

## **Biochemical detection of insecticide resistance mechanism in the populations of *Aedes aegypti* – A dengue and chikungunya vector**

### **Executive Summary**

Development of resistance by vectors has been a major problem in controlling communicable diseases transmitted especially by mosquitoes. Careful scrutiny of the current information about vector resistance shows that the full effect of this phenomenon on control efforts is not clearly known. Researching every resistance problem may not be practical, yet control measures have to be selected, at the time of emergency. Monitoring the resistance development and the mechanisms associated with it is important at each place as it may involve different and complex pattern. In this regard, one of the significant advancement in the mosquito genetics has been the investigation of enzyme variability by electrophoretic technique.

Multiple molecular forms of enzymes or isozymes are commonly found in most organisms. Their profiles are providing answers for many basic questions pertaining to evolution, genetics and development. The technique involves the electrophoretic separation and specific staining of enzyme bands and allows the examination of variation in protein production by genes. Further, understanding the more applied but analogous problems involved in insecticide resistance management depend on the ability to measure allelic frequency in different populations. Quite a few studies on insecticide resistance coupled with electrophoresis have been reported so far. Hence, it is apparent that this technique may help us in relating the detoxifying enzymes to resistance/tolerance to insecticides. Isozyme polymorphism as evident from gel electrophoresis could be used as 'biochemical markers' in studying the genetics of insecticide resistance in the absence of any visible markers. Carboxylesterases, phosphatases and dehydrogenases are the group of enzymes that take part in the detoxification process. Polymorphism is a notable characteristic of insect esterases. They can be classified in to two groups, carboxylesterases and acetylcholine esterases. The former is involved in the detoxification of organophosphates and pyrethroids. Depending on their activity, carboxylesterases are designated as A-esterases and B-esterases. Chakraborti and others from Pune have studied the esterases in 5 different population of *Ae. albopictus* from the field and compared it with lab strain. Rodriguez and others have found increased intensity in a A-esterase band of *Ae. aegypti* from Santiago de Cuba associated with pyrethroid and organophosphateresistance. There are instances where pyrethroid resistance could be associated with increased band intensity of A-esterase, B-esterase and G-6-PD in *An. stephensi*. Further, Gad *et al.*, have revealed esterase polymorphism in two *Ae. caspius* forms from Egypt by electrophoresis.

Further, monooxygenase or the mixed function oxidases (MFO) are the enzymes that give protection to a variety of insecticides in arthropods. Metabolic detoxification is associated with changes in monooxygenase activity, producing pyrethroid-specific resistance (Berge *et al.*, 1998). This complex involves a reductase and one or more cytochrome P-450s and requires NADP as cofactor. G6PD generates this cofactor for monooxygenases. Hence an increase in the

activity of MFO's will be reflected in the activity of G6PD. An increase in the activity of this enzyme is the most versatile mechanisms of resistance in insects. Oxidation mediated by monooxygenase is considered to be the major pathway for pyrethroid detoxification though to a limited extent esterase hydrolysis is also possible. Qualitative and quantitative increase in the activity of G6PD was found to be the mechanism involved in the deltamethrin selected *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Similar association was noticed in the deltamethrin exposed *An. stephensi* at Mysore. Like-wise, role of monooxygenases in resistance has been found in deltamethrin selected *An. minimus* at Thailand. However, MFOs were found to play only a minor role in pyrethroid resistance in *Boophilus microplus* at Brisbane, Australia.

In light of these informations, the present investigation was taken up to assay and compare the susceptibility status of *Ae. aegypti* larvae collected from different areas of Mysore city and surrounding places against synthetic pyrethroids and to analyze the isozyme variations of A-esterase, B-esterase, Glucose-6-Phosphate Dehydrogenase, Acid Phosphatase and Alkaline Phosphatase in order to work out the allelic frequencies in the populations of *Ae. aegypti* from Mysore city and surrounding places. Further, the enzyme assays of A-esterase, B-esterase Glucose-6-Phosphate Dehydrogenase, Acid Phosphatase and Alkaline Phosphatase were also undertaken by spectrophotometers to understand the genetic resistance mechanism involved as *Ae. aegypti* populations from Mysore city and surrounding places. These investigations were aimed to learn the genetic differentiation if any, between the populations and also to correlate the insecticide susceptibility difference with the biochemical data.

Thus in the present investigation a total of five enzymes, which have direct or indirect role in overall fitness were studied. The overall result indicates that all the three groups (I, II & III) of enzymes may have certain role in conferring relatively more fitness in terms of tolerance to insecticide tested in *Aedes aegypti*, rural populations. This reiterates the idea that biochemical approach for the detection of insecticide resistance/ tolerance will help us in integrated vector management (IVM) in rural areas, where these species might have had an earlier exposure to agricultural insecticides. The overall profiles of isozyme studies also indicate that, out of five enzyme loci examined in two populations of *Aedes aegypti*, the isozymes of Est-A and Est-B have revealed significant variation in the allelic frequency. On the other hand, certain alleles could be seen only in one population and absent in the other. In addition, monomorphism was found for *Aedes aegypti* - APH and - AcPH enzymes. The knowledge on diversity, systematics with a base line data on susceptibility to insecticides along with their biochemical genetics will be helpful while undertaking any control measures in future in rural areas. Identification of resistance mechanisms will be helpful to reveal the cross-resistance spectrum facilitating the choice of alternative insecticides and allows monitoring of foci with endemicity. So these two populations of *Aedes aegypti* from urban and rural habitats have presented differential isozyme profile and enzyme activity with significant susceptibility variation to deltamethrin.