PRELIMINARY SURVEY OF CIGARETTE BUTTS' DISPOSAL BEHAVIORS IN SELECTED PUBLIC PLACES ON PENANG ISLAND AND THE EVALUATION OF THEIR LARVICIDAL, OVIPOSITION AND FITNESS ALTERING ACTIVITIES AGAINST DENGUE VECTOR *AEDES AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE)

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PRELIMINARY SURVEY OF CIGARETTE BUTTS' DISPOSAL BEHAVIORS IN SELECTED PUBLIC PLACES ON PENANG ISLAND AND THE EVALUATION OF THEIR LARVICIDAL, OVIPOSITION AND FITNESS ALTERING ACTIVITIES AGAINST DENGUE VECTOR *AEDES AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE)

by

SUDHA D/O RAJASAYGAR

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

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LIST OF SYMBOLS

S.E	Standard error
^{o}C	Celsius
%	Percentage
±	Plus minus
>	Greater than
<	Less than
$	Dollar
®	Registered trademark

LIST OF ABBREVIATION

Ae.	*Aedes*
DCBs	Discarded cigarette butts
CBs	Cigarette butts
ANOVA	Analysis of variance
cm	Centimeter
DF	Dengue fever
DHF	Dengue haemorrhagic fever
mL	Mililiter
df	degree of freedom
S.E	Standard error
L1	First instar
L2	Second instar
L3	Third instar
L4	Fourth instar
h	hour
1CBSol	One cigarette butt solution
2CBSol	Two cigarette butt solution
3CBSol	Three cigarette butt solution
MPMD	Matrix of pairwise differences
Inc.	Incorporated
WHO	World Health Organization
MHLG	Ministry of Housing and Local Government

US EPA	U.S. Environmental Protection Agency
USDA	US Department of Agriculture
UNDP	United Nations Development Programme
ITC	International Tobacco Control
ANR	Americans for Nonsmokers Rights

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TINJAUAN AWAL TINGKAHLAKU PELUPUSAN PUNTUNG ROKOK DI BEBERAPA TEMPAT AWAM TERPILIH DI PULAU PINANG DAN PENILAIANNYA TERHADAP VEKTOR DENGGI *AEDES AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE) KE ATAS AKTIVITI-AKTIVITI LARVICIDE, OVIPOSISI DAN PENGUBAHSUAIN KECERGASAN

ABSTRAK

Tesis ini ialah kajian mengenai penggunaan rokok, persepsi tentang nasib puntung rokok (CB), cara pelupusan dan bebanan disebabkan CB di tempat-tempat awam. Kesan hasil buangan puntung rokok terhadap perkembangan *Aedes aegypti* dan juga terhadap tahap kecergasan progeninya. Secara keseluruhan, penggunaan tembakau adalah tinggi dan tahap kesedaran tentang cara perlupusan CB dengan cara yang tidak sepatutnya kian berleluasa. Sebanyak 900 puntung rokok yang mewakili 31 jenama dikutip secara konsisten di sekitar kawasan tempat makan (24.44%), masjid (20.44%), lot kedai (28.55%), dan pusat membeli belah (26.55%). Bahagian puntung rokok yang tidak terbakar ditemui di kebanyakan tempat dan ada yang mempunyai ukuran panjang melebihi 5 cm. Marlboro merupakan jenama yang paling kerap ditemui. Kajian tahap toksik di dalam makmal menunjukkan bahawa ketidakupayaan *Ae. aegypti* meningkat, tetapi tindak balas mortalitinya adalah pelbagai mengikut tahap perkembangan. Pada fasa awal perkembangan, larva *Ae. aegypti* mati pada kadar yang lebih tinggi dengan kepekatan CB yang paling tinggi (2CBSol and 3CBSol). Di dalam 1CB mikrokosmo, kemandirian larva meningkat seiring dengan perkembangan larva tetapi dengan kehadiran kepekatan CB yang paling tinggi (3CBSol), kadar mortaliti adalah tinggi walaupun pada tahap fasa akhir perkembangan. Proses perubahan larva ke peringkat pupa berlaku di setiap peringkat larva yang telah terdedah kepada larutan CB, tetapi kejayaan perubahan dari larva ke peringkat pupa tersebut terhad apabila rawatan CB digunakan ketika fasa awal

perkembangan. Kajian entomologi ini juga melibatkan penyiasatan sama ada CB mempunyai kesan sampingan secara tidak langsung terhadap *Ae. aegypti*. Tindak balas oviposisi berlaku pada kadar yang lebih tinggi dengan kehadiran CB. Walaubagaimanapun, mikrokosmo yang mengandungi 5CBSol kurang menarik bagi betina gravid mungkin disebabkan oleh halangan dari bahan toksik dan bau. Substrat oviposisi dengan rendaman CB juga menyediakan persekitaran yang baik untuk kematangan telur manakala telur yang matang dengan kehadiran lindi CB menetas pada kadar yang ketara walaupun perkembangan berlaku di dalam substrat yang di lembabkan dengan kepekatan CB yang paling tinggi. Kesemua betina yang terdedah kepada CB menghasilkan telur: walau bagaimanapun, fekunditi progeni menurun secara relatif terhadap induk terutamanya dalam kalangan betina yang terhasil dari larva mikrokosmo yang mengandungi 1CBSol. Jangka hayat menjadi lebeh pendek bagi jantan dan betina dewasa yang terhasil dari larva yang terdedah pada CB. Hasil kajian menunjukan bahawa CB memberi kesan secara langsung dan tidak langsung terhadap larva dan Ae. aegypti dewasa secara langsung mempunyai potensi untuk mengurangkan saiz populasi nyamuk. Penemuan ini dibincangkan dalam konteks menukarkan hasil buangan CB kepada salah satu strategi untuk mengawal nyamuk vektor sebagai pendekatan yang dapat membantu mengurangkan bebanan CB dan kos berkenaan dengan strategi pengawalan vector terkini.

PRELIMINARY SURVEY OF CIGARETTE BUTT' DISPOSAL BEHAVIORS IN SELECTED PUBLIC PLACES ON PENANG ISLAND AND THE EVALUATION OF THEIR LARVICIDAL, OVIPOSITION AND FITNESS ALTERING ACTIVITIES DENGUE VECTOR *AEDES AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE)

ABSTRACT

This thesis reports cigarette consumption, perception about DCB (discarded cigarette butts) fate, methods of disposal as well as CB burden in public places. The effects of the CB waste on the development of *Aedes aegypti* and on the fitness of its offspring. Overall tobacco use was high, level of awareness about DCB fate inadequate and careless disposal widespread. A total of 900 butts representing 31 brands were consistently picked up from the ground near a restaurant area (24.44%), mosque (20.44%), shop lot (28.55%) and shopping mall (26.55%). Un-burnt tobacco part was found in most littered butts, with portion reaching 5 cm in some cases. Marlboro was the commonest butt brand. In the laboratory, butt toxicity study revealed increased vulnerability of *Ae. aegypti*, but mortality responses varied considerably with developmental stage. At the early phases of development, larvae died at much greater rates in the presence of the highest CB concentrations (2CBSol and 3CBSol). In 1CB microcosms, larval survival gradually increased as development progressed. But, in the presence of the highest CB concentration (3CBSol), mortality was high even during the late developmental phases. Pupation occurred among survivors from all larval stages following their exposure to each of the CB solutions, but success was much limited when CB treatments were applied during early developmental phases. The entomological study also involved investigating whether CB has indirect effects on *Ae. aegypti*. Oviposition responses occurred at greater rates in the presence of CB. However, microcosms holding 5CBSol were less attractive to gravid females, presumably due to deterrent effects exerted by toxicants or odour. CB-soaked oviposition substrates also provided good environments for egg maturation—eggs that matured in the presence of CB leachates hatched at appreciable rates even when development occurred in substrates moistened with the highest CB concentration. All females that have been exposed to CB as larvae produced eggs; however, the fecundity of offspring tended to be reduced relative to that of parents, especially among females that derived from larvae which were maintained in microcosms holding 1CBSol. Also longevity was shorter for both male and female adults that resulted from survivors after CB exposure. The results indicate that CB waste exerts direct and indirect effects on *Ae. aegypti*, both of which have potential to reduce the population size of the mosquito. These results are discussed in the context of turning CB waste into a mosquito vector control strategy, an approach that can help to reduce both CB burden and cost associated to current vector control strategies

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Mosquitoes are the most crucial group of arthropods because of their medical and veterinary importance as they play an important role as pest and vectors of the most acute febrile human diseases. Mosquitoes serve as vectors of a variety of pathogens and parasites including viruses, bacteria, fungi, protoctistants and nematods (Clements, 1992) which lead to several dangerous diseases such as dengue, filarioses, yellow fever, malaria, encephalitis (Ang *et al.*, 2010; Goma, 1966; Muspratt, 1956; Richard & Davies, 1977) and Chikungunya (Robinson, 1953). According to Clement (1963), the blood sucking habit makes adult mosquitoes prone to acquire pathogens and parasites from one vertebrate host to pass them to another. In Malaysia, *Aedes* are the major concern because they transmit dengue virus (Lee, 2000) and *Aedes aegypti* is known to be the primary vector of dengue due to its high antropophilism, increased domesticity and multiple feeding behavior during a single gonotrophic cycle (WHO, 1997).

Worldwide, dengue virus infection cause more human morbidity and mortality than any other arthropod-borne virus. It infects up to 50 million people each year, causing more than 20,000 deaths (Burke & Monath, 2001). The fastest spreading mosquito-borne viral is dengue fever (DF) and it is believed to become the next global public health threat (Bast, 2010). Dengue can develop into dengue haemorrhagic fever (DHF), where one out of twenty cases is fatal without proper treatment. The disease is now endemic in more than 100 countries including Africa, the Americas, the East Mediterranaen, South-east Asia and the Western Pacific. Not only increasing number of cases as the disease is spreading to new regions, but explosive outbreaks are occurring (WHO, 2009a).

In Malaysia, The first report of dengue fever (DF) was from Penang which was described by Skae in 1902. By the 1960's dengue has become an endemic disease in Malaysia and the first laboratory confirmed dengue haemorrhagic fever (DHF) was reported in November 1962 in Penang Island (Sazaly & Norazizah, 2002). The 1973 outbreak was the first major national DF and DHF outbreak after the report from Penang in 1962. According to the Ministry of Health Malaysia, there were 54 deaths and a case fatality ratio of 5.6/100 (Rebecca, 1992). In 2008, over 48,000 cases were notified to the Ministry of Health and over 3000 cases were reported in the first 2 weeks of 2009 with 8 deaths (Ang *et al.*, 2010). While in 2010, dengue fever has infected 35,533 people which resulted in 107 deaths (WHO, 2010a).

Large scale control activities have been carried through insecticide spraying of adults and larval habitats and through public health sanitation programs which result in limited success (Dieng *et al.*, 2011). Currently, there are no effective vaccines & prophylactic drugs have been discovered to protect against dengue (Chaturvedi *et al.*, 2005). Lately, the development of lethal ovitrap and the improvement of adult and larval surveillance devices have been the focal point in controlling dengue vectors (Fay & Eliason, 1966; Ritchie *et al.*, 2003). The lethal ovitrap designed to kill *Aedes* through water-filled black cups containing an insecticide-treated ovistrip (Perich *et al.*, 2003). The ovitrap was first produced in the United States as a surveillance tool for *Aedes aeypti.* It has been used for detecting and monitoring *Aedes* population even when the population density was low

(Service, 1993). It is also known to be safe, economic and environmental friendly (Chan *et al*., 1977).

In 2011, Dieng *et al*. (2011) discovered that the toxic discarded cigarette butts capable to help in controlling dengue vectors. The study shows that discarded cigarette butts provide favorable site for egg deposition and reduce the survival of young larvae by attracting ovipositing females to habitats containing decaying discarded cigarette butts. This method allows in promoting the development of lethal ovitrap incorporating DCBs lethal ovitrap which could be a practical, new method for combating *Aedes* vectors from breeding in artificial containers and has important environmental significances.

1.2 Research objectives

The aim of this work is to study on reducing the environmental impact of CB waste by recycling it into a control tool against dengue vectors. This study involves the investigation of CB discarding behavior, diversity and abundance of CBs in different public sites on Penang Island, the direct effects of CBs on larval development of *Ae. aegypti*, the indirect effects on key adult life traits—oviposition, egg hatching responses, longevity of adult survivors following larval exposure to CB.

As such the objectives of the study are:

1) To examine the cigarette butt discarding behavior and the degrees of awareness about CB litter among smokers in Penang. The types and abundance of cigarette remnants discarded in public places of the Island
2) To examine whether CBs can alter development of the immatures of *Aedes aeypti* mosquitoes—larval and pupal stage.
3) To determine whether *Ae. aegypti* modifies its oviposition responses in reaction to presence of different CB remnants levels in its habitats and the sub-lethal effects of CBs on some fitness traits affecting vectorial capacity—hatchability of eggs, fecundity and longevity of *Ae. aegypti.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification of *Aedes* mosquito

Classification of *Aedes albopictus* (Knight & Stone, 1977)

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Subfamily: Culicinae

Genus: *Aedes*

Species: *aegypti*

2.2 Distribution of *Aedes aegypti*

Aedes aegypti originated in Africa but is now found in tropical and subtropical regions throughout the world. The invasion of *Ae. aegypti* in Malaysia was first noted by Leicester (1908) and Stanten in (1974). The distribution of this mosquito in Peninsular Malaysia, Sabah and Sarawak has been well documented (King, 1978; Lee, 1991).

Aedes aegypti is commonly found in urban areas especially in the densely populated districts (Rudnick, 1966) and it breeds in domestic and peridomestic water container consisting of clean waters (Service, 1992).

2.3 Biology of *Ae. aegypti*

Ae. aegypti has a pattern consists of two straight lines surrounded by curved lyre-shaped lines on the side of the thorax (Cheong, 1986; Goddard, 1993). It is a medium size mosquito, approximately between 4 to 7 mm, usually males are smaller than females (Rios & Maruniak, 2004). *Ae. aegypti* is a holometabolous, meaning it undergoes complete metamorphosis to complete its life cycle with distinct egg, larva, pupa and adult stage (Goma, 1966). The first three stages are passed in water but the adult is an active, flying insect that feeds on blood and plant nectar (Burges & Cowan, 1993).

2.3.1 Egg biology

The egg of *Ae. aegypti* are found to be cigar-shaped, shiny jet black (Linley, 1989) and elongate-oral in shape (Goma, 1966). They are blunt anteriorly and more tapering posteriorly (Estrada-Franco & Craig, 1995). The outer chorion of the egg is bounded by small reticulations (Goma, 1966). According to studies carried out by Institute of Medical Research, Malaysia, the females are ready to oviposit 4-5 days after copulation. The females are able to lay an average of 100 eggs (Lee, 2000) and these eggs are laid in a batch singly (Kettle, 1984; Rhodain & Rosen, 1997) above the water line on damp substrate such as mud or leaf or on the inside of stagnant water in tree holes (Service, 1995).

The eggs are at first white and soft when laid but later become black and hard (Christopher, 1960; Schlaeger & Fuchs, 1974). Like other species of Culicine group, the eggs are protected by a rigid proteinaceous shell that reduces water loss and allows gas exchange. Eggs can withstand desiccation for longer period of time, remain dry for months but still remain viable and hatch when submerged in water (Service, 1995). The eggs may require several flooding before hatching (Hawley, 1988). Oxygen tension also greatly affects egg to hatch (Hawley, 1988). Besides egg flooding, low oxygen tension is also a contributing factor for hatching (Hawley, 1988). Low oxygen tension due to colonizing of micoorganisms on the egg surface, induce an increase in microbial activity, which in turn stimulates hatching (Edgerly, 1993).

2.3.2 Larval Biology

According to Lee (1991), *Aedes* larvae require clear, but not necessarily clean water to grow and develop. The mosquito undergoes larval and pupal development in natural (rock, pools, tree holes, leaf axils) and artificial (water tanks, blocked drains, decorative pots, discarded tyres and food or beverages containers) freshwater collection in the urban and peri-urban environment (WHO 2009; WRBU, 2011; Weaver & Reisen, 2010). The larvae are capable of adapting to an exceptionally wide range of confined water sources (Lee, 1990).

The mosquito larvae are not found in turbulent water as they are unable to withstand wave action (Bates, 1970). The larvae are quite mobile and will quickly dive to the bottom when the water surface is suddenly disturbed but will return back to the surface shortly (Potter & Beavers, 2005). They move about either by jerking of the body or by slower propulsion aided by the mouth brushes (Michigan Mosquito Control Association, 2002).

Based on Christopher (1960) statement, the mosquito consists of four larval instars and the larval moult or ecdysis at the end of each instar. One of the first sign that shows ecdysis is about to occur is when the appearance of dark bands across the thorax caused by the circularly wrapped lateral hairs of the next instar shining through the cuticle. The newly hatched larvae are only about 1 mm in length but increase twice the size during the instar. The first instar possesses the egg-breaker which is located on dorsal side of the head. The egg-breaker helps the larva cuts its way out of the egg shell by simply forcing off a circular cup from the anterior end of the egg.

The second instar larva after ecdysis is almost the same length as a fully grown instar, but it is heavier and the swollen head is enormous. The larva grows ranging in length from 2 mm to 3 mm during the instar (Christopher, 1960).

During the third instar, larva become even larger and to a large extent the head measurement is more variable than any other stage, since they are large and small headed forms. This character is shown more conspicuously because the pre-ecdysis stage is long, thin and is larger than the previous instar (Christopher, 1960).

The fourth instar at the corresponding size become much stouter due to the development of the thoracic imaginal buds and fat-body accumulation. The tail comb-spine is the most important structure during this instar. The rudiment of the pupal respiratory trumpets can be seen in this instar (Christopher, 1960).

2.3.3 Pupal Biology

The fourth and final instar larva moults to become non-feeding, yet active pupa. The pupa is the second aquatic stage in the mosquito life cycle. They are comma shaped and dark in colour (Service, 1995). The pupa is white in colour when first emerged but in short time shows pigment changes (Christopher, 1960). It spends most of the time in the water-air interface taking in oxygen through the respiratory trumpets (Service, 1995). The pupal stage is short, usually 2 to 3 days long (Lee, 2000) depending on the temperature (Goma, 1966). All organs of adult stage are present in an incomplete state. The pupa usually rests at the water surface due to a large air space placed between the future adult's insect's wings (Goma, 1966).

2.3.4 Adult Biology

Aedes aegypti is easily recognized by silvery-white lyre-shaped pattern of scales on its scutum (Rueda, 2004). The clypeus of *Ae. aegypti* which is covered by silvery white stripe scale patches (Rueda, 2004) unlike clypeus of *Ae. albopictus* is bare and entirely black (Becker *et al.*, 2003).

The newly emerged adult rests on the surface of water for short period of time in order to allow itself to dry and harden its body parts. The wings have to spread out and dry completely before it can fly (Rahman, 2008). Mating and blood feeding of inseminated females occur within 24 to 36 hours after emergence.

Both females and male adult mosquito feed on nectar which is used as energy sources but only females feed on blood (Clements, 1992). In anautogenous mosquito including *Aedes* the blood meal stimulates development and reproduction of ovary and provides energy resources (Clements, 1992). The blood meal initiates the egg development in female mosquito. According to Clement (1992), mosquito do not take blood meal first day of adult life due to the slow progress of the mouth parts hardening which are not strong to penetrate skin of the host.

In laboratory studies, the type of nourishment given to females affects their longevity. Hien (1976), in a laboratory study observed that if provided only water, most females lived only 5-7 days. According to Udaka (1959) and Gubler (1970), females provided with both blood and sugar live longer compared to those given only sugar or only blood. This statement contradicts with the report by Gao *et al.* (1984), which indicates that females with access to both blood and sugar which were required to repeated oviposit did not live as long as those provided only sugar.

The flight range of *Aedes* mosquito is shorter compared to other genera of vector mosquitoes such as *Culex*, *Anopheles* and *Mansomia*. The flight distance of adult normally around 50 m to 200 m from breeding source (WHO, 1995). *Aedes aegypti* females rarely visit more than 2 or 3 houses during their lifetime because being domesticated the dispersal of this mosquito is usually limited (Rhodain & Rosen, 1997).

Adult mosquito frequents a wide range of habitats in search of resting places. Mosquito are generally found in high humidity and relatively static air areas. Most mosquitoes prefer to rest in dark places and avoid light during the day time (Goma, 1966). According to Rodhain and Rosen (1997), *Ae. aegypti* prefers to rest inside the houses where it is dark, such as, on clothing, furniture and other semi-permanent articles (Macdonald, 1957).

Once a female mosquito is fully engorged, it flies to a shaded area until her eggs are fully developed. Then, the gravid female begins to search a suitable place to lay its egg. Egg laying activity is known as oviposition. Normally, engorgement to oviposition takes 2 to 5 days (WHO, 1995). The average number of eggs oviposits after a single blood meal ranged from 42 to 88 eggs per blood meal for the first gonotropic cycle (Gubler & Bhattacharya, 1971; Ito, 1959; Mori, 1979).

In laboratory studies, *Ae. aegypti* prefers to lay eggs above water surface on a dark, rough and vertically oriented substrate (del Rosario, 1963; Hien, 1976; O'Gower, 1957). Dark-coloured water appears to be more attractive than clear water. Leaf, fresh grass, infusion of dried grass, egg and larval holding water are attractive to ovipositing females (Gubler & Bhattacharya, 1971). In Malaysia, a field study showed that red or black painted glass ovitraps found to be more attractive than yellow, green or blue traps and very few eggs were laid in white or unpainted (clear) traps (Yap, 1975).

Vision plays an important role in adult female mosquito biology including location of hosts, food sources, mates, resting sites and oviposition sites (Allan *et al.*, 1987). Many studies have been carried out on host-seeking visual parameters of shape, size, contrast colour, light intensity and movement (Bentley & Day, 1989; Burkett & Butler, 2005) while only a few studies have explored which of these parameters are attractive to gravid adult female (Hawley, 1988). Diurnal mosquitoes are believed to have the capability to distinguish different colored light across the visible spectrum. Burkett and Butler (2005) found that host-seeking mosquito fed more on blood baited lighted targets of 600 nm, followed by blue-green targets of 500 nm and blue targets of 450 nm. Human body emanations such as heat, moisture, carbon dioxide act as mosquito attractants in locating and recognizing the hosts (Goma, 1966; Service, 1996). Bar-Zeev *et al.* (1977), found that attraction to body emanations from is due to lactic acid which is found in muscle, blood and skin of vertebrates (Mahler & Cordes, 1971).

Aedes aeypti is primarily a daytime biter (Estrada-Franco & Craig, 1995). Ho *et al.* (1973) noted that its diurnal biting behavior exhibits bimodal peaks which occur in mid-morning and late afternoon hours. According to Lee (2000), *Aedes aeypti* bites mainly indoor unlike *Ae. albopictus* which bites both indoor and outdoor. The female mosquito acquires the dengue virus while feeding on the blood of an infected person. It normally requires an incubation period of 8 to 10 days where the virus multiple in the midgut and escape to the salivary gland and finally enters saliva the causing infection during probing of the next human host. In man, the period of incubation covers from 5 to 7 days. *Aedes* mosquito is capable of transmitting the dengue virus immediately from an infected person to another individual by a change of host when its blood meal is interrupted.

2.4 Ovipositing behavior

In the population dynamics of mosquito vectors, oviposition has become an essential feature as it underlines the production of next generations and thus population maintenance (Bentley & Day, 1989; Dieng *et al.*, 2007). Females of *Aedes* mosquito has the potential to access habitats for oviposition as microhabitat quality is often the major determinant for larval development (O'Malley, 1990). For example, Dieng *et al.* (2003) have carried out an experiment on the oviposition of *Ae. albopictus* where oviposition sites were different sized-container with or without leaf detritus and they observed that oviposition was greater in large-sized container and in the presence of leaves especially those that decay rapidly. Based on the ovipositional responses, they suggested this behavior appears to reduce competition among their offspring for food because large containers provide spacious environment with more food sources which if known to be positive effects on larval development (Lord, 1998; Mori, 1979). Besides that, Fish and Carpenter (1982) found that *Aedes* mosquito larvae fed on decaying leaf litters which act as the source of all nutrients they need for development. It has been said that mosquitoes are quite discriminating in selecting sites for egg deposition (Bentley & Day, 1989). In Japan, ovipositing females of *Ae. aegypti* deposited eggs preferentially in open-type holes when compared to small-type holes as small-type holes were less protective during drought season (Tsuda *et al.*, 1994) and this behavior could be an adaptation to prevent unsuccessful egg development and better survival of their offsprings (Dieng *et al.*, 2011). Another study on *Aedes* mosquito elicited that females preferred to deposit eggs in hall-filled containers rather than fully filled ones as overflow events tend to occur more rapidly in fully filled containers during rainy days. This obseravtion could likely be an adaptation carried to prevent the loss of their immature stages, since overflow of habitat pose detrimental effect on survival of mosquito larvae (Paaijmans *et al.*, 2007). In Brazil, Pamplona *et al.* (2009) noticed reduced oviposition responses from *Ae. aegypti* in habitat harboring larvivorous fish. The females exhibit such behavior in order to prevent from laying eggs in habitat with high densities of larval conspecifies. However, *Ae. aeypti* females showed enhanced oviposition response when the habitat holds a low larval density, particularly larval conspecific are unhealthy (Serpa *et al.*, 2008; Zahiri & Rau, 1998). In a field work, where substrate with conspecific eggs were used as oviposition sites, Chadee *et al.* (1990) observed an increase in oviposition response in habitat with low egg density.

Gravid females of *Aedes* mosquito exhibit a special behavior known as "skip" oviposition which occurs when females deposit their eggs in several oviposition sites instead of depositing their entire clutch at one site (Chadee & Corbet, 1987; R. W. Fay & Perry, 1965; Rozeboom *et al.*, 1973). The term "skip" oviposition was first used by Mogi and Mokry (1980) to describe the behavior of *Wyeomyia smithii* Coquillet females that distributed their eggs from a single batch across several pitcher plants. Similar behavior has been observed in laboratory conditions that *Ae. aeypti* does not deposit their eggs from the same gonotrophic cycle in many containers as oviposition sites (Fay & Perry, 1965).

2.5 Hatchability

In mosquito, there are several biological and environmental cues that induce egg hatching activity. Previous studies have shown that microbial growths were shown to stimulate eggs of *Ae. aegypti* to hatch (Gjullin *et al.*, 1941; Judson, 1960). According to Gillett *et al.* (1977), larval grazing on microbial surface growth may weaken the hatching stimulus and delayed the egg hatching activity. In a related work, researchers found that increased larval densities inhibited hatching of *Aedes* eggs. Rozeboom (1934) reported that no hatching was found when eggs were submerged in sterile water but hatched when the medium was injected with bacteria. In *Ae. triseriatus*, gradual depletion of dissolved oxygen by the mircobes resulted in a hatching rate of 99% whereas the rate was minimal when the reduction of dissolved oxygen was rapid (Novak & Shroyer, 1978). High humidity increases viability of *Aedes* eggs as they hatched readily under such conditions (Hardwood & R., 1959). In a study, about 62 % of *Ae. aegypti* eggs remained viable for more than a year at

0% relative humidity (Buxton & Hopkins, 1927). In the laboratory, the magnitude of egg hatching inhibition is influenced by interactions among resource availability, larval stages and density (Livdahl, 1982). The egg hatching inhibition may be adaptive for the egg because the adverse influence of large larvae on small may be severe through competition for food resources (Livdahl, 1982) and cannibalism of newly hatched larvae (Koenekoop & Livdahl, 1986). In Japan, Tsuda *et al.* (1991) evaluated the growth pattern of laboratory populations and changes in population density and they observed death of young larvae after hatching and the larval population decreased. Thus, they suggested that competitive interaction, for instance compete for food and space to survive, might have occurred within and between cohorts of hatched larvae. In the similar mosquito, some studies have documented that the presence of mature larvae inhibits hatching of eggs (Gillett, 1959; Livdahl, 1982). It has also documented that high larval density adversely influence several components of mosquito such as fitness, larval survivorship (Juliano, 1998; Seawright *et al.*, 1977), development rate (Dye, 1982), adult longevity (Reiskind & Lounibos, 2009) and female fecundity (Steinwascher, 1982). Dieng *et al.* (2007), discovered that embryo hatching also induced by availability of food source. Once eggs are laid by the females, the egg or embryonic development proceeds rapidly into first-stage larva and it is of critical importance for this newly hatched larva to find nutrient sources for growth and development (Clements, 1992; Gillet, 1971). The completion and the quality of these processes largely depend on the nutritional environment (Dieng *et al.*, 2007). Studies have documented that high food level positively correlated with survivability, developmental period, adult size and fitness (Dieng *et al.*, 2002; Mori, 1979).

2.6 Fecundity

Genetic background, environmental conditions and interactions are factors that influence the reproductive success of an individual. The environmental conditions experienced by parents are recognized to have a profound impact on their offsprings success for instance nutrient deprivation in parent results in less well-provisioned and hence produces small sized offspring (Grech *et al.*, 2007). Such parental effects enable the parents, especially mother to adjust their maternal investment into their offspring according to the environment experienced during the larval or adult life in order to optimize fitness of offspring (Lynch, 1983; Pieters & Liess, 2006). Pieters and Liess (2006), working with *Daphnia magna*, a freshwater crustacean, observed that mothers kept in poor conditions produced offspring with better fitness, in term of, size, survival, fecundity or resistance to parasites, suggesting that environmental stress (poor condition) experienced by the mother increased per offspring investment in the few offspring they were able to produce. Thus, there are many other maternal effects that can affect offspring traits such as development time, survival and fecundity. For example, offsprings that are reared under low food condition were found to imbibe larger blood meals and produce more eggs when their parents had also experienced the low food environment. Such alteration, consumption of larger blood meals size and increased fecundity, found in daughters may arise to compensate for maternal provisioning and expected decreased longevity in low food environment (Grech *et al.*, 2007).

2.7 Longevity

Variety of factors in the field and laboratory contribute to the variation in mosquito longevity. An adult mosquito found in the field has a shorter life expectancy than the one reared in laboratory condition. Under laboratory condition, the maximum longevity of the females were recorded more than 100 days in which *Ae. albopictus* and *Ae. aegypti* were given ample amount of sugar and blood meals while being kept at a constant and suitable temperature (Christopher, 1960; Hawley, 1988), whereas in the field they are bound to many risk factors such as quality of environment, climate, and predation (Westbrook, 2010), reducing their life span to less than a month. Indeed, life span of females was longer when compared to males at all temperatures, even with constant temperatures (Calado & Navarro-Silva, 2002; Joshi, 1996). Predators and their defensive behavior can cause an increased in mortality rate in wild population of *Ae. aegypti*, thus if predators are an important source of mortality, household practices and mosquito control measures that decrease the predator populations could lengthen the life span of *Aedes* mosquito. Longevity also may be affected by the effect of size on daily survival rate addressing the effect of larval nutrition indirectly and other influences on larvae (Fox, 1998; Nandi & Raut, 1985; Strickman *et al.*, 1997; Sulaiman *et al.*, 1990). Larvae derived from high quality and quantity food sources tended to emerge with a large body size. In addition large mosquito, especially females have a tendency to imbibe large amount of blood meals, produce more eggs and live longer which in turn, resulting in greater vectorial capacity (Araujo *et al.*, 2012; Briegel *et al.*, 2002). In general, any cues that increase the life span of female *Aedes* mosquito will likely shorten the extrinsic incubation period of the dengue virus (Watts *et al.*, 1987) and increase the risk of dengue transmission (Strickman, 2006).

2.8 Ovitrap

Ovitrap, also known as oviposition trap (Pratt & Jacob, 1967; Tham, 2000) is a simple device which functions by attracting the female *Aedes* mosquito to lay their eggs into the device. The development of ovitrap in a community has aided in controlling *Aedes* population and effectively reduces *Aedes* population in an area (WHO, 1995). The following instructions should be considered before placing an ovitrap (Evans & Bevier, 1969; Pratt & Jacob, 1967).

1) Place the trap at ground level and if possible avoid disturbance by children and pets.
2) Place the trap where it is protected from home lawn sprinklers or excess rainwater.
3) Place it close to adult resting sites such as shrubbery or junk or trash accumulation area.
4) Place the trap in partial or total shaded area, if possible near any containers in order to avoid direct sunlight.
5) Place it at the back of the house because there are more mosquitoes breeding places and shelters.
6) Place the trap in visible area where the mosquito can see the trap.
7) Place it away from piles of tires because *Aedes* mosquito is more attracted to tires.

Historically, monitoring of *Aedes aeypti* and *Aedes albopictus* were achieved through the use of ovitraps (Focks, 2003). The skip-oviposition behavior and environment condition most likely affects the number of eggs that can be found in each ovitrap (Hawley, 1988). Previously, Fay and Perry (1965) were the one who came up with the ovitrap and later on it was improved by Fay and Eliason (1966). Ovitrap is a black container which consists of partially filled tap water that holds a vertical wooden paddle with one rough side (Dibo *et al.*, 2005). The presence of eggs on the ovitrap paddles reveals existence of mosquito in an area (Pratt & Jacob, 1967). According to Tham (2000), ovitrap can be used to access *Aedes* population fluctuation over a long-term period and give insights relative changes in the adult female populations.

Ovitraps are usually collected every seven days to remove the eggs on the paddles and replace with water for the next sampling (Jacob, 1969). In most survey, a longer trap exposure (a week) is suitable (Ritchie, 1984) because it is economical, efficient and more sensitive (Fay & Eliason, 1966; Ritchie, 1984; Tham, 2000) and increases the statistical reliability of the data (Ritchie, 1984). Trapping every week provides continuous trapping because it reduces bias due to unpleasant weather condition (Fay & Eliason, 1966) and increases the percentage of positive ovitraps (Frank & Lynn, 1982).

There are different types of oviposition traps created to attract the gravid females. In many studies, the technique is simplified by modifying the ovitrap. Pratt and Jacob (1967), used oviposition jar which is made of flint glass and painted in black and followed by a pressed wood paddles. After some time, glass jar are increasingly being replaced by plastic cups, used ovitrap made from black plastic beakers containing hardboard paddles and found out it is equally attractive as glass jar for *Aedes* oviposition. Besides that, different types of flower vases also were used as oviposition sites for *Aedes* mosquito (Vezzani & Schweigmann, 2002). Chan *et al.* (1971), noted that the most common outdoor habitat of *Ae. albopictus* in Singapore was discarded tin cans and he made use of condensed milk tins painted black as a convenient ovitrap.

2.9 Control of *Aedes aegypti*

According to Yap *et al.* (2003), the control of mosquito has been practiced since ancient time in order to reduce man-mosquito contact and consequently human suffering. The lack of vaccines and specific drug treatment, rapid urbanization and the increase number of viruses and vectors throughout the world require development of alternative method to control the most common disease which is dengue (Paupy *et al.*, 2003). The only option is by controlling the vectors to combat dengue outbreaks. Emmel (1991), stated that larval control would be the most effective way to control mosquito population. The best way to control *Ae. aegypti* and other *Stegomyia* species is by limiting the availability of larval habitats (Estrada-Franco & Craig, 1995).

Methods of mosquito control include environmental management, biological control, chemical control (Estrada-Franco & Craig, 1995; Goma, 1966; Yap *et al.*, 2003) and physical barrier and personal protection (Emmel, 1991; Reither & Gubler, 1997).

2.9.1 Environmental Management

The environmental management concepts for mosquito control spans a wide range of measures which are created to inhibit mosquito population and the risk of disease transmission. WHO (2011), stated that in order to control *Aedes aegypti* and *Aedes albopictus* and reduce human-vector-virus contact, the required method for environmental management are source reduction, solid waste management, modification of man-made breeding sites and improve house design. According to WHO, there are three basic categories of environmental management for vector control:

- Environmental modifications: long-lasting physical transformation of land, water and vegetation aimed at reducing vector habitats.
- Environmental manipulation: temporary changes in vector habitat as a result of planned activity to produce unfavorable conditions for vector breeding involving the management of “essential” and “non-essential” container and management or removal of “natural” breeding sites.
- Changes to human habitation or behavior to reduce human man-vector-virus contact.

Environmental management of mosquitoes may include a broad range of interventions, such as proper water storage containers and management practices to reduce stagnant water (for example: covering domestic water storage, managing construction sites, mosquito-proofing of overhead tank or underground reservoirs, cleaning flower pots and ants trap), modifications to sewage works, routine collection and proper dispersal of certain item of urban waste (tin, glass, bottles, buckets, coconut shell), training of health department personnel and community activities, and preparation and distribution of educational leaflets about mosquito to communities (Becker *et al*, 2003; WHO, 2011).

Environmental management concepts may sometimes be difficult to implement. The efficacy of environmental management methods, for example source reduction, may not give a simple linear correlation with the number of mosquito being removed from the breeding sites (Becker *et al.*, 2003). Gu *et al.* (2006) argued that source reduction not only reduce the number of adult mosquito production, but also prolong the cycle duration by extending delays in locating egg deposition site. Newton and Reiter (1992), noted that source reduction that provided a long-term reduction of *Aedes* population, were actually more effective and sustainable compared to insecticide treatment in combating dengue outbreaks.

The initial cost of environmental management may be high but if long-term cost effectiveness is considered, then it is cheaper compared to other control measures in combating dengue diseases (Becker *et al.*, 2003).

2.9.2 Biological control

Biological control is defined as the reduction of target population of *Aedes* mosquito by the use of predators, parasites, pathogens, competitors or toxins from microorganisms. Biological control involves natural and applied biological control. The reduction of mosquitoes through naturally occurring biotic agents is known to be natural biological control. Whereas, applied biological control is intervention planned by human to add biological agents to a oviposition sites. Potential biological control agents are pathogens and predators (Woodring & Davidson, 1996).

The most effective biological control agents are larvivorous fish and microbial agent. The best predators of the *Aedes* mosquito is the so-called mosquito fish, *Gambusia affinis* and the common guppy, *Poecilia reticulate*. These two fish are effective predators as their upward-facing mouth enables them to engulf mosquito larvae on the water surface and high tolerance to pollution, salinity and variations in temperature (Becker *et al.*, 2003). The biocide *Bacillus thuriengiensis* H-14 and *Bacillus sphaericus* are very effective in combating *Aedes* mosquito. *Bacillus thuriengiensis* was discovered to produce toxic product that kills the mosquito larvae after ingestion of the product. This application does not harm the non-target organisms in the mosquito breeding sites (Becker *et al.*, 2003).

The benefit of biological control methods is that there is no chemical contamination of the environment, particularly against target organisms and the self-dispersion of some agents into sites that could not easily be treated by other means. While, the disadvantages are the expense and task of the rearing organisms on a large scale, difficulty in applying and their limited utility in aquatic habitat such as pH, temperature and organic pollution may exceed the limited requirement of the agents (WHO, 1999).

2.9.3 Physical barrier and personal protection

Physical control includes the use of surface layer such as monomolecular films or polystyrene beads that prevents immature stages of mosquito from breathing at the water surface and decrease the egg deposition on the water surface (Curtis *et al.*, 1990; Reiter, 1978). Personal protection is the reduction of human-mosquito contact such as the use of bed nets or curtains impregnated with insecticide and repellants. The treated bed nets or curtain may act as insecticidal trap by attacking and killing the *Aedes* mosquito (MacCormack & Snow, 1986; Schreck & Self, 1985).

Repellants are widely available but expensive (WHO, 1997) and only provides a short-term effect (WHO, 1995; 1997).

2.9.4 Chemical control

In the 1940s, the chemical era was introduced with the discovery of organic insecticide, ever since then chemical control has been the most popular approach in controlling *Aedes* mosquito (WHO, 1996; Yap *et al.*, 2003). The insecticides belong to four major classes, they are, organochlorines, organophosphates, carbamates and the pyrethroids and a special class, the insect growth regulators (IGRs) (Becker *et al.*, 2003). Chemical control involves adulticides and larvicides.

The larval and adult stage habitat of the *Aedes* mosquito is different; therefore the approaches to control, types of insecticide and formulation used may differ. Before a chemical control is carried out, surveillance evaluation and data analysis must be considered as they are very important (WHO, 1996).

Chemical control using insecticides alone will seldom become the choice of the control method for the community because of the cost involved, necessity of continuous input and safety and environmental factors (Yang, 1982).

2.9.4.1 Adulticides

Adulticides are chemical compounds that are used against adult stage of insects (Hoel, 2005). Adulticiding mainly involves aerial, truck and hand-held