CHAPTER 2

LITERATURE REVIEW

2.1 Mosquitoes

Mosquitoes are flies in the family Culicidae. Over 3,000 known species of mosquitoes exist worldwide. Adult mosquitoes are characterized by having long, slender, needle-like mouthparts (proboscis), antennae, and legs. Their narrow wings are often covered with minute scales. Fine scales cover the mosquito body and vary in coloration from white, silver, or gold, to very dark. Pattern arrangement and scale coloration are often useful for identifying mosquito species. Despite their delicate appearance, mosquitoes are aggravating pests of humans and other animals. Bites from mosquitoes can cause severe discomfort. The resulting intense itching is due to an immunological reaction to mosquito saliva injected into the bite wound.

2.2 Biology and life cycle

2.2.1 Egg

A freshly laid egg is light in colour and darkens within a few hours. Mosquito eggs are oval and about $1/40^{th}$ of an inch (0.635mm) long. Eggs are either deposited singly or as an egg raft depending on the type of mosquito. A standard egg raft is about 1/4 inch (6.35mm) long and contains 100-200 eggs. Some species of mosquitoes lay their eggs singly and deposit them directly on water or floating aquatic vegetation. Others will lay their eggs on moist soil that is subject to periodic flooding, or above the water line in natural and artificial containers. The number of single eggs laid per batch varies within and between mosquito species and can range from 60 to 200.

2.2.2 Larvae

Larvae will emerge from eggs within 2-3 days when environmental conditions are ideal. All mosquito larvae go through four developmental stages called instars. First instars are barely noticeable to the human eye. Last larval instars of some species can be approximately 1/2 inch (12.7mm) long. Larvae move through the water in a serpentine motion. When they sense a shadow or movement in their habitat, larvae will quickly dive to the bottom to avoid the source of disturbance.

Mosquitoes have four distinct developmental stages: egg, larva, pupa, and adult. Immature stages of mosquitoes require water to complete their life cycle. Mosquitoes are poikilothermic (cold-blooded) animals, thus their rate of development and other aspects of their physiology are temperature-dependent. As the temperature increases, their development time shortens.

Larvae have a well-formed head and lack legs. Upon careful inspection one can distinguish the wider thorax from the long and slender abdomen. A tube-like structure, called a siphon, is located at the tip of the abdomen. Larvae use the siphon to breathe air from the water surface. Larvae possessing a siphon (Culicine) hold their body roughly at a 45-degree angle from the surface. Species of mosquitoes lacking a respiratory siphon (Anopheline) hold their body horizontal to the water surface. They obtain air through openings located on the dorsal surface of the abdomen. A few species will bore their siphon into stems of aquatic plants to obtain oxygen.

Mosquito larvae can be found in a wide variety of habitats, including temporary floodwater and snowmelt pools; more permanent water habitats like marshes, swamps, lagoons, and ponds; stagnant waters; and natural and artificial containers. Shallow water is ideal for larval survival because there is less turbulance and wave action. Upper water movement interferes with the surface feeding of some mosquito species, and in most species, it hinders the larvae and pupae from obtaining oxygen at the air-water interface. A deep-water environment prevents bottom-feeding water from reaching food that has accumulated at the lower levels of the water column. Water quality in larval habitats can vary from fresh to saline to high in organic wastes. Different species can tolerate and thrive in water with varying degrees of organic content.

Mosquito larvae eat a variety of dead and living organisms, including detritus, algae, bacteria, and fungi. Some mosquito species are predaceous and feed on other mosquito larvae and small invertebrates. Depending on water temperature, crowding, and food availability, the larval stage is typically completed in 5-6 days (Clements, 1992; Foster & Walker 2002).

2.2.3 Pupae

Mosquito pupae are active when compared to other insect pupae. Pupae move in a somersault fashion through the water. Pupae breathe through tubes located on the thorax and will remain at the water surface unless they are disturbed. This non-feeding stage can be completed in as few as 2-3 days (Clements, 1992; Foster & Walker 2002).

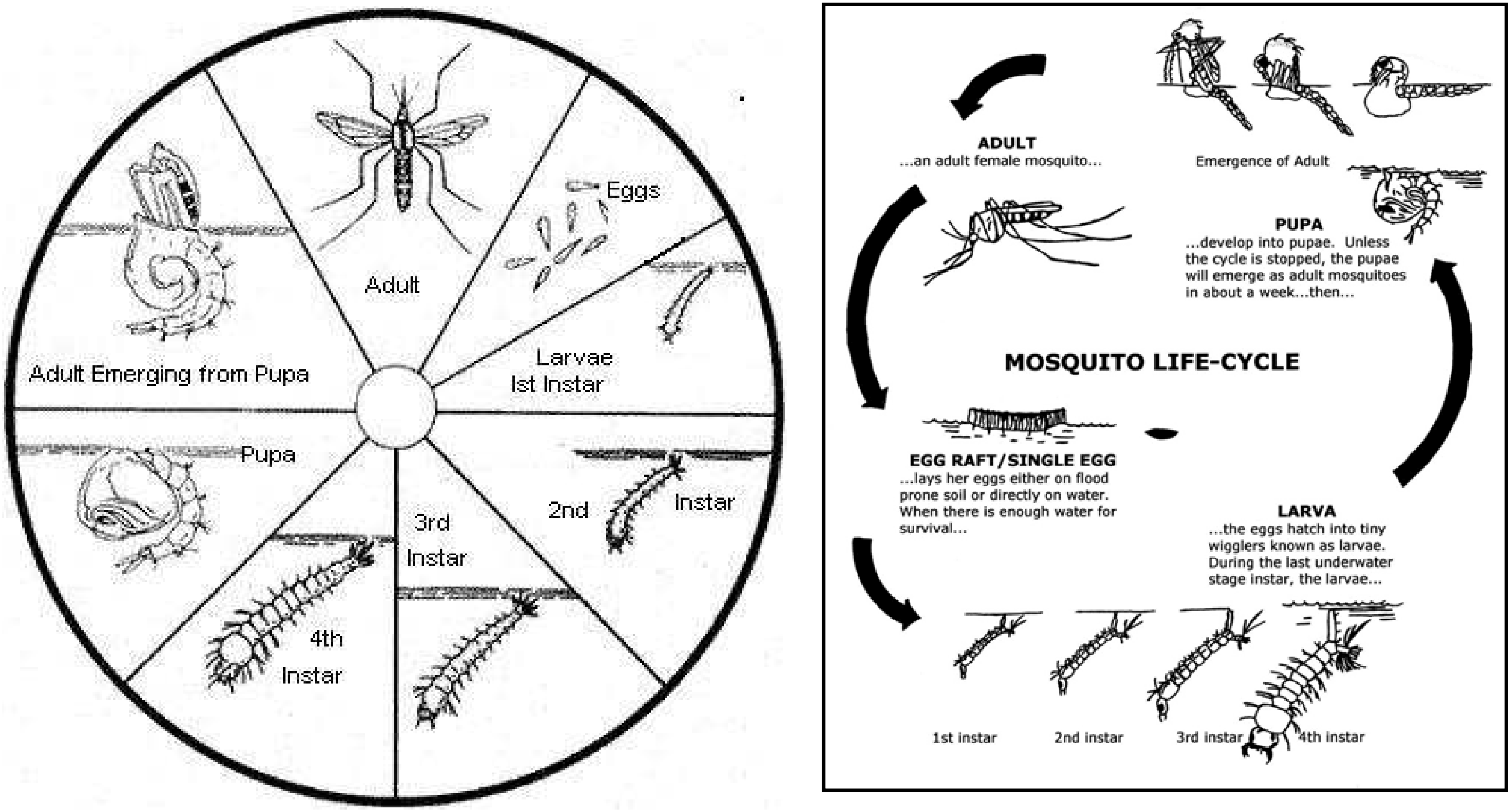
2.2.4 Adults

Both male and female mosquitoes feed on sugar sources such as plant nectar and honey dew, but only females feed on blood. Female mosquitoes use proteins and lipids from the blood meal to develop a batch of eggs. There are a few exceptions to this rule. Some species are autogenous, meaning a female is able to utilize energy reserves from the larval stage to produce eggs without a blood meal.

Many species of mosquitoes are specific in their host preference for birds, mammals, or cold-blooded vertebrates such as reptiles and frogs. Consequently, various mosquito species use a wide variety of cues to find a suitable host, often involving a variety of complex interactions. This complex of cues is still not fully understood by scientists. These cues can be either odors emanating from skin or breath, or visual cues such as movement or contrast of a potential host with the surrounding background. Carbon dioxide is a major cue and is often used as bait in mosquito traps.

Several hundred compounds found in human breath, secretions, and sweat glands have been identified and vary in their degree of attractiveness to female mosquitoes.[6] Some of the more attractive odors include: carbon dioxide, alanine, lysine, lactic acid, and estrogen. Some chemicals emanating from a potential host may be more important for certain species of mosquitoes. In other mosquito species, these very same odors may play a secondary role, no role, or act to repel mosquitoes (Clements, 1999).

The distance that mosquitoes can fly is difficult to generalize because mosquito species vary in their flight range. Some species can fly long distances (up to 10 km), but most do not travel more than several yards (Clements, 1992; Foster & Walker 2002;& Service, 1997).



(a) (b)

Source : West Umatilla Vector Control District 3005 South 1st Street, Hermiston, Oregon 97838

Figure 2.1: (a) The Life Cycle of *Aedes* mosquito and (b) the life cycle of *Culex* mosquito

2.3 Insecticide resistance

2.3.1 Insecticide resistance in vector mosquitoes

There has been a large increase in the number of insecticide-resistant culicine species and also an increase in the geographical areas involved. According to the 22nd report of the WHO Expert Committee on Insecticides, (WHO,1976) 41 species exhibiting resistance, 35 are resistant to DDT, 26 to dieldrin and 49 to organophosphorus and carbamate compounds. About 17 culicine species are resistant to all three groups of insecticides. The increase in the number of species with multiple resistance to organophosphorus compounds is of particular importance, since these constitute the main group of insecticides used for larviciding, which is the principal attack measure against most of these species.

Resistance is defined by the WHO as "the development of an ability in a strain of an organism to tolerate doses of toxicant, which would prove lethal to a majority of individuals in a normal (susceptible) population of the same species.

The development of resistance in insects to acutely toxic insecticides generally occurs by selection of rare individuals in a population that can survive the insecticide. It is pre-adaptive and not a mutational effect. This implies that it is an inherited trait. Most commercial insecticides are designed to be poor mutagens and their use results in an intense chemical selection (high dose, high toxicity) which is not conducive to genetic alterations, but allows survival of pre-adapted (i.e. resistant) individuals. Insects become resistant either: behaviourally (avoid exposure to a lethal dose) or physiologically (find ways to survive a normally lethal dose).

Resistance of *Aedes aegypti* to chlorinated hydrocarbons is general in tropical America and South-East Asia. Resistance is rapidly increasing in Africa and in the Pacific Islands, mainly to dieldrin. Organophosphorus resistance has been recorded in the field in a number of places in tropical America and in South Vietnam, but multiple resistance was not detected and the resistant strains could not be colonized. The reports of organophosphorus resistance in New Caledonia, Malaysia, Congo and Thailand should be confirmed, as the tests are not yet conclusive (WHO, 1976). Dengue vector, *Aedes albopictus* is now resistant to DDT and dieldrin in several countries of South-East Asia and the Western Pacific. It is also resistant to malathion in South Vietnam and to fenitrothion in Madagascar. Organophosphorus resistance has also appeared in *Culex pipiens pipiens* in Egypt, Israel and France and in *Culex pipiens pallens* in the Republic of Korea and Japan, a species which has now shown resistance to a number of organophosphorus compounds.

2.4 Insecticide resistance mechanism and causes of resistance

Recent years have seen important advances in the understanding of the mechanisms causing resistance, despite the fact that studies on the subject seem to be less fashionable and a consequent decline in the number of scientist investigating it. The progress made has mainly been due to the improved techniques available, these include improved possibilities for genetic analysis in some insect species, such as the housefly, *Culex p. fatigans* and *Aedes aegypti* with the increased identification of marker genes. For these species, it has been possible to locate an increasing number of resistance genes on the chromosome maps. There have been improved opportunities for biochemical studies, mainly comprising micro-techniques for the study of insecticide detoxication or enzyme inhibition, such as the use of isotope-labeled insecticides, chromatography and spectrophotometric techniques.

2.4.1 Genetic basis of resistance

It is most important to know whether a certain type of resistance is monogenic or due to several genes. Moreover, assigning certain types of resistance to specific loci on certain chromosomes has been helpful in distinguishing between different mechanisms of resistance and has been used as a valuable tool in biochemical research on the effects to the different factors involved.

There are many examples of strains of insect in which resistance is due to a single gene. On the other hand, other types of resistance have been shown to be due to the interaction of a number of genes. This type of resistance tends to develop after a long period of insecticidal pressure. It seems to be facilitated by the accumulation of the contributing genes through successive selection with a number of different insecticides, each of which confers some cross-resistance.

A distinction between monogenic resistance and these more complex types are important since the former type generally shows a simpler pattern of cross-resistance and is more vulnerable to counter measures, such as the addition of synergists. The analysis of field strains with a relatively complex genetic background has been very useful, since the influence of a single resistance gene when present along with, or another in combination with other resistance genes has shown in many cases the importance of interaction.

2.4.2 Biochemical basis of resistance

Insecticide resistance mechanisms (as opposed to insecticide avoidance behaviors important in the control of malaria vectors) have a biochemical basis. The two major forms of biochemical resistance are target-site resistance, which occurs when the insecticide no longer binds to its target, and detoxification enzyme-based resistance, which occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST) prevent the insecticide from reaching its site of action. An additional mechanism based on thermal stress response has been proposed (Patil *et al*., 1996), but its importance has not been assessed.

Many causes of resistance have been defined, although there are several types that defy an explanation in biochemical terms. An altered site of action, as a cause of resistance has been definitely established with cholinesterase inhibitors. In this case, a mutant acetyl cholinesterase is produced that is inhibited more slowly by the insecticides than the normal enzyme in susceptible strains. Generally, this provides resistance to a large number of compounds, but the factor of insensitivity (speed of inhibition in susceptible over that in resistant strains) depends on the particular compound.

Although same inhibitors escape being affected, this type of resistance is generally very serious because of the extensive cross-resistance conferred by it. The genes for this resistance seem to be extremely rare but are nevertheless arising in more species after prolonged selection.

2.4.2.1 Increased detoxication

The following enzymes or classes of enzymes are now known to be of importance (WHO, 1976; WHO, 1992):

- DDT-ase (DDT – dehydrochlorinase), affecting DDT and several analogues.
- Hydrolase, affecting phosphate esters or carboxylic ester groups in organophosphorus compounds and in some pyrethroid
- Gluthathion-S- transferase, affecting organophosphorus compounds

- Oxidases, affecting carbamates, organophosphorus compounds, DDT and its analogues as well as pyrethroids.

The detoxication enzymes in resistant strains are generally more efficient (i.e., have a changed substrate specificity) and are not produced in higher amounts. The group of oxidases is of special importance since various oxidases can attack a wide variety of insecticides. In the housefly, the genetic effects in oxidases appear to be complex since several genes on at least two chromosomes can be involved, probably affecting both oxidase structure and the amount of oxidase produced. The nature of the compounds that can be attacked and consequently the type of cross-resistance brought about, can be quite obsecure. Although carbamates are esters, no hydrolytic detoxication mechanisms have been found, and hydrolytic action on phosphoric esters is generally only slow.

For all detoxication enzymes, inhibitors are now known that can overcome detoxication, at least experimentally, by their action as synergists. There are some inherent difficulties in the application of synergists in practice, owing to their high cost, instability and possible hazards. Synergism, if it can be further developed, hold some promise for over coming resistance in future (WHO, 1992).

2.2.2.2 Target-site mechanisms

Alterations of amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective. The target of organophosphorus (OPs) (e.g., malathion, fenitrothion) and carbamate (e.g., propoxur, sevin) insecticides is acetylcholinesterase in nerve synapses, and the target of organochlorines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath. DDT-pyrethroid cross-resistance may be produced by single amino acid changes (one or both of two known sites) in the axonal sodium channel insecticide-binding site (Miyazaki *et al*., 1996 & Williamson *et al*., 1996). This cross-resistance appears to produce a shift in the sodium current activation curve and cause low sensitivity to pyrethroids (Vais *et al*., 1997). Similarly, cyclodiene (dieldrin) resistance is conferred by single nucleotide changes within the same codon of a gene for a γ-aminobutyric acid (GABA) receptor (Ffrench-Constant *et al*., 1993). At least five point mutations in the acetylcholinesterase insecticide-binding site have been identified that singly or in concert cause varying degrees of reduced sensitivity to OPs and carbamate insecticides (Mutero *et al*., 1994).

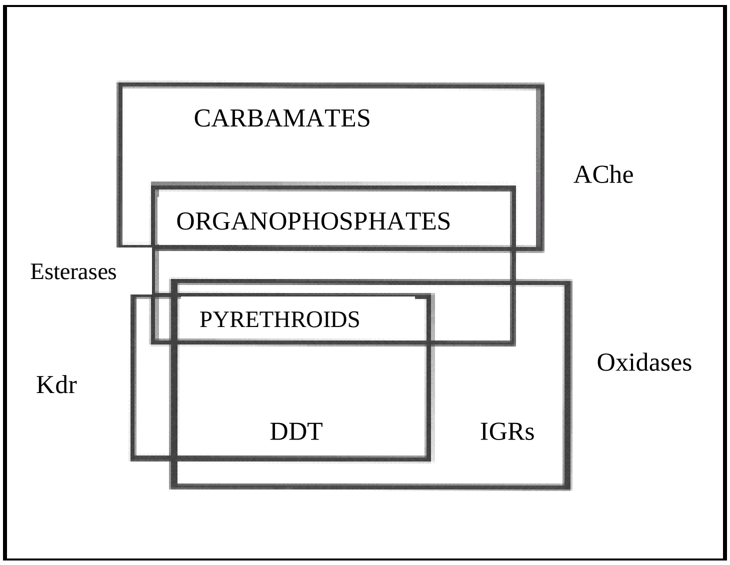
2.4.3 Reduced penetration

Reduced penetration is another, though less important, mechanism of resistance. Generally it is important because it increases resistance brought about by other factors. High resistance, as already mentioned, can result from the interaction of factors, some of which have little influence when present alone (WHO, 1976; WHO 1992).

2.4.4 Cross-resistance

Resistance due to the selection by a certain insecticide of one or more genes will generally extend to other compounds; this phenomenon is called cross-resistance. Perhaps the best known example is resistance to all the members of chlorinated cyclodiene group, which is due to one gene with a characteristic resistance spectrum to all members of the group, irrespective of which one was used for selection (WHO, 1976).

Unfortunately, with the development of more cases of resistance this simple picture has now become much more complex and consequently, predictions about cross-resistance spectra are much more difficult. One obvious reason for this is that different groups of genes can be selected with one insecticide. Under these circumstances, the different patterns of cross-resistance will depend on the particular genes that happen to be represented in various cases. Even when a single resistance gene is present, however, the pattern need not be as well defined as in the case of the cyclodieaes. This is because several alleles of the gene can exist, with differences in their cross-resistance spectra; for example, different alleles of the acetylcholinesterase gene produce enzymes with very different rates of inhibition for different insecticides. Our knowledge at present does not allow for predictions as to the nature of possible chemicals that will not be affected. The same applies to oxidation enzymes already mentioned.



Source: Emerging Infectious Diseases, adapted from Brogdon & McAllister (1998)

Figure 2.2: Cross-resistance relationships of commonly used classes of insecticides

Despite these deficiencies, the efforts to determine the cross-resistance spectra of various mechanisms should be intensified. This is of obvious practical significance, while simultaneously contributing to better understanding of nature of resistance mechanisms.

2.4.5 Life cycle dependent resistance

The level of resistance in insect populations is dependent on the amount and frequency of insecticides used, and the inherent characteristics of the insect species selected. For example, for decades tsetse flies have been controlled by DDT treatment but resistance never developed in this species; the same is true of triotomid bugs. In both species the major factor influencing insecticide resistance development is the life cycles for the bugs and the production of very small numbers of young by tsetse. Mosquitoes, in contrast have all the characteristics suited to rapid resistance development including short life cycles and abundant progeny (Hemingway, 2003).

2.5 Resistance of vector mosquitoes to insecticides

2.5.1 Malathion

Malathion is an organophosphorus compound which is neurotoxic. It inhibits production of acetylcholinesterase thereby resulting in the continuous stimulation of the insects leading to paralysis and finally death (Cynamide Internationale, 1975). Hydrolisis of malathion in most insects undergo two different pathways (Perry, 1966) :-

a) due to action on phosphatase enzyme at P-S, C-S linkages yielding di-alkyl phosphates and triphosphates
b) due to hydrolysis of diethyl succinate moiety by carboxylesterases yielding the non-toxic mono and the dicarboxylic acid derivatives of malathion.

Studies showed that resistance mechanisms against malathion were due to the increased production of the detoxifying enzymes (esterases) and insensitive acetylcholinesterase (Perry, 1966; Brown and Brogdon, 1987; Denholm and Rowland, 1992).

2.5.2 Permethrin

Since last two decades WHO has considered permethrin as a candidate insecticide for vector control. Permethrin [(3 – phenoxyphenil) methyl (±) cis / trans 3 – (2- dichloroethenyl) 2,2 – dimethyl cyclopropane carboxylate] is a synthetic pyrethroid which is known to exhibit two types of effect on insects. It is first being an intial rapid knock down (kd) effect and secondly randering the insects motionless and a subsequent lethal effect. Permethrin is relatively non-target organisms and it is considered safe for addition to drinking water (WHO, 1985). Permethrin has also been used for treating mosquito nets and clothes to prevent mosquito bite (Schreck *et al.*,; 1984; WHO, 1989).

2.5.3 Temephos

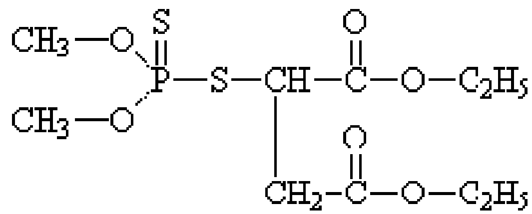
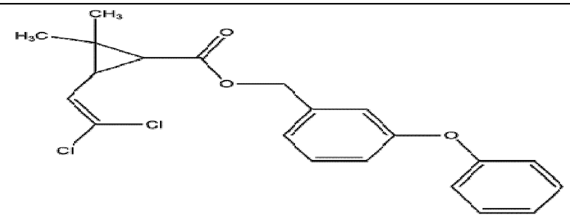
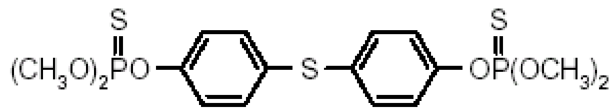
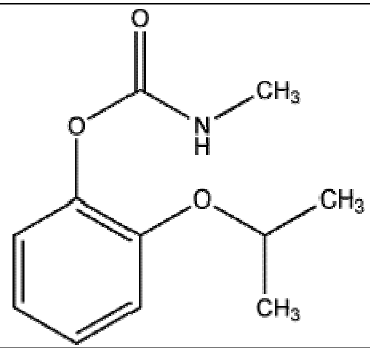
Temephos or Abate® is an organophosphorus compound characterized by very low toxicity to warm-blooded animals and other non-target organisms. In addition, temephos is safe for controlling mosquito larvae in drinking water because it is neither accumulated in the treated water nor harmful to man (Laws, 1967).

Although Abate® reduces *Aedes* larval population significantly, the possible development of larval resistance to temephos cannot or should not be overlooked. Studies in Malaysia indicated that field-collected *Aedes aegypti* larvae were becoming more tolerant to temephos (Lee, *et al.*, 1984 and Lee, 1990). In Surinam, Hutson (1985) also exhibiting abnormally low susceptibility level to temephos when tested according to WHO standards. If high level of resistance develops, temephos must be replaced by an effective alternative agent which like temephos, is relatively non-toxic to non-target organisms and exhibits a reasonable period of persistency, especially when used in water storage containers.

2.5.4 Carbamate

This class is easily identified by the presence of carbamic acid group (-OC (O) NH_2). In 1951, CARBs, (isolan, dimetan, pyramat, pyrolan) were introduced by the Geigy Chemical company in Switzerland, but were not well-received then due to their low effectiveness and high cost. By changing the chemical structure, the first successful carbamate, carbaryl was introduced in 1956. Two special characteristics which made this chemical a success then were: (1) Its relatively low mammalian toxicity; (2) Its broad spectrum for insect control. Since then, many CARBs have been synthesized and are used widely. Today, CARBs such as propoxur and bendiocarb are widely used in the public health and household sectors especially for cockroach control (Lee, 1998)

Table 2.5.1: The insecticides used in this study

Common name	Chemical name	Group	Empirical formula	Chemical structure
Malathion	S-1,2-bis(ethoxycarbonyl) ethyl O,O- dimethyl phosphorodithioate	Organophosphate	$C_{10}H_{19}O_6PS_2$	
Permethrin	(±)-(cis-trans)-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	Pyrethroid	$C_{21}H_{20}Cl_2O_3$	
Temephos	Tetramethyl O,O'- thiodi-p-phenylene phosphorothioate	Organophosphate	$C_{16}H_{20}O_6P_2S_3$	
Propoxur	o-Isopropxyphenyl methylcarbamate	Carbamate	$C_{11}H_{15}NO_3$	

2.6 Mode of action of insecticides

The way in which a chemical acts upon an insect to kill is its mode of action. There are five groups of chemical used in insect control physical poisons, protoplasmic poisons, metabolic inhibitors, nerve poisons and molting inhibitors or insect growth regulators (IGR).

Organophosphate, carbamate, cyclodiene DDT, and more recently pyrethroid insecticide have been the major classes of chemical used in mosquito control (Vaughan *et al*., 1997). This range of chemical classes affect the insect nerve system has been stated in Table 2.2.

Table 2.2 : Mode of action of insecticides (Quoted from William and John, 1998).

Insecticide	**Mode of action**	**Mechanism of resistance**
Chlorinated hydrocarbons	Affection permeability of nerve membrane for cyclodienes and related chemicals (primarily) GABA Cl^- channels); for DDT and anologues (voltage-gated Na^+ channels	Kdr factors, esterases Metabolism (dehydrochlorinase); penetration
Carbamates and organophosphates	Bind to acetylcholinesterase	Altered acetylcholinesterase, PSMO* , penetration, etc.
Pyrethroids	Affection permeability of nerve membranes	PSMO metabolism; penetration; Kdr; esterases

PSMO* - microsomal cytochrome P450 polysubstrate monooxygenases

The organophosphate and carbamates inhibit an enzyme (acetylcholinesterase) that usually breaks down the neurotransmitter acetylcholine at the synaptic cleft. This leads to a change in neural pattern and the insect ultimately dies. Acetylcholine has been identified as one among several possible transmitter substances in insects. As soon as acetylcholine is being released into synaptic cleft, the enzyme acetylcholinesterase begins to break it down. Without the normal breakdown of neurotransmitters in the synaptic cleft region, normal neuronal functioning is greatly impaired or stopped. A strong interest in cholinergic synopsis because many insecticide (i.e., carbamates and organophosphates) act on the cholinergic system. These insecticides act as inhibitors of acetylcholinesterase, the enzyme that removes the acetylcholine from the synaptic region, thus interfering which normal neurotransmission (William & John, 1998).

2.7 Biochemistry of esterase enzyme

2.7.1 Esterase

Esterases in insects are a large, ubiquitous group of enzymes that are able to metabolize a wide variety of substrates. Among them are esterases able to catalyze the cleavage of the different types of ester bonds (Healy *et al*., 1991) found in OP and carbamate insecticides (Hutson & Roberts 1985). It is therefore not surprising that elevated levels and mutated forms of esterases feature in mechanisms of resistance to these insecticidal groups. Resistance of medically important insects to chemical insecticides in Malaysia is reviewed here. High resistance to insecticides was found in mosquitoes, notably *Culex quinquefasciatus* but in other mosquitoes, various degree of resistance mechanisms in Malaysian insects is due to the elevated level of non-specific esterase. Studies here indicated that the level of this enzyme was directly proportional to the degree of resistance. Thus, the detection of these enzymes is indicative of the resistance status of an insect. Based on these findings, a rapid test for the detection of insecticides due to increased level of non-specific esterase in the mosquitoes has been developed (Lee *et al*., 1998

Early studies tried to correlate distribution of larval esterase with toxicity of various larvicides. Chen & Sudderuddin (1978) conducted a correlation study for the larvae of *Culex quinquefasciatus* and *Aedes aegypti*. Some correlation between *in vivo* toxicity (LD_{50}) and *in vitro* esterase inhibition was observed. Results also showed *Aedes aegypti* which were generally more tolerant to organophosphates and carbaryl showed higher carboxylesterase activity as evident from value obtained from densitometric readings. From then on, the possible role of detoxifying enzymes in correlation with resistance level was taken into consideration

2.7.1.1 Non-specific esterase

Non-specific esterase are a group of enzymes or isoenzymes present in the haemolymph or juvenile mosquitoes that catalyze the hydrolysis of ester linkage irrespective of chain length or solubility (Downer, 1978).

The non-specific enzymes are demonstrated by the use of certain synthetic compounds as substrates, the action of the enzyme altering the substrate to produce a visible change. Most widely used are the naphthol esters, which are hydrolyzed with an appropriate diazonium salt, the naphthol released from its ester linkage by the enzyme then couples with diazonium salt to produce a dark coloured, insoluble dye. The esters commonly used are those of the short-chain carboxylic acids such as naphthol acetate or butyrate, and naphthol phosphates. Enzymes hydrolyzing the former are called *esterases*, (Charles, 1965) while those acting on the phosphate esters are termed acid phosphatases or alkaline phophatases, depending upon the pH at which they are most active.

Obviously, there is some overlap among these three groups of non-specific enzymes (some of the esterases are phophatases). Also, the natural substrates upon which most of them act are not known. Which of the many known hydrolytic enzymes are present among the non-specific esterase and phosphatases is by no means completely determined. A tentative list would include the lipases, cholinesterases, nucleotidases, glycerophosphatases, creatine phosphatase and probably many others. However, most of the non specific esterase and phosphatases have not yet been identified. Non-specific enzymes are not entirely valid or meaningful because some of the esterase display activity toward a number of synthetic substrates in vitro, whereas in vivo they may have a high degree of specificity.

2.7.2 Biochemical enzyme determination microassay

2.7.2.1 Microplate assay

Conventional detection of resistance is based on insecticide susceptibility tests which one dosage and mortality bioassay are not designed to detect underlying resistance mechanisms. Biochemical approach can be used to determine whether or not individual posses a mutant resistance allele. As resistance is often based upon increased enzymatic detoxication of an increased or reduced sensitivity of a target enzyme to inhibition by the insecticide. Therefore the advantage of using biochemical microplate enzyme assay technique is the resistance can be detected at a very low frequency in an individual samples.

Biochemical test would have important advantages, especially if a complete field kit for testing biochemical resistance possessed the attributes as cited by Brown & Brogdon (1987) :-

a) detects levels of resistance and it's mechanism(s) involved,
b) permit analysis of single insects,
c) permit multiple assays from single insect,
d) fast and accurate,
e) adaptable for most or all insects in providing all needed assessment information in the field and
f) usable in undeveloped areas so the equipment is simple, inexpensive and easy to carry.

Different biochemical studies have already been undertaken to determine mechanisms of resistance and its frequency from field collected populations of mosquitoes. The biochemical assays with appropriate substrate and indicator can detect both mechanism and resistance level; and allows computation of the proportion of resistant individuals in the field population.

Studies undertaken by Hemingway *et al*., (1986) showed that resistance towards organophosphate and carbamates in 3 populations of *Anopheles nigerrimus* from different areas of Sri Lanka appeared to be due to an altered acetylcholinesterase mechanism. The resistant frequency correlates with the use of agricultural pesticides; being highest in the region where the greatest selection pressure would be expected to be operatic.

Brogdon *et al*., (1988) used simple microplate assay technique to determine the frequency of insecticide resistance in single mosquito and to study the distribution and localization of organophosphate and carbamate resistance in field populations of *Anopheles albimanus* where such resistance caused by agricultural pesticides has long been assumed to be widespread.

Areas of complete susceptibility to orgnophosphate and carbamates were observed as well as areas where resistant phenotypes represented up to 98%. The mechanism of resistance identified were elevated levels of esterases and insensitive acetylcholinesterase mechanisms. Absorbance value cut-off determined from the susceptible insect population where minimal or no insecticidal application served as the reference point to determine resistance.

In Malaysia, Lee (1990) developed a simple biochemical method to detect elevated levels of esterase in organophosphate resistant individuals of field collected *Culex quinquefasciatus*. Quantified differences on colour intensity between the susceptible and resistant strains were done visually. Microplate assays using different substrate with appropriate coupling reagent showed that resistance mechanism of *Culex quinquefasciatus* was mainly due to elevated levels of esterase which correlated directly with malathion resistance (Lee *et al*., 1992).

2.8 Native Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (Native SDS - PAGE)

Complementary to biochemical assays are electrophoretic studies to determine different kinds of esterases responsible for resistance in particular insect. Based on the assumption that these enzymes have undergone biochemical modification, it is reasonable to assume that at least in some cases, they will be electrophoretically different from normal enzymes. (Georghiou & Pasteur, 1978).

Existence of clear relationship between the levels of tolerance of organophosphate (malathion) and the intensity of esterase bands was shown by refined horizontal electrophoretic separation techniques in polyacrylamide gels (Field *et al.*, 1984). Large number of esterase bands (up to 12) with significantly higher densities were observed from the field tolerant and highly laboratory-selected strains as compared to the susceptible ones.

2.9 Vector Control

In the absence for an effective vaccine for immunization against dengue, suppression of the vector population remains essential particularly in endemic areas. Ideally this should be achieved by source reduction based on environmental sanitation and public health measures such as introducing or improving piped water supply, regular clean-up campaigns, legislature and public health education (WHO, 1976).

Insecticidal control is encouraged only in areas where sanitation areas have not been effective or during outbreaks of disease, and may be aimed at larvae or adults. The methods for controlling the adults have been reviewed (Chow *et al*., 1977). They include residual spraying, thermal fogging and ultra-low volume (ULV) applications. Residual spraying has been carried out mainly with organochlorines such as DDT, dieldrin and organophosphate such as fenthion (Mottar-Sanchez *et al*., 1976). This method has not been very effective particularly with the development of resistance to the organochlorines.

Thermal fogging of insecticide (mainly organophosphates such as malathion, fenitrothion and chlorpyrifos and sysnthetic pyrethroid, bioresmethrin) is now widely used for *Aedes* control (WHO 1976, Phanthumachinda *et al*., 1976). The fog is rapidly dispersed in conditions of rain, wind and high temperature. Thus for maximal effectiveness, particularly in Malaysian conditions, it should be carried out in the early morning or evening. One limitation of the method is the possible thermal decomposition of the insecticide. It is also difficult to confine the fog to precise areas. Despite these limitations it is an useful method (Chow *et al*., 1977).

ULV application is the most economical and effective of the three methods. It involves the application of undiluted or partially diluted insecticides mainly malathion or fenithrothion at low volume. ULV applications may be made aerially or on the ground. The advantage of this method is that relatively small quantities of solvent are required, making it economical particularly for large areas (Pant & Mathis, 1973; Pant *et al*., 1974).

An important aspect of vector control is the constant monitoring of vector distribution and densities in the field and periodic determination of their susceptibility to insecticides currently in use or which are to be introduced as alternative control agents (WHO, 1976; Chow *et al*., 1977). Such measures would allow evaluation of control programs and provide useful baseline data for mapping out further control measures particularly in emergency situation. This is important since insecticide resistance constitutes the single biggest problem in vector control (WHO, 1976).