

# CHAPTER 7

## ***RESEARCH HIGHLIGHTS***

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- In the study, *Cx. quinquefasciatus* populations were surveyed and collected from six districts of northern West Bengal: Alipurduar, Coochbehar, Darjeeling, Jalpaiguri, Malda and Uttar Dinajpur. Except for Alipurduar, *Cx. quinquefasciatus* larvae were collected from 3 sites each from a particular district.
- Though survey was done in Kalimpong district as well, mosquito larvae were scarce and as such, adequate number of mosquito larvae for performing the experiments was not available.
- Throughout the study sites, *Cx. quinquefasciatus* larvae and pupae were mostly collected from cemented and earthen drains from residential areas and from drains along the highways.
- In the larval habitat of *Cx. quinquefasciatus*, larval stages of drain flies, chironomids and *Anopheles sp.* were also found to co-exist in a comparatively lesser number.
- In larval bioassay test against temephos (0.02 ppm), almost all of the studied populations showed susceptibility with 98.5-100% mortality except TFG (92.31% mortality) and SHM (80% mortality).
- However, 3 populations *i.e.*, TFG, SHM and SLG showed resistance to NVBDCP recommended temephos dose (0.0125 ppm) with 85.71%, 44.23% and 88.2% mortality respectively. Other four populations, COB, DPG, DLK and ISL showed incipient resistance to temephos with 92.3% – 96% mortality.
- The  $LC_{50}$  and  $RR_{50}$  values of the studied populations were significantly higher than that of SP with highest  $RR_{50}$  value (11) in SHM. Moreover, the site-specific diagnostic dose

calculated for the study sites ranged from 0.008 ppm in JPT in Jalpaiguri district to 0.138 ppm in SAM in Malda district.

- Susceptibility assays against 3 synthetic pyrethroids used, showed that all of the populations under study showed resistance to pyrethroids except JPT in Jalpaiguri district with 95.65% mortality against permethrin, thereby depicting incipient resistance or unconfirmed resistance towards permethrin.
- MEK and COB in Coochbehar district showed severe resistance to deltamethrin with mortality as low as 7.69%. Similar resistance to lambda-cyhalothrin was observed in the studied populations with lowest mortality of 4% in MLT of Malda district. Resistance to permethrin – a Type I pyrethroid was also found with lowest mortality percent of 7.69 in SHM.
- Adult bioassays performed against DDT revealed severe resistance in *Cx. quinquefasciatus* to DDT in all of the studied populations with even zero mortality in HCP and MLT populations of Malda district. Similar resistance was also observed against propoxur – a carbamate insecticide and malathion – an organophosphate.
- *Cx. quinquefasciatus* populations from six different districts of northern West Bengal in the present study showed multiple resistance to seven different insecticides belonging to four insecticide class with populations even showing zero mortality rate against DDT, propoxur and malathion in few study sites.
- $KDT_{50}$  and  $KDT_{99}$  values of *Cx. quinquefasciatus* populations in the study were found to be many folds higher than that of SP. CPR in Uttar Dinajpur district showed the lowest  $KDT_{50}$  value for deltamethrin and lambda-cyhalothrin. Similarly, JPT in Jalpaiguri district

showed lowest KDT<sub>50</sub> value for permethrin and APD in Alipurduar district had lowest KDT<sub>50</sub> value for DDT.

- Highest KDT<sub>99</sub> values were recorded in MEK, TFG, SAM and SHM for deltamethrin, permethrin, lambda-cyhalothrin and DDT respectively. In none of the studied populations KDT<sub>50</sub> was recorded to be below 60 minutes.
- The use of two synergists – 4% PBO and 10% TPP showed a non-significant ( $p \leq 0.05$ ) increase in the mortality rate of most of the studied populations. Exposure to PBO prior adult bioassay in JPT restored the susceptibility status to permethrin and deltamethrin. However, in rest of the studied populations, susceptibility to insecticides could not be restored with the use of 4% PBO.
- Similarly, the exposure to TPP prior susceptibility assays in the studied populations of *Cx. quinquefasciatus* could not restore susceptibility to the insecticides used. TFG in Coochbehar district showed a decrease in the mortality when exposed to deltamethrin following PBO exposure.
- Quantitative enzyme activity showed highest activity of  $\alpha$ -CCEs in HCP with 9.535 mM mg protein<sup>-1</sup> min<sup>-1</sup> and in BDN with 9.140 mM mg protein<sup>-1</sup> min<sup>-1</sup> while a lowest activity of 0.743 mM mg protein<sup>-1</sup> min<sup>-1</sup> and 0.749 mM mg protein<sup>-1</sup> min<sup>-1</sup> in MLT and SAM respectively. SAM also showed least activity (0.3321 mM mg protein<sup>-1</sup> min<sup>-1</sup>) of  $\beta$ -CCEs while highest activity was recorded in ISL (4.537 mM mg protein<sup>-1</sup> min<sup>-1</sup>). A maximum of  $\approx 49.66$  fold higher  $\alpha$ -CCEs activity and  $\approx 22.46$  fold higher  $\beta$ -CCEs activity was observed in the studied field populations when compared to SP.

- In the present study,  $\approx 4.42 - 48.65$  fold higher activity of CYP<sub>450S</sub> activity than that of SP was observed with activity ranging from  $0.274 \text{ nM mg protein}^{-1} \text{ min}^{-1}$  in COB to  $3.016 \text{ nM mg protein}^{-1} \text{ min}^{-1}$  in DLK.
- Quantitative GSTs assay showed GSTs activity to be a maximum of  $\approx 80$  folds higher than SP. Highest activity of GSTs was recorded in SHM ( $18.12 \times 10^{-3} \text{ mM mg protein}^{-1} \text{ min}^{-1}$ ) and lowest in JPT ( $76.68 \times 10^{-5} \text{ mM mg protein}^{-1} \text{ min}^{-1}$ ).
- Based on the relative mobility (R<sub>m</sub>) of individual bands on polyacrylamide gel, highest number of  $\alpha$ -CCEs isozymes was recorded in SHM, FLB, MLT, SAM and CPR while only a single isozyme for  $\alpha$ -CCEs was observed in MEK and HCP. Similarly, highest  $\beta$ -CCEs isozymes were observed in ISL while DPG, HP, MLT, SAM and CPR expressed only a single  $\beta$ -CCEs isozyme.
- The isozyme bands obtained for both  $\alpha$ -CCEs and  $\beta$ -CCEs varied in staining intensity showing the presence of esterase isozymes in different amount. Furthermore, none of the studied populations showed all of the isozymes for both  $\alpha$ -CCEs and  $\beta$ -CCEs.
- L1014F mutation was observed in all of the studied populations and maximum mutated homozygote (F/F) frequency of 30% was observed in MLT while was absent in TFG and FLB. Highest heterozygote genotype frequency (L/F) of 35% was recorded in CPR.
- L1014S mutation was observed in a comparatively lower frequency with mutated homozygote genotype (S/S) frequency ranging from 5% - 10%. Highest heterozygote genotype (L/S) frequency of 30% was found in TFG. The wild susceptible homozygote genotype (L/L) frequency was the highest observed genotype frequencies in *Cx. quinquefasciatus* populations under study and ranged from 35% – 65%.

- Resistant allele (F) frequency was found to be highest in MLT (37.5%) and highest resistant allele (S) was found in MEK, TFG, FLB, HCP and MLT. L1014F and L1014S mutations were not observed in SP.