BIONOMIC OF *AEDES* SPECIES AND THE SUSCEPTIBILITY TO INSECTICIDES IN PENANG ISLAND, MALAYSIA

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by

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ABSTRACT

A field study was conducted in Flat Hamna (FH), Kampung Sungai Gelugor (KSG) and Kampung Tanjung Tokong (KTT) for a period of 14 months from January 2010 until February 2011. The objectives of this study are to determine the population and distribution of *Aedes* mosquito and the resistance status among the larvae and adult mosquitoes against selected insecticides that have been used in the vector control activity in Penang Island. The result showed that *Ae. albopictus* was a dominant species in Flat Hamna, Kampung Sungai Gelugor and Kampung Tanjung Tokong. It was found at higher frequency in both indoor and outdoor compared to *Ae. aegypti.* In indoor, overall *Ae. albopictus* accounts high number of larvae collection in Flat Hamna (28.59±1.10), Kampung Sungai Gelugor (19.94±1.59) and Kampung Tanjung Tokong (24.10±1.80) as compared to *Ae. aegypti*. However, *Ae. aegypti* was preferred to breed outdoor with 186% higher than indoor. A negative correlation was found between the ovitrap index and temperature in Flat Hamna and Kampung Sungai Gelugor. Whereas, positive correlation was recorded between ULV activity with number of collected larvae from Flat Hamna and Kampung Sungai Gelugor except from Kampung Tanjung Tokong. *Aedes albopictus* FH strain showed incipient resistance scale to all commonly used insecticides against permethrin, deltamethrin and malathion which caused 93%, 97% and 87% of mortality respectively using WHO adult bioassay diagnostic dose kit. KSG strain also started to develop resistance towards permethrin, whereas the KTT strain was still susceptible to all insecticides. However, statistically significant differences were detected between all insecticides tested and localities ($F(2,6) = 4.798$, $p = 0.008$). The highest KdT_{95} of 42.26 min occurred in KTT strain to deltamethrin but showed no significant difference compared to other insecticides ($F(2,6) = 3.316$, $p = 0.107$). The highest RR_{95} value of was detected in the FH strain against permethrin with 1.93 fold. Larval bioassays were also carried out to determine the lethal time (LT_{50} and LT_{95}) and resistance ratio (RR_{50} and RR_{95}) to temephos and *Bacillus thuringiensis israelensis* (Bti). The result showed that *Ae. albopictus* from FH, KSG, and KTT were susceptible to *Bti* and temephos. This study found that the abundance of larvae would be increased after two or three month following the rainy season with a high number of OI. As conclusion the application of insecticide in vector control in Penang on selected areas still relevant and effective tool to control *Aedes* mosquito populations.

CHAPTER ONE

INTRODUCTION

Aedes aegypti and *Ae. albopictus* are the species that currently widespread throughout tropical to temperate regions. They are principle vector to transmit the dengue viruses (Guzman & Isturiz, 2010; Cheung & Fok, 2009). The distribution of *Ae. aegypti* and *Ae. albopictus* overlap between each other in Malaysia and both can spread the dengue viruses (Rozilawati *et al*., 2007). These mosquitoes can be found in man-made and artificial containers in urban, suburban and rural areas in tropical and subtropical regions (Juliano *et al.,* 2004; Honorio *et al*., 2009). Natural habitat and containers as tree holes and bamboo stumps usually inhabits by *Ae. albopictus* larvae (Marques *et al*., 2001; Joshi *et al*., 2006)

Monath (1994) found that the distribution and the abundance of the mosquitoes were closely related with human and vector ecology. *Aedes aegypti* has made an extraordinary evolution with their behavior to coexist with the human life. Study in Africa has found that the *Ae. aegypti* a forest species feeds on rodent and wild animals has been evolved into the highly domesticated species. The mosquitoes followed human in their journey, later on breed in artificial container that has been used for storage water, and also flying rarely more than 50 meters from their convenience habitats (Monath, 1994).

Climatic variable such as humidity, temperature and precipitation also can affects the distribution, abundance, development and survivorships of *Aedes* mosquitoes (Hopp & Foley, 2001). Mosquito life stage, hatching and larval survivor depends on the temperature conditions whereas mosquitoes mortality depends on the relative humidity (Carrington *et al*., 2013; Almeida Costa *et al*., 2010; Hopp & Foley, 2001). Currently, research on dengue, yet do not have the entomological measure to predict the risk of the transmission of dengue virus between human and mosquitoes. The situation of the transmission and dengue control program is based on assumption that the increasing in adult *Ae. aegypti* and *Ae. albopictus* population will increase the risk of virus transmission (Palihawadana, 2013; Scott & Morrison, 2003).

However, Alshehri (2013), have found the strong correlations between the mosquitoes density with the reported dengue cases in Jeddah, Saudi Arabia. This finding also been supported by Barrera *et al*., (2011), which he has found the positive significant correlation between the number of dengue cases with the number of female *Ae. aegypti* and number of eggs per ovitrap. Although the result is not a perfect measure for dengue cases, but the sensitivity make it used as a good monitoring method for mosquitoes abundance (Ayanda, 2009; Dibo *et al*., 2008).

During the dengue outbreak, the insecticide control has been frequently and intensively used as the best way to control adult mosquitoes and to determine the transmission of viruses. Space sprays (fogging) and Ultra Low Volume (ULV) are the easy and fast application to be use around the house and outbreaks areas. The advantages are it can kill the mosquitoes at the same time of the treatment, immediate knockdown and only required a small amount of chemical (Floore, 2001).

However the common insecticides have been used for a long time and the effectiveness of the chemicals are questioned (Chen *et al*., 2005). In some areas, the resistance has been occurred and linked into the failure of dengue control program. In Thailand, the organophosphate and pyrethroid is mainly used through the spraying and had caused the pyrethroid resistance among the mosquitoes (Pimsamarn *et al.,* 2009). According to Lee (2000) the adult mosquitoes were highly susceptible to malathion but showed some resistance towards permethrin and DDT. This is the major problem which makes the control activity of dengue becoming more difficult.

According to Eldridge (2008), the resistance occurs when the mosquitoes can avoid the lethal effects of the insecticides with development of ability to tolerate with the dosage of toxicity. The vector may become resistance due to repeated exposure of a same chemical with a similar mode of action (WHO, 2002) and also when the under-dose of chemical always been used for a long period of time (Eldridge, 2008). According to Nazni (2009), the study using the adult mosquitoes strain MyWT (originated from Selangor) in Malaysia as showed, the resistance decreased in order: DDT > propoxur > fenitrothion > malathion > lambdacyhalothrin > permethrin > cyfluthrin.

In Malaysia, the Dengue Control Program has been enforced since 1973 (Singh, 2000; Rudnick & Lim, 1986) under the Epidemiology Unit, Public Health Service, Ministry of Health. In which integrated with epidemiological surveillance, environmental sanitation, integration with primary health system, social community and social mobilization. In vector control program, the activities include the inspection of all houses in each locality where the dengue cases were previously reported. During the visit, the vector control staffs will be looking for the potential and breeding sites of mosquitoes, and also give educational information to the resident.

In the afternoon, all inspection house around 200 meter radius within the reported dengue case's house will be sprayed with insecticide to reduce and kill the mosquitoes (Singh, 2000). Fogging will be conducted using ULV machine and thermal fog machine, both inside and outside of the house. Insecticides that used in this activities are permethrin, malathion, sumithion (Lee, 2000). For the long-term effective program, the monitoring of mosquitoes resistance should be implemented in all agencies and organization to ensure the best insecticide are used to prevent the failure of mosquitoes control in the future (Pimsamarn *et al*., 2009).

To bring Dengue Program Control to success, the current distribution and population of *Aedes* mosquitoes in infected areas and the relationship with the number of dengue cases should be studied. It's also useful to study the resistance status of *Aedes* mosquitoes to confirm that commonly use insecticides in the vector control program by Ministry of Health are still reliable to control mosquito populations in Malaysia especially in Penang.

1.0 RESEARCH OBJECTIVE

In my thesis, I have outlined three objectives to understand the current pattern of *Aedes* mosquito abundance in relation to dengue cases. Also, to investigate the effectiveness of current uses insecticides to control the population of *Aedes* mosquitoes adult and larvae. My specific objectives are:

i. To study the *Aedes* mosquitoes abundance in relation to the reported dengue fever (DF) cases and the effect of physical parameter (temperature, rainfall and relative humidity).

ii. To study the resistance status on *Aedes* field strain adult mosquitoes on permethrin, deltamethrin and malathion insecticides with comparable to laboratory strain.

iii. To examine the effectiveness of two selected larvicides; temephos and *Bti* on the current level of *Aedes albopictus* larvae using current dose used by the Penang Health Department.

CHAPTER TWO

LITERATURE REVIEW

2.0 Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF)

Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) are the most important viral vector-borne disease in the world. The first major epidemics of dengue was occurred in Asia, Africa and North America between 1779 and 1780 (Pepper, 1941; Hirsch, 1883; Rush, 1789). In Asia, the epidemic of DHF was expanded widely from Southern Asian countries to India, Sri Lanka, Pakistan and East of China (Gubler, 1998).

In 1779, there was also an epidemic reported in Batavia (Jakarta), Indonesia and Cairo, Egypt and similarly looked like pandemic of dengue-like illness that begun around the first 18^{th} century. The epidemic in Philadelphia in 1980 was reported as dengue fever (Carey, 1971). The dengue occurs in over 100 countries throughout the Americas, Southern Europe, North Africa, Mediterranean, Asia and Pacific regions now a day (Malavige *et al*., 2004). There has been estimated around 390 million (95% credible interval 284-528) dengue infection per year with 96 million (67-136) manifest dengue infections (Bhatt *et al.*, 2013)

DF and DHF are the main causes of the hospitalization and death with a case fatality rate between 0.3% to 5% among children in Southern Asian regions (Halstead, 2000; Madarieta *et al*., 1999). All the infections were reported in urban populations where the density of dwelling is congested and the distance of vector to fly quite short which create an ideal condition of the dengue virus transmission. It's also spread in rural areas due to the increased transport contact, mobility and spread of urbanization (Guha-Sapir & Schimmer, 2005). Dengue viruses belong to the family *Flaviviridae* and genus *Flavivirus*, which contains approximately 70 viruses (Westaway & Blok, 1997). They are closed related group between the viruses, which are sharing the same structure, genome arrangement and protein types. The *Flaviviruses* are relatively small around 40-50 mm and spherical structure approximately 50 µm in diameter consisting of a single strand, positive sense RNA genome located inside a nucleocapid, which is turn surrounded by a protein and a lipid envelope (Uddin *et al*., 2005).

Dengue is one of the most-borne viral disease and become a major public health problem in developing countries such as Malaysia, Puerto Rico, US Virgin Islands, Mexico, Brazil, Samoa and Guam. Approximately around 120 million peoples travel to the tropical and subtropical regions with two billion people live in the developing countries (Banu *et al*., 2014). About 2.5-5.0% of people are at risk of contracting with dengue virus annually and the mortality rate approximately 2.5% (Mahmood *et al*., 2009). Fifty million cases of dengue fever (DF) and several hundred thousand cases of dengue hemorrhagic fever (DHF) with 20000 death each year were reported annually (Suaya *et al*., 2007).

There was four antigenically viruses of the family *Flaviviridae*, named DEN-1, DEN-2, DEN-3 and DEN-4 (Uddin *et al.*, 2005; Gubler, 1998). Human can be affected with the viruses as many as four times with every singles serotypes in their whole life. Guzman *et al*. (2002) had found that the severity of the dengue fever depending on the interval between the initial DEN-1 infection and the secondary of DEN-2 infection as the type of the virus, especially on DEN-2 cases (Nisalak *et al*., 2003).

There are four serotype of dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4) and antigenically very similar each other but different enough to elicit only transient partial cross-protection after infection by each one of them (Henchal & Putnak, 1990; Monath, 1990). The serological diagnostic test becomes difficult with the common group of the epitopes on the envelope protein that result of the extensive cross-reaction (Gubler, 1998). All of four serotype of dengue viruses have shared a common antigenic determinants and make the identification using the classical serological test become difficult (Henchal & Putnak, 1990). Once infected with the dengue virus, it will provide a lifelong immunity to the virus and the person can be infected with three or four serotype of viruses for the whole of their life (Gubler, 1998) and also can make the situation become worse (Halstead 2000; Madarieta *et al*., 1999).

In human, dengue virus will cause the spectrum of illness from mild febrile to sever fatal hemorrhagic disease (WHO, 1997). The incubation period of the viruses is around 3 to 14 days with an average of 4 to 7 days (Tomashek, 2011; Sabin, 1952). The strain of the serotype, immune status of the patient, age and genetic background influenced the proportion of infected patients during the epidemic transmission (Thomas *et al*., 2008; Rosen, 1977; Barnes *et al*., 1974). The symptom of DF at the beginning are overlapping in nature and hardly been differentiable immediately after infection. There are divided into 'Dengue Syndrome' and will encompass with the following Dengue Classical Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (WHO, 2000; Rathor, 2000).

The symptom can be characterized by the sudden onset of fever and other nonspecific symptom as frontal headache, retro-ocular pain, vomiting, nausea and vomiting, joint pains, rash and weakness (Malavige *et al.*, 2004; Hayes & Gubler, 1992; Waterman & Blok, 1989). The high index of the suspicion is based on period, population and place (DGHS, 2000). Some patients may show symptom of anorexia and mild sore throat with the body temperature increase to 102-105^{o}F and might last for a few days. Usually it can recover within 12-24 hours later. For young children, they will show respiration symptoms including cough, rhinitis and sore throat (Halstead *et al*., 1969). Epistaxis (nose bleeds), microscopic hematuria and skin hemorrhages (Teng *et al*., 2003) usually happens in adults, however gastrointestinal bleeding, menorrhagia, bleeding from other organ have been rarely occurring (Kuberski *et al.*, 1977).

Patient has increased capillary fragility, petechia (purpura), bleeding by any injection, hematemesis and also with thrombocytopenia with platelet less than 100,000/mm^3 and hematocrit rise $\geq$ 20% after DF infection (Khan & Rahman, 2011; Teng *et al.*, 2003). Specific treatment will be given to the patient with a fluid and electrolyte replacement. Other treatment such as using aspirin and salicylates should be avoided in term of the diminished number of platelets and other functions (Lashley *et al*., 2007; Trying *et al*., 2002).

Dengue Shock Syndrome (DSS) is a new disease associate with Dengue Haemorrhagic Fever (DHF) with one or more of the symptoms like hypertension, restlessness, cold clammy wall, rapid weak pulse, narrow pulse pressure (<20mm of Hg) and profound shock (Suma, 2010; Rathor, 2000). Patient may become shock from blood loss without early diagnosis and proper treatment (Sumarmo, 1983; Sumarmo *et al*., 1983). Until now, there is no vaccine available for dengue viral (Kumar *et al*., 2012), prevention with man-vector combat is the best solution for long term activities (Malavige *et al*., 2004)

2.1 Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF) in Malaysia

The first cases in the Peninsular Malaysia were reported back in 1902 and the first DHF was reported in 1962 in Penang. Between 1962 and 1964, there were 61 cases including five fatalities were confirmed by dengue virus isolation and serology (Rudnick, 1986) and increased to 67 reported cases in 1965 (Lucas, 1967). Parameswaran (1966) was reported that in November 1962 until July 1963, almost 41 dengue haemorrhagic fever patients admitted to General Hospital, Pulau Pinang.

Whereas, three mild cases reported in Kuala Lumpur between 1963-1965 and became endemic throughout the country since then (Singh, 2000). The worst dengue cases outbreak in Peninsular Malaysia was documented in 1974 and 1984 (Chandra *et al*., 1992; Aiken *et al*., 1980). In 1973 until 1974, Johor and Selangor were the most serious state infected with dengue outbreak. Penang, Malacca, Negeri Sembilan and Terengganu also reported with a big number of dengue cases. All of four dengue viruses serotypes were detected in all the states (Aiken & Leigh, 1978). Most of the cases from Selangor were reported from the "new village" which it has been a new settlement place established between 1948 until 1960 due to the anti-communist military operation and the squatter settlement in Kuala Lumpur areas. However, Johor was also reported with the highest incident of DHF which occurred in a small village with densely populated (Aiken *et al*., 1980)

According to AbuBakar *et al.* (2002), all of four dengue virus serotypes were isolated in Malaysia after the first dengue outbreak in 1960-1969 with DEN-4 being as predominant (53.1%) serotype isolated from DF patient. In 1970 the DEN-2 has become the major serotype replaced the DEN-4. Since then, in between 1971 to 1985 all of four serotype presented with none of the serotype becomes a dominant. Starting in 1986 until 1996 the DEN-3 has become a dominant serotype isolated from DF /DHF patient. However from 1996 until 2000 the DEN-3 has been replaced with DEN-2 as a dominant serotype with a major isolated from the patient.

In year 2014, 108,698 cases were notified with 215 deaths with an increment of the 151% cases compared to 43,346 cases reported in 2013. The increased number of cases was contribute by the people movement from one place to other, changes in dengue serotype from DEN 2 to DEN 1, climatic factors and lacking in human awareness about dengue (MOHM, 2015). Dengue strategic plan for the Asia Pacific region was launched for a time period of 2008 until 2015 with a targeting to reduce the incidence rate of cases by at least 20% annually (WHO, 2008).

2.2 Dengue situation in North East, Penang

Based on dengue data (**Figure 2.1**), showed the number of cases in 2013 was found throughout the year, with a seasonal variation demonstrated and peak number of dengue cases reported in December (101 cases) followed by November (57 cases) and January (35 cases). During the period (January 2013 to December 2013) there were nine outbreaks areas. However, in 2014 (**Figure 2.2**), the number of dengue cases was increase to 299 cases with 19 outbreaks. There was a high number of dengue cases was registered which are more than 59% from the 2013.

In 2014 the number of cases has been highly increased compared with 2013 cases (**Figure 2.1)**. We are expecting more cases will be reported when there was a changes in the climatic scenario with a heavy and continuous rainfall during the year. In the middle of the year 2014, we started to get many dengue cases in a scattered area. The massive surveillance, search and destroy and space-spraying activity have been planned. However, the activity cannot be done due to the rain started in the morning until the night. The numbers of pending case to control become higher and we have to use an Ultra Low Volume (ULV) in the early morning as our prevention cautions. All the activity only can be initiate after the rainy season.

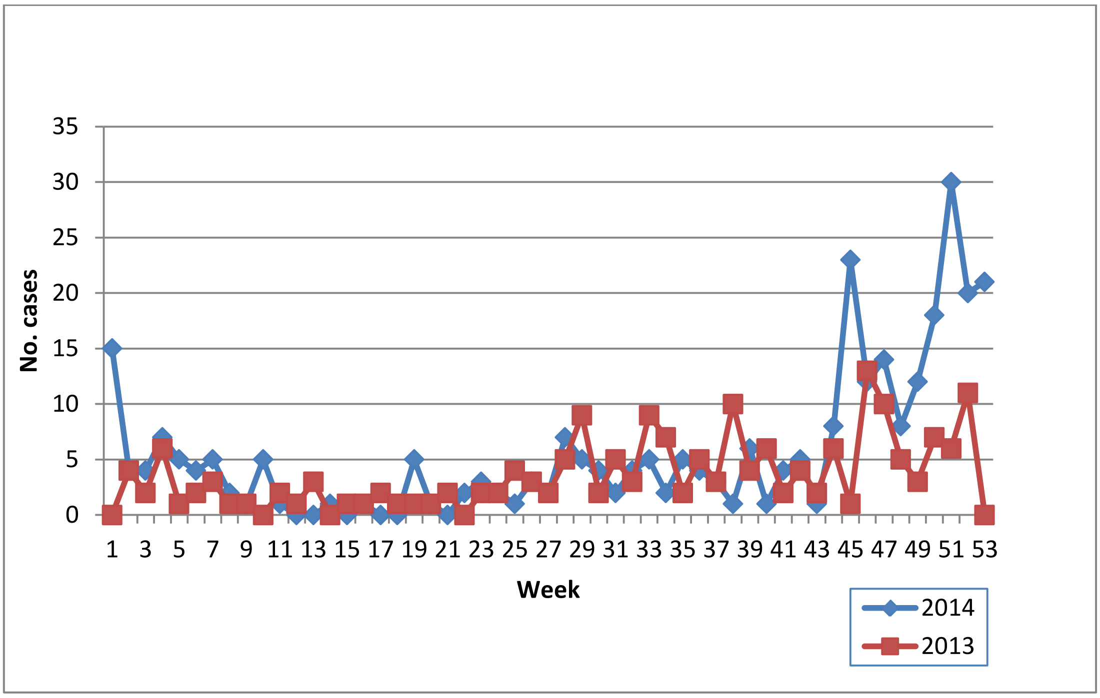

Figure 2.1: The comparison of dengue cases reported in 2013 and 2014 in Northeast District, Penang.

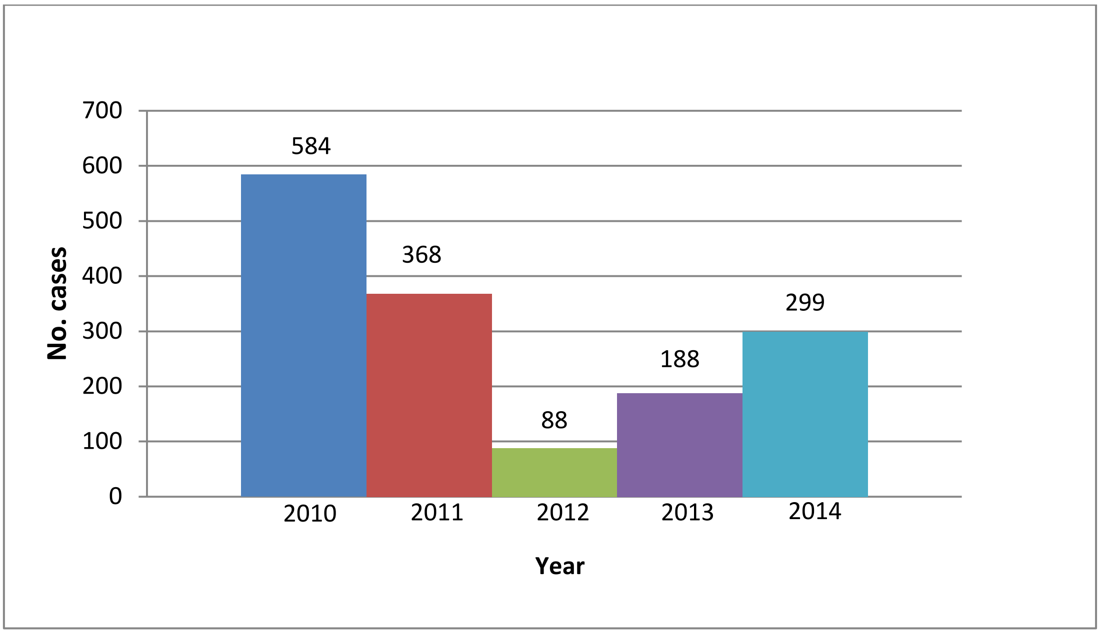

Figure 2.2: Dengue fever cases in Northeast, Penang by year (2010-2014)

2.3 Relationship Between the Incidence of Dengue Cases and Dengue Vector

In dengue virus there is no entomological measure to predict the risk of human infection as an Entomological Inoculation Rate (EIR) for malaria (Smith *et al*., 2001). Kuno (1997) was reported that the virus infection in *Ae. aegypti* is typically very low and difficult to screen compared to EIR or its equivalent. A positive correlation was found between *Ae. aegypti* indices and sero prevalence of dengue antibody, which the lower number of mosquito density prevented dengue transmission in the community (Figueroa *et al*., 1982). The DF transmission threshold is expected to be different in association with temperature and human population also fluctuation of mosquitoes in environment to transmit the viruses (Focks, 2003; Focks *et al*., 1995) and difficult to observe and very complex (Scott *et al*., 2003).

Periodic epidemic affected thousands of people and over hundred DF cases were reported each year, particularly during rainy season when the density of mosquitoes becomes higher. The density and distribution of mosquito were significantly influenced by environment conditions (Focks *et al.*, 1993; Rueda *et al*., 1990). Mosquito breeding sites consist of any types of water holding containers, tree holes or leaves, discarded bottles and tires (Christopher, 1960) will increase the mosquito populations. Mosquito survival rates depend on temperature in the presence of water and relative humidity affected adult mosquito mortality (Hopp & Foley, 2001).

In Thailand, the number cases increased after the onset of the Southwest Monsoon rains in May and epidemic peak usually occurs in July or August (Jatanasen, 1979; Jusatz, 1975). The number of cases was decreased in October after the monsoon rain and it similarly with the report from Burma and Philippines (Baltazar, 1979; Khai Ming, 1974). In Kelurahans, Indonesia was reported in October until December 1997 preceding epidemic peak had a combined mean House Index (HI) of 25% (10-50.8%). Based on the data, receptive dengue transmission and *Aedes* infestation was highly 'permissive' during the epidemic season. With limited flight range of mosquito and human blood feeding behavior to concentrate in dense areas will increase the epidemic virus transmission (Van Bentham *et al*., 2005; Russell *et al*., 2005; Harrington *et al*., 2005).

2.4 *Aedes* as Vector

Aedes albopictus, Ae. polynesiensis and several species of *Ae. scutellaris* also been known to attribute in an epidemic dengue outbreaks. Each of these species has their own geographically distribution and less efficient in transmitting the dengue virus than *Ae. aegypti* (WHO, 2009; Halstead *et al*., 2008). The dengue virus will be transmitted to human by infected mosquitoes (Angel & Joshi, 2008; Fink *et al*., 2006) which ingested blood from a person with dengue viruses (Uddin *et al*., 2005)

Aedes aegypti is one of the most efficient mosquitoes vector as a host for arboviruses especially dengue virus (Rezza, 2012; Vasilakis & Weaver, 2008; Hubalek, 2008) which extremely anthropophilic, frequent blood feeding behavior, long life span and always stay closed to human (Medical Research Institute & Dengue Cordination, 2011; WHO, 2009). In Asia, *Ae. albopictus* also play the same role as an urban vector to spread the viruses (Vazeille *et al*., 2003). Generally *Ae. aegypti* distribution always be correlated with DHF and *Ae. albopictus* will be matched with the dengue occurrence (Yap, 1975)

In Malaysia, *Ae. aegypti* and *Ae. albopictus* are the common species and easy to be found in every part of the place (Honorio *et al*., 2009; Rudnick *et al*., 1965). *Aedes albopictus* have been found to breed outdoors in all types of natural containers (Thavara *et al.*, 2001; Foo *et al.*, 1985) and also one of the important dengue vector in rural and suburban areas (Leroy *et al*., 2009). A study has been found that *Ae. albopictus* can also breed in all man-made containers same as *Ae. aegypti* (Paupy *et al.*, 2009; Kwa, 2008). With the uncontrolled urbanization, *Ae. aegypti* becomes more dominant and has been reported breeding in natural containers (Vazeille *et al*., 2003; Strickman & Kittayapong, 2002). It's easily spread because of good transportation routes, urban development and at low altitude (Leisnham & Juliano, 2009). *Aedes aegypti* and *Ae. albopictus* can co-exist and found together in same container even in outdoor and indoor. It's hard to differentiate the preferred breeding site between these two species (Chen *et al*., 2006; Bracks *et al*., 2004)

A study in Pingtung found that a positive male of *Ae. aegypti* in a natural surrounding showed either transovarial of venereal transmission caused the minimum infection rate of 11.76 per 1000 male mosquitoes (Jen Ten *et al*., 2007). Whereas in Singapore the minimum infection rate of *Ae. aegypti* and *Ae. albopictus* were 13.3 and 21.5 per 1000 mosquitoes respectively (Kow *et al*., 2001). The mosquitoes that infected with dengue virus also transmit the virus to 20 humans in just few days (Getis *et al*., 2003; Putnam & Scott, 1995).

In the tropic zone with warm climates, eggs may develop in two days whereas in cooler temperate the development of the eggs can be reached until a week (Foster & Walker, 2002). Nelson (1968) has found that the eggs of *Ae. aegypti* can survive for a month and withstand desiccation up to one year, unfortunately the eggs can hatch once submerged in water (Toma *et al.*, 2011). However, it's still unclear how the virus can maintain in nature either its transfer vertically from an infected to her offspring or from infected male to female during copulation. All of the strategies are considered as an important role in maintaining the virus in the population of nature surrounding (Joshi & Sharma, 2001; Thenmozil *et al*., 2000; Rosen, 1987). If the frequency of cleaning the container is more than a week, it will make the situation of *Ae. aegypti* control program becomes worst with the increasing number of the mosquitoes (Bohra & Adrianasolo, 2001).

2.5 Surveillance of mosquitoes

A good surveillance programme is designs to avoid infestation as much less costly than eradication after the infestation occurred. The surveillance are based on the oviposition taken by ovitraps. The oviposition technique founds to be more economical, sensitive, easy and rapid to determine the abundance of Aedes mosquitoes in the field (Fay and Eliason, 1966)

2.5.1 Oviposition

Oviposition behavior in mosquitoes relies on several factors such as pheromones, water chemical compositions (William *et al.*, 2008) and also with the presence of the predators in the water (Bently & Day, 1989). The adult mosquitoes highly preferred to lay eggs into containers with a rough texture, dark colors and horizontal opening. They also breed in artificial water containers which were found nearly inhuman dwelling (Aldstadt *et al*., 2010; Lenhart *et al*., 2005).

A lot of consideration need to be reviewed including the size of the containers, the quality of the water, the type of container, opening of the containers and also the shape to of container to attract the female mosquitoes to deposit their eggs as much as possible (Chua *et al*., 2004). This selection of breeding site has an impact on their life and progeny. They have to choose the right place that can maximize their survivability. Large containers always had been considered due to lot of foods for their larvae and high humidity for adult to rest (Harrington *et al*., 2008).

A chemical that has been released by the larvae of the same species inside the containers attracted female mosquitoes to breed more than once in the same location (Zahiri *et al*.,1997). They also attracted by the dark color water (Gubler, 1971). Usually *Ae. albopictus* do not oviposit whole of their eggs in one container (Rozeboom *et al*., 1973). There are about 98% of mosquitoes laid their eggs in the same container when there is no other choice of container to breed in (William, 2008; Harrington & Edman, 2001).

2.5.2 Ovitrap

Ovitrap is a container (cup shape), filled with water within one inch from the tops and placed it together with wooden or velour paper paddle projecting above the water surface. The ovitrap sampling technique has provided useful data on the abundance and temporal distribution of *Ae. aegypti* and other container-inhabit mosquitoes (Iriarte *et al*., 1991; Ritchie, 1984). It's can also be used to monitor the effect of control technique involving source reduction and insecticide testing (Lenhart *et al.*, 2005). The number of the eggs can be used to monitor the density of *Aedes* mosquito around that area (Zeichner & Perich, 1999). Ovitrap is one of the sensitive tools to detect the mosquitoes index at low population in nature (Regis *et al.*, 2008; Wan-Norafikah *et al*., 2009). Once the Ovitrap Index of mosquito becomes low at about 1% it's can possibly reduce and eliminate the potential of epidemic dengue outbreaks (Kay & Nam, 2005; Pontes *et* al., 2000).

Ovitrap has been used for the first time in the United State as a surveillance of *Ae. aegypti* (Fay & Eliason, 1966; Fay & Ferry, 1965). In 1969, the first documented study was done in Singapore Paya Lebar Airport and still can detect the number of mosquitoes at the lower significant number (Chan, 1973). The ovitrap can also be autocidal control by using the paddle impregnated with insecticide and turn it as "lethal ovitrap" (Ritchie *et al.*, 2008). The using of adhesive strip on the paddle will replace the normal paddle and make it as "sticky ovitrap" (Ritchie *et al.*, 2003; Ordonez-Gonzales *et al.*, 2001).

Ovitrap use for vector surveillance has become a current trend in a dengue endemic country. This method shows better assessment of the mosquito density compared to a surveillance of larvae (Polson *et al*., 2002; Ai-Leen & Song, 2000; Braga *et al*., 2000) and also more sensitive and cheaper in a cost of operational (Braga *et al*., 2000; Rawlins *et al*., 1998). The ovitrap index shows a direct prediction and estimates of the feeding and oviposit activities of the mosquitoes (Hoeck *et al*., 2003)

2.6 Physical factors influencing mosquito populations

Temperature, rainfall and relative humidity were physically influenced the abundance of the mosquitoes and found to be linked with the biology and density of *Aedes* mosquitoes (WHO, 2012a). The dengue incidence fluctuates with climatic conditions and also associated with the increased of the temperature and rainfall. However, the relationship to the precipitation also depending on the local characteristic and whether breeding sites are maintained predominantly by rain (Wu *et al*., 2007).

2.6.1 Rainfall

Climatic factor is one of the factors that associated with the effectiveness of *Ae. aegypti* in transmitting the virus (Hales *et al*., 2002). With large amount of rainfall, the density of eggs and larvae will become higher (Surendran *et al*., 2007; Vezzani, 2004) and increase the number of mosquitoes breeding site and their density (Aiken, 1980).

A study in Nicaragua found that the infestation of *Ae. aegypti* was very high with the average house index of 12.5 and 7.7 and Breteau Index 18.6 and 13.9 in 1997 and 1998 associated with rainfall (Harris *et al*., 2000). Whereas, in Salta, Argentina *Ae. aegypti* oviposition activities was very high during throughout the year especially in March which received a high rainfall (Maceili & Campos, 2003). For the small peak period (March/April) of mosquito population in, it's always depend on the domestic storage of water and the large peak (September/ October) depends on monsoon and make the breeding containers and breeding sites become extended to every place in India (Katyal *et al*., 2003).

2.6.2 Temperature

Several studies found that temperature can accelerate the mosquito into an adult and increase the mosquito survival rates (Neto & Silva, 2004; Alto & Juliano, 2001; Tun-Lin *et al*., 2000). The combination of the temperature and water will increase the egg viability reduce the extrinsic incubation period and increase the replication rate of the virus (Neto & Silva, 2004; Watts *et al*., 1987). Surveillance on the feeding pattern of mosquito found that the outbreak of dengue with very high during hot-dry and rainy season (Pant & Yasuno, 1973).

The increasing of vectorial efficiency associated with the duration of the extrinsic incubation period was influenced directly by environment temperature (Kramer *et al*., 1983). The mosquito incubation period with 32-35^{o}C are about seven days and the incubation period at $<30^{o}$C is 12 days or longer (Reiter, 1998). The development period of *Ae. aegypti* mosquito eggs and larvae increased when the temperature dropped (Vezzani & Carbajo, 2008). Larval survival increased to 60% under 35^{o}C and dropped to 23% at 37^{o}C with a small fluctuations (Carrington *et al*., 2013). The temperature also affects the breeding, biting and longevity of the mosquitoes and the ability of the mosquitoes to transmit the dengue viruses. A study with stochastic population dynamic model have shown that almost all the mosquitoes in Buenos Aires, Argentina depend freely on the seasonal temperature and yearly temperature (Otero *et al*., 2006).

2.6.3 Relative Humidity

Aedes aegypti survival rates also depending on moisture. Study by Rezende *et al*., (2008) reported that the *Aedes* eggs can survive desiccation under dry conditions for months and even a year. For development of egg and to make it hatch, water is crucial requirement and also one of the important factors for development of larvae, survival and for vapor pressure deficit (Christopher, 1960). Canyon (1999) has found that, the eggs of *Ae. aegypti* can be able to survive which depending on humidity pressure. The result showed that the mortalities of the eggs become higher around 39% in a low humidity after 19 days and with a high humidity the mortality rate becomes low at around 6-9%.

2.7 Control of vector mosquitoes

Ultimately, the prevention and controlling the dengue outbreak will depend on the effectiveness of vector control activities to eradicate mosquito populations.

Resources used in the prevention and treatment of dengue fever are not available and need to complete with other disease for the limited resources available (Stahl *et al.,* 2013). Therefore, control of dengue vector is very important to reduce the infection of dengue fever. Control activities are involved the combination of micro stratification, multi disease approach with local authorities, community mobilization and integration of vector control activity (Van den Berg *et al.,* 2012). However, in the practice of dengue control management many challenges still exist includes lack of strategy to respond against dengue outbreaks, rapid urbanization, sanitation and bad waste disposal management in the population (Chang *et al.,* 2011).

The effective strategy to control and manage the dengue fever transmission by mosquitoes can be done using biological, chemical, active case detection and environmental control. The best preventive control is to reduce the population and distribution of mosquitoes. It's requires to reduce the *Aedes* index of *Ae. aegypti* and *Ae. albopictus* to the lower level until the virus transmission is hardly to occur. It will be reduced the incidence of dengue infection from the mosquitoes (McConnell & Gubler, 2003).

The public health unit has to provide several strategies to control the mosquitoes. The best control strategy is an integrated control. The integrated vector control program utilizes the most suitable combination among the alternative control program including the chemical, environmental and biological control. The confirmation of the transovarial transmission of dengue virus, integrated program to kill adults and larval mosquitoes should be introduced in vector control to disrupt and abort the transmission of the virus (Yap *et al*., 2000). Without a proper knowledge, the impact of the integrated vector control strategy would not be fully utilized.

2.7.1 Biological control

A biological control agent usually only affected on the specific target organisms. One of the most extensively used in mosquito control is a larvivorous fish. *Gambusia affinis*, *Poeciliidae* and *Cyprinodontidae*, *Nothobranchius guentheri* (killfish) is common larvivorous fish species that used in the control of mosquito larvae (Matias & Adrias, 2010). The other bio-agent has been tested but hardly to practice such as predaceous mosquito *Toxorhynchites amboinensis*, predacious copepods, the parasitic nematode *Ramanomermis* and the fungus *Tolypocladium cylindrosporum* (Annis *et al*., 1990; Munstermann & Leiser, 1985; Soares, 1982) due to limitation factors.

In biological control program there is no chemical contamination impact on the environment, which all of the compounds has specificity against the target organism. They do not attack on non-targeting species. The biological program also has disadvantages which it can increase the expenditure to grow the biocontrol agent. They also have limitation which it can be only use in water and depending on some factors such as temperature, pH status and the water pollution and can reduce the effectiveness of the agents (WHO, 1997).