ABSTRACT

Three strains of Culicine species, namely *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes albopictus* were bioassayed to determine resistance development to malathion (OP), temephos (OP), permethrin (pyrethroid) and propoxur (CARB). Two methods were i.e., WHO procedures of larval bioassay to determine the susceptibility of lethal concentration (LC) and adult bioassay to determine the lethal time (LT). These mosquito strains were bred in the Insectarium, Division of Medical Entomology, IMR. Thousands of late fourth instar larvae which survived the selection pressure to yield 50% mortality of malathion, permethrin and temephos were reared and colonies were established from adults that emerged. Larvae from these colonies were then subjected to the subsequent 10 generations. Selection pressure at 50% - 70% mortality level was applied to the larvae of each successive generation. The results showed that LC_{50} for both malathion (F61 – F70) and permethrin (F54 – F63) resistant *Culex quinquefasciatus* increased steadily to the subsequent 10 generations indicating a marked development of resistance. The adult female malathion resistant strain have developed high resistance level to malathion diagnostic dosage with resistance ratio 9.3 to 9.6 folds of resistance. Permethrin resistance ratio remained as 1.0 folds of resistance at every generation. It was obvious that malathion resistance developing at a higher rate in adult females compared to permethrin. Female adults exposed to 2 hours of exposure period for propoxur 0.1% showed presence of cross-resistance among the both strains of mosquitoes towards propoxur and it was indicated by 70%- 100% mortality at 24 hours post-recovery period. *Aedes aegypti* shown degree of potency or effectiveness to larvae of when comparison made on its resistance ratio (RR) in ascending order permethrin > malathion > temephos.

It is recommended that temephos is a promising chemical larvicidal agent for the control of *Aedes aegypti* larvae. In contrast, malathion and permethrin were the effective adulticide agent for the control of adult *Aedes aegypti.* There was some degree of a cross-resistance relationship against propoxur in these three strains.

Interestingly, the LC_{50} for temephos selected *Ae. albopictus* larvae fell well within the diagnostic concentration recommended by WHO 1992, i.e. 0.02 mg/L. Permethrin selected *Ae. albopictus* exhibited overall mean ± S.E. for LC_{50} 0.28 ± 0.01 and this indicated *Ae. albopictus* was more tolerant to permethrin 14 fold than temephos and 2.5 fold than malathion. Percentage mortality of selected adults were lesser or can be defined as developed cross-resistance against propoxur for malathion and temephos strain and permethrin selected strain found to be moderately resistant to propoxur. In none of the strains did the LT_{50} approach the World Health Organization (1992) recommended diagnostic dosage. Adult bioassay results for *Ae. albopictus* exhibited permethrin as the most potent insecticide to produce high level of mortality rate in adults.

Microplate assay was performed to measure levels of non-specific esterase enzyme in all the three species of selected strains. There is no correlation observed between the LC_{50} values of malathion and permethrin and non-specific esterases in larval stage of *Cx. quinquefasciatus*. The present study also exhibited no correlation between the resistance ratios of LT_{50} and mean esterase activity in female permethrin strain. Malathion and permethrin selected *Ae. aegypti* and *Ae. albopictus*, the frequency population of female replicates distributed below the resistance threshold and defined non-specific esterase was not associated in malathion and permethrin resistance in adult female of *Ae. albopictus*.

The polyacrylamide electrophoresis gel detected 4 distinct esterase bands in *Cx. quinquefasciatus* malathion and permethrin strain, *Ae. aegypti* selected strains revealed 6 bands labeled as E1, E2, E3, E4, E5 and E6; and 5 regions of esterase bands were detected in *Ae. albopictus* named accordingly E1, E2, E3, E4 and E5. E3 esterase band in *Cx. quinquefasciatus* was very heavily stained when compared to susceptible strain indicating that E3 could be responsible in the resistance mechanism due to malathion (OP). There was also a noticeable increase of band intensity with regards to the esterase activity from one instar to another. It is concluded that non-specific esterase band patterns at different life stages demonstrated a direct relationship between levels of enzyme activity and resistance development in these strains. In *Ae. aegypti* and *Ae. albopictus* selected strains the esterase activity in relation to the band intensity found to be in low level in all the developmental stages, thus suggesting non-specific esterase not playing a role in resistance to malathion and permethrin in these strains.

This comprehensive study has provided information and detailed knowledge about pattern of resistance development, identified potential resistance mechanisms and techniques for their detection; and these information can help to formulate potential strategies for resistance management.