CHAPTER 1

GENERAL INTRODUCTION

1.1 Scope of study

In Malaysia as in many other tropical countries, vector-borne diseases still pose serious public health problems. The most important vector-borne diseases are malaria, dengue fever, dengue hemorrhagic fever, filariasis and Japanese encephalitis. Many anopheline species are important malaria vectors. *Aedes aegypti* and *Aedes albopictus* are involved in dengue transmission in Southeast Asia. Several species of *Mansonia*, *Anopheles*, *Culex* and *Aedes* are vectors of Brugian and Bancroftian filariasis (Vythilingam *et al*., 1992).

Culex quinquefasciatus is one of the most common mosquitoes found in human habitations in the Tropics and Subtropics of the world. In most of its range female are intensely anthropophilic and feed actively only at night and it causes nuisance (Richard & David , 1959). In laboratories in many parts of the world *Wuchereria bancrofti* has been found to mature in *Culex quinquefasciatus* to the infective stage. This mosquito is the only vector of Bancroftian filariasis in the New World and an important vector in many parts of the Old World, including tropical Africa. It is not good vector of *Wuchereria malayi* and in areas where that parasite predominates, *Culex quinquefasciatus* may not be an important vector of filariasis.

There are about 434 species of mosquitoes in Malaysia belonging to 20 genera. However, the genera *Anopheles*, *Aedes*, *Culex* and *Mansonia* are of medical entomological importance. Dengue virus infection continues to present a serious health problem in many tropical areas of the world including Malaysia. Dengue viruses are

transmitted to humans through the bite of infected mosquitoes. For many years, members of the subgenus *Stegomyia*, especially *Aedes aegypti* and *Aedes albopictus* (Skuse) have been recognized as the primary vectors of dengue (Boromisa *et al*., 1987 ; Rohani *et al.*, 1998). Despite control effort in suppressing mosquito populations in Malaysia, cases of both the classical dengue fever and dengue haemorrhagic fever are on the rise in our country. Statistics of Ministry of Health Malaysia in the annual report for the year 1999, indicating a total of 10,146 dengue fever and dengue haemorrhagic fever with 37 deaths was reported in 1999, as compared to 27, 373 cases with 58 deaths in 1998 (a decrease of 62.9%). (Ministry of Health Malaysia, 1999). In situations where the role of mosquitoes as vectors of threatening disease is minimal, their status as a nuisance is still prevalent (Yap *et al*., 1997).

The control of these vectors relies largely on the use of chemicals which include organochlorine, organophosphate, carbamate compounds and recently the synthetic pyrethroids. Residual insecticide spraying is still the most widely used method of vector control in antimalarial programmes worldwide (Davidson, 1981; WHO, 1984). However, long term use of insecticides can lead to development of resistance.

Resistance is defined as an inherited characteristic that imparts an increased tolerance to a pesticide or group of pesticides, such as that resistant individuals survive a concentration of the compound(s) that would normally be lethal to the species on the basis of this definition, the proportion of survivors (heterozygotes in the first place, but including homozygotes as selection progress) can be looked upon as reflecting the frequency of the gene or genes that code for particular resistance mechanisms and thus confer resistance. The World Health Organization (WHO) defines *resistance* as “the development of an ability in a strain of an organism to tolerate doses of toxicant which

would prove lethal to the majority of individuals in a normal (susceptible) population of the species " (WHO , 1957).

Apart from this term of definition for resistance, it is known as an inheritable capacity developed in a population of normally susceptible mosquitoes. It is not a characteristic acquired during their life-time, nor can it be induced in a population by completely sublethal doses of the insecticide. It derives from the selective effect of exposure that kill or disable a portion of these population, the subsequent generation originating from the survivors (WHO, 1976; WHO, 1980). Among these survivor are those which carry pre-adaptation (genes or rather gene alleles) for resistance to that insecticide. With repeated applications generation after generation, the population comes to consist mainly of individuals carrying those pre-adaptations or genes, by a process of Darwinian selection. A population is usually termed "resistant" only when it has reached a level that results in a control failure in the field with the recommended dosage of the insecticide, and when a marked divergence from normal has been confirmed by a standard test of a sample of the insects (WHO, 1976; WHO 1980; WHO, 1986 & WHO, 1992).

The development of mosquito resistance to chemical insecticides is making the control of mosquitoes and the diseases they transmit more difficult (Vythilingam *et al*., 1992). Mosquito resistance to chemical insecticides widely used to control them is a major global problem today. Insecticide resistance is especially serious in disease vector and nuisance mosquitoes occurring at least 83 anopheline and Culicine species (Georghiou & Pasteur 1978). Such resistance when widespread may adversely hamper vector control programmes, rendering them highly ineffective as a tool for control. The emergence of insecticide resistance in these vectors has necessitated the development of

resistance detection techniques (Lee *et al*., 1992). Towards this end, standard resistance test kits were produced by the World Health Organization (WHO, 1981). These tests, though are easy to use especially with the inclusion of diagnostic dosages, are often time-consuming, requiring a large number of mosquitoes and limited number of insecticides or impregnated papers for testings (Lee & Tadano, 1994).

The present WHO standardized bioassay which is based on insect survivorship following exposure to an insecticide, has been widely used for the past 2 decades and this test gives and indication of development and trends of resistance. However, several short-comings of the technique have prompted the development of biochemical assay methods. Biochemical techniques are essentially based on the detection and qualification of enzymes known to be responsible for resistance. The importance of electrophoretic studies is demonstrate the esterase pattern of strains of the mosquito. Chen & Sudderudin 1987, suggested that the level of insecticide tolerance was found to be related directly to the number of esterase bands.

The principal factors on which the development of insecticide resistance in insect populations depends on various aspects. If the genetic potentiality for development of resistance to a given insecticide is present, the rate at which development proceeds will depend on certain obviously important factors such as the frequency of resistance genes and their dominance, the selection pressure and the previous history of exposure to insecticides. Also involved are ecological influences such as the isolation, inbreeding and reproductive potential of the insect population.

The rate of development of resistance in previously unselected populations is usually very low at first, during the period when the frequency of major genes for resistance is gradually increased and the genetic background is progressively organized

toward greater fitness in the contaminated environment. After this initial phase, the rate at which resistance develops accelerates rapidly, often leading to failure of control measures. The more intense the selection pressure, the more rapid the development of resistance, provided that the number of survivors is large enough to maintain genetic variability. Furthermore, it appears that the type of insecticide and hence the type of resistance mechanism involved is also an important determination of the rate of development of resistance. In Table 1 the more important factors influencing resistance development in field population of insects are noted, (WHO,1976).

Table 1.1: Factors influencing development of resistance to insecticides in a population

FACTORS INFLUENCING DEVELOPMENT OF RESISTANCE TO INSECTICIDES IN A POPULATION
Genetic 1. Presence of resistance (R) genes and ancillary genes (genetic potential) 2. Frequency of R genes 3. Number and combination of R genes 4. The degree of resistance due to an R gene or combination of R genes 5. Dominance of recessiveness of R genes 6. Fitness of R genotypes
Operational 7. Selection pressure: (a) proportion of the population exposed to selective dosages (b) generations exposed (c) mortality (plus infertility of survivors) due to insecticide 8. Stages exposed (eggs, larvae, adults before or after mating or egg-laying) 9. Insecticide(s) used 10. Type(s) od application (exposure route, residual or nonresidual etc.) 11. Previous exposure to insecticide and resistance development
Biological 12. Generations per year 13. Relative isolation of the population (inbreeding dispersal, migration) 14. Size, growth rate and breeding structure of the population (including reproductive potential and fluctuations in numbers) 15. Variance of ecological conditions in time (e.g., seasonal) and space 16. Natural selection : intensity, types and fluctuations (including co-adaptation) of R genotypes

Source: Board on Agriculture National Research Council (1986)

Adapted from Georghiou & Taylor (1976a) & (1976b)

1.2 Objective of Study

1) To identify resistance mechanism through insecticide selection-based approach by the use of WHO standardized larval bioassay and adult bioassay test for the determination of susceptibility and resistance of vector mosquitoes.

2) To verify the lethal concentration (LC_{50}) for mosquito larvae and mean knock down of lethal time (LT_{50}) for adult mosquitoes of three different strains by using probit analysis.

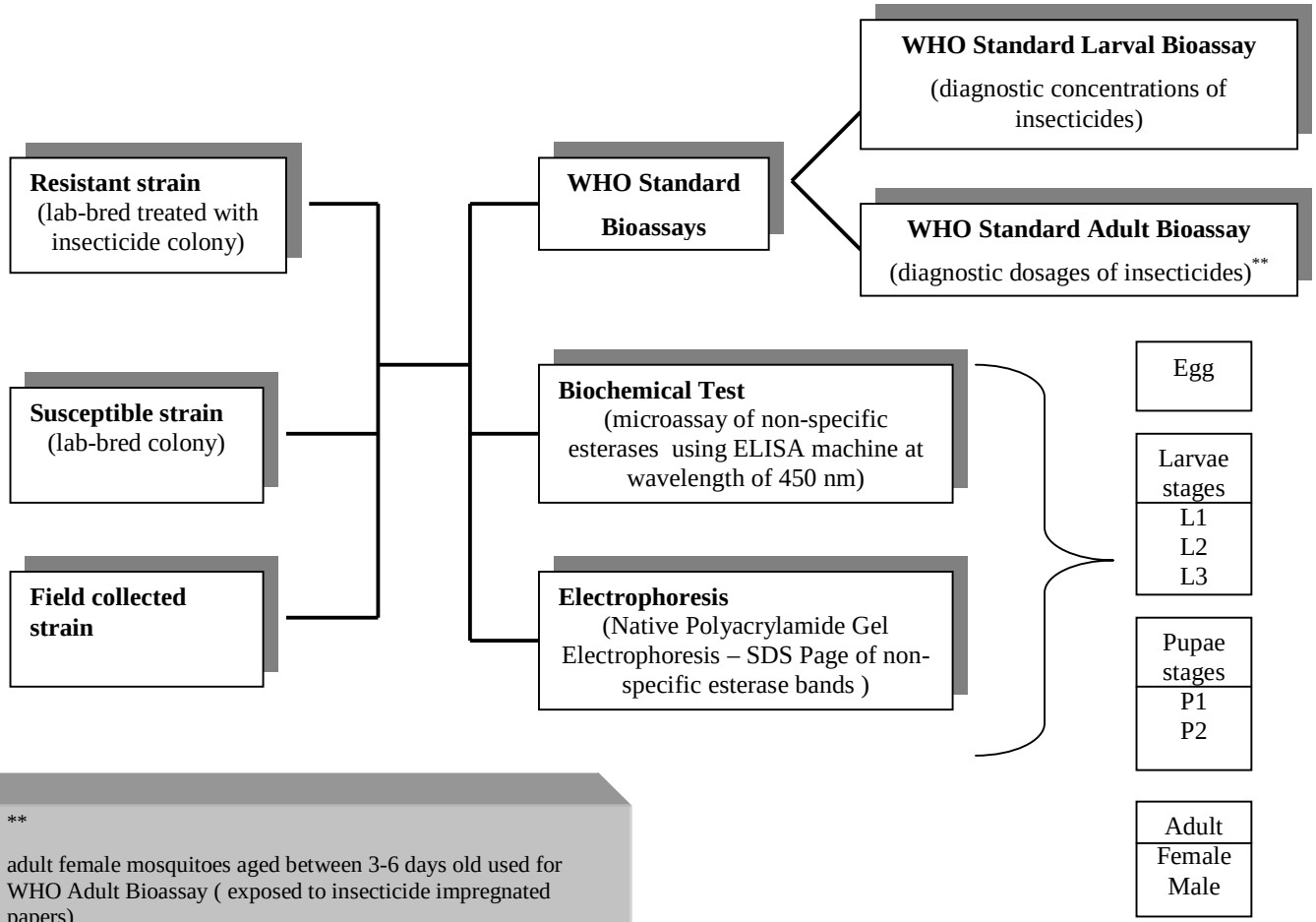
3) To estimate level of non-specific esterase activity in resistant (selection pressure strain), susceptible (lab-bred colony) and field caught strains of mosquitoes by using optical density reading with ELISA Reader Dynatech MR 5000 at 450 nm.

4) To identify the presence of non-specific esterase activity in different developmental stages and sexes of mosquitoes, respectively egg, larvae, pupae, adult male and adult female in *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*.

5) To compare non- specific esterase activity among resistant strain (selection pressure strain- treated with insecticides), susceptible strain (lab-bred strain) and field caught strain of *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus.*

6) To analyze the pattern of inheritance of non-specific esterase bands between developmental stages and sexes among the 3 different strains of mosquitoes respectively egg, larvae, pupae, adult male and adult female in *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus*.

Figure 1.1: Schematic of Experimental Design for the Comparison of Esterases Between Life Stages and Sexes of Resistant And Susceptible Strains of Vector Mosquitoes



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adult female mosquitoes aged between 3-6 days old used for WHO Adult Bioassay (exposed to insecticide impregnated papers)

WHO Standard Exposure Time (Hours)

Insecticides	*Culex*. sp (hrs)	*Aedes*. sp (hrs)
Malathion	1	1
Permethrin	2	1
Propoxur	3	1