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Understanding biochemical mechanisms conferring organophosphate and pyrethroid resistance in *Spodoptera litura* (Feb.)

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ABSTRACT
The army worm *Spodoptera litura* is one of the most damaging pests of cotton, tobacco and castor in India. Synthetic pyrethroids and organophosphate pesticides are used for controlling this pest. Due to continuous use of these chemicals there are reports of insecticide resistance among *S. litura*. In the present study we analyzed the mechanisms involved in the development of pyrethroid resistance and organophosphate resistance in *S. litura* using biochemical marker enzymes. The result shows an increased carboxylesterase activity, GST and AChE activity in organophosphate and pyrethroid treatments in field population as compared to laboratory populations. The data presented in this work shows the occurrence of pyrethroid and organophosphate resistance in *S.litura* may confer through estrases and glutathione complexes.

Introduction

Spodoptera litura (fabricius) (Lepidoptera: Noctuidae) is a polyphagous insect pest of a variety of crops in South Asia (Holloway, 1989), found to cause more than 26-100% yield loss in groundnut, castor, tobacco, and cotton (Dhir *et al*., 1992; Rao *et al*., 1993. Earlier studies on insecticide resistance shows that monooxygenases are the single most important contributors of resistance in *S.litura* (Armes *et al*., 1997; Kranthi *et al*., 2002). Insecticide resistance is one of the major problems for the control of agriculturally and medically important pest (Zlotkin, 2001). Resistance to organophosphate and carbamates pesticides has been reported in *Helicoverpa armigera* and the army worm *Spodoptera litura* in India (Kranthi *et al*., 2001). Insecticide resistance in lepidopteran involves two important biochemical mechanisms, (i) metabolic resistance, and (ii) target site insensitivity. Metabolic resistance to organophosphates and synthetic pyrethroids mainly depends on estrases and glutathione enzyme complexes (Hemingway, 2004).

Among estrases, acetylcholine esterase is major target of organophosphate (OP) and carbamates insecticides (Corbett, 1974). Carboxylesterase (CarE) is another enzyme which plays an important role in OP detoxification by cleaving the esters and is known to be a powerful player in conferring OP resistance among insects (Tang *et al*., 1990; Crow *et al*., 2007). Insect Glutathione S-transferase (GSTs) primarily confers resistance to OP and synthetic pyrethroids (SP) via biotransformation of xenobiotics, and excretion of the conjugated product (Vontas *et al*., 2002).

Till date studies on insecticide resistance in *Spodoptera litura* has largely been confined to the baseline susceptibility data and lethality estimates of different insecticides commonly used in the field (Verma *et al*., 1971, Ramakrishnan *et al*., 1984; Mukherjee and Shrivastava, 1970). However the mechanisms leading to the development of resistance has not been explored in this insect which can provide vital understanding in managing insecticide resistance in *Spodoptera litura*. Hence the present study was aimed at understanding the importance and the extents to glutathione and estrases enzymes complexes in confer insecticide resistance among larval *Spodoptera litura*.

Materials and methods

Insects

Field population of *Spodoptera litura* larvae were collected from Castor (*Ricinus* communis) field and maintained in the laboratory. The population was maintained on castor leaves, larval diet and honey as adult food. This stock served as field population (FP) for the present study. Laboratory maintained stock (10 generations) without insecticide exposure and maintained castor leaves served as susceptible stock population (SP).Both the culture were maintained at 26±1°C and 65±10 % RH; 16:8 LD. For adult moths honey solution with sucrose was provided as diet.

Bioassay

Bioassays were performed on 3rd instars larvae using the leaf dipping method (Shelton *et al*, 2000). Two pesticides, Dichlorvos (50% EC) and λ-cyhalothrin (5% EC) Atul Ltd, Gujarat; Syngenta India Ltd, Mumbai, purchased from market were used in the present study. Pesticides concentrations (five) were prepared (0.01, 0.1, 1.5, 2, 5 ppm). Castor leaf were cut (9 cm, diameter) sizes and dipped in respective concentrations for 30 seconds, air dried and kept in bioassay container (9cm, diameter).10 third instar larvae were released for each concentration and three replicates were maintained for each concentration. In control treatment the larvae were treated with water. After 24hours of exposure the surviving larvae from each treatment were pooled together and were used for subsequent biochemical analysis

Sample preparation

Larval homogenate was prepared for each treatment by grinding 30 larvae in ice- cold 50mM sodium phosphate buffer (pH 7.5). The homogenate was centrifuged at at10, 000 rpm for 20 min at 4ºC. The supernatant were stored at -80ºC and used as enzyme source.

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Biochemical assay

Total Protein estimation

The total protein of homogenate was estimated by Lowry *et al*., (1951) method, using bovine serum albumin (BSA) as a standard to construct the standard curve.

Carboxylesterase Assay

Carboxylesterase activity was measured by the method of Kranthi, (2005). 100µl of enzyme solution from untreated control (deionizedwater) and treated larvae were added to the tubes containing (100µl 0.3 mM α- napthyl acetate as a substrate, 4.8ml of 40mM PB pH 6.8) was added to the test tubes and incubated in dark for 20 minutes at room temperature. After gentle shaking, 1ml of staining solution (1% fast blue BB salt in phosphate buffer [40mM pH 6.8] with 5% sodium dodecyl sulphate (SDS) was added to each tube and incubated at 20ºC for 30 minutes the absorbance was recorded at 590 nm. The enzyme activity was calculated from α- napthol standard curve. Each sample was measured in triplicate to minimize error.

Acetylcholinesterase Assay

Acetylcholine esterase (AChE) activity was measured using acetylcholine-iodide as a substrate according to (Ellman *et al*., 1961). 200 µl of enzyme stock and 100µl of (0.075 M Acetylthiocholine-iodide), 240µl of 0.1M phosphate buffer (pH 7.4) were added and incubated for 15 min at 27ºC, and then 500µl of 0.1M eserine was added and mixed. The change in absorbance was measured at 412 nm.

Glutathione S-transferase Assay

Activity of Glutathione S-transferase (GST) was carried using the method of (Habig *et al*., 1974). 50 µl of 50mM (CDNB) and 150µl of reduced glutathione (GSH) were added to 2.79 ml phosphate buffer (40mM pH 6.8). 10µl of enzyme stock was then added. The mixture were gently shaken and incubated for 2-3 minutes at 20ºC and change in absorbance was measured at 340nm up to 5 min and the enzyme activity in terms of µmol of CDNB conjugated min^{-1} mg of enzyme $protein^{-1}$ was calculated using the extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Statistical analysis

Significance among enzyme activities of AChE, CarE and GST were subjected to statistical analysis using One Way Analysis of Variance (ANOVA) with Bonferronii multiple comparison tests.

Results

Carboxylesterase (CarE) activity

There is a significant increase (P=0.05) in carboxylesterase activity in field collected field population (FP) as compared to laboratory reared population (SP). In dichlorvos and λ-cyhalothrin there was 2.8 and 10 fold increase in carboxylerase activity respectively (Table1). Acetylcholinesterase (AChE) activity

Acetylcholinesterase activity also showed a marked increase of 2.5 and 3.1 fold in Dichlorovos and λ-cyhalothrin treatment respectively (Table 2).

Glutathione –S-Transferase (GST) activity

GST levels were also significantly different in field populations as compared to laboratory population. λ-cyhalothrin (FP) treatment shows 2.3 fold increases in activity, while 1.3 fold increase in GST activity was observed in dichlorvos (FP) as compared to their respective laboratory population (Table 3).

Discussion

Insecticide resistance in lepidopteran is a major concern throughout the world. Several lepidopteran species viz *Helicoverpa armigera*, *Spodoptera litura*, and *Plutella xylostella* have been reported to have developed resistance to several classes of insecticides (Denholm *et al*., 1998). Among the three enzyme biomarkers tested carboxylesterase activity showed significant difference among field population as compared to laboratory population. This suggests that Carboxylesterases are one of the dominant enzymes involved in pesticide detoxification. Earlier studies also suggest that higher carboxylesterase activity do contribute to increased capability of insects to tolerate neurotoxic insecticides (Newcomb *et al.*, 2007; Wheelock *et al.*, 2008). Other studies also confirm a strong correlation between higher esterase activities with the development of resistance in insects (Yang *et al*., 2004; Gao *et al*., 1998; Xu *et al*., 1999). Carboxylesterases are also known to involved in conferring pyrethroid and indoxacarb resistance among lepidopteran insects (Sayyed and Wright, 2006; Hemingway, 2000; Wu et al., 2011).

Acetylcholinesterase (AChE) is a key enzyme which is target of organophosphate and carbamate insecticides (McCaffery, 1999; Gunning and Moores, 2001). AChE insensitivity is a known mechanism which confers resistance in lepidopteran insects (Baek et al., 2005; Yu et al., 2003; Yoo et al., 2002) in the present study we found higher AChE activity in pyrethroid treatments as compared to organophosphate treatment. These suggest that AChE enzymes are involved predominantly in detoxification of pyrethroid insecticides.

Glutathione S-transferase (GST) is known to be involved in conferring organophosphate resistance in houseflies (Motoyama and Dauterman, 1975). GST is also responsible detoxification of several classes of insecticides organophosphate, carbamate, pyrethroid and chlorinated hydrocarbons in *Plutella xylostella* (Sun et al., 2001; Wu et al., 2004; Yang et al., 2009). In *H.armigera* GST is known to confer resistance to insecticides (Rajkumar, 2003) in the present study higher GST activity in field population (FP) showed a high activity exposed to λ-cyhalothrin, and dichlorvos treatment (FP), supports the hypothesis that GST may play a role in combination with estrases enzyme complex in conferring organophosphate and pyrethroid resistance in *Spodoptera litura.* Further studies on analysis of gene expression profiles of these enzyme complexes can further improve our understanding as to how these insects develop resistance to a wide array of insecticides.

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Table 1: CarE activity in mass homogenates of *S.litura* exposed to Organophosphate, Synthetic pyrethroid

Treatments	carboxylesterase activity (µM mg protein^{-1} min^{-1})		Fold increased in activity field & susceptible
	Field strain(FP)	Susceptible strain(SP)	
Control(water)	0.35±0.05*	0.13±0.01	2.10
Dichlorvos	0.49±0.13*	0.26±0.03	2.80
λ-cyhalothrin	2.24±0.34*	0.21±0.02	10.0

All values are mean values; * significant at p=0.05 level [One Way ANOVA]

Table 2: Activity of acetylcholinesterase in mass homogenates of *S.litura* exposed to Organophosphate, Synthetic pyrethroid

Treatments	AChE activity (µM mg protein^{-1} min^{-1})		Fold increased in activity field & susceptible
	Field strain(FP)	Susceptible strain(SP)	
Control(water)	0.43±0.13*	0.20±0.21	2.15
Dichlorvos	0.31±0.21*	0.12±0.13	2.58
λ-cyhalothrin	1.36±0.68*	0.41±0.02	3.11

All values are mean values; * significant at p=0.05 level [One Way ANOVA]

Table 3: Activity of glutathione S-transferase in mass homogenates of *S.litura* exposed to Organophosphate, Synthetic pyrethroid

Treatments	GST- activity (µM mg protein^{-1} min^{-1})		Fold increased in activity field & susceptible
	Field strain(FP)	Susceptible strain(SP)	
Control (water)	0.73±0.03*	0.40±0.01	1.82
Dichlorvos	0.81±0.01*	0.62±0.23	1.30
λ-cyhalothrin	0.96±0.12*	0.41±0.02	2.34

All values are mean values; * significant at p=0.05 level [One Way ANOVA]