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STUDIES ON ACETYLCHOLINESTERASE AND ITS POSSIBLE ROLE IN INSECTICIDE RESISTANCE OF MAJOR PEST OF TEA, *Helopeltis theivora* FROM DARJEELING FOOTHILLS PLANTATION

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AUTHOR'S CONTRIBUTION

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Tea, *Camellia sinensis* (L.) O. Kuntze is a perennial and monoculture crop growing over extensive areas of Darjeeling hills, Terai and the Dooars regions. It provides an inexhaustible resource for colonization of the tea mosquito bug, *Helopeltis theivora* Waterhouse that cause substantial damage to the tea crop. Insecticides like organophosphates and pyrethroids are regularly applied to control this pest. Acetylcholinesterase (AChE) act by binding to the neurotransmitter (acetylcholine) in some synapses of the nervous system in many pests. The objective of this study was to investigate the quantitative and qualitative differences in the acetylcholinesterase in cerebral ganglion of this sucking bug, and then to compare between specimens that maintained in laboratory conditions and those collected from pesticide exposed tea plantations. A significantly high level of activity of the acetylcholinesterase was evident in the cerebral ganglia homogenate of the pesticide-exposed individual. Comparison of isozyme profiles also showed a common basic pattern with only one acetylcholinesterase band with R_m value of 0.13 that was observed both in pesticide exposed as well as laboratory maintained individual. The field-collected specimens showed deeply stained band indicating an intensive formation of AChE, which tells us much about the insecticide resistance level of this sucking pest. AChE based detection technique would be helpful for easy detection of the pesticide resistance status of this tea pest in near future.

Keywords: Acetylcholinesterase; Darjeeling; *Helopeltis theivora*; pesticide.

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1. INTRODUCTION

Tea growing region has its own distinctive pest fauna though they may be found in other areas as well. More than a thousand species of arthropods are known to attack tea all over the world though only about 300 insects and mites are recorded from India Muraleedharan et al. [1].

The damage potential and recurrence of many pests are well known from tea plantations for a long and one of the major sucking pest, *Helopeltis theivora* that attack most of the tea cultivar from this region, Anonymous [2]. Organophosphates and synthetic pyrethroids are regularly applied to control the tea mosquito bug but still it's a difficult pest to control. O'Brien [3] showed acetylcholinesterase, which acts by binding to the neurotransmitter (acetylcholine) in some synapses of the nervous system is the major target site both for organophosphates and carbamates. Oppenoorth [4] also showed that reduced sensitivity of acetylcholinesterase (AChE) to these insecticides and has been expressed in a number of insects, such as *M.domestica*, in the Colorado potato beetle, Walsh et al. [5] and in the fruit fly, *Drosophila melanogaster*, Mutero et al. [6]. So, as this pest shows resurgence and recurrence despite pesticide treatments, a study on the occurrence of AChE in cerebral ganglion homogenate in both the pesticide exposed and unexposed one was contemplated and by detecting the pesticide resistance status, this knowledge-based may be utilized in designing control programmes of this tea pest as strains or biotype from this region in near future.

2. MATERIALS AND METHODS

2.1 Insect Collection and Maintenance

Adult tea mosquito bug (200-250 nos) was collected from pesticide exposed tea plantations at Dooars and Terai regions of North Bengal. Specimens for laboratory-maintained ones was collected from bio-organic tea plantations of Darjeeling foothills areas and kept on Tocklai variety for two generations. The rearing was done at 27 ± 2^0 C; 72 ± 2 % R_H with a photoperiod of (L: D) 12:12 in transparent containers (30 x 30 cm) with supply of fresh tea twigs from the experimental tea plot maintained organically.

2.2 Enzyme Extraction and Gel Electrophoresis

Adult *H. theivora* (both laboratory reared and pesticide exposed) was dissected and their cerebral ganglion was kept in ice-cold sodium phosphate buffer (0.1 M, pH 7.0) with the help of sterilized scissors and needle. It was then homogenized individually in fresh sodium phosphate buffer containing 0.01 M each of EDTA (Ethylene Diamine Tetra Acetic Acid) and 0.5% Triton X-100. The volume of the buffer was adjusted to produce similar protein concentration in the homogenates of each individual. The homogenate was centrifuged at 12,000g for 10 min at 4^0 C. The supernatant of this preparation was stored at -20^0 C for future use. Native polyacrylamide gel electrophoresis was performed in a vertical electrophoresis unit by using 8% separating and 5% stacking gel with a discontinuous Tris-glycine buffer system. 15 µl of sample homogenate prepared from head region (brain) of this pest was loaded in each lane. Acetylcholiesterase activity was marked according to the method of Lewis and Shute [7]. The gel was preincubated with a mixture of 65 ml 0.1 M sodium phosphate buffer (pH 6.0) and 0.05 g acetyl thiocholine iodide. Then 5 ml 0.1 M sodium citrate was added to the gel buffer and the same was shake well. After that 10 ml of 30 mM copper sulphate ($CuSO_4$) was added. Finally 10 ml 5 mM potassium ferricyanide was added in the reaction mixture and was shaken well. The incubation was completed when the background of the gel turned a yellowish brown. After incubation, the gel was washed for 1 hr with three changes of distilled water and then the gels were photographed and the relative migration of acetylcholinesterase bands in the zymograms was determined by the Kodak digital science 1D Image Analysis Software, version 2.0.3. Relative mobility (R_m) was calculated as: distance migrated by the specific bands (cm) / distance migrated by the marker dye (cm).

2.3 Quantitative Assay of Acetylcholinesterase

Acetylcholinesterase activity was determined by the method of Ellman et al. [8] with some modification. 100 µl of the enzyme solution was added to a test tube containing 2.86 ml of 0.1 M sodium phosphate buffer (pH 7.5) and the mixture was then incubated at room temperature for 5 min. To this 10 µl of 0.01 M 5,5' – dithiobis (2-nitrobenzoic acid) (DTNB) was mixed. After 10 min incubation of the above mixture 30 µl of the acetylthiocholine iodide in phosphate buffer was added and the change in absorbance was determined at 412 nm. The change in absorbance was taken every 1 min for a period of 12 min. The increase in absorbance over 5 minutes period was considered for calculation. The test was replicated five times. All the chemicals used for the experiments were analytical and research grade, EDTA (Ethylene Diamine Tetra Acetic Acid) (Merck), Triton X-100 (Himedia), acetyl thiocholine iodide (Sigma), copper

sulphate (Himedia), potassium ferricyanide (Himedia), 5,5' – dithiobis (2-nitrobenzoic acid) (DTNB) (Himedia), Bovine serum albumin (Himedia). The protein concentrations of the all the above supernatants were determined by the method of Lowry et al. [9]. Bovine serum albumin was used as standard.

3. RESULTS AND DISCUSSION

In *H. theivora* acetylcholinesterase quantity in the homogenate of cerebral ganglia showed a significant difference between the laboratory-maintained and pesticide-exposed individuals in the field (Table 1).

Electrophoregram of *H.theivora* showed formation of a single band (R_m value 0.13) from the homogenate of cerebral ganglia (Fig. 1). The band-intensity was low in laboratory-maintained ones and notably high in the pesticide-exposed specimens. Aldridge and Reiner [10], Silver [11], Oppenoorth [4], Siegfried and Scott [12] showed that organophosphate (OP) and carbamate insecticides act by inhibiting acetylcholinesterase in many vertebrates and invertebrates. AChE is an important regulatory enzyme responsible for the termination of synaptic nerve impulse transmission in cholinergic nerve synapses in animals. In insects, AChE is the target site of organophosphate and carbamate insecticides immobilizing the formers' function and causing death of the exposed insect, Eto [13]. The key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system is the acetylcholinesterase (AChE). Wang et al. [14] showed that organophosphate insecticides, target the AChE and irreversibly inhibit the enzyme by phosphorylating a serine hydroxyl group within the enzyme active site. In the present study the quantity of acetylcholinesterase in the homogenate of cerebral ganglia of *H. theivora* showed a significant difference between the laboratory-maintained and pesticide-exposed ones (Table 1). The zymogram of the acetylcholinesterase of *H. theivora* also showed a single band formation with a higher intensity in the pesticide-exposed individual as compared to the laboratory-maintained ones (Fig. 1).

Table 1. Quantities of acetylcholinesterase of *H.theivora* (mean ± SD) maintained in laboratory on TV clone and pesticides exposed in field

H.theivora	**Acetylcholinesterase quantity μ mol $^{-1}$. min $^{-1}$. mg of protein $^{-1}$**
	Homogenate of cerebral ganglia
Laboratory-maintained	0.050 ± 0.005a
Pesticide-exposed	0.077 ± 0.006 b

Means followed by the different letters in the column are significantly different at p>0.001using t-test

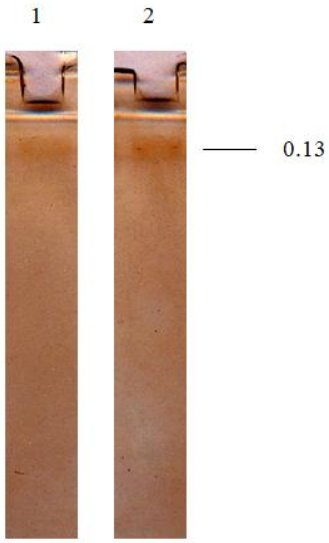

Fig. 1. Zymogram of acetylcholinesterase in the homogenate of cerebral ganglia of *H.theivora*
Laboratory-maintained: Lane 1
Pesticide-exposed: Lane 2

4. CONCLUSION

Based on the above finding that a high level of AChE molecules occurs in the pesticide-exposed individual in comparison with the laboratory maintained one so, we might suggest that a different mechanism may be associated with pesticide resistance of this pest. A large number of available catalytic sites may bind and scavenge toxic molecules like organophosphates and carbamates, still leaving enough free AChE for proper functioning of the nervous system to survive the pest in pesticide exposed field conditions. In a similar finding by Park and Kamble [15] showed high acetylcholinesterase activity was also observed in the head of the German cockroaches resistant to pesticides. So, enhanced occurrence of this enzyme, even at band level, speaks for development of a greater tolerance in the *Helopeltis* populations that are exposed to pesticide spray in tea plantations.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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