STUDIES ON THE MOSQUITO FAUNA IN AN URBAN AND SUBURBAN AREA IN PENANG AND THE LABORATORY EFFICACY OF MOSQUITO COILS CONTAINING DIFFERENT ACTIVE INGREDIENTS AGAINST SELECTED VECTOR MOSQUITOES

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By

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LIST OF ABBREVIATION

Ae.	*Aedes*
An.	*Anopheles*
Ma.	*Mansonia*
Cx.	*Culex*
KT	Knockdown time
min	Minute
TPI	Taman Permai Indah
KPG	Kampung Pasir Gebu
OI	ovitrap index
RH	relative humidity
^{0}C	Celsius
%	Percentage
$\pm$	plus minus
SE	Standard error
Temp	Temperature
P	Significant
R	Correlation

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KAJIAN TERHADAP KEPELBAGAIAN FAUNA NYAMUK DI KAWASAN BANDAR DAN PINGGIR BANDAR DI PULAU PINANG DAN KEBERKESANAN LINGKARAN UBAT NYAMUK YANG BERLAINAN KANDUNGAN BAHAN AKTIF TERHADAP NYAMUK VEKTOR TERPILIH

ABSTRAK

Satu kajian terhadap kepelbagaian fauna nyamuk di kawasan bandar dan pinggir bandar di Pulau Pinang telah dijalankan selama 14 bulan iaitu di Taman Permai Indah (kawasan pulau) dan Kampung Pasir Gebu (semenanjung). Dengan mengunakan teknik perangkap umpan lembu dan tangkapan menggunakan umpan kaki, telah didapati bahawa sebanyak 675 (22.80%) *Culex gelidus* yang merupakan spesis terbanyak di Kg Pasir Gebu diikuti oleh 514 *(*17.37%) *Anopheles peditaeniatus,* 383 (12.94%) *Anopheles sinensis,* 254 (8.58%) *Mansonia uniformis*, 252 (8.52%) *Anopheles subpictus*, 192 (6.49%) *Anopheles campestris* dan 135 (4.57%) *Anopheles vagus.* Nyamuk-nyamuk tersebut telah didapati lebih tertarik kepada umpan lembu berbanding umpan manusia. Penyampelan populasi telur di luar kediaman juga telah dijalankan dengan menggunakan perangkap telur atau ovitrap. *Aedes albopictus* merupakan spesis *Aedes* yang paling banyak di kawasan ini. *Aedes egypti* dan *Culex quinquefasciatus* juga telah didapati berada di dalam ovitrap yang sama tetapi hanya pada peratusan yang rendah. Keputusan ini menunjukkan bahawa *Aedes albopictus* adalah vektor denggi utama di kawasan kajian. Satu korelasi yang kuat telah didapati antara jumlah hujan dan populasi telur di kedua-dua kawasan kajian (r=0.982 dan r=0.918). Jumlah telur yang dikumpul pada musim hujan yang rendah adalah lebih banyak berbanding musim hujan yang tinggi. Nisbah antara nyamuk jantan dan betina yang terhasil juga menghampiri satu (TPI= 0.93 ± 0.33 dan KPG= 0.97 ± 0.42). Keberkesanan formulasi lingkaran ubat nyamuk terpilih telah dijalankan di makmal terhadap empat vektor nyamuk terpilih (*Aedes aegypti, Aedes albopictus, Culex quinquefasciatus* dan *Anopheles sinensis*). *Anopheles sinensis* merupakan spesis yang paling rentan terhadap semua formulasi, manakala *Culex quinquefasciatus* merupakan spesis yang paling rintang berdasarkan kepada keputusan nilai KT_{50}, KT_{95} dan kadar kematian. Strain nyamuk di makmal juga telah didapati lebih rentan berbanding strain nyamuk dari lapangan.

STUDIES ON THE MOSQUITO FAUNA IN AN URBAN AND SUBURBAN AREA IN PENANG AND THE LABORATORY EFFICACY OF MOSQUITO COILS CONTAINING DIFFERENT ACTIVE INGREDIENTS AGAINST SELECTED VECTOR MOSQUITOES

ABSTRACT

A study of the mosquito fauna in an urban and suburban area in Penang Island was carried out for 14 months namely in; Taman Permai Indah (on the island) and Kg Pasir Gebu (mainland). Using the cow baited trap and bare leg catch techniques, it was found that *Culex gelidus* totalling 675 (22.80%) was the most abundant in Kg Pasir Gebu followed by 514 *(*17.37%) *Anopheles peditaeniatus ,* 383 (12.94%) *Anopheles sinensis,* 254 (8.58%) *Mansonia uniformis*, 252 (8.52%) *Anopheles subpictus*, 192 (6.49%) *Anopheles campestris* and 135 (4.57%) *Anopheles vagus.* The mosquitoes were more attracted to cow than human. Outdoor ovitrap surveys were also carried out in the urban and suburban sites and it was found that *Aedes albopictus* was the most abundant *Aedes* species in this area, even though a small percentage of *Aedes aegypti* and *Culex quinquefasciatus* was found to breed simultaneously in the same ovitrap. This indicated that the main dengue vector is *Aedes albopictus*. A strong correlation was found between rainfall and number of eggs in both of the study sites(r=0.982 and r=0.918). The eggs collected were more abundant during low rainfall (dry season) than during higher rainfall (wet season). The ratio between males and females that emerged from the eggs collected was also close to one (TPI=0.93$\pm$ 0.33 and KPG=0.97$\pm$0.42). The effectiveness of five selected mosquito coil formulations was also studied against four selected vector mosquitoes (*Aedes aegypti, Aedes albopictus, Culex quinquefasciatus* and *Anopheles sinensis*). *Anopheles sinensis* was the most susceptible against all the formulation, whereas *Culex quinquefasciatus* was found to be the most tolerant species against all the formulation based on the KT_{50}, KT_{95} and mortality values. The laboratory strain mosquitoes were also more susceptible than the field strain.

CHAPTER ONE

GENERAL INTRODUCTION

Mosquitoes are small insects belonging to the family Culicidae of the order Diptera. Mosquitoes are unquestionably the most important vectors of diseases (Brenda *et al*., 2000).They are important because of the effects on human welfare by direct annoyance as nuisance biters and most of all due to the role they play in the transmission of diseases (Service, 1993). Mosquitoes are still a persistent problem in Malaysia. According to Abu & Salmah (1990), in Malaysia including Sabah and Sarawak, there are 431 species representing 20 genera of mosquitoes.

Studies on the distribution and relative abundance of mosquitoes which frequent houses in urban/suburban areas indicated that *Culex quinquefasciatus* (Say), *Aedes albopictus* (Skuse) and *Aedes aegypti* (Linnaeus) are the most abundant (Yap, 1975; Yap *et al.,* 1978; Yap & Thiruvengadam, 1979; Yap *et al.* 1990a,b). Mosquito surveillance therefore plays an important role in formulating a good control programme (Service, 1993).

Mosquito-borne diseases such as dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most important arthropod-borne viral diseases of public health significance. Their geographical spread is increasing: only five countries documented dengue in the 1950's but to date there are more than 100 countries around the world reporting the incidence of DF and DHF (Guha-Sapir & Schimmer, 2005). Several important factors also have influenced the epidemiology of dengue.

Aedes (Stegomyia) albopictus (Skuse), 1894 known as the Asian tiger mosquito and *Aedes (Stegomyia) aegypti* (Linnaeus), 1762 (Diptera: Culicidae) are the principal dengue vectors and to date have become the main vectors in the transmission of dengue and dengue haemorrhagic fever in the tropical and subtropical regions (Smith, 1956; Rudnick *et al.*, 1965; Hammond, 1966; Knudsen, 1995). The distribution of *Ae.aegypti* and *Ae.albopictus* in Peninsular Malaysia has been well established (Lee, 1990).

In a study in an endemic dengue area in Selangor by Chen *et al.* (2005), it was found that mixed breeding of *Ae. aegypti* and *Ae. albopictus* occurred in the same container outdoors and indoors. Therefore, both mosquito species play an important role of in the transmission of the dengue virus. Furthermore it was reported by Lee & Inder (1993) that *Ae. aegypti* and *Ae. albopictus* are incriminated as dengue vectors in Malaysia.

Aedes albopictus is indigenous in tropical Asia but presently the distribution is world wide. The high incidence of dengue is closely associated with the abundance of the vectors. It was also reported that the abundance of the vectors is associated with environmental factors such as rainfall, temperature and relative humidity (Okogun *et al.*, 2003), while the wet seasons are associated with the higher prevalence of mosquito borne diseases.

Several control measure are available in combating mosquitoes. One of the most widely used mosquito control approaches is personal protection. The usage of household insecticides is the most favoured personal protection method used by consumers. Among them, mosquito coil is still widely used in

Southeast Asia. It is important to test the effectiveness of coils being used to avoid resistance development in mosquitoes and other side effects.

Therefore, this study was conducted to look at the mosquito fauna in selected urban and suburban areas in Penang Island and to determine the efficacy of several mosquito coil formulations against selected vector species. The general objectives of this study are:

- To determine the composition and seasonal abundance of mosquitoes in a selected urban and suburban areas in Penang Island.
- To determine the density, distribution and other physical parameters relating to the fluctuations of *Aedes albopictus.*
- To determine the laboratory efficacy of several formulations of mosquito coils against laboratory and field strains of *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus* and *Anopheles sinensis.*

CHAPTER TWO

LITERITURE REVIEW

2.0 Introduction

2.1 Mosquitoes

Mosquitoes are placed in the family Culicidae, suborder Nematocera of the order Diptera, the true flies (Barry & William, 1996). Culicidae contains 3500 species which are divided into three subfamilies: Toxorhynchitinae, Anophelinae and Culicinae (Knight & Stone, 1977). *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* are genera of mosquitoes that are of medical importance because of their habit of biting humans for blood (Service, 1995a; Abu Hassan &Yap, 1999).

2.2 Medical importance of mosquitoes

Mosquitoes are very successful vectors. Some species are capable of transmitting diseases such as dengue, yellow fever, chingkungunya and Japanese encephalitis (viruses), malaria (protozoa) and filariasis (nematode). *Aedes* are of major concern in Malaysia because they transmit the dengue virus (Lee, 2000). There are other species that are also of major concern as vectors such as *Anopheles sp.,* mosquitoes that transmit malaria and filariasis (Sulaiman, 2000), *Culex* sp. which transmit the Japanese encephalitis and urban filariasis (Adanan *et al.,* 2000), and *Mansonia* sp. which are known vectors of filariasis (Chang, 2000). Some other arboviruses that can be transmitted by mosquitoes are Eastern Equine Enchephalitis *(Coquilletidia perturbans),* Ross River, Murray Valley Encephalitis (*Culex annulirostris*), Sindbis, West Nile Virus (*Cx. univittatus*), Venuzuelan Equine encephalitis, St. Louis Encephalitis, Rift Valley Fever (*Cx. pipiens*), Western Equine Encephalitis *(Cx. tarsalis*), Japanese Encephalitis (*Cx. tritaneiorhynchus*), yellow fever (*Ae. aegypti, Ae. africanus, Ae. simpsoni, Haemagogus sp*.) and La Crosse Encephalitis (*Aedes triseriatus*)(Monath, 1988).

2.3 Dengue

In tropical countries around the world, dengue is one of the most common viral diseases spread to humans by mosquitoes. Tens of millions of cases of dengue fever and up to hundreds of thousands of cases of dengue hemorrhagic fever occur each year. Globally an estimated 2 billion people are at risk of dengue while over 100 million people a year are infected with about 100,000 deaths (Gubler, 1997; CDC, 2005). Dengue remains of great public health importance in many tropical countries, causing considerable morbidity and significant mortality.

Dengue occurs in subtropical and tropical countries in the world (CDC, 2004). The spread of dengue is now considered a worldwide problem, since the global prevalence of dengue has grown dramatically in recent decades (WHO, 2002).

Dengue is a disease caused by a retrovirus belonging to the family of Flaviviridae, genus *Flavivirus* (Urdaneta *et al.,* 2005). It is transmitted by a mosquito vector of the genus *Aedes*. There are 4 serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4) and all are co-circulating in Malaysia (Gubler & Clark, 1996; Abubakar & Shafee, 2002). In 2005, dengue was the most important mosquito-borne viral disease affecting humans; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission (CDC, 2005).

In Malaysia, the first reported DHF cases was in Penang in 1962 (Rudnick *et al.*, 1965) while classical dengue was first reported in 1901-1902 in Penang by Skae (1902). Major outbreaks were reported in 1974, 1978, 1982, 1990 and 1995 (Lam, 1993; Poovaneswari, 1993; Hairi *et al.,* 2003). Since then, the disease has become endemic throughout the country (Singh, 2000). Up until November 2005, there were 3098 cases reported in Penang, with 7 deaths (MOH, 2005). In the last decade, cases of dengue have become more severe (Hairi *et al.,* 2003). The incidence rate of dengue has increased from 8.5 to 123.4 per 100, 000 respectively in 1988 and 1998 (Chua *et al.,* 2005). The infection is predominant in urban areas where 61.8% of the total population lives and the rapid industrial and economic development created many man made opportunities for *Aedes* mosquito breeding (Teng & Singh, 2001).

Dengue vaccines have been touted as the most effective control measure for the disease (Lam, 1994). However no licensed vaccine is available to date. As there is no effective vaccine to prevent and no specific treatment for dengue, vector control remains the best strategy to prevent the disease. In Malaysia, four strategies are applied: (1) Anti-larval measures; (2) Anti-adult measures; (3).Health education and (4) Enforcement of the Destruction of Disease Bearing Insects Act (DDBIA) (Hairi *et al.,* 2003). Vector control is the only option currently available to contain dengue outbreaks (Arunachalam, 1999).

2.4 *Aedes* as vectors

Aedes aegypti and *Aedes albopictus* are important vectors of dengue in Malaysia (Vythilingam *et al.*, 1999). According to Macdonald (1956), in Peninsular Malaysia (known as Malaya before), *Ae. albopictus* is a very common species and its breeding preferences overlapped those of *Ae. aegypti*. Reid (1954), Smith (1956), Rudnick *et al.* (1965) and Hammond (1973) have also reported that *Ae. aegypti* and *Ae. albopictus* are dengue vectors and to date have become the main vectors in the transmission of dengue and dengue haemorrhagic fever in tropical and subtropical regions worldwide (Yap *et al*., 1994**;** Knudsen, 1995; CDC, 2004). The distribution of *Ae.aegypti* and *Ae.albopictus* in Peninsular Malaysia is well established (Lee, 1990). A recent taxonomic review proposed to elevate the subgenus *Stegomyia* to the rank of genus. The new nomenclature proposed for these two species are, *Stegomyia albopicta* (Skuse), 1894 and *Stegomyia aegypti* (Linnaeus), 1762 (Inform'ACTION, 2005). In the present work, and to avoid confusion, we will continue to use the first nomenclature i.e. *Aedes albopictus* and *Aedes aegypti*.

The classification of *Aedes aegypti* and *Aedes albopictus* is shown below (Knight & Stone, 1977):

Aedes aegypti (Linnaeus, 1762)	*Aedes albopictus* (Skuse, 1894)
Kingdom: Animalia	Kingdom: Animalia
Phylum: Artropoda	Phylum: Artropoda
Class: Insecta	Class: Insecta
Order: Diptera	Order: Diptera
Family: Culicidae	Family: Culicidae
Subfamily: Culicinae	Subfamily: Culicinae
Genus : *Aedes*	Genus : *Aedes*
Species: *aegypti*	Species: *albopictus*

Whilst *Ae. aegypti* is entirely domestic, *Ae. albopictus* has been found breeding both in and around dwellings (Vythilingam *et al*., 1999). Both species are very adaptable to both tropical and temperate climate (Hawley, 1988). Both species are container breeders and both may be found together (Vythilingam *et al*., 1999). They are also capable of using a wide range of suitable container habitats. The most typical habitats are artificial containers, tree holes and bamboo stumps near human dwellings (Hawley, 1988). At the beginning of the 20th century, *Aedes aegypti* was found only in coastal towns (Daniels, 1908; Leicester, 1908) and by 1920, it had already moved inland and was found in Kuala Lumpur (Vythilingam *et al.*, 1992).

According to Rudnick *et al.* (1965), from their studies on dengue studies in Malaysia between1962-1964 showed that *Ae. aegypti* was dominant in urban areas, whereas *Ae. albopictus* is abundant in the suburban, rural and forested areas. Sulaiman *et al.* (1991), in their study on the distribution and abundance of *Ae. aegypti* and *Ae. albopictus* in endemic areas of dengue/dengue haemorrhagic fever in Kuala Lumpur, indicated that *Ae. albopictus* was more dominant than *Ae. aegypti.* Many researchers also reported that *Ae. aegypti* was more common in urban areas (Ho & Vythilingam, 1980; Lee, 1991; O'meara *et al*., 1993; Lee, 2000) but the study conducted by Rohani *et al.* (2001) indicated that *Ae. albopictus* was dominant in both rural and urban areas. Both *Ae. aegypti* and *Ae. albopictus* are found in Malaysia, though *Ae.aegypt*i is not an indigenous species (Rudnick *et al.,* 1965).

The distribution of *Ae. aegypti* and *Ae. albopictus* in Peninsular Malaysia is well established (Lee, 1990) and has been found to overlap. Along with the establishment of *Ae. albopictus*, a decline in the density of *Ae. aegypti* has occurred in sites where their distributions overlap (Black *et al*., 1989; Nasci *et al*., 1989; Smith *et al*., 1990; Hobbs *et al*., 1991; O'Meara *et al*., 1992; 1993). The establishment and spread of *Ae. albopictus* in the U.S is also associated with a reduction in the abundance and the range of the yellow fever mosquito *Ae. aegypti* (Hawley 1988; Hanson *et al.*, 1993).

Studies on the dispersion studies of *Aedes aegypti* conducted by Harrington *et al.* (2005) showed that in outdoor releases of males and females, the majority of recaptures were made in the house adjacent to their outdoor release location. The maximum dispersal distance detected was about 556–594 meters for females and 400–456 meters for males, while in indoor releases of females, the majority of recaptured mosquitoes (77%) were collected in the house from which they were released and the maximum dispersal distance detected was about 52 meters from the release site. Other studies on dispersion, conducted by Honório *et al.* (2003), have shown that *Aedes aegypti* and *Aedes albopictus* can be found as far as 800m.

In Southeast Asia, *Ae. albopictus* has been incriminated as a secondary vector of dengue while *Ae. aegypti* as the principal vector of the dengue viruses (Sulaiman *et al.,* 1996). *Aedes albopictus* inhabits all of Southeast Asia and parts of temperate Asia, where it transmits the dengue fever virus, *Dirofilaria immitis* (dog heartworm) and other pathogens (Hanson *et al.*, 1993). In addition to its ability to transmit yellow fever and dengue viruses, *Ae. albopictus* also is a competent laboratory vector of viruses endemic to the United States, including eastern equine encephalitis, La Crosse Encephalitis, St. Louis Encephalitis, Western Equine Encephalomyelitis Viruses, Eastern Equine Encephalitis Virus and Jamestown Canyon Virus (Shroyer, 1986; Scott *et al*., 1990; Mitchell, 1991; Mitchell *et al.,* 1992; Grimstad *et al.*, 1997; Moore & Mitchell, 1997).

2.4.1 Biology of *Aedes albopictus*

All mosquitoes urdergo complete metamorphosis to complete their life cycle. For *Aedes* sp. they only need clear water, but not necessarily clean water to complete their life cycle (Lee, 1990).

2.4.1.1 Eggs

About 48-72 hours after the females take a blood meal, they begin laying eggs. *Aedes albopictus* is a container-inhabiting species which lay its eggs in any water-containing receptacle in urban, suburban, rural and forested areas. The primary immature habitats of this species are artificial containers such as tyres, flower pots, cemetery vases, and even in natural containers such as tree holes, bamboo pots and leaf axils. This mosquito prefers to lay its eggs above the water surface on the dark rounded vertical surface. They deposit them just above the water line on damp substrate, such as mud or leaf or on the inside of tree holes (Service, 1995a). Different from other species, *Aedes* oviposit their eggs singly and are black in colour. Like other species in the Culicine group, the eggs are elongated and protected by a rigid, proteinaceous shell that minimizes water loss but permits gas exchange. The eggs can withstand desiccation, remain dry for months but still remain viable and hatch when soaked in water (Service, 1995a). According to Lee (2000), one female can deposit 102 eggs.

However newly deposit eggs cannot withstand desiccation (Kettle, 1990). The embryo needs time to develop, hence the eggs needs to be dried slowly (Kettle, 1990). After the embryo is fully developed, it can withstand desiccation for a few months. Several physical factors affect the egg hatching such as water temperature and oxygen pressure.

2.4.1.2 Larvae

Once the eggs hatch, the first instar larvae will emerge. The larvae require water to develop, no larvae can withstand desiccation. All stages of the larval instar (1^{st}, 2^{nd}, 3^{rd} and 4^{th}) are bottom feeders and only use their siphons to breath at the water air interface (Lee, 1990). Depending on the temperature and the availability of food, *Ae. albopictus* can complete its larval development between 5 to 10 days.

The *Ae. albopictus* larvae can be identified by several taxonomic characters. A particularly useful characteristic is the nearly complete saddle found in the early instar specimens of *Ae. albopictus* as well as the late instar larvae. The lateral hairs on the saddle are useful because they can be observed in living specimens without special orientation. The lateral hairs are double in *Ae. albopictus*. The four long caudal hairs of the dorsal brush in *Ae. albopictus* are also a useful character because they can be discerned at very low power. However, it should not be used as the sole character to identify this species.

Aedes albopictus is an opportunistic container breeder that is capable of utilizing natural as well as artificial container habitats. It has the ability to adapt to an exceptionally wide range of confined water sources. The mosquito is known for its ability to survive in very small collections of water, requiring only a depth of 1/4" to complete its life cycle. Larval habitats of the population discovered included discarded tires, 50 gallon drums, plastic buckets of various sizes, dishpan, plastic drinking cups, crushed aluminium beverage cans and cemetery vases (Lee, 1990; Rohani *et al.*, 2001).

2.4.1.3 Pupae

All pupae are aquatic, comma shaped and dark in colour. They are non-feeding aquatic forms. They spend most of the time at the water –air interface taking in air through the respiratory trumpets. If disturbed they swim up and down in the water in a jerky fashion (Service, 1995a). The life span of the pupae is between 2 to 3 days.

2.4.1.4 Adult

In 24 to 48 hours the pupae will emerge into adults. The adult body is divided into the head, thorax and abdomen. The head bears a pair of compound eyes and antennae as well as mouth parts. A pair of jointed legs is formed on each segment of the thorax. A pair of wings is found on the last 2 thoracic segments. The abdomen is composed of ten segments.

Adults males and females *Ae. albopictus* are covered with shiny black scales with distinct silver white bands on the palpus and tarsi. Its most striking taxonomic character is the band of silver scales forming a distinct stripe on the dorsal surface of the thorax and head. When they are full grown, the adults emerge from the pupae in the water and after resting on the water surface, they fly away to search for hosts to blood feed. *Ae. albopictus* is a very aggressive daytime biter with peaks generally occurring during the early morning and late afternoon. It feeds on a large number of hosts including man, domestic and wild animals and this generalized feeding behaviour contributes to its vector potential. Only female adults feed on animal blood while male adults feed on plant juice. A female mosquito has to obtain blood meal for eggs development. Habitats of the females can be permanent stagnant water, flowing water, temporary stagnant water or containers. Generally male mosquitoes only survive about one week but the females can live up to two to three weeks.

2.5 Other mosquito of medical importance

Other than *Aedes*, several species are of medical importance because of their habit of biting humans for blood meal such as *Anopheles*, *Culex*, *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* (Service, 1995a; Abu Hassan & Yap, 1999).

2.5.1 *Culex quinquefasciatus*

Culex quinquefasciatus is a medium size brownish mosquito. This species transmits bancroftian filariasis and is predominantly found in the tropics and temperate regions (Sharma, 2001). *Culex quinquefasciatus* larvae breed and thrive abundantly in stagnant dirty water (Mak, 1986; Hidayati *et al*., 2005). However at times it is also found together with *Aedes* in clear water. West Nile Virus has also been isolated from *Cx. quinquefasciatus* in Mexico (Darwin *et al.*, 2005); and Lousiana (Marvin *et al.*, 2005).

This mosquito is regarded as a nuisance in Malaysia. However, owing to the rapid urbanization and unplanned growth of cities, the risk of urban bancroftian filariasis transmission will also increase since this mosquito is a vector of urban bancroftian filariasis in other countries (Lee, 2005). *Culex quinquefasciatus* is a night biter. In Malaysia, though urban bancroftian filariasis has been eliminated; cases have been detected in migrant workers from endemic areas. Hence, the re-introduction of urban bancroftian filariasis is possible in the presence of *Cx. quinquefasciatus* (Lee, 2005).

2.5.2 *Culex gelidus*

Culex gelidus is a paddy field breeder in the countryside. It is highly zoophilic in nature (CRME, 1989*)* and prefers to bite large animals such as cattle and pigs rather than humans at night (Miyagi & Toma, 2000). *Culex gelidus* can be easily recognised by the white scales on the thorax. It rests inside houses, cattle sheds and tents.

According to Lee (2005), *Culex gelidus* is also found in India, China, Thailand, Indonesia, Timor and Irian Jaya. It has been reported as a veracious biter of humans indoors and having a preference for larger domestic animals with little preference for human (Colless, 1959) The larval stage can be found in freshwater ground pools, rivers, marshes and containers, dirty water and sometimes with considerable organic matter (Craig *et al*., 2005).

Culex gelidus is primarily a vector of *Wuchereria bancrofti*, chikungunya virus and getah virus. It is apparently refractive to *Dirofilaria immitis* (Dog heartworm) and *Brugia malayi* (Malayan filariasis) (Miyagi & Toma, 2000). *Culex gelidus* can also transmit the Japanese enchephalitis virus (JE). The JE disease remains endemic in several countries in Southeast Asia including Malaysia (Miyagi & Toma, 2000).

2.5.3 *Mansonia uniformis*

Mansonia uniformis, is a mid-sized mosquito of mottled brownish appearance. Adult *Ma. uniformis* appears to be active mostly at night, but also bites during the day in or near shelter. They can disperse a few kilometres from their habitats and readily attack humans as well as other animal including birds (Clements 1999). This mosquito has a highly antropophilic nature and only enter houses to feed (Iyenger, 1938; Wharton, 1962 and Mahapatra *et al*., 1995).

The breeding sites characteristics for this species include open swamp forest, neglected rice fields, blocked drains, rivers, canals and neglected ponds in urban and rural areas (Chang, 2000), whereas the main host plants are floating aquatic vegetation such as *Eichornia*, *Salvinia,* and swamp grasses.

Mansonia uniformis is primary a vector of *Wuchereria bancrofti* (bancroftian filariasis), *Brugia malayi* (malayan filariasis) and *Brugia pahangi* (tropical eosinophilia), chikungunya virus was also isolated from this species (Chiang & Loong, 1985; Miyagi & Toma, 2000).

2.5.4 Subfamily Anophelinae

Anopheles is the only genus in this subfamily which is medically important being the sole vector in the transmission of malaria. *Anopheles* mosquitoes breed in permanent bodies of fresh water with an abundance of aquatic plants that provide protection from fish and other predators. Eggs supported by floats are laid singly on the water surface. *Anophel*es mosquitoes can be distinguished from *Aedes* and *Culex* mosquitoes in several ways (Plate 2.1), as follows:

Identification of larvae:

1. Absent of siphon
2. Hair no 1 is modified like fan (palmate hair on abdomen)
3. *Anopheles* larvae float parallel to the surface of the water as opposed to hanging down at an angle.

Identification of adults:

1. *Anopheles* have patterned wings,
2. Adults rest on surfaces with their head lower that the abdomen while *Aedes* and *Culex* species rest with the head and abdomen parallel to the surface
3. The scutellum is rounded, (Culicine scutellum is trilobed).
4. Adult *Anopheles* females have palps that are almost as long as their proboscis

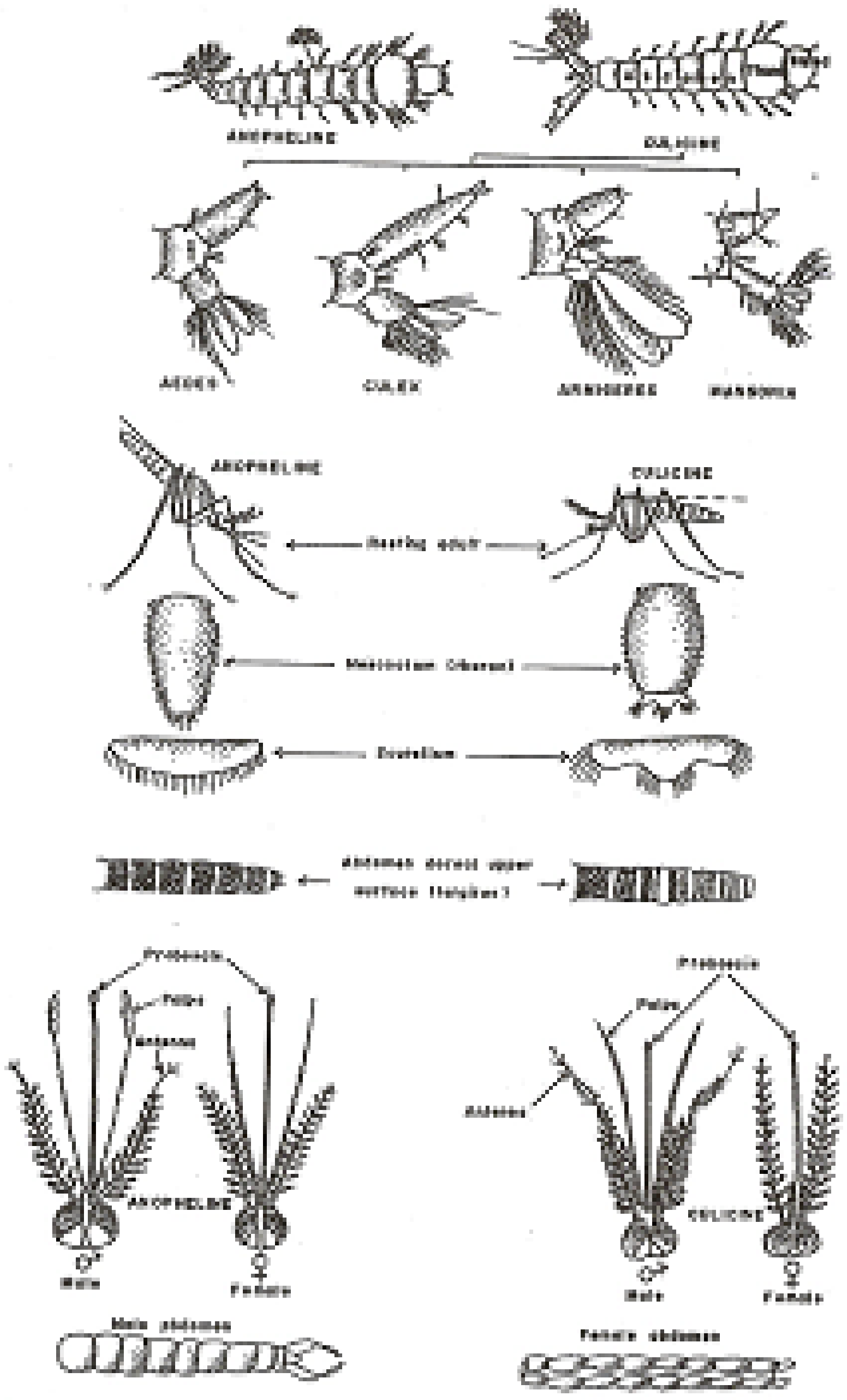

Plate 2.1: The characteristics used for identifying mosquitoes of general importance (Source: IMR. Entomological teaching charts)

2.5.4.1 *Anopheles peditaeniatus*

Anophele peditaeniatus is largely zoophilic and the adults are found abundantly around cattle sheds (Zairi, 1990). In India and Malaysia, adult *An. peditaeniatus* can always be distinguished by the long hind tarsal pale bands, a long dark mark on vein 5, the line of white scales along the remigium and the bare humeral cross vein (Reid, 1968). *Anopheles peditaeniatus* is one of the commonest species found in rice field (Zairi, 1990). Aside from being numerous in rice fields, larvae are found in swamps and grassy ponds (Reid, 1953). This species has been recorded in India, Sri Lanka, Myanmar, Thailand, Indochina, China, Malaysia, Indonesia and the Philippines (Knight & Stone, 1977).

It can be readily infected with *Wuchereria bancrofti, Brugia malayi* and *Dirofilaria immitis* but rapid development of calcified cysts around dead worms indicates poor vector potential (Reid *et al.*, 1962; Wharton *et al.,* 1963). Furthermore, according to Wharton *et al.* (1963), the proportion biting man in nature was probably too small to pose a real danger as a vector of human diseases. Therefore it is also not considered as a disease vector in Malaysia (Zairi, 1990).

2.5.4.2 *Anopheles sinensis*

Anopheles sinensis is generally regarded as a zoophilic and exophilic species although it can also bite human but, only outdoors and after dark (Reid, 1953). *Anopheles sinensis* is not a vector in Malaysia (Reid *et al*., 1962), however it is a vector of malaria in other Asian countries such as Japan, China and Korea (Ohmori & Otsuru, 1960; Ho *et al*., 1962; Kim, 1974). *Anopheles sinensis* is also a vector of brugian filariasis and bancroftian filariasis (Chiang & Loong, 1985; Miyagi & Toma, 2000).

The distribution of *An. sinensis* ranges from Japan and Korea through central and southern China, Taiwan, Hong Kong, Vietnam, Cambodia, Peninsular Malaysia and Singapore, west ward to the Union of Myanmar (Burma) and Assam, but it is absent from the rest of India (Knight & Stone, 1977; Zairi, 1990). It breeds in open grass ponds, especially in rice fields (Zairi, 1990).

2.6.4.3 *Anopheles campestris*

In Malaysia, *Anopheles campestris* is probably the most antropophilic and endophagic of all anopheline mosquitoes (Zairi, 1990). This species was formerly identified as a dark winged form of *An. barbirostris* (Reid, 1947). In Malaysia, although 75 species of *Anophele*s have been recorded, only 9 have shown to be vectors of malaria (Rahman *et al*. 1997, 2002) including *An. campestris.* This species can also be a potential vector of filariasis by transmitting *Brugia malayi* mainly in swampy rice-field terrain (Chiang & Loong, 1985; Miyagi & Toma 2000). The larvae commonly breed in corners of rice fields and burrow pits in coconut plantations, and sometimes found in slightly brackish water (Chow, 1970; Chooi, 1985).

2.5.4.4 *Anopheles vagus*

Anopheles vagus is a zoophilic species *(*Wharton, 1953*).* It is abundant in houses and cow sheds and rest inside cars or small boats. This species is closely related to *An. subpictus* except that the apical pale band of the palps is usually broader, the subapical dark band narrower and the tip of the proboscis usually has an obvious pale mark (Reid, 1968). It is not considered as a vector in Malaysia, however, it has been found naturally infected by malaria parasite in India and also considered as a secondary vector of bancroftian filariasis (Lee *et al*., 1983; Rao, 1984). It is distributed throughout India, Sri Lanka, Andaman Islands, Myanmar, Thailand, Indo-China, China, Malaysia, Indonesia, Papua New Guinea, the Philippines and the Marianas Islands (Knight & Stone, 1977). According to Covell (1944), the larvae are typically found in small freshwater pools and puddles and also in brackish water.

2.5.4.5 Anopheles subpictus

Anopheles subpictus is generally regarded as a zoophilic species but only a small proportion feeds on man (Zairi, 1990). Many studies have reported that *An. subpictus* is primarily zoophilic, more attracted to bovine than human (Roy, 1943; Covell, 1944; Collins *et al*., 1979). It was experimentally infected with malaria parasite but its role in transmission is undelianeated. However, *An. subpictus* appears to be a malaria vector on the coast of Southeast India (Panicker *et al.*, 1981) and in Indonesia as a major vector of bancroftian filariasis (Lee *et al*., 1983). *Anopheles subpictus* is distributed in India, Nepal, Pakistan, Afganistan, Iran, Sri Lanka, Myanmar, Thailand, Cambodia, Malaysia, China, Indonesia, Maldives, Papua New Guinea and the Marianas Islands (Knight & Stone, 1977). The larvae are found both in fresh and brackish water (Zairi, 1990). It has also been found together with *An. aconitus* in rice fields and with *An. sundaicus* in lagoons (Sundararaman *et al.,* 1957; Soerkirno *et al.,* 1983). However *An. subpictus* is confined to the coast in brackish water even though the habitats sometime overlap with *An. indefinitus* (Zairi, 1990).

2.6 Physical factors

According to WHO (2000), many countries in Asia experienced unusual high levels of dengue and/or dengue haemorrhagic fever in 1998, the activity being higher than in any other year (Andrew *et al.,* 2000). Since laboratory experiments have demonstrated that the incubation period of dengue 2 virus could be reduced from 12 days at 30^0C to 7 days at 32-35^0C in *Aedes aegypti* (Watts *et al*., 1987), changes in weather patterns, may be the major contributing factor to the high incidence of the disease.

Temperature, rainfall and relative humidity are physical factors that influence the abundance of the mosquitoes. According to Lee (1990), with no changing seasons in our country's weather, therefore there is no significant difference in larval numbers throughout the year. However, indoor temperature may provide a suitable condition for *Aedes* breeding. In general, insects are exceedingly sensitive to temperature and rainfall regiments and tropical and temperate species frequently show great variations in seasonal abundance (Samways, 1995). In tropical and subtropical climates, *Ae. albopictus* is abundant all year round; however, in temperate climates such as the Midwestern United States, Japan and Argentina, the active season for the larval stages is limited to late spring through early fall, with larval abundance greatest in July- August (Mori & Wada, 1978; Toma *et al.,* 1982). The temperature fluctuations affect the mosquito populations and allow *Aedes* proliferations only between September and April (deGarin *et al.*, 2000).

2.6.1 Rainfall

Rainfall is the most important factor that affects *Aedes* breeding (Khim, 2003). Reproduction of *Ae.aegypti* populations in tropical and subtropical zones occurs all year round and their abundance can either be associated with rainfall regimens (Moore *et al*., 1978; Chadee, 1991, 1992; Kalra *et al*., 1997; Micieli & Campos, 2003) or no asoociation is observed (Shepped *et al.*, 1969, Barrera *et al.*, 1997). Generally, *Aedes* breeds after rain, not during raining days. With heavy rainfall, water in containers will overflow, and consequently larvae cannot survive in it (Lee & Cheong, 1987). In the study on adult females of *Aedes* *albopictus* in Kuala Lumpur, the highest peak can be seen in September, the lowest in May, these situations are closely related to rainfall (Sulaiman & Jeffrey, 1986). According to Chan *et al.,* (1971a) in a study in Singapore there was a few high and low peaks for *Aedes albopictus* adult female population between March, June-July and November-December. The larvae and pupae are higher after two months of high peak of adult population.

2.6.2 Temperature

Mosquitoes are sensitive to temperature changes as immature stages in its aquatic environment and as adults. If the water temperature rises, the larvae take shorter time to mature (Rueda *et al*., 1990) and consequently there is a greater capacity to produce more offspring during the transmission period. Adult female mosquitoes digest blood faster and feed more frequently in warmer climates, thus increasing transmission intensity (Gillies, 1953). However, warming above 34^0C generally has a negative impact on the survival of vectors and parasites (Rueda *et al*., 1990).

2.6.3 Relative humidity

High relative humidity can give high hatching rates. With 100% humidity the eggs can hatch on filter papers. It is important to allow slow desiccation of eggs as the embryo takes time to develop prior to the drying process. The low relative humidity also gives negative impact on egg hatching (Horsfall, 1956). With the tropical weather in this country, the high relative humidity has little impact on eggs development (Manorenjitha, 2005).