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Determination of Mechanism of Insecticide Resistance Through Enzyme Estimation in Tobacco Caterpillar, *Spodoptera litura* (Fab) in Cotton

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ABSTRACT

The activity of acetylcholine esterase (AchE) in the third instar larvae of *Spodoptera litura* was 0.994 ± 0.06 µ moles / min / ml of enzyme in Guntur strain while it was 0.742 ± 0.03 µ moles / min / ml of enzyme in Prakasam strain. However the activity of carboxylesterase (CE) was 274.99 ± 4.41 n moles / min / mg protein in Guntur strain while it was 227.48 ± 3.95 n moles / min / mg protein in Prakasam strain. The glutathione *S*-tranferase (GST) activity was 0.047 ± 0.01 µ moles / min / mg protein in Guntur strain while it was 0.039 ± 0.01 µ moles / min / mg protein in Prakasam strain. Higher levels of these three enzyme assays were found in Guntur strain compared to Prakasam strain of *S. litura*.

Key words : Acetylcholine esterase (AchE), Carboxylesterase (CE), Glutathione *S*- tranferase (GST), *Spodoptera litura*.

Cotton (*Gossypium hirsutum* L.), is the important commercial crop of India and is considered as a major agricultural commodity with export potential worth Rs. 38,000 crores. India is the largest producer of cotton in the world contributing nearly 15 per cent of global production. Cotton is highly susceptible to insects; especially to the larvae of lepidopteran pests, which is impacting cotton production. The total loss due to damage to cotton crop is estimated to be more than Rs.1200 crores. Fifty percent of the total insecticides consumed in the country are used only on cotton crop. The chemical control to suppress these insect pests are proving the enzyme activities of different strains are important in contributing the insecticide resistance (Devonshire, 1977).

MATERIAL AND METHODS

The present investigation was carried out in the Department of Entomology, Agricultural College, Bapatla, Guntur district, Andhra Pradesh during two seasons *viz.*, *kharif,* 2007 and *kharif*, 2008-09. The estimation of Acetylcholine esterase (AchE), Carboxylesterase (CE) and Glutathion - *S*- transferase (GST) was done using the third instar larvae of *S.litura* of Guntur and Prakasam districts as per the procedure outlined by Kranthi (2005).

Sample preparation

The whole insect was homogenized in 0.05 M phosphate buffer, pH 7.2 containing 0.5 % triton x-100 and 2mM EDTA. The sample was centrifuged at 10,000 rpm at 4ºC for 20 min and the supernatant was used as enzyme source. The protein concentration was determined by the method of Lowry *et al*. (1951) by using Bovine Serum Albumin as standard.

Procedure for AchE estimation

100 µl of the enzyme, 10 µl of the DTNB and 30 µl of acetylcholine iodide was added to 2.86 ml of sodium phosphate buffer (0.1M, pH 8.0) in a sample (4ml) cuvette. 10 µl DTNB and 30 µl of acetylcholine iodide solutions to 2.96 ml sodium phosphate buffer (0.1M, pH 8.0) in another 4 ml cuvette, was used as blank in a double beam spectrophotometer. The increase in absorbance in the sample cuvette at 412 nm for 30 min against the blank was recorded. The AchE activity was calculated by the following formula

$$\text{AchE activity in } \mu \text{ moles/min/ml of enzyme} = \frac{\Delta E \times 1000 \times 3.0}{1.36 \times 10^4 \times 0.10}$$

Where
ΔE = Change in absorbance per minute
3.0 = Total volume of reaction mixture (ml)
0.1 = The volume of enzyme (ml)
1000 = Factor to obtain µ moles
1.36×10^4 = Molar extinction coefficient of the chromophore at 412 nm

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Procedure for CE estimation

5 ml of 1 mM á – naphthyl acetate solution (substrate solution) was mixed with 1 ml enzyme homogenate and incubated for 30 min at room temperature. 1 ml of coupling reagent (Fast blue B salt solution + sodium lauryl sulphate solution) was added to stop the reaction. A red colour was developed immediately and changed to fairly stable blue colour was measured at 600 nm against a reagent blank. The amount of á-naphthol released by carboxylesterase in the sample was assessed using the standard graph. The standard graph was prepared by using á-naphthol working standard solutions (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml). They were pipetted out into a series of test tubes. The volume was made upto 5 ml with 20 mM phosphate buffer in all the test tubes. The mixture was incubated at room temperature for 30 minutes. The reaction was stopped by addition of 1.0 ml coupling reagent and the absorbance was measured at 600 nm against a reagent blank. The amount of á-naphthol released by carboxyl esterase was assessed by using the standard graph. The standard graph was obtained by plotting the concentration of á-naphthol (µg) on the X-axis against absorbance on the Y-axis. Specific activity (SA) of the enzyme can be expressed as n mol. of á-naphthol released min^{-1} mg of $protein^{-1}$ by using the following formula

$$SA = \frac{\mu g \text{ of } \alpha\text{-naphthol released}}{144} \times \frac{1}{30} \times \frac{1000}{\mu g \text{ of protein}} \times 1000$$

Where 144 is M.wt of substrate

Procedure for GST estimation

50 µl of 50 mM CDNB, 150 µl 50 mM reduced glutathione was added to 2.77 ml phosphate buffer (100 mM, pH 6.5, 0.1 mM PTU). 30 µl of enzyme stock was added to the above mixture. The contents were gently shaken and incubated for 2-3 minutes at 25ºC. The contents were transferred into a 4 ml cuvette and placed it in the sample cuvette slot of the spectrophotometer. 3 ml of reaction mixture without the enzyme was taken in a 4 ml cuvette and placed it in the reference slot of the spectrophotometer. The absorbance was noted at 340 nm. The increase in absorbance over 5 minutes was noted for calculations. The enzyme activity was calculated by the following formula

$$\text{CDNB – GSH conjugate formed in } \mu \text{ moles } min^{-1} \text{ } mg^{-1} \text{ protein} = \frac{\text{ABS (increase in 5 min)} \times 3 \times 100}{9.6 \times 5 \times \text{protein in mg}}$$

9.6 = Millimolar extinction coefficient between CDNB – GSH conjugate and CDNB

RESULTS AND DISCUSSION

During the present investigations enzyme activity in the third instar larvae of *S. litura* was also estimated to understand the mechanism of insecticide resistance acquired by the test insecticide. The enzymes *viz*., esterases (Acetylcholine esterase and Carboxyl esterase) and Glutathione *S*- transferase were estimated in the third instar larvae of *S. litura* in Guntur and Prakasam strains during *kharif*, 2008-09. The activity of Acetylcholine esterase activity (AchE) in the third instar larvae of *S. litura* was 0.994 ± 0.06 µ moles / min / ml of enzyme in Guntur strain while it was 0.742 ± 0.03 µ moles / min / ml of enzyme in Prakasam strain (Fig.1). However the activity of Carboxyl esterase (CE) was 274.99 ± 4.41 n moles / min / mg protein in Guntur strain while it was 227.48 ± 3.95 n moles / min / mg protein in Prakasam strain (Fig.2). Pertaining to activity of Glutathione *S*- transferase (GST) it was found to 0.047 ± 0.01 µ moles / min / mg protein in Guntur strain while it was 0.039 ± 0.01 µ moles / min / mg protein in Prakasam strain (Fig.3 & Table.1) The data pertaining to enzyme activity in third instar larvae of *S. litura* clearly indicated higher levels of activity of all the three enzyme assays in Guntur strain which was more resistant

Fig.1 Acetylcholine esterase activity

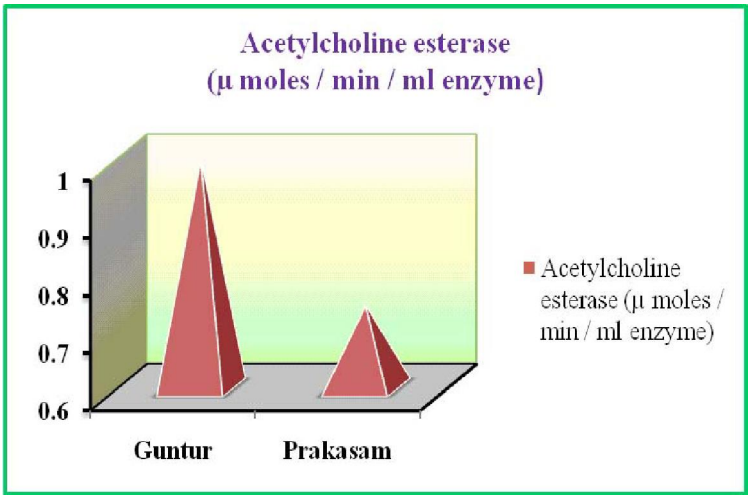

Fig.2 Carboxyl esterase activity

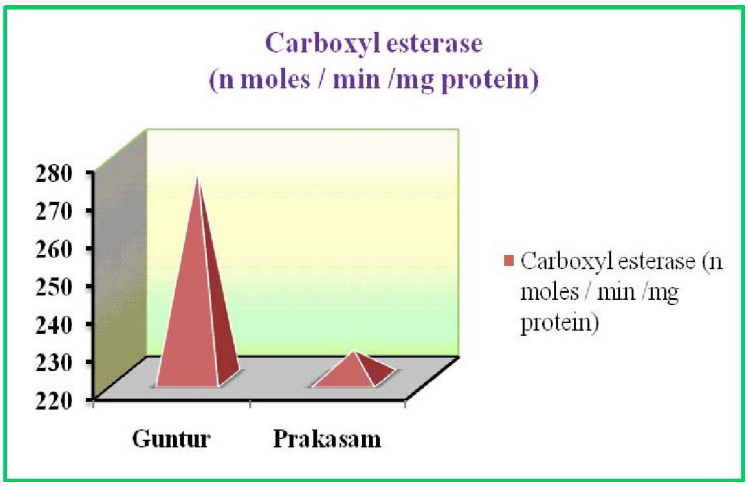

Fig.3 Glutathione –*S*- transferase activity

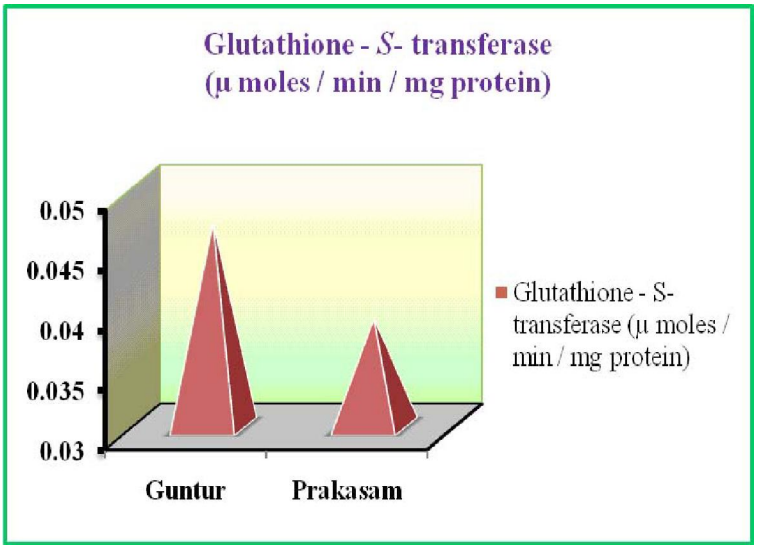

Table 1. Comparison of enzyme levels in *S. litura* populations collected from Guntur and Prakasam districts.

Strain	Enzyme activity		
	Acetylcholine esterase (μ moles / min / ml enzyme)	Carboxyl esterase (n moles / min / mg protein)	Glutathione – *S* – transferase (μ moles / min / mg protein)
Guntur	0.994 ± 0.06	274.99 ± 4.41	0.047 ± 0.01
Prakasam	0.742 ± 0.03	227.48 ± 3.95	0.039 ± 0.01

compared to Prakasam strain during both the seasons. The results pertaining to the activities of enzymes in the present study are in accordance with Devonshire (1977) who reported the esterase activity of different aphid strains ranging from 0.21 ± 0.003 to 7.13 ± 1.46 μ mol of naphthol / h per mg of aphid. The esterase activity in *H. armigera* was also reported by Mehrotra and Phokela (1986); Kranthi *et al*. (1997) and Yang *et al*. (2005). Janarthanan *et al*. (2003) reported the CE activity ranging from 92.03 ± 33.64 to 1820.48 ± 118.46 n mol / min / mg protein in *S. litura* conforming the present findings. In the present investigation, the enzyme activity was found less compared to the earlier reports (Tripathy and Singh, 1999 and Tripathy and Singh, 2000) which may be probably due to the fact that the low insecticide usage in the cotton fields which brought down the resistance levels significantly. The glutathione *S*- transferase (GST) activity of third instar larvae of Guntur and prakasam strains was 0.047 ± 0.01 and 0.039 ± 0.01 μ moles / min / mg protein, respectively. The present investigation is in accordance with Yu (1982) who reported the GST activity of *S. frugiperda* ranged from 17.8 ± 3.4 to 189.0 ± 13.5 n mol DCNB conjugated / min / mg protein. Yang *et al*. (2005) reported the GST activity in midgut of sixth instar larvae of *H. armigera* from susceptible (YS) and resistant (YS-FP) strains with 14.9 ± 0.48 and 21.1 ± 0.93 n mol DCNB conjugated / min / mg protein, respectively conforming the present findings. However Kranthi *et al*. (1997) who reported the GST titres ranging from 93-312 μ mol / min / mg protein in *H. armigera* populations which are concurrent with the present findings. This may be probably due to high selection pressure of the insecticides which impart higher levels of resistance hence high levels of enzyme activity was observed during 1993-96. The present results are indicating low activity of GST which may be due to reduction in the selection pressure of the insecticides, ultimately resulting in decreased levels of insecticide resistance in *S. litura*.

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