RECENT ECOLOGICAL, PHYSIOLOGICAL AND PROTEIN PROFILE OF THE DENGUE VECTOR POPULATION IN PENANG ISLAND, MALAYSIA

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By

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LIST OF SYMBOLS AND ABBREVIATIONS

AI	Air Itam
ANOVA	Analysis of variance
B	Buckets
BI	Breteau Index
BM	Batu Maung
BP	Balik Pulau;
BSA	Bovine serum albumine
CB	Coomassie blue
CHIKV	Chikungunya virus
CID	Container identity
cm	Centimetre
D	Drums;
*d0*FWMs	Females derived from wild mosquitoes
*d5*FWMs	Females after five generations from *d0*FWMs
DC	Drum covers;
DENV	Dengue virus
DF	dengue fever
df	Degree of freedom
dH_2O	Distilled water
DHF	Dengue haemorrhagic fever
dia	diameter
DNA	Deoxyribonucleic acid
DSS	dengue shock syndrome
EIP	Extrinsic incubation period

EP	Earthen pots
EPPC	Empty paint cans;
ETOH	Ethanol
FTZ	Free Trade Zone
FWMs	Wild outdoor mosquitoes
GA	Gonotrophic activity
GCs	Gonotrophic cycles
GIS	Geographical Information System
GL	Gelugor
gm	Gram (s)
GPS	Global Positioning System
h	Hour
HI	House Index
HMLs	High moisture levels
HRF	Heavy rainfall
IL	Instar Larvae
IN	Indoor
JL	Jelutong
Jln	Jalan
kDa	Kilo Dalton
Kg.	Kampung;
Kg. TT	Kampung Teluk Tompoyak;
KOH	Potassium Hydroxide
l	Litre
L2	Second instar larvae
L3	Third instar larvae

L4	Fourth instar larvae
LC	Large containers
LD	Day-light
L-DOPA	L-3,4-dihydroxyphenylalanine
LRF	Light rainfall
m	Meter (s)
m^2	Square meter (s)
mA	Milli ampere
Max	Maximum
MB	Mixed breeding
MEG	Moisture exposed egg,
MeOH	Methanol
min	Minute
mL	Millilitre
MLs	Moisture levels
mm	Millimetre
mRNA	messenger ribonucleic acid
MW	Molecular weight
n	Total individuals/numbers
OU	Outdoor
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate-buffered saline
PlC	Plastic containers
PC	Positive container
PCI	Premise condition index

PS	Plastic sheets
PVC	Poly vinyl carbon
rpm	Revolution Per Minute
RU	Rural
RW	Rain water
SC	Small containers
SD	Sungai Dua
SDS	Sodium Dodecyl Sulphate
SE	Standard error
Sg.	Sungai
SH	Spontaneous hatching
SPSS	Statistical Package for Social Science
ST	Silver staining
SU	Suburban
SW	Store water
TIEL	Timing of initial egg laying
ul	Micro litre
UR	Urban
USM	Universiti Sains Malaysia
VG	Vitellogenin
Vn	Vitellin
WC	Wet container
WHO	World Health Organization
®	Registered
µg	Microgram
°C	Celsius degrees

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1. **Saifur R.G.M.**, Dieng, H., A. Abu Hassan, M.R. Che Salmah, T. Satho, F. Miake, A. Hamdan. 2012. Changing Domesticity of *Aedes aegypti* in Northern Peninsular Malaysia: reproductive consequences and potential epidemiological implications. *PloS One* (**in press**), IF = 4.411.

2. Dieng H., **R.G.M. Saifur**, A. Abu Hassan, M.R. Che Salmah, M. Boots, T. Satho, Z. Jaal, and S. Abu Bakar. 2012. Unusual developing sites of dengue vectors and potential epidemiological implications. *Asian Pacific Journal of Tropical Biomedicine* 2:228-232.

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CIRI-CIRI TERKINI EKOLOGI, FISIOLOGI DAN PROFIL PROTIN POPULASI VEKTOR DENGGI DI PULAU PINANG, MALAYSIA

ABSTRAK

Kekurangan pengetahuan berkenaan faktor-faktor berisiko dan interaksi di antara mereka merupakan punca utama yang dihadapi untuk mengatasi masalah denggi. Kawalan vektor denggi yang berkesan memerlukan pemahaman yang dalam tentang ekologi, fisiologi dan komponen-komponen molekular populasi dinamik. Berdasarkan pemahaman ini, kajian dijalankan di lapangan dan juga di dalam makmal untuk menentukan keadaan populasi vektor dan hubung-kait faktor-faktor yang terlibat dalam kemandirian, kesuburan dan kematian. Pemantauan larva sepanjang tahun di 9 kawasan penduduk yang mewakili kawasan bandar, pinggir bandar dan luar bandar di Pulau Pinang menunjukan populasi vektor yang tinggi (BI= 79.6 dan HI = 44.4) bagi kedua-dua nyamuk, *Ae. aegypti* dan *Ae. albopictus*. Lebih banyak kes denggi dilaporkan dari kawasan dominasi *Ae. aegypti* yang mempunyai pecahan peratusan populasi vektor tertinggi (60%) di kawasan bandar, peratusan sederhana ke rendah di kawasan pinggir bandar dan tidak terdapat di kawasan luar bandar. Mereka menunjukkan kecergasan dan kebolehan membiak yang sama di dalam dan luar rumah (7-9 kitaran gonotropik). *Aedes albopictus* merupakan satu-satunya vektor di kawasan luar bandar, dijumpai di dalam dan luar rumah, dominan di kawasan sub bandar dan masih lagi perlu bersaing dengan *Ae. aegypti* di habitat luar rumah di kawasan bandar. Ia memperoleh populasi tinggi di kawasan luar bandar dengan meningkatkan kitaran gonotropik (sehingga 14 kitaran) oleh individu yang berhabitat di dalam rumah bersama dengan peningkatan aktiviti gigitan pada waktu malam.

Kawasan luar bandar mengeluarkan jumlah bekas pembiakan tertinggi dengan indeks bekas 55.4, diikuti oleh indeks bekas 42 dan 33, masing-masing di kawasan

pinggir bandar dan bandar. Dua puluh dua peratus premis dikenalpasti sebagai premis utama (>2 bekas positif dalam rumah) yang meliputi 45% daripada bekas yang mengandungi larva. Bekas tong, plastik atau tangki simen dan tin cat kosong (bekas jenis 1, 2 dan 4) mengandungi populasi larva nyamuk terbesar dan dikenalpasti sebagai bekas utama. Bekas yang paling kerap ditemui di ketiga-tiga kawasan kajian ialah tin cat kosong dan tong. Bekas lain yang berpotensi sebagai tempat pembiakan untuk *Ae. aegypti* dan *Ae. albopictus* ialah bekas penyimpanan air dan kepingan plastik di kawasan bandar, pelbagai penutup bekas di kawasan pinggir bandar dan baldi di kawasan luar bandar.

Hujan sederhana (>50mm) pada awal monsun menyebabkan penambahan habitat untuk pembiakan, memulakan penetasan telur dan menarik vektor nyamuk untuk bertelur di dalam bekas berair baru tersebut. Bekas-bekas pembiakan ini akan kekal menghasilkan populasi vektor yang banyak sehingga BI 295, walaupun ketika kadar hujan rendah, tetapi akan berkurang ketika hujan lebat disebabkan oleh air melimpah keluar dari bekas pembiakan. Hujan yang berterusan ketika bulan March sehingga Disember mengekalkan kelembapan (HMSs; 66% dan 72%) di kawasan pembiakan *Aedes*. Ini membantu menambahkan vektor populasi dengan memastikan penetasan telur yang tinggi yang mempunyai perkembangan embrio yang sepatutnya, terutamanya bekas luar rumah. Populasi vektor dikekalkan ketika musim kering dengan pembiakan dalam bekas dalam rumah. Dengan ini, ia berkemungkinan membolehkan tranmisi denggi yang tinggi sepanjang tahun. Selain itu, SDS-PAGE menunjukkan kehadiran enzim spesifik atau protin ketika penetasan telur (~7 kDa), peringkat pupa dan nyamuk dewasa yang baru muncul (~200 kDa) yang berkemungkinan mempunyai kesan kawalan terhadap penetasan telur, proses menjadi pupa, perkembangan sayap nyamuk dewasa.

RECENT ECOLOGICAL, PHYSIOLOGICAL AND PROTEIN PROFILE OF THE DENGUE VECTOR POPULATION IN PENANG ISLAND, MALAYSIA

ABSTRACT

Inadequate knowledge regarding the risk factors and their interaction is the key problem to face dengue threat. An efficient control of its vectors requires a deep understanding of ecological, physiological and molecular components of their population dynamics. In view with this inference, the study was conducted both, in the field and the laboratory to explore the vector population situation and the interrelated factors involve in their survival, fecundity and mortality. A yearlong larval surveillance in 9 residential areas representing from urban, sub-urban and rural habitats in Penang Island indicated abundant vector population (BI = 79.6 and HI = 44.4) including both, *Ae. aegypti* and *Ae. albopictus*. The higher numbers of dengue cases were reported from *Ae. aegypti* dominated areas that comprised a greater proportion (60%) of the vector population in urban areas, moderate to low in suburban areas and absent from rural areas. They showed equal preference and fitness for breeding indoors and outdoors (7-9 gonotrophic cycles). *Aedes albopictus* is the only vector in rural areas, found equally both indoors and outdoors, dominant in suburban areas and still competing with *Ae. aegypti* in outdoor habitats in urban areas. It attained a high population in rural areas through increased gonotrophic activity (up to 14 cycles) by indoor habiting individuals together with increased night time biting activity.

Rural area produced the highest number of breeding containers with a container index of 55.4 followed by 42 and 33 in suburban and urban areas, respectively. Twenty two per cent of premises were identified as key premise (>2

positive container in a house) that accounted 45% of infested containers. Drums, plastic or cement tanks and empty paint cans (container types 1, 2 and 4) harboured the largest share of mosquito immature population and identified as key containers. The most common containers found in all three study areas were empty paint cans and drums. Other potential containers were water reservoirs and plastic sheets in urban area, different container covers in suburban area and buckets in rural area for both *Ae. aegypti* and *Ae. albopictus* breeding.

An early moderate rain (>50mm) at the beginning of the monsoon create a numbers of breeding habitat, initiate hatching of old eggs and attract vector mosquitoes to lay their eggs in newly generated containers. These breeding containers are maintained later even in the low rain and produce enormous vector population up to a BI of 295, which reduce due to the over flushing of the breeding containers during the heavy rain. Frequent rainfall during March to December in a year ensures sufficient moisture (HMSs; 66% and 72%) in the *Aedes* breeding habitats. It helps to amplify vectors population by ensuring high egg hatchability with proper embryonic development, especially in outdoor containers. The vectors population is maintained in the dry months by indoor breeding containers. Thus, uphold a high level of dengue transmission possibility throughout the year. Furthermore, SDS-PAGE indicates some enzymes or proteins with a specific molecular weight near to egg eclosion (~7 kDa), pupation and adult emergence (~200 kDa), they may have controlling effects on egg hatching, pupae formation and wing development in adults.

CHAPTER 1

INTRODUCTION

1.1 Background

Mosquito-borne diseases are still a big threat to public health systems, worldwide. These include dengue of which incidence and spread are occurring at ever fast rates with 50 million infections and over 20,000 - 30,000 deaths yearly (WHO, 2006; Kroeger and Nathan, 2006; Kouri *et al.*, 2007). The disease has since spread over almost all tropical areas (Gubler, 2006), thus posing a threat to 55% of the world's population present in over 124 countries (Beatty *et al.*, 2007). In Asia and the Pacific this ratio increases to 70% (WHO, 2009a). This includes Malaysia where dengue is on the rise: from 2009 to 2010, the number of cases has risen from 33,684 to 40,152 with 118 deaths (WHO, 2010). In the early phase of 2011, 2,471 cases were recorded (WHO, 2011) and this may be just a curtain-raiser for more dengue incidences.

Effort to control dengue has mainly involved insecticide spraying programme, but this strategy has proven incompetent (WHO, 1999) due to development of resistance by its vector mosquitoes (Rodríguez *et al.,* 2002, 2007; Flores *et al.*, 2006; Strode *et al.*, 2008; Marcombe *et al.*, 2009a & 2009b; Polson *et al.,* 2011) and environmental health hazards. The other control strategies such as using natural insecticides (Amusan *et al.*, 2005; Chung *et al.*, 2010), biological agents (Scholte *et al.*, 2007; Becnel & White, 2007; Lapied *et al.*, 2009; Pelizza *et al.*, 2010; Ansari *et al.*, 2011) and vaccine has recorded little success at the field level (WHO, 2009b), while the use of sterile insect technique (Benedict and Robinson 2003; Catteruccia *et al.*, 2009; Nolan *et al.,* 2011) and genetically modified mosquitoes (Atkinson *et al.*, 2007; Wilke *et al.*, 2009; Bargielowski *et al.*, 2011;

Wise de Valdez *et. al.,* 2011) are still controversial and an intensely-debated topic in the scientific world (Alphey *et al.,* 2010). Therefore, vector control remains the only viable method to prevent dengue transmission (Deen, 2004; Guzman *et al.,* 2004). Thus, better understanding of the breeding behaviour and reproductive characteristics of vectors mosquitoes *i.e.,* egg bio-ecology are relevant.

Malaysia has a long history of dengue incidence, which started at the beginning of the 20th century with the first dengue outbreak in Penang in 1902 (Skae, 1902; Daniels, 1908). Considered endemic in Malaysia, dengue occurred in many areas regardless of the urbanization level. *Aedes aegypti* and *Ae. albopictus* are known vectors for this disease. *Aedes aegypti,* originated and migrated from the African forest, was initially found only on the coast of Peninsular Malaysia (Daniels, 1908; Leicester, 1908; Stanton, 1920) then gradually moved inland and completed its spread by 1990 (Smith, 1956, Lee and Hishamudin, 1990). *Aedes albopictus* is known as the Asian tiger mosquito and is an indigenous species in Malaysia. Both species are incriminated dengue vector in this country. The early history of dengue epidemics in this region (Smith, 1956) broadly followed that of the invasion by *Ae. aegypti* and was restricted to the urban centre (Hammon *et al.,* 1960; Chew *et al.,* 1961; Rudnick and Chan, 1965). Now both species are capable to maintain dengue viruses in the immature stages through transovarian transmission (Yap, 1984; Chan and Counsilman, 1985; Lee *et al.,* 1997; Rohani *et al.,* 1997).

In Penang, previous larval surveillance of *Ae. aegypti* have shown considerable changes in its population densities. During the mid-1950’s, Macdonald (1956a) recorded a house index of 26, which increased in subsequent years. Concomitant to these increases, a minor dengue fever outbreak occurred in 1962-64 (Rudnick *et al.,* 1965) and a major one in 1973-74 (Cheong, 1978) in the city of

Georgetown. Through this latter outbreak which involved dengue haemorrhagic fever cases, the prevalence of *Ae. aegypti* attained a house index of 41. In late 1970s, the larval population densities of this mosquito have drastically dropped (Cheong, 1978; Cheong, 1986; Lee *et al.*, 1989; Lee and Hishamudin, 1990), presumably owing to the continuous control programmes launched since the 1973-74's outbreak. In 1975, an ovitrap surveillance conducted in Penang by Yap (1975) revealed a high prevalence of *Ae. albopictus* compared to that of other mosquitoes, including *Ae. aegypti*. The progressive decreases of *Ae. aegypti* population combined with the occurrence of dengue infection cases and the increased population size of *Ae. albopictus* led to the incrimination of the latter species in the transmission of dengue in Penang Island (Rozilawati *et al.*, 2007; Nur Aida *et al.*, 2008).

The occurrence of disease transmission has been often associated with the population density of the insect vector. There is a density level, called threshold below which transmission is low to nonexistent. Thus, accurate measurement of this threshold is central to strategies aimed at predicting and managing mosquito-borne-diseases including dengue. Addressing the development and implementation of management strategies, Russell *et al.* (2005) argued for the need to fulfil what they called "essential prerequisite," which they considered to be the identification of key species and accurate and reliable information on their breeding habits, distribution as well as their dispersal potential.

A substantial body of research works had been done to understand the breeding behaviours and preferences of dengue vectors. Overall, *Ae. aegypti* is considered to breed, especially in urbanized areas, whereas *Ae. albopictus* prefers rural areas and to some extent, suburban (Teng *et al.*, 1999). In Malaysia, both species have been found indoors and outdoors regardless of level urbanization

(Cheong, 1967; Lee and Hishamudin, 1990; Lee, 1991; Sulaiman *et al.*, 1991). *Aedes aegypti* is highly anthropophilic (Huber *et al.*, 2008) and prefers to feed during the day and to rest inside houses (Scott *et al.*, 1993a; WHO, 1999). Female *Ae. aegypti* show a preference for laying their eggs in domestic containers (Hawley, 1988), but may also use rainwater-accumulating containers present in peridomestic environments (Chan *et al.*, 1971a; Pamplona *et al.*, 2009). *Aedes aegypti* is believed to lack the marked domesticity while *Ae. albopictus* is known as an opportunistic and aggressive biter with a wide range of the host, including humans and a variety of vertebrates (Niebylski *et al.,* 1994; Tandon and Ray, 2000). The variability in domesticity, an important factor in maintaining constant and close contact between a disease vector and its host, has rarely been investigated in the dengue vector community of Penang.

Successful landing on a vertebrate host generally leads to the uptake of a blood meal. In mosquitoes, blood feeding, a process during which a female mosquito can acquire blood proteins necessary for egg production appears as a phenotypic expression of reproductive investment (Roitberg *et al.*, 1993). The level at which these proteins are present in the midgut of the female is influential to its reproductive output. Indeed, increases in both number and size of blood meals result in increased individual egg mass and number of eggs (Leisnham *et al.*, 2008). The act of blood feeding is also the time during which, the female can transmit and/or pick up pathogens. As such, a greater risk of disease transmission is predicted with an increased frequency of host-infected female contacts.

In recent years, the economic impact of dengue management through vector control, hospitalization and medication has drastically increased worldwide. In Southeast Asia, mean annual cost of dengue control per 1,000 population varied between US$15 and US$2,400 from 1998 to 2000 (Shepard *et al.,* 2004). These figures are expected to increase with expanding new areas with dengue vectors and amplifying new cases. Many factors could help to overcome this trend. One possibility would be to identify the most productive habitats and conduct direct larval control efforts as suggested by Gu and Novak (2005). Although a diversity of container is used as breeding sites by dengue vectors, container productivity varies considerably. Addressing this issue, Tun-Lin *et al.*, (1995a) has considered those holding large numbers of pupae as key containers. Furthermore, they defined properties with three or more containers infested with larvae or pupae as key-premises, believed to play a key role in population maintenance (Chadee, 2004). Clearly, identifying and targeting key containers and premises have the potential to help reduce the population abundance of the targeted vector and presumably disease occurrence. By specifically directing control to such productive sites, the amount of insecticide to be used can be reduced and thus the cost implication of vector control.

Dengue vectors use various aquatic habitats, including natural and artificial containers as breeding sites. Their larvae have been collected from a wide range of containers such as water reservoirs, discarded tins, plastic containers, car parts, brick holes, dead leaves on the ground, tree holes and rock pools (Hawley 1988; Sota *et al.*, 1992; Simard *et al.*, 2005). In South America, small miscellaneous containers, buckets, drums were found to be highly productive (Focks and Chadee, 1997; Maciel-de-Freitas *et al.*, 2007). In many parts of Southeast Asia, drums and water reservoirs used for washing or drinking purposes were also reported to harbor high densities of the immature stages of dengue vectors (Bang and Pant, 1972; Chareonsook *et al.*, 1990; Kittayapong and Strickman, 1993; Thavara *et al.*, 2001; Sebastian *et al.*, 1990; Ishak *et al.*, 1997; Tsuda *et al.*, 2002). In both geographical areas, these container habitats were considered as key breeding sites. In some parts of Malaysia (Cheong, 1986), many larval breeding sites have been identified, but in Penang Island, there is still no definitive answer as to which containers are key sites for the breeding of dengue vectors.

As any mosquito breeding site, container habitats mediate cues that influence the oviposition behaviour of mosquito vectors, including dengue vectors (Isoe and Millar, 1996). In general, the females of Aedine mosquitoes, including those forming the dengue vector community in Penang Island prefer to deposit their eggs preferentially on moist substrates. Such substratum is generally located at sites where there has been standing water previously (Hill *et al.*, 2006) and where flooding will likely occur at some time in the future (Hill *et al.*, 2006). In fact, freshly oviposited eggs must retain sufficient moisture for successful embryonation (Strickman, 1980). Thus variability in larval eclosion in response to moist variation is predicted.

The prevalence of the larvae in these habitats depends largely on rainfall, which is therefore, the major water source (Fish and Carpenter, 1982). Although evidence exists that rainfall is responsible for the abundance of *Ae. albopictus* (Lo and Narimah, 1984), heavy rains have negative effects on the egg population (Hornby *et al.*, 1994). Therefore, it is likely that there is a trade-off between sufficient rainfall and habitat population. This is because heavy rainfall could create new habitats and the overflowing of existing ones; which may off-set the quality of the older habitats. As Malaysia has a year-round equatorial climate and high levels of both sunshine and rainfall (Ahmad *et al.*, 2006), the breeding sites may be subjected to constant overflow and drying events that trigger large variations in moisture conditions within the habitats. Larvae and pupae of mosquitoes, including *Aedes* live in water and reach the air-water interface from time to time to obtain oxygen (Paaijmans *et al.,* 2007). During heavy rains, the rain drops hit the water surface in the containers, thus splashing some water out of the containers. During splashing of water, the larvae and pupae in the container could be swept out. As such, direct negative effects of heavy rains in the population size of dengue vectors are expected.

The relationship between larval eclosion and post oviposition moisture conditions is very close. This has been well documented in *Aedes* mosquitoes (Buxton and Hopkins, 1927; Gjullin *et al.,* 1950; Horsfall, 1956). Egg hatchability in dengue vector mosquitoes has also been shown to largely depend on moist levels just after oviposition. Hardwood and Horsfall (1959), working with *Ae. aegypti*, reported increased hatchability when embryos were pre-conditioned in highly moistened environments. In a related work, Dieng *et al.* (2006a), working with *Ae. albopictus* embryos, found that those reared in a high moisture environment hatched at a higher rate when compared with their counterparts submitted to a drier environment. As in most insects, embryogenesis in *Aedes* mosquitoes is a biochemical process characterized by many metabolic events, including protein synthesis and enzymatic activities. In the well-studied *Drosophilla melanogaster*, known to exhibit a similar embryonic development with *Ae. aegypti* (Bate and Arias, 1993; Vital *et al.*, 2010). The protein levels in young *D. melanogaster* embryos are correlated with glycogen content (Gutzeit *et al.*, 1994), thus suggesting protein synthesis variations, as embryo development proceeds with a pace. Furthermore, the amount of carbohydrates was shown to decrease from late oocyte stages until after 2 h of embryogenesis, and increases up to the blastoderm stage, during later development (Yamazaki and Yanagawa, 2003). In *Ae. aegypti*, Li and Christensen (1993) observed increasing hatchability as embryonic phenol oxidase content increased. Concerning these reports, it seems likely that there is a link between moisture uptake during embryogenesis and changes in protein synthesis.

1.2 Objectives

The primary goal of this thesis is to provide a greater understanding of the behavioral ecology of dengue vectors and some key physiological and molecular traits underlying their population maintenance and increase potential, all of which outlines potential risks and control perspectives.

Specifically, this thesis embarked to:

1) To survey the dengue vector (DV) population in the vast majority of residential areas of Penang Island and determine their seasonal patterns;
2) To identify the breeding location preferences and key containers for DVs;
3) To investigate the potential reproductive and epidemiological implications of particular observed breeding behaviour (s);
4) To investigate the effects of key seasonal parameter (s) i.e., rain on population density and oviposition behaviours;
5) To assess the effects of key microhabitat parameter (s) i.e., moisture on oviposition and egg hatch responses;
6) To characterize proteome evolution during embryogenesis relative to moist conditions

It is expected that by achieving these different specific objectives, we will have a useful background on the dengue vector population of Penang that could form the basis of a sound dengue management not only on the Island, but also areas with similar conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Global dengue situation

Dengue is the most prevalent and rapidly spreading mosquito-borne viral disease in the world. The incidence has increased 30-fold, expanding into new geographic areas (Franco *et al.,* 2010; Ross, 2010) which causes 75% of the current global disease burden (WHO, 2009b). It has a worldwide distribution and is spread over almost all tropical and subtropical countries (Gubler, 2006) predominantly in urban and semi-urban areas. About 55% of the world's population (3.46-3.61 billion) over 124 countries are at risk of dengue (Beatty *et al.*, 2007). In Asia and the Pacific this ratio increases into 70% and approximately 1.8 billion populations are at risk of this disease (WHO, 2009b). The incidence is increasing with an estimated 50 million new dengue infections every year. It causes a significant health, economic and social burden globally and was estimated for 2001, which was equals to 528 disability-adjusted life years (DALY is a new measure of the burden of disease. It is the combination of "time lived with a disability and the time lost due to premature mortality") (Cattand *et al.,* 2006).

2.2 Dengue causing agents

Dengue is caused by a small single-stranded RNA virus with four closely related distinct serotypes (DENV-1 to DENV-4), which belongs to the genus *Flavivirus,* family Flaviviridae (Holmes and Burch 2000; Wilder-Smith and Schwartz, 2005; Morens, 2009). Each serotype further can be divided into three to five different genotypes which has made it difficult to determine the mechanisms involved in the pathogenesis of dengue viruses. However, DENV-1 serotype was first isolated in 1943 and the other serotypes were isolated between 1944 and 1957 (Kimura and Hotta, 1944; Hotta, 1951; Hotta, 1952; Kuno, 2007). The viruses are thought to be originated from forest non-human primates (Rudnick, 1986). But the current dengue virus strains which are circulating within human populations are different from forest strains (Wang *et al.,* 2000). The "Asian" genotype, DENV-2 has been focused to provoke the most severe form of dengue (Messer *et al.*, 2003; Vazquez-Prokopec *et al.,* 2010; Tchankouo-Nguetcheu *et al.*, 2010). But overall, DENV-1 and DENV-3 have been identified to cause severe disease at primary infections as well as newly emerging types of dengue viruses in Europe and Africa (La Ruche et al. 2010; Gautret et al. 2010) while DENV-2 and DENV-4 are found to be involved in frequent dengue outbreaks at secondary infections (Leitmeyer, 1999; Vaughn, 2000; Vaughn *et al.,* 2000; Fried *et al.*, 2010; Murphy and Whitehead, 2011).

2.3 Transmission of dengue

The dengue viruses are taken up by vector mosquitoes during the blood-meal from an infected patient and multiply in its mid gut. Then it affects other cells and/or infects the salivary gland. After an incubation period of about 7-14 days, which depends on the mosquito strain, virus genotype, and environmental factors such as humidity and temperature (Black *et al.,* 2002; Watts *et al.,* 1987; Salazar *et al.*, 2007), viruses are transmitted to the other hosts during blood meals. Once a mosquito is infected with the virus, it is infected for life (Lee, 2000). People infected with the dengue viruse "maintain an infective viremia for up to 7 days during the febrile period" (Weinstein *et al.,* 1995). The important contributing factors in the infection of mosquitoes by the dengue virus is the level of immunity to the circulating virus serotype in the local human population (Halstead, 1990).

2.4 Dengue classification and symptoms

Dengue is defined by experts as a disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome (WHO, 2009b). It has a wide clinical spectrum with severe and non-severe clinical symptoms (Rigau-Perez *et al.*, 1997). Symptoms of dengue fevers vary from non-symptomatic infection to severe dengue haemorrhagic form (WHO, 2009b). The common symptom of a probable dengue infection may be flu-like illness with high fever, nausea, vomiting, rash, severe headaches, muscle and joint pains without or with different warning signs, i.e., abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness etc. Severe dengue may cause severe plasma leakage leading to dengue shock syndrome (DSS), severe bleeding or severe organ impairment.

WHO (1997) classified symptomatic dengue virus infection into three categories: undifferentiated fever, dengue fever (DF) and dengue haemorrhagic fever (DHF). The last one was further divided into four severity grades, with grade III and IV known as DSS. But widely used classification is DF/DHF/DSS (WHO, 1997; Bandyopadhyay *et al.*, 2006). The symptoms also can be divided on the basis of age (Halstead, 1980). Infants and children are with undifferentiated febrile illness or mild febrile disease with maculopapular rash. Older children and adults are usually with fever, headache, myalgia, and gastrointestinal symptoms, often terminating with a maculopapular rash. Primary infection of dengue is thought to induce lifetime protective immunity to the infecting serotype (Halstead, 1974). Individuals suffering an infection are protected from clinical illness with a different serotype within 2-3 months of the primary infection but with no long-term cross protective immunity.

2.5 Dengue vectors in the world

Aedes aegypti (Linnaeus) is the principal vector of dengue virus, chikungunya virus and yellow fever virus (Kow *et al.*, 2001; Gubler, 2002; Lambrechts *et al.*, 2010) while *Ae. albopictus* (Skuse, 1894) serving as a secondary vector of dengue in tropical as well as temperate regions of the world (Weaver and Reisen, 2010), including Japan, Seychelles, Hawaii, and Reunion Island (Harinasuta, 1984; Gratz, 2004; La Ruche et al. 2010). The later one is likely to be a more important vector of chikungunya in the countries bordering the Indian Ocean (Reiter *et al.*, 2006; Vazeille *et al.*, 2007; Delatte *et al.*, 2008a, 2008b), in Central Africa (Leroy *et al.*, 2009; Paupy *et al.*, 2009) and in Europe (Charrel *et al.*, 2008). *Aedes albopictus* is also a potential vector of yellow fever, Ross River virus (Knudsen, 1995; Russell, 2002), La Crosse encephalitis virus (Gerhardt *et al.*, 2001), and possibly Japanese encephalitis virus (Hawley, 1988). It has been reported as a very efficient laboratory vector of West Nile virus (Niebylski *et al.*, 1992; Sardelis *et al.*, 2002) and eastern equine encephalitis virus (Turell *et al.*, 1994). It is also a natural vector of *Dirofilaria immitis* (canine heartworm) in Italy (Cancrini *et al.*, 2003).

2.6 Dengue vectors in Malaysia

In Malaysia, dengue and chikungunya infections are both transmitted by *Ae. aegypti* and *Ae. albopictus* (Rudnick, 1965). In Southeast Asia *Ae. albopictus* has been repeatedly incriminated as a vector during dengue outbreaks (Jumali *et al.*, 1979; Shroyer, 1986). Both of the species are also capable of transovarian and venereal transmission of dengue virus (Rosen *et al.,* 1978; Lee *et al.,* 1997) and are equally efficient (Jumali *et al.,* 1979), but sometimes *Ae. albopictus* are shown to be more efficient (Rosen *et al.,* 1985). In Malaysia several workers have experimented the transovarial transmission of the dengue virus in both *Ae. aegypti* and *Ae. abopictus,* and detected the virus in field collected larvae (Rohani *et al.,* 1997; Joshi *et al.,* 2002; Rohani *et al.,* 2005). These findings confirmed the maintenance of virus in the immature stage through transovarian transmission.

2.7 Distribution of dengue vectors in Malaysia

Both of the dengue vectors, *Ae. aegypti* and *Ae. albopictus* are present in Malaysia since 1902 when DF was first reported (Skae, 1902). *Aedes albopictus*, the Asian tiger mosquito is indigenous and originated in the tropical forest of Southeast Asia and available in urban, sub urban and rural areas in Malaysia (Rudnick *et al.,* 1965). They breed both indoors and outdoors in a variety of containers as well as in ovitraps (Lee, 1991; Norzahira *et al.,* 2011). *Aedes aegypti*, which is thought to be imported from Africa (Tonn *et al.,* 1969; Gubler, 2008), was domesticated and spread into Asia through commerce and colonization. It was gradually introduced into Malaysia during the 19th century (Smith, 1956). At the beginning it was found only on the coast (Daniels, 1908; Leicester, 1908) which gradually moved inland (Stanton, 1920) and completed its spread in Peninsular Malaysia, Sabah and Sarawak by 1990 particularly in urban areas, both inside and outside houses (Smith, 1956; Hii 1977; Cheong, 1978; Chang and Jute, 1982; Lee and Hishamudin, 1990; Lee, 1991). At the beginning, more than half of the *Ae. aegypti* breeding was reported from outdoor containers (Macdonald, 1956b), and maintained as such (Lo and Narimah, 1984), and now they show equal preference to breed in both outdoor and indoor containers and ovitraps (Lee, 1991; Lee, 1992; Norzahira *et al.,* 2011). Mixed breeding is also a common phenomenon in this region. In a recent ovitrap study, Rozilawati *et al.* (2007) found 6-15% mixed breeding of *Aedes* mosquitoes in an outdoor location in Sungai Dua, an urban area of Penang Island. Chang and Jute (1994) reported 9% mixed breeding mainly in outdoor containers from an urban housing area in Sarawak, Malaysia.

The history of having dengue vector(s) in Penang Island is very old since the first dengue case was identified here in 1902. A high density of *Ae. aegypti* was reported in the early surveillance conducted by Macdonald (1956b), the house index then was 28 which increased to 41.1 during the first major outbreak in 1974/75 in Malaysia which was reduced to 0.89 in the last nationwide surveillance in 1988-89 (Lee and Hishamudin, 1990). However, the distribution of *Ae. aegypti* in Penang Island was confined in the city centre, Georgetown and its fringes (Yap, 1975; Yap and Thiruvengadam, 1979; Rozilawati *et al.*, 2007). The previous all ovitrap studies were reported the abundant *Ae. albopictus* population with a small percentage of *Ae. aegypti*. Phon (2007) found that *Ae. albopictus* dominated in both indoor and outdoor in Penang Island where *Ae. aegypti* prevailed only in the urban settlement (Lorong Mahsuri), and has begun to spread slowly to the south-western part of Penang Island. Nor Adzliyana (2006) found only *Ae. albopictus* in her ovitrap surveillance in USM campus. But, the nationwide larval surveillances since 1954 were reported comparatively low density of this species. Cheong (1967) found a breeding index of 10.6% for *Ae. albopictus* and in last nationwide surveillance in 1988/89 reported HI of 0.22 for the same species in Penang Island (Lee and Hishamudin, 1990). This contradictory result with the ovitrap surveillances may due to the small scale sampling in the nationwide larval surveillances, which may not reflect the real picture of the field. On the other hand, ovitrap surveillances do not focus on container and real vector distribution information. So there is a gap of information about recent distribution of these mosquitoes based on larval occurrence in natural and artificial containers.

2.8 Distribution of *Aedes aegypti* in the world

The principal dengue vector *Ae. aegypti* is widely distributed in the tropical and subtropical countries mostly between latitude 35^0N and 35^0S commonly within 1000 metres from the breeding sites (WHO, 2009b). It was introduced into America during colonial times (Tabachnick, 1991). A hemisphere-wide initiative in 1947 eliminated *Ae. aegypti* from Colombia (1952) to Mexico (1963) but re-infested after 1967. Nowadays, it has invaded the whole American continent from the United States, the Caribbean, Central and South America, down to Chile (Gubler and Trent, 1993; Christophides *et al.,* 2004). It is distributed in altitudes ranging from 2,200 m above sea level in Colombia.

The sub-Saharan Africa is considered to be a native geographic region for *Ae. aegypti* (Mattingly, 1957), infecting all countries and occurs in a broad range of environments, from sylvan to urban. In West Africa, this species has been responsible for historic epidemics of Yellow Fever Virus (Monath, 1991; Barrett and Higgs, 2007) and CHIKV (Thonnon *et al.,* 1999).

At the beginning of the 20^{th} century *Ae. aegypti* was abundant in southern Europe (Curtin, 1967; Aitken, 1954). It was common in Spain until the 1950's (Rico-Avello, 1953). In Italy it was very common up to World War II (Romi *et al.,* 2008). It was last seen in northern Italy in 1971 (Callot and Delecolle, 1972). After 1950s, *Ae. aegypti* disappeared from almost in the Europe and neighbouring countries (Schaffner *et al.,* 2001).

2.9 Distribution of *Aedes albopictus* in the world

Aedes albopictus, the Asian tiger mosquito, is indigenous to both tropical and temperate regions of East Asia. It has spread and expanded its range in the recent decades from as far north as Beijing, China, at 40º latitude in Asia to Africa, the America, Europe and Australia (Benedict *et al.,* 2007; WHO, 2009b). Due to the high biological adaptability and the ability to overwinter in embryonic diapause, they spread rapidly and colonize in different areas in the world (Mogi, 2011). In America, it was first reported in 1985 from Houston, Texas in the United States (Hawley *et al.,* 1987), dispersed northwards and spread over 23 states by 1995. It was simultaneously introduced into Brazil in 1986 and later into the Southern-Mexican state of Chiapas (Martinez and Estrada, 2003).

In Europe dengue was first recorded in the Mediterranean area in 1778 particularly in Spain (Angolotti, 1980). The vector mosquito *Ae. albopictus* was first recorded from Albania in 1979 (Adhami and Reiter, 1998) which later spread to other European countries around the Mediterranean Sea such as in Italy (Sabatini *et al.,* 1990; Dalla Pozza and Majori, 1992; Carrieri *et al.,* 2003), France (Schaffner and Karch, 2000), Serbia and Montenegro (Petrić *et al.,* 2001), Belgium (Schaffner *et al.,* 2004), Switzerland (Flacio *et al.,* 2004), Greece (Samanidou *et al.,* 2005), Spain (Aranda *et al.,* 2006), Croatia (Klobučar *et al.,* 2006), Slovenia, and Bosnia and Herzegovina (Scholte and Schaffner, 2007).

In Africa, *Aedes albopictus* was first reported in 1990 from South Africa (Cornel and Hunt, 1991) and in 1991 in Nigeria (Savage *et al.,* 1992). In recent years, it has spread to several Central African countries (Paupy *et al.,* 2009) where it occurs in most towns up to a latitude of 6^0 N (Simard *et al.,* 2005) and suspected to transmit DENV and CHIKV in Cameroon (Peyrefitte *et al.,* 2007) and Gabon (Paupy *et al.,* 2010).

2.10 Breeding habitats of dengue vectors

Aedes mosquitoes prefer to breed in different types of natural and artificial containers holding clean and rain water. Eggs are laid on the moist container walls and resistant to desiccation for months, and larvae emerge when eggs submerged in water. *Aedes aegypti* is strictly domiciled, prefer less vegetation and biting indoor except for some African strains (Sucharit *et al.,* 1978; Foo *et al.,* 1985; García-Rejón *et al.,* 2011). They breed in a wide range of artificial containers in the domestic environment (Kyle and Harris, 2008), unusual habitats such as rock holes (Parker *et al.,* 1983), tree holes (Anosike *et al.,* 2007; Tubaki *et al.,* 2010; Mangudo *et al.,* 2011) but not in leaf axils (Burkot *et al.,* 2007) in outdoor habitats.

The aggressive anthropophilic and daytime biting *Ae. albopictus* is widely known outdoor breeder and commonly found in a wide range of natural and artificial containers (Hawley, 1988; Forattini *et al.,* 1998b; Richards *et al.,* 2008; Bartlett-Healy *et al.,* 2011) including a number of unusual habitats such as ground pools (Forattini *et al.,* 1998a), water pools, cement floors, 20 stories above the ground (Ishii, 1987; Nathan and Knudsen, 1994).

2.10.1 Breeding habitats of dengue vectors in Malaysia

A large variety of commonly found containers are the breeding sources of dengue vector in Malaysia. Any artificial container, coconut husk, bamboo stump, or tree hole is quite likely to contain *Ae. albopictus* and *Ae. aegypti* immature (Ho *et al.*, 1973). *Aedes albopictus* reproduce both in artificial and natural breeding sites in Malaysia, and usually found at forest fringes, in secondary forests, and in green areas in towns (Abu Hassan, 1994; Macdonald, 1956b).

Several nationwide larval surveillances indicated different preferential breeding containers by this vector. Macdonald (1956b) reported ant-traps, earthenware jars and bathtubs as the key containers since they comprised more than 90% of total positive containers in Malaysia. Cheong (1967) identified them to produce about 70% of the positive containers. He identified the main indoor breeding containers as ant-traps (26.5%) and earthenware jars (25.2%) while the outdoor containers were earthenware jars and storage drums. In addition, tires and flower pots were preferential breeding habitats. However, two decades later miscellaneous containers such as buckets, basins, bowls etc. (27.8%) and concrete tanks (21.2%) were found as preferred *Ae. aegypti* breeding sites. There were no breeding detected in ant-traps, tires and flower pots (Lee and Cheong, 1987). In subsequent nationwide surveillances in 1984-85 and 1988-89, concrete tanks were among the indoor containers, while and earthenware jars and miscellaneous containers were among the outdoor containers, which played major roles in the increase of vector population (Lee, 1990; Lee, 1991).

Some regional surveillance identified different key vector breeding containers in different places. In the urban areas of Selangor, Peninsular Malaysia, the main indoor breeding containers reported were the bathroom tanks whilst in both urban and rural areas, the preferred outdoor breeding containers were earthenware jars (Ho and Vyihilingam, 1980). The favourable breeding sites for *Ae. aegypti* in a new village in the suburbs of Kuala Lumpur were water storage containers, i. e. drums

(20%), concrete tanks (21.3%) and earthen jars (21.3%). Whereas *Ae. albopictus* preferred to breed in tins (15.8%), drums (15.8%) and various other containers (47.4%) including buckets, basins, bowls, frying pans, etc. In the residential housing estates, concrete tanks (33.3%) and flower vases (33.3%) were identified as *Ae. aegypti* breeding habitats, while earthen jars (25%) and flower pots (25%) identified as *Ae. albopictus* breeding sites (Lee and Cheong, 1987).

In east Malaysia, Chang and Jute (1982) found discarded tin cans (43.1%) as the most abundant breeding containers in all villages followed by plastic cups, pans or bowls (14.8%) in Lundu district, Sarawak. The discarded containers produced 57.9% of *Ae. albopictus*. Chang (1993) found *Ae. albopictus* breeding in 38% of the septic tanks surveyed in housing areas in Kuching, Sarawak. In the town of Sibu, the most preferred outdoor breeding habitats reported were plastic cups and used tires while indoor habitats were ant traps and flower vases (Chang and Jute, 1994).

Lam (1989) observed *Ae. albopictus* breeding in domestic septic tanks in Ipoh, Malaysia. Abu Hassan *et al.* (2005) identified construction sites (flooded floor, floor recessed opening, basement flooded floor, drains and water tanks) as a potential *Ae. aegypti* and *Ae. albopictus* breeding sites. In Johor the potential breeding sites were flower pots and pails in urban area, bowls in suburban area and, flower pots, vases and tyres in rural area (Nyamah *et al.*, 2010). Little is known about the specific and key breeding container(s) for *Aedes* in Penang Island.

2.10.2 Breeding habitats of dengue vectors in other countries

In Singapore ant-traps were identified as the most common indoor and earthenware jars were the most common outdoor breeding habitats for *Ae. aegypti* followed by earthenware jars, bowls, tanks, tin cans and drums (Chan *et al.*, 1971a).

In Samui Island, Thailand, the larval habitats are distinctly separate, i.e., *Ae. aegypti* preferred to breed in earthen jars and concrete water storages while *Ae. albopictus* breed in coconut husks and coconut floral spathes (Thavara *et al.,* 2001). In another study in Thailand, water jars were reported as the main important breeding containers of *Ae. aegypti*, while broken cans and plastic containers were the preferred breeding habitats of *Ae. albopictus* (Chareonviriyaphap *et al.*, 2003). In Indonesia, drum was found to be the most common outdoor breeding habitats of *Ae. albopictus* in the hill and mountain areas where as *Ae. aegypti* bred mostly in earthen jars in the village area (Ishak *et al.,* 1997). In central Laos *Ae. aegypti* was breeding mainly in water jars, cement tanks, drums and discarded containers (Tsuda *et al.,* 2002). Rainwater tanks contained many immature *Ae. aegypti* in Queensland (Tun-Lin *et al.*, 1995a). Small miscellaneous containers, buckets and outdoor drums were highly productive in Trinidad (Focks and Chadee, 1997). In Rio de Janeiro, water tanks and metal drums were generally the most productive container types, sometimes holding together up to 65% pupae (Maciel-de-Freitas *et al.*, 2007). There are some unusual containers such as rock holes producing *Ae. aegypti* (Parker *et al.*, 1983).

2.11 Replacement of dengue vectors

Competition is a widely known natural phenomena which causes competitive displacement. It can occur among the members of the same species which is known as 'Intra-specific competition', and 'inter-specific competition' occurs between individuals belonging to different species. The physical factors including environmental modification and biological factors (cannibalism, predation, active interference (e.g., inhibition of mating, feeding and ovipostition), disease, parasitism, and genetic drift) may contribute to species displacement (Moore and Fisher, 1969).

As a result of competition exotic species may colonize in new regions and new environmental conditions and compete with the pre-existing species (Carrieri *et al.*, 2003). But, the different species cannot simultaneously occupy the same niche (De Bach, 1966). Moreover, spatial and ecological coexistence of *Ae. aegypti* and *Ae. albopictus* has been documented in several parts of the world which is thought to result in competitive interaction. The larvae sometimes share common developmental sites (Braks *et al.*, 2003; Simard *et al.*, 2005; Chen *et al.*, 2006b) under the influence of environmental factors (Rey *et al.*, 2006; Tsuda *et al.*, 2006). In South American and Southeast Asian areas they segregate in different habitats as the two species are sympatric.

2.12 Replacement of *Ae. albopictus* by *Ae. aegypti*

On the basis of the principle of competitive displacement, there is a current belief that inter-specific competition has replaced the indigenous *Ae. albopictus* by the immigrant *Ae. aegypti* in cities of Asia (Service, 1992; Rudnick *et al.*, 1967). Macdonald (1956a) and Gilotra *et al.* (1967) reported competitive displacements of *Ae. albopictus* in laboratory experiments. The former author noticed comparatively more *Ae. aegypti* adults emerged than *Ae. albopictus* during mix rearing in earthenware jars and in tree-holes. Macdonald (1958) documented *Ae. aegypti* as the more successful domestic species to some extent in Malaya which was found only in coastal towns at the beginning of the 19^{th} century and established itself as the most common urban mosquito both inland and along the coast within 60 years.

Rudnick (1965) showed evidence of progressive replacement of the ‘competing’ native *Ae. albopictus*. He reported that *Ae. aegypti* has almost replaced *Ae. albopictus* in Bangkok. Stanton (1920) stated that "Within the past ten years, *Ae.*

aegypti has replaced *Ae. albopictus* in Kuala Lumpur. These two cities represent the extremes, with Manila, Singapore, and Penang representing intermediate stages". In a nationwide larval surveillance in Malaysia, Lee (1991) observed inter-species competition and reported that *Ae. aegypti* is beginning to edge out *Ae. albopictus* and the competition started with the use of household insecticide products which deter the mosquitoes from breeding in houses. Gould *et al.* (1968) also found evidence of the displacement of *Ae. albopictus* by *Ae. aegypti* on the Koh Samui Island, Thailand. *Aedes aegypti* was found in an advantage position in mixed rearing with *Ae. albopictus* by delaying larval development (Lee, 1994; Moore and Fisher, 1969). Rearing using different detritus resources (e.g. dead insects, yeast, and liver powder) showed approximate competitive equality or even an advantage for *Ae. aegypti* (Barrera, 1996; Daugherty *et al.,* 2000).

There are some contradictory reports for instance in early studies indicating the replacement of *Ae. albopictus* by *Ae. aegypti* in Southeast Asia. Lamborn (1920) reported that *Ae. albopictus* was the most abundant species in Kuala Lumpur. Reid (1954) and Rudnick (1965), stated without supporting data, that *Ae. albopictus* was more numerous than *Ae. aegypti* in Kuala Lumpur. A similar discrepancy appears in the reports of Senior-White (1934). He stated that *Ae. aegypti* appeared to have gradually replaced *Ae. albopictus* in Calcutta during the present century according to a relative prevalence of the two species in 1931 and 1933 and on the data reported by Brunetti in 1907 and by Paiva in 1912. The authors of these reports made their claims on the basis of the relative density of the two species and from their own observations which was conducted with a bias for one or the other species. Senior-White (1934) made the conclusion on the results of his indoor catches of adult *Ae. aegypti* and *Ae. albopictus* carried out in the early morning at 11 stations. It is well known that *Ae. aegypti* is predominantly an indoor mosquito while *Ae. albopictus* is predominantly an outdoor one. The method of collection of the two species by Senior-White was undoubtedly biased for *Ae. aegypti*. A biased collection method probably also accounts for Stanton's (1920) observation in Kuala Lumpur, as has been suggested by Reid (1954).

However, it is quite possible that this phenomenon can be brought about by progressive urbanization which tends to increase artificial containers and reduce naturally occurring containers, the amount of vegetation and outdoor shade for *Ae. albopictus* breeding (Hawley, 1988).

2.13 Replacement of *Ae. aegypti* by *Ae. albopictus*

Aedes albopictus spread during the 2nd World War from Asia to the Pacific Islands and rapidly reduced the range and abundance of *Ae. aegypti* throughout most of south-eastern USA (Hobbs *et al.,* 1991; O'Meara *et al.,* 1995) presumably by inter-specific competition (Rozeboom and Bridges, 1972; Juliano and Lounibos, 2005). Other likely reasons identified for this replacement include, (a) sterile offspring due to inter-specific mating (b) losing fitness of one species due to parasitic infection brought in with its counterpart and; (c) superiority of one species in larval resource competition (Lounibos, 2002).

Invasion of *Ae. albopictus* and displacement of *Ae. aegypti* have been documented in many countries around the world (Savage *et al.*, 1992; Pan American Health Organization, 1993; Knudsen, 1995) especially in south-eastern U.S.A. and Brazil (Lounibos, 2002; Juliano and Lounibos, 2005), and is suspected in La Reunion and Mayotte (Bagny *et al.,* 2009a, 2009b). It has been listed as one of the world's worst invasive species by the World Conservation Union (Lowe *et al.,* 2000). The outcome of competitive interactions has not yet been studied between this two species in an African context.

Local disappearance or decline of *Ae. aegypti* that followed the spread of *Ae. albopictus* in south-eastern states of North America has been well documented (O'Meara *et al.*, 1995). According to Usinger (1944), both species were introduced into Hawaii, and by 1892 *Ae. aegypti* was widespread whereas *Ae. albopictus* did not become numerous until 1902. In 1911, the two species were in equal proportion but in 1912, *Ae. albopictus* outnumbered *Ae. aegypti* by 4 to 1. In 1913, *Ae. aegypti* was found to outnumber *Ae. albopictus* by 2 to 1. *Aedes albopictus* was dominant again in 1914, by 12 to 1, and continued to be dominant in 1915 and in 1926. During the dengue epidemic of 1943-44, as many as 85 % of the day-time mosquitoes were *Ae. albopictus* and only 15% were *Ae. aegypti*. In Guam, *Ae. aegypti* was the dominant species in 1945 but, following a control programme, this species was not found in 1948 and 1951 whereas *Ae. albopictus* was common (Hull, 1952). Hu (1953) claimed that "*Ae. albopictus* is more versatile and has replaced *Ae. aegypti* in Guam and Honolulu."

There were some laboratory experiments reported the competent characteristics of *Ae. albopictus* over *Ae. aegypti* and *Aedes triseriatus*. *Aedes albopictus* had greater survivorship, resource-harvesting ability, higher metamorphic success, rapid eggs hatching and emergence in the presence of the predatory larvae of the native mosquito *Toxorhynchites rutilus* (Lounibos *et al.,* 2001; Yee *et al.*, 2004). *Aedes albopictus* was found capable of exploiting artificial microhabitats even in case of food scarcity and had greater efficiency in converting food into biomass which helps to bring rapidity in larval development (Carrieri *et al.,* 2003). It is also