6. Summary

- The defoliating caterpillars, *Buzura suppressaria* (looper), *Eterusia magnifica* (red slug) (Lepidoptera) and the sucking bug, *Helopeltis theivora* (tea mosquito) (Hemiptera) and the mite, *Oligonychus coffeae* (red spider) (Acari: Tetranychidae) are reported as major tea pests of the plantations of Darjeeling foothills, Terai and the Dooars, with their regular incidence in the tea growing areas of North-East India.

- Herbivore insects possess an assemblage of enzymes that on one hand regulate their digestive activity as hydrolyzing enzymes and on the other hand constitute defence against chemical toxicants as oxidoreductases and detoxifying enzymes.

- Among the **digestive enzymes** the quantity of amylase detected in the salivary and midgut homogenate was found to be higher in midgut of *B.suppressaria* as compared to almost equal quantity of amylase at salivary and midgut levels in *Et.magnifica*.

- *H.theivora* showed enhanced levels of amylase both in salivary gland and midgut. Amylase could also be detected in the whole body homogenate of *O.coffeae*.

- In *B. suppressaria* the midgut protease activity was significantly higher than that of the salivary gland whereas in *Et.magnifica* almost equal protease activity in oral and midgut regions was recorded.

- The protease activity was evident in salivary gland and midgut of *H.theivora*. The protease activity was found in the whole body homogenate of *O.coffeae*.

- In *B.suppressaria* the lipase activity is relatively higher than that of *Et.magnifica* both at salivary and midgut levels and in *H.theivora* a very low activity of lipase was registered. In *O.coffeae* the lipase activity was also found at a low key.

- Among the **oxidoreductases**, the catalase quantity of *B.suppressaria* was found to be marginally higher in salivary gland homogenate as compared with the midgut homogenate but in larvae of *Et.magnifica* almost similar quantity of catalase was observed both at salivary and midgut levels.

- The catalase present in salivary gland homogenate of *H.theivora* initially showed more activity as compared to that of midgut, and in *O.coffeae* the catalase was detected in whole body homogenate.
- In *B.suppressaria* as well as in *Et.magnifica* the peroxidase in salivary gland homogenate showed less quantity than that of the midgut.

- The peroxidase quantity was found to be similar both in the salivary and midgut homogenate of *H.theivora*. Peroxidase could also be detected in *O.coffeae* at an enhanced level.

- The apparent lack of activity of polyphenol-oxidase in both salivary and midgut homogenate indicated that *B.suppressaria* could possibly use peroxidases as the only enzyme to metabolize tea phenolics, however in *Et.magnifica* polyphenol-oxidase was detected in both saliva and midgut homogenate indicating a different strategy of oxidizing than the former lepidopteran pest species. In *H.theivora*, level of polyphenol-

oxidase was apparently less than that of the peroxidase and in *O.coffeae* polyphenol-oxidase could also be detected in the whole body homogenate.

- Among the **detoxifying enzymes**, a significantly high quantity of the general esterases (EST) in salivary gland homogenate and midgut homogenate of the pesticide-exposed *B.suppressaria* and *Et.magnifica* larvae, collected from conventional plantation (field), over unexposed ones could be registered. This difference possibly indicated a greater esterase-based detoxifying activity in the field-collected specimens.

- Comparison of isozyme profiles for fifth instar caterpillars of laboratory-reared *B.suppressaria* and *Et.magnifica* showed that in the salivary gland homogenate of *B.suppressaria* eight esterase bands were present whereas in midgut homogenate, thirteen bands were present; whereas in *Et.magnifica* the salivary gland homogenates showed two esterase bands and midgut homogenates showed eight bands.

- In *H.theivora* the quantity of esterases was always significantly higher in the midgut than that in the salivary gland independent of tea clone on which they were reared. A marked difference in general esterase quantity was noted between the conventional (pesticide-exposed) and laboratory-reared (pesticide unexposed) female *O.coffeae*.

- The esterase bands developed from midgut homogenate of *H.theivora* were three and could be identified as slow-moving, medium-moving, and fast-moving and bands with a higher staining intensity in pesticide-exposed specimens than the laboratory-reared ones. Analysis of esterase bands showed that the pesticide-exposed female *O.coffeae*

possessed 3 major co-migrating bands whereas the laboratory-reared ones showed only one.

- In *B.suppressaria*, *Et.magnifica* and *O.coffeae* no significant difference in the quantity of glutathione *S*-transferase (GST) was observed between laboratory-reared specimens and pesticide-exposed ones. Moreover, in the qualitative assay, no band formation was detected in laboratory-reared and pesticide-exposed ones, thus suggesting absence of any role of GST in pesticide detoxification in these species.

- In *H.theivora* significantly high glutathione *S*-transferase quantity in specimens from the field-collected population was estimated as compared to laboratory-reared specimens. The banding pattern in PAGE also showed a single low-intensity band in laboratory-reared specimen as compared to a parallel band with high intensity in field-collected specimens.

- Acetylcholinesterase (AChE) quantified from the homogenate of cerebral ganglia of four major pests in question showed a significant difference between the laboratory-reared and pesticide-exposed ones. The zymogram of the acetylcholinesterase of *B.suppressaria* and *Et.magnifica* showed a single band formation with a higher intensity in the pesticide-exposed larvae as compared to the laboratory-reared individuals.

- In *H.theivora* it was also evident that quantity of acetylcholinesterase that binds with the organophosphates and carbamates, was significantly higher in the specimens collected from field compared to the laboratory-reared ones. Electrophoregram pattern of acetylcholinesterase indicated notably high band-intensity in the pesticide-exposed field specimens and low intensity in unexposed ones. Both quantitative and qualitative

analysis of the homogenate of *O.coffeae* showed that the quantity of acetylcholinesterase in the pesticide-exposed specimen was significantly higher as compared with the laboratory-reared ones.

- The insecticide inhibition studies with organophosphate showed that all the pesticide-exposed specimen of the tea pests in the field showed partial or no inhibition of esterase bands *vis-a-vis* in the laboratory-reared individuals all the bands were completely or largely blocked resulting in disappearance of the electrophoretic bands when treated with field-recommended doses of organophosphate pesticides.

- The inhibition studies with the same pesticide also showed partial or no inhibition of AChE band in the pesticide-exposed individual while they disappeared in the laboratory-reared ones.
- The digestive enzymes commonly found in the salivary and mid gut of the tea pests in question are therefore of interest in understanding their feeding relation to host (tea) as well as in devising methods of non-conventional pest management. The defense enzymes work by oxidation, reduction, hydrolysis or conjugation of molecules. The oxidoreductase enzymes are of great value because of their involvement not only in defensive but also in processing the secondary metabolites of the host plant. Many such enzymes involved in detoxification pathways act on a broad array of substrates found as plant allelochemicals and chemical pesticides. The present investigation is contemplated to evaluate the character and quantity of digestive, oxidoreductase and detoxifying enzymes of the four arthropod pests of tea so that the knowledge-base may be utilized in designing control and resistance management programmes of these tea pests in future.