Chapter – 2.
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The vector potential of a mosquito strain to dengue viruses is the result of the interactions of environmental conditions and factors affecting their abundance. Some of the important factors are blood feeding behaviour of the mosquito, its survival and ability to support the virus multiplication (Gubler, 1988). The cycle of dengue virus in mosquitoes is a complex phenomenon wherein the virus first binds to the receptors in the midgut cells and multiplies. In some events it escapes multiplication in the gut and reaches the haemocoel directly. This is possible in case of leaky midgut. After multiplying in the gut cells, by budding it is released into the haemocoel and salivary glands. Here again the entry of the virus is receptor mediated. Once it is established in the salivary glands it is likely to be transmitted to the susceptible host by bite (Hardy, et al, 1983). Studies on the mechanism of the susceptibility of Ae. aegypti mosquitoes to chikungunya virus have revealed similar decimation of virus in the mosquitoes (Mourya, et al, 1998a). On the other hand studies on the development of malarial parasite in the mosquitoes have shown that there are several innate immune responses offered by the vector, Anopheline mosquitoes which determine the successful completion of parasite cycle (Dimopoulos, et al, 1998). It is suggested that during the process of the virus crossing the gut barrier and reaching the salivary glands, the mosquitoes also offer some defence responses. Studies on the selection of both chikungunya and dengue virus-susceptible and -refractory strains of mosquito have shown that virus susceptibility is genetically controlled and it is polygenic and recessive (Miller and Mitchell, 1991; Mourya, et al, 1994b).

The virus susceptibility trait in the mosquitoes is quantitative and it is difficult to select a homozygous susceptible mosquito strain, since several genes are involved in the expression of these characters. Many of these genes are said to have cis and trans control in their expression (Tabachnick, 1994). It has been demonstrated in the past that different geographic strains of the species show variations in their susceptibility to oral infection of dengue viruses (Gubler, et al, 1979). The role of dengue virus in the transmission patterns and pathogenesis is poorly understood (Gubler and Kuno, 1997). Recent phylogenetic analysis on dengue based on the variations in the nucleotide
sequences encoding for the envelope glycoproteins have shown several variations in
dengue virus genomes within each of the four dengue serotypes (Chungue, et al, 1993). In an interesting study by Falcoz, et al, (1999) at Tahiti island, oral susceptibility to
dengue-2 virus of Ae. aegypti was highly differentiated within the samples from the same
island. Two geographical areas on the island were distinguishable by their topography. The Ae. aegypti from the Westcoast showed high heterogeneity in the dengue infection
rates while the Eastcoast area has relatively homogeneity in infection rates. Ecological
features as well as particularities in human activities may explain the differentiation of
infection rates of these two areas. The Westcoast area covers almost 75% of the Tahiti
population. It is also an important urban centre with an international airport, where as the
Eastcoast is more or less forested and less inhabited. Control measures are undertaken
predominantly on the Westcoast because of its economic importance. Sporadic use of
insecticides resulted in the reduction in the available breeding sites for Ae. aegypti in the
Westcoast. It seems that Ae. aegypti females of different genotypes frequented these
restricted habitats. This has reflected in the heterogeneity of dengue virus infection rates
in this area. Use of insecticides in the area can drastically affect the microenvironments
of the breeding habitats of mosquito. As a result of insecticide pressure the susceptible
individuals usually die while the survivors tend to generate insecticide resistance
(Georghiou and Taylor, 1976). Besides this the larval density is also affected and more
feeding resources are available to the surviving larvae as a result of less competition.
This may result in a genetic selection of Ae. aegypti strains with increased tolerance
against the insecticides and perhaps having more susceptibility towards dengue virus
(Suminochitrapon, et al, 1998). The insecticide application pressure is less on the
Eastcoast of Tahiti Island because of its forested area, less urbanisation and low
economic importance. The Ae. aegypti populations from this area show homogenous
infection rates to dengue virus. The study at Tahiti Island indicates that changes in the
microenvironment of Ae. aegypti can affect the virus-vector interactions in case of
dengue viruses.

Expression of any gene, whether in the susceptibility of mosquitoes to virus or
any other normal function is due to the interactions of the intrinsic and extrinsic
(environmental) factors (James, 1992). The environment plays important role in
determining the transmission of dengue viruses. However, the exact influence of
microenvironment on the transmission dynamics is difficult to define. The effects on the
microenvironment of Ae. aegypti may affect its biology. Such as the density of larvae,
the nutritional status, which in turn influences its size, survival and vector competence (Hardy, et al, 1997). Among the environmental factors, temperature and insecticides are major factors, which can have strong impact on the population dynamics of mosquitoes (Tabachnick, et al, 1994). These limiting factors can drastically alter the genetic structure of mosquito population. Repeated exposures to insecticides can select insecticide resistance genes from the population. Such selection of the physiological parameters may add in to the susceptibility of this population to virus.

In a study at Bangkok it was found that the size of *Ae. aegypti* females might be one of the factors influencing their infectivity (Suminochitrapon, et al, 1998). Using a rearing procedure that produced three distinct size classes of mosquitoes, they could demonstrate that more of the larger mosquitoes were infected than small mosquitoes. These results suggest that insect control measures that reduce the density of larvae in individual containers may create larger mosquitoes. These larger mosquitoes are more easily infected and may exacerbate dengue transmission. The influence of conditions in the larval habitat on the susceptibility of adult *Ae. aegypti* to dengue infection not only complicates risk assessment, but also has important practical implications for vector control. Larger mosquitoes are more easily infected, live longer and get bloodmeal more successfully (Sumanochitrapon, et al, 1998). In an another study on mosquito size and virus infection, the authors came to the opposite conclusion (Grimstad and Haremis, 1984). The smaller *Ae. triseriatus* (Say) infected more easily with La Cross encephalitis virus. The observation on *Ae. aegypti* and dengue virus contrast sharply with those on *Ae. triseriatus* and La Cross virus, suggesting that each mosquito-virus system has its own particular characteristics.

The present study incorporates an important parameter with reference to *Ae. aegypti* susceptibility to insecticides and dengue multiplication and transmission in the mosquito. In India, *Ae. aegypti* and associated dengue has now spread to different parts of the country, involving both urban and rural areas (Banerjee, 1996). Reports of severe dengue fever outbreaks and occasional association with dengue haemorrhagic fever (DHF) has given it a serious dimension. The public health authorities tend to control vector borne diseases with the use of pesticides and these control measures are likely to generate insecticide resistance in mosquitoes (Mourya, et al, 1993a). There is now an awareness that this genetic selection of *Ae. aegypti* because of pesticides can indirectly increase the dengue perceptions in the country.
Recent studies on the mechanism of insecticide resistance have revealed that differential splicing of glutathione s-transferase genes are responsible for incurring resistance in *Anopheles gambiae* (Ranson, *et al*, 1998). It can be postulated at this juncture that such gene expression may also occur with virus susceptibility genes in the mosquitoes due to the interaction of environmental factors. It would be important to study whether gradual exposure to pesticides also tends to select out *Ae. aegypti* population in a similar way like *An. gambiae*. Can such changes in the microenvironments at larval and adult stages of *Ae. aegypti* influence their biological fitness also, which can indirectly affects their susceptibility to viruses? Since the use of chemical pesticides is still the method of choice for control of vector borne diseases, it is important to study the associated aspects that are likely to affect the vector-virus interaction.

2.1 Review of relevant Literature

The literature on the possible genetic selection of mosquito strains for virus susceptibility because of the environmental pressures is conspicuous by its absence. Comparatively very few studies have been carried out on these aspects. Similarly very few studies have been carried out on the possible effects of virus on the susceptibility status of its mosquito hosts to insecticides.

Whether the presence or absence of a viral infection in a mosquito vector can vary its susceptibility to the pesticide is not known. However, some studies have been conducted on the effect of dengue virus in *Ae. albopictus* with respect to its susceptibility to malathion (Rawlins, *et al*, 1988). The results indicate that dengue virus did not affect the susceptibility of invertebrate host to pesticides. In another study of similar kind it has been shown that esterase enzymes in *Ae. aegypti* that are responsible for insecticide resistance in mosquitoes get elevated due to CHIK virus infection (Mourya, *et al*, 1995). The exact effect these viruses can produce in enzyme elevation and associated insecticide resistance in mosquito is not known. Studies have shown that in certain rural areas of Maharashtra State, residual spraying of DDT has altered the susceptibility of mosquitoes to virus (Mourya, *et al*, 1993a). Attempts were made by Lee, *et al*, (1997) to study the possible association between malathion exposure and dengue susceptibility in *Ae. aegypti* were negative. It is expected that during a chemical control operation against *Ae. aegypti* both the infected and -uninfected mosquitoes are
exposed equally to insecticide in nature. Laboratory studies have indicated that the mosquitoes collected after the control operation show higher susceptibility to virus than the population before the insecticide exposure (Mourya, et al, 1993). Whether there is a possibility of dual selection of virus susceptibility along with insecticide resistance is a matter of scientific interest. It is known that susceptibility of mosquitoes to dengue virus is polygenic, therefore it is quite natural that during such possible selection some genotypes must be getting selected. Studies carried out on the possible effect of sublethal dosages of insecticide on the susceptibility of mosquitoes to dengue and chikungunya virus was carried out (Lee, et al, 1997; Mourya, et al, 1994b). Those adult, \textit{Ae. aegypti} mosquitoes survived the exposure to malathion were membrane-fed on a feeding suspension containing blood-virus mixture. After an incubation period of 10 days the vectorial capacity of the surviving adults was studied. It appeared that there was no apparent correlation of virus infection rate and exposure to malathion dosages (Lee, et al, 1997). It is thus obvious that as long as mosquitoes survived the initial sublethal effects of malathion they could get infected and supported the development of virus. Contrary to this, studies by other workers have shown that sublethal treatment with various insecticides has little to moderate effect on the susceptibility of some Culicine and \textit{Anopheleine} mosquitoes to filarial and malarial parasite (Gaaboub, and Busvin, 1976; Prasittisuk and Curtis, 1982; Vernick, \textit{et al}, 1989). In a similar study the effect of sublethal dosages of DDT on the vectorial capacity of mosquitoes to Brugia pahangi was investigated (Gaaboub and Busvine, 1976). It was reported that there was a significant reduction of infection rate in the susceptible strain after exposure and facilitated infection rate in the refractory strain. All these findings are relevant in assessing the effects of control programs against dengue vector. Incomplete fogging or improper dosing of malathion can cause exposure of vectors to sublethal dosages resulting in selection of heterozygotes which can contribute to indirect selection of certain genes responsible for insecticide susceptibility. Besides these in such situation dengue infection will continue to occur inspite of regular malathion application (Lee, \textit{et al}, 1997).

Isofemale lines of \textit{Ae. aegypti} raised by oral infection selection for susceptibility and refractivity to chikungunya virus was studied for the concomitant effect of sublethal dosages of insecticides, which did not show significant association between these parameters. Similarly there was no difference in their fecundity. However, another strain of \textit{Ae. aegypti}, which was selected for DDT resistance, showed some increase in virus susceptibility with insecticide selection pressure. Biochemical
analysis revealed that there was an increase in glutathione s-transferase and esterase activity in this strain (Mourya, et al, 1995).

It is generally accepted that sublethal effects of insecticides such as DDT, Carbaryl and Fenitrothion affect the biological processes and reproductive parameters of public health pests. It is also reported that sublethal doses of Insect Growth Regulators have adverse effects on egg hatching, immature development time and adult reproductive potential of the vector mosquitoes (Vasuki, 1992). As with insecticides, arboviral infection in mosquitoes is known to cause some pathologic changes that may affect survival, fecundity and feeding behaviour. (Platt, et al, 1997). Some parasites manipulate their host behaviour that enhances their own reproductive success. A study by Patman and Scott (1995) suggested that once Ae. aegypti become infected with dengue virus they remain infective throughout their life. Their probing response increases but it reduces their engorging capacity. This increases their transmission capacity and they become more efficient disseminators of dengue virus.

A number of biochemical, immunological and molecular techniques are now available for analysing the effects of insecticide exposures on the mosquito population in relation to pathogens transmitted by them. These techniques have specific application, and if used in combination can generate large amount of data on these aspects employing even individual mosquitoes (Hemingway and Karunaratne, 1998).

2.2 Scope of this work

In this thesis, I have reported the results and analysis of the studies carried out to investigate:

- Insecticide susceptibility status of some strains of Ae. aegypti.
- Vector competence studies with four dengue virus serotypes in insecticide resistant and susceptible strains.
- Enzymatic studies on the interaction of dengue virus-vector relationship in Ae. aegypti.
- Studies on the effect of virus in Ae. aegypti in association with insecticide resistance.
- Studies on the biological fitness in Ae. aegypti strains.