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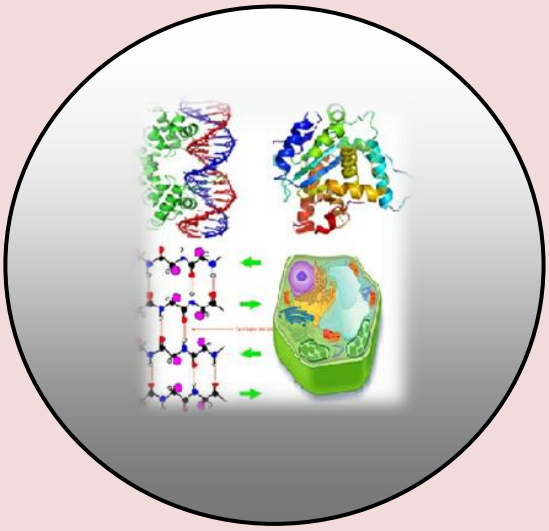

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RESEARCH PAPER

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Possible Role of Acetylcholinesterase in Insecticide Resistance of Major Lepidopteran Tea Pest, *Buzura suppressaria* Guenee from Darjeeling Foothills, India

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ABSTRACT

North Bengal provides a typical agro climate for cultivation of tea, *Camellia sinensis* (L.) O. Kuntze and the plantations cover a wide area of Darjeeling slopes and its adjoining plains. The folivorous pest that is dominant in this region causing substantial damage to the crop is the looper caterpillar, *Buzura suppressaria* Guenee. To control multiple generations of this folivore, insecticides like organophosphates and pyrethroids are regularly applied. The importance of acetylcholinesterase (AChE) was well established in many pests that act by binding to the neurotransmitter (acetylcholine) in some synapses of the nervous system. The objective of this study was to investigate the quantitative and qualitative differences in the acetylcholinesterase of cerebral ganglion of looper, and then to compare between specimens reared in laboratory and those collected from conventional plantations (pesticide-exposed). A significantly high activity of the acetylcholinesterase was evident in cerebral ganglia homogenate of the pesticide-exposed one. Comparison of isozyme profiles for fifth instar caterpillar of looper showed a common basic pattern with only one acetylcholinesterase band with R_m value of 0.168 was observed both in pesticide exposed as well as laboratory reared ones.

In the present study the field-collected larvae showed deeply stained band indicating an intensive formation of AChE. This may be due to enough acetylcholinesterse in cerebral ganglia to protect it by offering a large number of alternative sites of phosphorylation and therefore reduce the amount of OP available to bind AChE. So, the status of AChE of *B. suppressaria* has been revealed in the present study tells us much about the insecticide resistance level of this pest. Techniques based on AChE analysis would help easy detection of the pesticide resistance / tolerance status of this pest.

Key words: *Buzura suppressaria*, Acetylcholinesterase, Tea, Darjeeling and Pesticide.

INTRODUCTION

Tea, *Camellia sinensis* (L.) O. Kuntze is grown as a monoculture over contiguous areas of Darjeeling hills and North–East India. The looper caterpillar, *Buzura suppressaria* is the major arthropod pest that attack most tea cultivars of Darjeeling foothills, Terai and the Dooars plantations of North-East India (Anonymous, 1994).

In spite of the use of synthetic pesticides, such as organophosphates and synthetic pyrethroids, the *B. suppressaria* remains a serious problem of tea and difficult pest to control. The major target site for both OPs and carbamates (O'Brien, 1960) is acetylcholinesterase, which acts by binding to the neurotransmitter (acetylcholine) in some synapses of the nervous system. Reduced sensitivity of acetylcholinesterase (AChE) to these insecticides is well-studied and has been expressed in a number of insects (Oppenoorth, 1985), such as *M.domestica* (Walsh et al. 2001), the Colorado potato beetle, *Leptinotarsa decemlineata* (Zhu et al. 1996), and the fruit fly, *Drosophila melanogaster* (Mutero et al. 1992). In the present study the electrophoregram of acetylcholinesterase (AChE) developed from homogenate of cerebral ganglia of *B.suppressaria* showed only one AChE band in both pesticide exposed and unexposed one. The high intensity in the exposed one indicates that a large number of available catalytic sites may bind scavenge toxic molecules, still leaving enough free AChE for proper functioning of the nervous system in pesticide-exposed field larvae. 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.

MATERIALS AND METHODS

Insect collection and maintenance: 200-250 larvae, of *B. suppressaria* was randomly collected from conventional plantations (maintained by synthetic pesticide spray) of eastern Dooars and western Terai of West Bengal State, India. Separate batch of specimens for laboratory rearing were collected from bio-organic tea plantations of Darjeeling foothills and maintained on Tocklai variety of Tea Research Association 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.

Enzyme extraction and Gel Electrophoresis: Fifth instar larva of *B. suppressaria*, was collected from laboratory colonies that were maintained separately on Tocklai clonal varieties, and from natural populations occurring in conventionally managed plantation (with synthetic pesticide spraying). Each larva was dissected and their cerebral ganglion was collected. Dissection was carried out with the help of sterilized scissors and needle in ice-cold sodium phosphate buffer (0.1 M, pH 7.0) which 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 10,000g for 15 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 the pest was loaded in each lane. Acetylcholiesterase activity was marked according to the method of Lewis and Shute (1966). 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).

Quantitative assay of Acetylcholinesterase: Acetylcholinesterase activity was determined by the method of Ellman et al. (1961) 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.

The protein concentrations of the all the above tissue supernatants were determined by the method of Lowry et al. (1951). Bovine serum albumin was used as standard.

RESULTS AND DISCUSSION

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

Table 1. Quantities of acetylcholinesterase of *B. suppressaria* (mean ± SD) reared on TV clone of tea in laboratory and those exposed to pesticides in field.

B. suppressaria	**Acetylcholinesterase quantity** μ mol^{-1}. min^{-1}. mg of protein^{-1}
	Homogenate of cerebral ganglia
Laboratory-reared	0.051 ± 0.009 a
Pesticide-exposed	0.095 ± 0.01 b

Means followed by the different letters in the column are significantly different at p>0.001 using t-test
The electrophoregram developed from the homogenate of cerebral ganglia of *B.suppressaria* showed formation of a single band (R_m value 0.168) (**Fig. 1**). The band-intensity was notably high in the pesticide-exposed specimens and low in laboratory-reared ones.

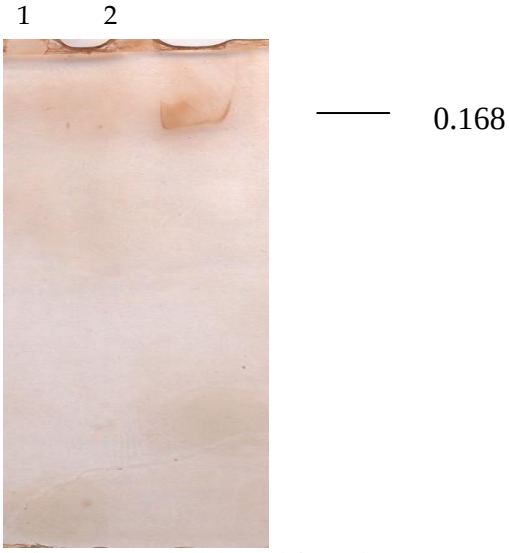

Figure 1. Zymogram of acetylcholinesterase developed from homogenate of cerebral ganglia of *B.suppressaria.*

Homogenate of cerebral ganglia of specimens:
Laboratory-reared: Lane 1
Pesticide-exposed: Lane 2

Many studies have revealed that organophosphate (OP) and carbamate insecticides act by inhibiting acetylcholinesterase in vertebrates and invertebrates (Aldridge and Reiner, 1972; Silver, 1974; Oppenoorth, 1985; Siegfried and Scott, 1990). 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, 1974). Acetylcholinesterase (AChE) is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system. Organophosphorous insecticides, target AChE and irreversibly inhibit the enzyme by phosphorylating a serine hydroxyl group within the enzyme active site (Wang et al. 2004). In the present study the quantity of acetylcholinesterase in the homogenate of cerebral ganglia of *B.suppressaria* showed a significant difference between the laboratory-reared and pesticide-exposed ones (Table 1).

The zymogram of the acetylcholinesterase of *B.suppressaria* showed a single band formation with a higher intensity in the pesticide-exposed larvae as compared to the laboratory-reared individuals (Figure 1). The difference in the AChE quantity of the pesticide-exposed larvae clearly indicated a higher concentration of AChE molecules in the exposed larvae. Based on the finding that a high level of AChE occurs in the pesticide-exposed larvae we might predict that a different mechanism may be associated with resistance. A large number of available catalytic sites may bind and scavenge toxic molecules, still leaving enough free AChE for proper functioning of the nervous system. In a similar finding, high acetylcholinesterase activity was also observed in the head of the German cockroaches resistant to pesticides (Park and Kamble, 2001). So, enhanced occurrence of this enzyme, even at band level, speaks for development of a greater tolerance in the *B.suppressaria* populations that are exposed to pesticide spray in plantations.

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