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Adaptive changes in detoxifying enzymes of looper pest (*Buzura suppressaria* Guen.) of tea in relation to specific tea clone and synthetic pesticide spray

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Abstract

Looper, *Buzura suppressaria* Guen. (Lepidoptera: Geometridae) is the major defoliating pest of tea in Darjeeling foothills, plains and NE India. Midgut esterase isozymes differed in looper populations maintained on TV-1 and TV-25 tea clones. The clone-based major difference was located in the 'Very slow moving band' group (VSM). While three of the VSM bands were absent in the pherograms of midgut of TV-1 looper population, they were prominently present in the midgut of TV-25 reared specimens. In looper populations of pesticide-treated plantations the esterase bands of VSM-group were more elaborate along with deep staining of slow moving (SM) and fast moving (FM) bands. The presence of VSM-bands both in TV-25-reared and pesticide-exposed loopers, collected from conventional plantations, implied a common detoxifying strategy based on esterases. Moreover, the enhancement of band intensity possibly reflected a greater tolerance or resistance of the loopers to synthetic pesticides. Spectrophotometric assay of glutathone S-transferase (GST) level in loopers collected directly from plantation also showed a higher activity (625 µM / min/ mg of protein) as compared to laboratory reared ones on TV-25 (333.3 µM / min / mg of protein) indicating a more tolerance or resistance in loopers occurring in conventional tea plantations.

***Key words**: Esterase isozymes, Glutathione S-transferases, Looper caterpillar, Tea clones*

1. Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze is a perennial monocultural crop, which provides a stable microclimate as well as a continuous supply of food to a large number of arthropod pests, of which the looper caterpillar (*Buzura suppressaria* Guen.) (Lepidoptera: Geometridae) causes a substantial loss of tea crop by heavy defoliation in the plantations of Darjeeling foothills and their adjoining plains. Defoliators can cause upto 40% crop loss (Banerjee, 1993).

To control depredation of various tea clones and multiple generations of *B. suppressaria,* regular spraying of synthetic insecticide is required. Host–plant can also influence the degree of insecticide susceptibility of herbivorous insects indirectly by inducing higher activities of insecticide–detoxifying enzymes or inhibiting these enzymes by limiting the energy availability to the insects to perform detoxification reactions (Brattsten, 1979). Different susceptibilities of insect pests maintained on specific plants have been related to different levels of metabolizing enzymes, presumably induced by the plants (Yu, 1982; Ambrose and Regupathy, 1992; Tan and Guo, 1996). Lindroth (1989) studied the effect of food plant on larval performance and midgut detoxification enzymes in larvae of luna moth, *Actias luna* and found that the kind of larval food plants affected the activities of soluble esterases, which were 1.8 fold higher in larvae that fed walnut, than in larvae that fed birch.

Many enzymes involved in detoxification pathways act on broad array of substrates, including both naturally occurring plant allelochemicals and synthetic pesticides (Gordon, 1961). Therefore, physiological adaptation of herbivores to host plant variety may lead to enhanced metabolism of pesticides as because the same detoxifying mechanism of host–plant allelochemicals may also be effective in detoxifying pesticides.

Esterase–mediated metabolic resistance is widespread and has been detected in almost all pests. Enhanced metabolism by esterases is a major mechanism to counter pesticide stress that has been detected in lepidopterans (Beeman and Schmidt, 1982) and in many other insect orders.

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Glutathione S-transferases are involved in metabolism of organophosphorus and organochlorine compounds and play a significant role in insect resistance to these insecticides (Motoyama and Dauterman, 1980; Clark *et al.*, 1986).

So far no information is available on detoxifying enzymes of *B. suppressaria* leading to its adaptation to tea variety and pesticides. So, the objective of this study was to determine the differences in the general esterase profiles of *B. suppressaria* when reared on two different Tocklai varieties of tea TV-1 (an early release) and TV-25 (a relatively late release from Tea Research Association). Attempts were also made to estimate GST activities by spectrophotometric method to find if a difference existed between *B. suppressaria* larvae reared in the laboratory in pesticide-free condition (on TV-25) and the conventional plantation collected specimens exposed to synthetic pesticide sprays.

2. Materials and Methods

2.1 Insect collection and maintenance

Larvae of *B. suppressaria* were collected from Terai tea plantations mainly having TV-25 as planting material. The collected specimens were separately reared on Tocklai clonal varieties (TV-1 and TV-25) for two generations at 27 ± 2^0 C; 72 ±2 % R^H and photoperiod of 13:11 hrs (L: D) in transparent containers (30 x 30 cm) with supply of fresh tea twigs.

Enzyme extraction was done from laboratory-reared fifth instar larvae of *B. suppressaria* and larvae of the same stage collected from natural populations occurring in conventionally managed plantation (maintained by organophosphate / carbamate / synthetic pyrethroid pesticide spraying).

2.2 Gel electrophoresis

Each larva was dissected and its midgut was collected. Dissection was carried out with the help of a sterilized scissors and needle in ice-cold sodium phosphate buffer (0.1 M, pH 7.0). The midgut was homogenized individually in fresh sodium phosphate buffer containing 0.01 M EDTA (Ethylene diamine tetra acetic acid) and 0.5% Triton X-100. 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. 15 µl of homogenate was dispensed into each well of the gel. Electrophoresis was carried out for 15 separate samples (replicates) from a population at constant current (10 mA) for about 1.5 hr on 8% native polyacrylamide vertical slab gel, using Tris –glycine (pH 8.3) as running buffer (Davis, 1964).

Gel was then stained for 30 min at 36^0 C for esterase with Fast Blue BB salt in 0.1 M phosphate buffer (pH 7.0) containing 0.03 M alpha napthyl acetate solution dissolved in acetone.

Relative migration of esterase bands and zymogram were determined by the Kodak digital science 1D Image Analysis Software version 2.0.3. Relative mobility (R_m) was calculated by:

Distance migrated by the specific bands (cm) / Distance migrated by the marker dye (cm)

2.3 Enzyme activity assay

Glutathione S-transferase activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as described by Habig *et al.* (1974). The assay consists of 50 µl of 50 mM CDNB, 150 µl 50 mM reduced glutathione to 2.77 ml phosphate buffer (100mM, pH 6.5). To this mixture 30 µl enzyme solution from each individual (n = 6) was added and then the change in the optical density was recorded for 6-7 min at 340 nm in UV-Vis double beam spectrophotometer and increase in absorbance over 5 min was considered. For each assay a control without the enzyme was used. Activity was calculated with an extinction coefficient of 9.6 mM /cm for CDNB. Enzyme activity was expressed as µM / min / mg of protein.

2.4 Data analysis

Data generated from studies on electropherograms and enzyme assays were used to interpret: - (i) Host-plant (tea variety) induced variations in *B. suppressaria* larvae (loopers) (ii) Variation between the loopers that were laboratory reared (without exposure) and those occurring in natural population, exposed to spaying operation in tea plantations. (iii) GST enzyme activity of laboratory (TV-25) reared loopers and those occurring as natural population (exposed to synthetic pesticides).

3. Results and Discussion

General esterase pherogram appeared in three groups, i.e. fast moving bands (FM or EST-1), slow moving bands (SM or EST-2) and very slow moving bands (VSM or EST-3) on the basis of relative mobility (**Table 1**). These groups were distinctly separate from one another. In the midgut homogenates, VSM-group was present as five bands in TV-25-reared and field-collected loopers. However, the first three bands of VSM were not present in the midgut homogenate of TV-1 reared population.

A similar pattern, parallel to TV-25, was observed in field-collected looper population, in which the bands of VSM, SM and FM were deeply stained indicating presence of larger quantity of esterases (**Fig. 1a, b, c**).

Isozyme analysis have been applied to identify species, biotypes and host–specific populations in many insects such as aphids and egg parasitoids, *Trichogramma* spp. and others (Loxdale and Hollander, 1989). In the present study zymograms of

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Table 1 : Electrophoretic variation in relative mobility (Rm) of esterase isozyme bands of midgut homogenate of loopers reared on TV-1, TV-25 and of field collected specimens.

Esterase	TV-1	TV -25	Field collected population
VSM (EST- 3)	----	0.161	0.161
	----	0.184	0.184
	----	0.201	0.201
	0.332	0.332	0.332
	0.381	0.381	0.381
SM (EST-2)	0.430	0.430	0.430
	0.458	0.458	0.458
	0.481	0.481	0.481
	0.538	0.538	0.538
FM (EST-1)	0.614	0.614	0.614
	0.632	0.632	0.632
	0.672	0.672	0.672
	0.684	0.684	0.684

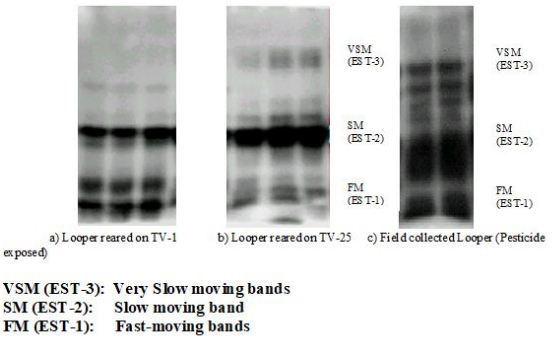

Fig. 1: Zymograms of midgut esterases of loopers fed and maintained on tea clones (a) TV-1, and (b) TV-25 and from (c) conventional plantations subjected to pesticide sprays. (Each lane represents pherogram of an individual looper)

esterase failed to show major differences in banding pattern except three additional "very slow moving bands" being present in the gut homogenate of the looper population reared on TV-25. It is known that herbivorous insects metabolize and detoxify insecticides using the same set of enzymes that are involved in the metabolism of ingested plant allelochemicals (Brattsten, 1979; Ahmad *et al*., 1986).

Three VSM band showed intense staining in the field-collected loopers that were exposed to pesticide treatments in conventional plantations. Such band intensity might be due to greater pesticide tolerance (*vis a vis* resistance). Similarly a higher midgut-esterase activity of *Plutella xylostella,* that were exposed to pesticides, was reported by Mohan and Gujar (2003). Further Maa & Liao (2000) also reported a higher activity of slow moving esterases in the same species under pesticidal stress. Resistant insects display a high level of non-specific esterase activity represented by intense esterase bands (Ono *et al*., 1994), which is also evident in malathion resistant *P. xylostella* (Maa and Chuang, 1983; Doichuanngam and Thornhill, 1989). EST-3 (very slow moving bands) appeared to be crucial for loopers utilizing the tea clone (TV-25) and for withstanding pesticidal exposures. So, this common set of bands (VSM) could be useful as markers in screening the populations of the pest for their resistance / tolerance status.

Furthermore, results also showed a significantly higher GST activity in the field-collected looper (625 µM/ min/ mg of protein) in comparison to laboratory reared on TV-25 variety (333.3 µM/ min/ mg of protein) (**Fig.2**). Thus suggesting that the GST activity might be involved besides esterases in detoxification of organophosphate / organochloride of the loopers collected from the conventional plantations. The role of GST in the degradation of xenobiotics and in development of organophosphate resistance was evident in *Plutella xylostella* (Ku *et al*., 1994) and housefly (Motoyama and Dauterman, 1975; Clark and Dauterman, 1982).

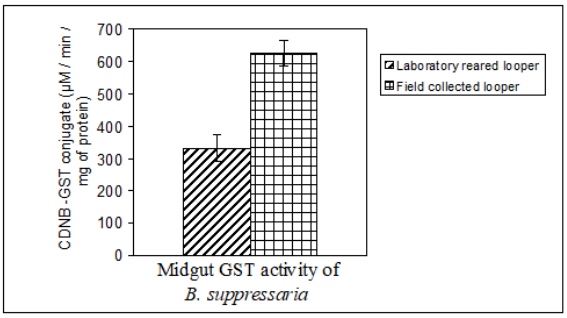

Fig. 2: Midgut GST activity of *Buzura suppressaria* (Looper caterpillar)

4. Conclusions

For Successful pest management strategies, it is important to know the influence of plant allelochemicals that can change the pattern of insecticide resistance. Appropriate dosage and quantity of insecticides can be determined for management of a pest if the host plant species or variety on which it is sustaining is considered. So, far

more efficacious and judicious application of pesticides, the pest response to a specific host plant at first needs to be understood thoroughly. Summarily, therefore, the present study brings to light that pesticide management of the loopers on TV-25 would be more difficult due to their acquisition of detoxifying enzymes than on TV-1. And, in general, a greater pesticide tolerance is developing in the natural population of loopers that are under constant exposure to pesticide spray in plantations.

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