus* after exposure to malathion and permethrin insecticides.
3. To investigate the 24 hours' current activity pattern of two common mosquitoes; *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes in urban Sg. Dua and sub-urban Batu Maung areas in relation with environmental effects.

CHAPTER 2

Literature Review

LITERATURE REVIEW

2.1 *Aedes* and Dengue

Aedes albopictus causes at least 22 arboviruses, notably dengue, which serves as a maintenance vector in rural area of endemic countries such as South-East Asia and Pacific islands (Gratz, 2004). Dengue fever was first reported in Malaysia in 1902 (Skae, 1902) and known as one of the major public health problems in Malaysia (Wallace et al., 1980). Malaysia is in the third ranked of the total number of reported cases of dengue disease among countries in the Western Pacific Region Office (WPRO) from period year of 1991 until 2007 (Yusoff, 2008). The dengue disease in Malaysia was reported rise abruptly from 46,681 in the year of 2014 to 62,648 in the year of 2015. According to the annual number of deaths, the deaths are increased from 87 in 2014 to 173 in 2015 in Malaysia (Ministry of Health Malaysia, 2015). Dengue infection in Malaysia is primary reported in urban areas in 2001 where 61.8% infected from the total of the country's population lives, as compared to only 34% infection in 1980 (Teng and Singh, 2001).

Dengue transmission involves interaction between peoples, mosquitoes, viruses and environmental factors. Human behavior is one of the crucial factors influencing the epidemiology of dengue fever, which affect the local vector habitat, and the control strategies that apply specifically depends on the socioeconomic context and behavioral characteristics of the populations (Service, 1992). Local human movement is a spatiotemporal driver for epidemic dengue transmission (Guzmán and Kouri, 2014). Beside, climatologic factors may play crucial role in dengue transmission (Keating, 2001). Based on a study by Thongrungkiat et al. (2003), DENV 1 infection was higher during hot season compared to cool and rainy seasons, while DENV 4 was higher during the rainy season. Changes in circulating serotypes may have an effect on the incidence and severity of dengue diseases, as major outbreaks tend to establish the switching DENV serotypes phenomenon in population (Abubakar and Shafee, 2002). Over the past few decades, major dengue disease outbreak involves cyclical pattern mainly viruses DENV-1, DENV-2 and DENV-3 (Abubakar and Shafee, 2002). This cycling correlated with the switching of the predominant DENV serotypes in the population (Mohd-Zaki et al., 2014).

Dengue virus transmission cycle works in its simplest form which only involve the ingestion of viremic blood by mosquitoes and passed it to the second susceptible human host. Mosquitoes need an incubation period of 8 to 10 days after feeding on a viremic from infected humans to ensure viral replication and internal dissemination in their body before the virus appears in the saliva and transmit during refeeding to another human. Female mosquitoes undergo more than one reproductive cycle during the extrinsic incubation period and have the opportunity to pass the dengue virus to their progeny (Monath, 1994). If the female adult mosquitoes do not live long until incubation period, the virus will not complete their development and cannot be transferred to new hosts (Aida et al., 2011). For disease transmission, the factors such as survivorship, fecundity and abundance of mosquitoes play crucial roles (Arunachalam et al., 2010).

2.2 *Aedes albopictus* (Skuse)

Aedes albopictus is classified under Family Culicidae, originated from forests and bred in natural habitat such as bamboo tree hole. However, recent studies have reported this species is increasingly found in urban cities (Higa et al., 2010), and has adapted very well in sub-urban and urban environments with larvae are able to breed in artificial containers such as in tires, and water storage containers (Bonizzoni et al., 2013).

Nowadays, many dengue infections are predominantly in urban areas, where 61.8% of the total human population live with the robust development of industrial and economic created more man-made breeder containers that suitable for *Aedes* mosquitoes oviposition site (Teng and Singh, 2001). Vazeille et al. (2001), found *Ae. albopictus* in Madagascar is more adapted to the urban environment compared to *Ae. aegypti* and considered to be an important vector for dengue in that area. This *Ae.* species are rapidly proliferating and take highly advantage of human transportation and take this chance to make contact between itself, humans and viruses.

Aedes albopictus found to be highly aggressive in biting human and might be involved in transmissions of viruses in human (Kamgang et al., 2012). Joshi et al. (2002), discovered nearly 40% of larvae and progeny has found in the presence of DENV from *Ae. albopictus* females that had been orally infected with DENV-2. Transovorial transmission of dengue virus has been reported to be depended on *Aedes* species that was infected, and seems that *Ae. albopictus* is more efficient in transmitting DEN-1 compare to *Ae. aegypti* (Günther et al., 2007).

2.3 Biology of *Aedes albopictus*

Mosquitoes undergo complete metamorphosis and have four different stages in their life cycle; egg, larva, pupa and adult (Goma, 1966; Figure 2.1). The first three stages live in water and adult is a flying insect that feeds on blood or sucrose (Burgess and Cowan, 1993).

2.3.1 Egg biology

The eggs of *Aedes* mosquitoes have elongate-oval in shape **(**Plate 2.1**)**. The eggs are superficially similar, shiny jet black (Linley, 1989). When newly laid, the *Aedes* eggs are white in colour and soft . Then later the eggs turn black and become quite hard (Christophers, 1960). The eggs are normally laid above the water line of breeding container. It can stand dryness for months, but still viable and hatch when they become flooded with water (Goma, 1966; Service, 1996).

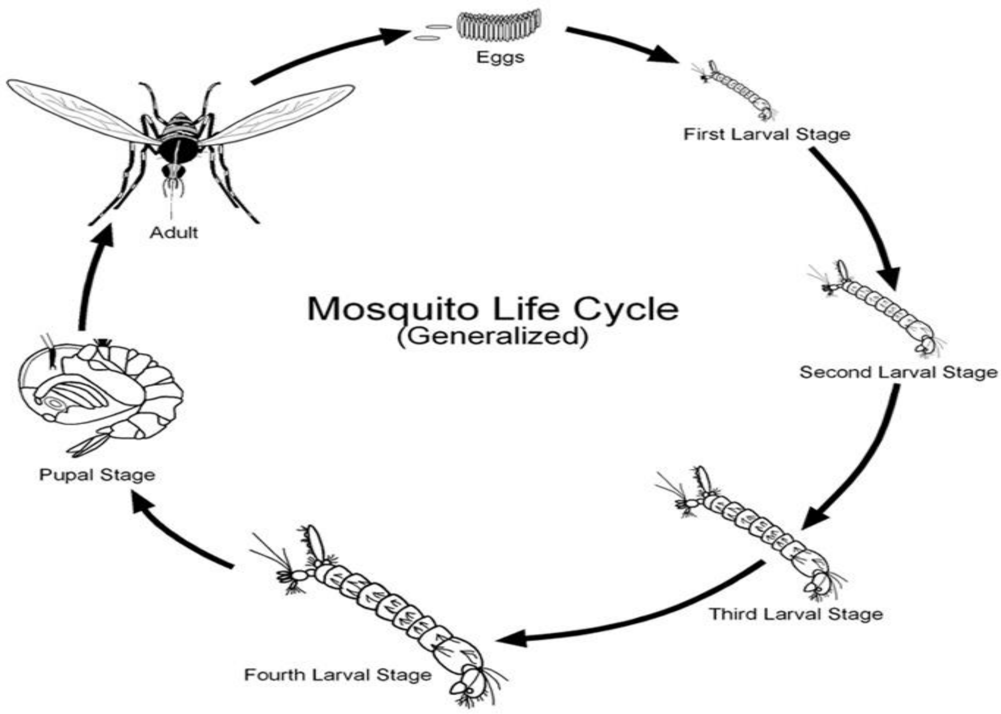

Figure 0.1 : *Aedes* Mosquito Life Cycle.

(http://extension.entm.purdue.edu/publichealth/insects/mosquito.html)

Plate 2.1 : Eggs of *Aedes albopictus.*

2.3.2 Larvae biology

Larvae of *Aedes albopictus* (Plate 2.2) undergo four larval stages that require five to 10 days to complete this stage . The duration of growth depends on temperature (Haridi, 1972) or larval diets (Hawley, 1988).

The first instar (L1) is recognized by the presence of an egg-breaker on the dorsum of the head and consists of a strongly sclerotized, pointed, curved and flattened cone set in an oval area of soft membrane. It is used to cut through the egg shell and allow the larva to escape by simply forcing off a circular cup from the anterior end of the egg (Christophers, 1960).

After ecdysis, the second instar (L2) larva, is much the same in length as the fully grown first instar, but is bulkier and the swollen head is enormous. The tracheal trunks are now enlarged and lined with taenidia and the terminal portions in the siphon have ballooned. During this instar the larva grows in length from 2 to 3mm (Christophers, 1960).

The third instar (L3) larva head measurement is more variable than in any other stage. It is very appropriate to have the ecdysis lines on the head capsule open for some time before ecdysis is definitely in progress. The pre-ecdysis stage is rather longer than the previous instar. The tail comb-spine is a prominent structure in this instar (Christophers, 1960).

The fourth instar (L4) larva at a corresponding size with much stouter due to the development of the thoracic imaginal buds and an accumulation of body fat. The fourth instar shows the rudiments of the pupal respiratory trumpets. The most prominent structure in this instar is the tail comb-spine (Christophers, 1960).

Plate 2.2 : *Aedes albopictus* Larvae.

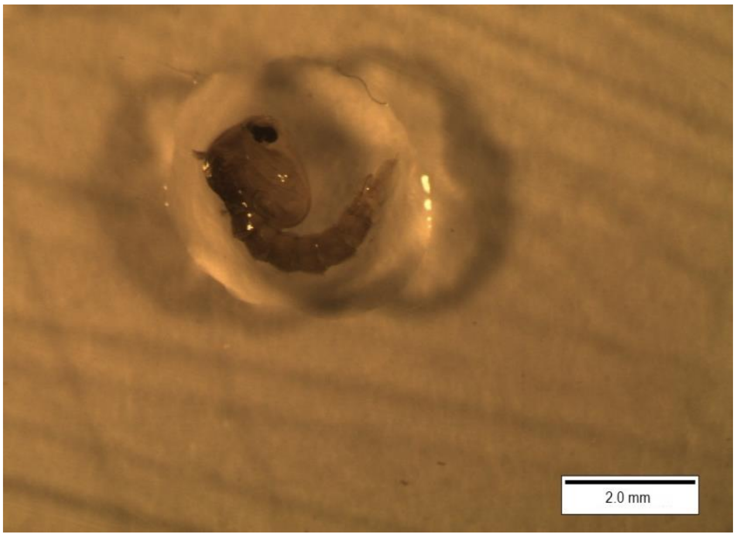

Plate 2.3 : *Aedes albopictus* Pupae.

2.3.3 Pupa and adult biology

Before mosquito emerge into adult, its undergo a pupal stage (Plate 2.3). The pupae will swallow air to expand the abdomen, thus open the pupal case and cause emergence of the head. Thus help the adult (Plate 2.4) to emerge freely. *Ae. albopictus* can easily be differentiated by the patterns of white scales on the dorsal side of the thorax (Plate 2.5). It has only a single broad line of white scales situated in the middle of the thorax (Sivanathan, 2006).

Adults will search a wide place with high humidity as their resting place. *Aedes* mosquitoes can fly around 50-100 meters from their nesting place to find food sources. *Aedes albopictus* is known as non-active migratory species, with a flight capability range of 800 m (Bonnet and Worcester, 1946; Honório et al., 2003). This *Aedes* species normally dispersed using human transportation, particularly on the ground transportation (Soper, 1967). Female adult is able to live within several weeks when have enough sources of sucrose. The life span for adult mosquitoes is between two weeks to a month depending on environmental conditions (Maricopa County Environmental Service, 2006). Female mosquitoes tend to live longer than male mosquitoes (Hawley, 1988).

Plate 2.4 : *Aedes albopictus* Adult.

Plate 2.5 : A single broad line of white scales located at the middle of *Ae. albopictus'* thorax.

2.4 Biting and resting behaviour of *Aedes albopictus*

Biting behaviour is related to host finding behaviour of the species. *Aedes albopictus* shows a highly mammalophilic and anthrophilic feeding pattern with 82% feeding on mammalian host and 68.5% of human blood (Sawabe et al., 2010). Besides, *Ae. albopictus* is known mainly as exophagic, which is a mosquito that feeds outdoors (Almeida et al., 2005). A study by Khan et al., (2014), showed *Ae. albopictus* displayed exophagic behaviour with index ranged from 0.17 to 0.25 for Endophagic and Endophilic Indexes by Ribeiro and Janz (1990).

Peak biting behaviour of *Ae. albopictus* in Southeast Asia is 'bimodal' which biting activities occur in the morning and evening twilight peak by female mosquitoes (Hawley, 1988; Hassan et al., 1996). Most often describe the part of the day such as diurnal, crepuscular, and nocturnal for foraging of bloodmeal time of a particular mosquito species (Petrić et al., 2014). *Aedes albopictus* reported rarely bites during night time (Hawley, 1988). Even it has low frequency, but continuous biting activity might happen throughout the night. This continuous activity may cause by the strong attraction from human bait which is unusually present in forested habitat (Amerasinghe and Ariyasena, 1991). *Aedes albopictus* feeds aggressively and opportunistically during the day on a broad range of potential host depending on their availability and the environmental condition (Bonizzoni et al., 2013).

The cavities (Burkett-Cadena et al., 2008), animal burrows and caves are examples of natural resting places for mosquitoes. Mosquitoes usually resting in man-made places such as barns, culverts and basements which provide darkness and moist environments that suitable for resting habitats (Crans, 1989). Mosquitoes resting behaviour have been classified into two categories; endophilic and exophilic.

Endophilic mosquito is defined as a mosquito that resting indoor (inside) human dwellings specifically during the time between the end of blood feeding and the onset of searching for eggs' laying site. While, an exophilic mosquito spends their resting time outside the human dwellings (Pates and Curtis, 2005).

Aedes albopictus mainly exophilic which resting outdoors (Almeida et al., 2005) and it has been found resting in clearing rubber plantations (Estrada-Franco and Craig, 1995). Besides that, outdoor mosquitoes also seek shelter in dry pots, at the bases of tree or in tree holes that provided shaded light and have their own specific microclimate (Haridi, 1972).

2.5 Survival and longevity of *Aedes albopictus*

The development period of *Ae. albopictus* started from egg stage to adult take 6.3 days. A faster development will favor high pupal population size, but the body size of pupae tends to taint because larvae may not have enough time to accumulate sufficient nutrient. However a quick development gives an advantage because effects at low levels of water-borne substances probably contain excretory product, toxic waste and may cause death in population (Bédhomme et al., 2005). The parity rate of mosquitoes is only 87% because some of female mosquitoes didn't complete one gonotrophic cycle. That higher parity rate of mosquitoes will possibility produced higher population density in the next generation. Higher hatching rate, a short aquatic stage life, and increased immature survival and fecundity attribute and uplift the population size of adult mosquitoes, which sustained a large population in the nature (Aida et al., 2011).

Few experiments constructed under laboratory condition with a constant temperature on *Ae. albopictus* have shown that higher temperature and precipitation directly affected mosquito growth (Muturi et al., 2011). Temperature will influence insect metabolism because of ectotherms effect, in which their vital activities are regulated by external temperatures. In Brazil, the density of *Ae. aegypti* were increased when exposed to lowest mean of temperature and the low rain index. However, the density of *Ae. albopictus* population increased after rain season and found associated with high temperatures (Lopes et al., 2004). This both *Aedes* species are container-inhabiting but differ in their biology and behaviour so that they occupy into different niches (Eisen and Moore, 2013). *Ae. aegypti* tends to be endophagic and endophilic compared to *Ae. albopictus* which is usually exophagic and exopihilic, thus prefers to take blood and resting outdoors (Delatte et al., 2010). Increased temperature and rainfall could lead to greater vector breeding site opportunities especially to *Ae. albopictus* which prefer living outside as rainfall introduces stagnant water to new areas (Haider and Turner, 2015). Increased temperature also results in increased probability of survival to adulthood and rapid larval growth and development of *Ae. albopictus* (Teng and Apperson, 2000).This condition may plays an crucial role in the life cycle of *Ae. albopictus* (Haider and Turner, 2015). While *Ae. aegypti* females found to survival longer during the dry winter than in the wet and hot summer (Maciel-de-Freitas et al., 2007). This mosquito may avoid excessive daytime heat by resting in cooler, shaded and indoors locations (Honório et al., 2009b).

Humidity in the environment may affect female mosquito's oviposition behaviour (Costa et al., 2010). Females will improve chances of survival in harsh conditions by shifting their oviposition to periods when eggs' survival is optimal. Female mosquitoes also considered the humidity stress and the availability of diet to ensure survival adaptations to their progeny (Angelica and Fong, 2008) and female mosquitoes was found living longer than males (Joshi, 1996). It’s also been proved by Löwenberg Neto and Navarro-Silva (2004) that females *Aedes* mosquitoes living 1.5 times longer than males under a constant temperature.

DENV virus can infect a variety of tissue within mosquito vectors especially the midgut and salivary glands (Salazar et al., 2007). Maciel-de-Freitas et al., (2011), observed females mosquitoes that infected with DENV significantly had lower survival rates and longevity compared to those uninfected mosquitoes. Besides that, their feeding-behaviour also affected as required more time during probing (Platt et al., 1997) and their locomotor activity had an increase of up to 50% compared to uninfected female mosquitoes (Lima-Camara et al., 2011). DENV virus capable to invade the mosquito's brain, which may modify the metabolism, behaviour and mosquito's physiology. Thus, may changing vectorial capacity and the pattern of disease transmission (Platt et al., 1997; Luz et al., 2011).

2.6 Mosquitoes trapping

Many trapping techniques and methods are used to assess the population of the mosquitoes for both larvae and adult stage. They are especially employed to study the population dynamics of mosquitoes and estimation of the population densities before or after insecticide application (Becker et al., 2003).

2.6.1 Larvae trap

Mosquito larvae occur in a wide range of habitats such as flowing streams, ponded streams, lake edges, swamps and marshes, shallow permanent or temporary ponds, and , natural containers, and artificial containers (Laird, 1988). Some simple techniques can be employed for the assessment of the larval population. Ovitrap represent a sampling technique that is widely used to trapping mosquitoes larvae and eggs stages. At the same time, it is used as indirect indices of adult abundance of container-breeding species. Ovitrap are black jars or tins filled with water and provided with hardboard paddle in which mosquitoes female lay eggs (Service, 1993). This trap is an inexpensive, simple to set up and operate (Facchinelli et al., 2007). Ovitraps have been used widely by researcher such as investigate the ecological parameter of *Ae. albopictus* and *Ae. aegypti* in relation to both eco-climatic factors (Mercado-Hernandez et al., 2006) and dengue inspection (Chen et al., 2005). In addition, it is also used to study the dispersal of dengue vectors (Liew and Curtis, 2004) and evaluation of the efficacy of vector control strategies (Vezzani et al., 2004).

The ovitraps were collected after 3 days introduced to that particular site. Particularly, the normal development of mosquitoes to reach adulthood is within 7 to 8 days. The water from the ovitrap cans brought back to the laboratory before larvae reach adult stage (Yap and Thiruvengadam, 1979). The larvae must be reared to 4^{th} instar or adult stages for identification of species (Ritchie et al., 2003). Recently, sticky ovitraps has been invented as new sampling methods (Ordóñez-Gonzalez et al., 2001). However, this method does not provide egg population instead it captures gravid females and act as adulticidal trap (Ritchie et al., 2003).

2.6.2 Adult trap

The standard method for adult trapping has involved the collection of mosquitoes that land on human volunteers or known as Bare-Leg Catch (BLC) and it have been used since long time ago. However, this method is nowadays considered unethical due to exposing volunteers to the risk of contracting non-preventable and potentially life-threatening diseases (Facchinelli et al., 2007).

Therefore, a new approach has been introduced to replace BLC technique such as CDC backpack aspirator (Clark et al., 1994) which can be employed to collect all species of the adult mosquito population, and indirectly providing data of mosquitoes abundance and vector-host contact. The CDC backpack aspirator permits indoor collection in favour to *Ae. aegypti* harborage sites (Rodriguez et al., 1995) and also collected a similar amount of *Aedes* as human-bait landing collections in a comparative study (Schoeler et al., 2004). However, this method not always ideal for *Ae. aegypti* surveillance as it weight makes tiresome to use, need a labor-intensive and its effectiveness varies between the operators (Williams et al., 2006; Facchinelli et al., 2007).

Light traps such as CDC miniature light trap (model 1912, John W. Hock Company, Gainesville, FL) and the CDC Fay-Prince Light trap (Fay and Prince, 1970) have been widely used to catch crepuscular and nocturnal host-seeking mosquitoes (*Culex* and *Anophele*s species), but using light as the only one attractive component are considered ineffective to capturing *Aedes* species (Farajollahi et al., 2009) because this species is primarily a diurnal host-seeking mosquito (Robertson and Hu, 1935) and not attracted to emitted light sources like the other species (Silver, 2008). In addition, these light traps were placed at 1.5 m above the ground and do not target *Ae. Albopictus* that usually host seek near the ground (Robertson and Hu, 1935). The uneffective of existing surveillance methods for *Ae. albopictus* has likely led to underestimations of population sizes and resulting troubling with assessing control methods (Farajollahi et al., 2009).

The BG-Sentinel (BGs) was originally developed to capture adult *Ae. aegypti* as a monitoring tool and as a method to reduce adult mosquito density which, possible to help to decrease the dengue transmission. Few studies have investigated the effectiveness of BG-Sentinel traps, where the BG-Sentinel trap collected more *Ae. albopictus* than CDC Fay-Prince Light trap and collapsible mosquito traps (CMT 20) in sub-urban Virginia (Meeraus et al., 2008). Besides that, a study by Bhalala and Arias (2009) also found the relative abundance of the West Nile Virus (WNS) infection in *Ae. albopictus* collected using BG-Sentinel traps and it was consistently higher than those in the program's routine of CDC Fay-Prince Light traps. Basically , this trap attempt to mimic convection currents created by a human body heat and apply attractive visual cues (BG Lure), then releases attractants odour through a large surface area to attract mosquitoes (Meeraus et al., 2008). The trap that placed on the ground is more effective for *Ae. albopictus* that usually host seek near the ground surface (Robertson and Hu, 1935).

2.7 Human lifestyles and their effects on mosquitoes

A study done by Russell et al., (2011) showed the use of insecticide residual surface (IRS) and insecticide impregnated net (INTS) recently has caused selective pressure on mosquitoes to become less endophilic and make vector species composition shifted from endophilic and anthropophagic toward more exophilic and exophagic. The hypothesis was proved by the introduction of impregnated nets in Kenya, which have reduced the number of indoor-resting *An. gambiae* by 94.6% and *Anopheles funestus* by 96.7% significantly (Bøgh et al., 1998). This control method has reduced the incidence of malaria infection effectively in 1995 (Choi et al., 1995).

The sub-urban and urban area distribution, aggressive behaviour of mosquitoes and wide viral susceptibility of *Ae. albopictus* may contribute to the transmission of dengue viruses (Francy et al., 1990). Honório et al. (2003), researched the ultimate factor that influence transmission of dengue virus showed that more toward human lifestyle (socio-economic) compared to the environmental factor in Texas. The resident people in Laredo (urban area) spend much of their daily life sequestered in sealed buildings with windows full glazed and always permanently closed to ensure air-conditioning work. Even infected mosquito gain entry to building with this condition, the artificially dry atmosphere lowers their survival rate and the cool temperature prolong the incubation period of their eggs which reducing the tendency of transmission of dengue virus (Honório et al., 2003).

Aedes aegypti was found to be more sensitive to light than *Ae. albopictus*. The increasing light intensity positively correlated with nocturnal host-seeking activity in both this *Aedes* species. Their host-seeking activity changes when the light intensity during the scotophase was changed daily from 0 to 100 lux. In the continuous dark conditions, mostly no activity peaks were recorded expect for the several minor peak in the morning (11:00 am) and evening (7:00 pm) for *Ae. aegypti*. While no activity was recorded for *Ae. albopictus* (Kawada et al., 2005). The level of activity drops rapidly following the onset of darkness suggested that light promotes activity or, less likely, that the absence of light reduces the activity. Light may have direct effect in determining the amount of activity and an indirect effect through setting the phase of the endogenous rhythm (Taylor and Jones, 1969).

2.8 Chemical control (Insecticides)

2.8. 1 Malathion

Malathion has been classified under organophosphorus type of insecticides. It has ability to inhibit acetylcholinesterase (AchE) which is a class of enzymes that catalyzes the hydrolysis of the neurotransmitting agent acetylcholine (Fukuto, 1990). Besides fenitrothion, malathion also been used widely in response to disease outbreaks which at the times when mosquito populations is in a high number (Vontas et al., 2012). Malathion commonly used as residual insecticides especially in control of malaria and Chagas disease (Unlu et al., 2014).

Malathion has been classified as an adulticiding control method to control adult mosquitoes. Adulticiding measures are not residual and need to be repeated on a weekly basis. The insecticides dispensed kill the insects by direct contact, therefore the fogging operator must ensure that all accessible areas must be fogged entirely. Beside using malathion, Ministry of Health Malaysia was reported to use a combination of pesguard-PS 102 and Vectobac 12As (Bti) applied by vehicle mounted with ULV machine, resulted more than 90% mortality of *Ae. aegypti* adult mosquitoes (Vector Control Unit, 2002). In Malaysia, fogging operation have been held in a specific formulation to control *Aedes* mosquitoes at construction sites, which is by using Malathion 96% TG, with dilution rate 1:25 with diesel for thermal fogging and undiluted 96% for ULV fogging (Vector Control Unit, 2002).

2.8.2 Permethrin

Pyrethroid has been used widely started in the 1970 after the development of photostable pyrethroid such as permethrin and fenvalerate. Permethrin revolutionized pyrethroids class, leading to their widespread use in pest management applications (Schleier III and Peterson, 2011). In additions, beside deltamethrin and cypermethrin. permethrin also been used to control adult *Aedes* species mosquitoes through mass spraying (fogging) during endemic seasons (Yaicharoen et al., 2005). Moreover, permethrin commonly used as a component in household insecticide products (Sathantriphop et al., 2006). Permethrin seems to be very effective against *Ae. albopictus* adults, and have been tested to all populations from various countries such as India, Malaysia, Thailand, Greece and Italy (Vontas et al., 2012).

Mode of action of pyrethroid is a chemical that causes neurotoxic. There a several ways of permethrin can enter the body of an organism such as rapid penetration through the epidermis followed by uptake by the blood and subsequent distribution throughout the body. This insecticide can attack the Center Nerve System (CNS) mainly through epidermis cells or directly via contact with sensory organs of the peripheral nervous system. Besides that, permethrin can enter through the airway into vapour phase such as usage in fogging activities, however the penetrations gave only a small contribution due to their low vapour pressure (Schleier III and Peterson, 2011).