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Variation in insecticide detoxification enzymes activity in *Spodoptera litura* (Fabricius) of different geographic origin

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Abstract

A study was conducted to establish insecticides metabolic enzymes activity among three different strains of *S. litura* (Noctuidae: Lepidoptera). Metabolic enzymes, viz., Carboxylesterase, Glutathione-S-transferase and Cytochrome P450 monooxygenase were estimated using standard methodology. Elevated level of CarE (28.09 μmol) observed in Sonapat strain, followed by Delhi strain (26.72 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ of protein. Varanasi strain registered enhanced GST activity (1.380 $\mu\text{mol min}^{-1} \text{mg}^{-1}$) and cytochrome P450 monooxygenase (19.31 $\mu\text{mol min}^{-1} \text{mg}^{-1}$). Variation in detoxification enzymes activity among *S. litura* strains could be attributed due to insecticide usage pattern.

Keywords: Insecticide resistance, biochemical mechanism, resistance monitoring, *Spodoptera litura*

1. Introduction

Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera), is one of the economically important pests of cauliflower in India and Asia. Currently, organophosphate and synthetic pyrethroid insecticides are used all over India to control this pest in Cauliflower [1]. However, the local variation in susceptibility is a widespread phenomenon, which known to occur frequently in different parts of the country predominantly in southern states and other regions where intensive control operations were carried out with insecticides. The indiscriminate use of broad spectrum insecticides is the major cause of failures of pest control [2, 3].

Susceptibility variations are underlined mainly by three important resistance mechanisms, namely decreased penetration, enhanced detoxification, and target-site insensitivity [4]. Of these, enhanced detoxification is the most important mechanism and occurs very often, which is dictated by enzymes, including carboxylesterase, glutathione-S-transferase and cytochrome P450 monooxygenase. The elevated activity of esterase is reported to be a major resistance mechanism in pyrethroid [5] and organophosphate resistant in insects [6]. Carboxylesterase is an important enzyme associated with organophosphate and pyrethroid resistance [7]. Glutathione S-transferase is specifically involved in organophosphate metabolism via conjugation [8]. The cytochrome P450 monooxygenases are important oxidative enzymes involve in pyrethroid detoxification [9]. Presently, synthetic chemicals are the major tool being used widely against this pest, and control failures noticed very often implicated the possibility of insecticide resistance [9, 12]. Therefore, it is of paramount importance to study the underlying metabolic resistance mechanism and susceptibility status to commonly used insecticide. The outcome of this investigation will give clear picture of detoxification enzymes associated with different strains and could be useful to monitor the field-evolved resistance and to develop viable alternative strategies for the successful management of this pest.

2. Materials and Methods

2.1 *S. litura* rearing

The study was conducted during 2011-13 at Indian Agricultural Research Institute (IARI), New Delhi. The sites selected for this study are the major cauliflower growing belt in India. The cauliflower is grown as early, mid and late season crop in this region and farmers are following intensive control operation against major pests. The larvae of *S. litura* were collected from cauliflower field of Indian Agriculture Research Institute, Delhi (28. 38° N, 78. 09° E); Sonapat, Haryana (28. 98° N, 77. 01° E); and Varanasi, Uttar Pradesh (25. 10° N, 82.

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52° E) and reared in the laboratory, (Insect Toxicology Laboratory, Division of Entomology, IARI, New Delhi) India giving artificial diet. The ingredients of the artificial diet with necessary supplements were prepared as described by Gupta *et al* [10] with slight modifications. The ingredients comprised of kidney bean powder (65g), wheat bran (55g), wheat germ (10g), ascorbic acid (4g), casein (3g), yeast powder (25g), methyl parahydroxybenzoate (0.4g), sorbic acid (0.92g), cholesterol (0.25g), streptomycin sulphate (0.1g), 35% formaldehyde (2 ml) and 3 drops of multivitamin, ABDEC (Park-Davis India Ltd). The diets were prepared using standard protocols and the insect cultures were maintained at 27±1°C, 60-65 % RH and a photoperiod cycle of 12L: 12D h. The seven-day old F₁ generation of field-collected larvae was used for the detoxification enzyme assay and insecticide bioassay.

2.2 Enzyme preparation

Uniformly weighed third-instar larvae ($n = 20$) were taken for the preparation of whole larval homogenate using the homogenization buffer (100 mM phosphate buffer, pH 7.0 containing 1 mM EDTA, 1 mM PTU, 1 mM PMSF and 20% glycerol). The larvae were homogenized thoroughly and centrifuged at 10,000 rpm for 20 min at 4 °C, and the supernatant was used as enzyme source for carboxylesterase, glutathione-S-transferase and acetylcholine esterase. For cytochrome P450, the enzyme solution was prepared from larval mid-guts. The dissected guts were pooled and grinded using homogenization buffer and centrifuged at 1000 rpm for 20 min at 4 °C. The collected supernatant was subjected to further centrifugation and served as the enzyme source.

2.3 Estimation of carboxylesterase activity in *S. litura* strains

Carboxylesterase (CarE) activity was measured using α -naphthyl acetate as a substrate by the method described by Asperen [11] and adopted by Kranthi [12] with slight modifications. The sample was prepared with 40 μ l of enzymatic source and was incubated with 5.0 ml of 0.3 mM α -naphthyl acetate for 20 minute at room temperature under dark with occasional shaking. One millilitre of the mixture of fast blue B and sodium dodecylsulfate (SDS) solution at 2:5 ratio was added to the sample and incubated for 20 min at 26 °C temperature. The absorbance value (OD) at 590 nm was recorded with UV-VIS spectrophotometer (ECI, Hyderabad) against a blank. Sample without enzymes served as blank. The activity of carboxylesterase was calibrated with α -naphthol standard curve.

2.4 Estimation of Glutathione S-transferase activity in *S. litura* strains

Activity of glutathione S-transferase (GST) was measured against substrate 1-chloro-2, 4-dinitrobenzene (CDNB) as described by Kranthi [12]. Reaction mixture of 2.77 ml phosphate buffer containing 100 mM, pH 6.5, 50 μ l of 50mM CDNB; 150 μ l, 50 mM reduced glutathione and 30 μ l enzyme solution (homogenates of twenty 3rd instar larvae) was used. The mixture was incubated for 2 to 3 min at 26 °C temperature. Change in the absorbance value (OD) was recorded with UV-VIS spectrophotometer for 5 min at 340 nm. The GST activity (μ mol mg⁻¹ min⁻¹) was then calculated using extinction co-efficient of 9.6 mM⁻¹ cm⁻¹.

2.5 Estimation of Cytochrome P450 monooxygenase activity in *S. litura* strains

Twenty, 3rd instar larvae, from each strain was dissected in phosphate buffer (100 mM, pH 7.0) containing 1.5% KCL. Larvae were placed individually in a dissection tray containing 10 ml of dissection buffer and held, stretched and fixed by using fine pins through the head and posterior region, with the dorsal side facing uppermost. After careful dissection, the food bolus was completely removed from the gut portion. The mid-guts were transferred to 1 ml of freshly prepared homogenization buffer (100 mM, pH 7.0) containing 1 mM each of EDTA, PMSF, PTU and glycerol. The buffer containing the guts was placed in an ice bucket and homogenized using homogenizer. The homogenate solution was transferred to 1.5 ml polypropylene tubes and centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was collected and the volume was brought up to 6 ml using cold homogenization buffer. Monooxygenase activity was estimated in a freshly prepared midgut homogenate using carbon monoxide different spectra, following reaction with sodium dithionite [12].

2.6 Estimation of Protein content

Protein contents of homogenates were determined using the Coomassie brilliant blue G-250 dye (CBBG) method as described by Bradford [13] and bovine serum albumin (BSA) was used as the standard. The protein content was measured at 595 nm absorbance in a spectrophotometer.

2.7 Statistical analysis

Activities of CarE, GST and cytochrome P450 in the three larval strains were subjected to analysis of variance (ANOVA) followed by Tukey's multiple mean comparison using statistical software SPSS v.16.

3. Results

3.1 Activity of carboxylesterase in *S. litura* strains

The activity of carboxylesterase (CarE) in 3rd instar larvae of *S. litura* collected from three different locations is given in Table 1. The larval strain of Sonapat showed significantly greater activity with 28.09 μ mol min⁻¹ mg⁻¹ of protein followed by Delhi larval strain of 26.72 μ mol min⁻¹ mg⁻¹ of protein. Varanasi larval strain showed the least activity of CarE (10.00 μ mol min⁻¹ mg⁻¹ of protein).

3.2 Activity of glutathione S-transferase in *S. litura* strains

The activity of glutathione S-transferase in Delhi, Sonapat and Varanasi larval strains of *S. litura* showed significant differences (Table 1). The activity towards the substrate CDNB was 1.155, 0.729 and 1.380 μ mol min⁻¹ mg⁻¹ of protein for Delhi, Sonapat and Varanasi larval strain, respectively. The relative activity of GST was 1.20 times more in Varanasi larval strain, while only 0.63 times in Sonapat larval strain as compared to Delhi larval strain.

3.4 Activity of Cytochrome P450 monooxygenase in *S. litura* strains

S. litura from Varanasi showed greater activity of (19.31 μ mol min⁻¹ mg⁻¹ of protein) cytochrome P450 followed by Sonapat (19.07 μ mol min⁻¹ mg⁻¹ of protein) and Delhi (17.15 μ mol min⁻¹ mg⁻¹) of protein strains (Table 1). The relative activity was only 1.11 and 1.13 times more in Sonapat and Varanasi strains, respectively, as compared to Delhi strain.

Table 1: Activity of CarE, GST and Cyt450 monooxygenase enzymes in strains of *S. litura* of different geographic origin

Strain	CarE (nmol/min/mg of protein) (\pm SE) ^a	Relative activity	GST(nmol/min/mg of protein) (\pm SE) ^a	Relative activity*	Cytochrome P450 (nmol/min/mg of protein) (\pm SE) ^a	Relative activity
Delhi	26.72 \pm 0.04 ^b	2.67	1.155 \pm 0.11 ^a	1.00	17.15 \pm 0.14 ^b	1.00
Sonepat	28.09 \pm 0.09 ^a	2.81	0.729 \pm 0.07 ^b	0.63	19.07 \pm 0.01 ^a	1.11
Varanasi	10.00 \pm 0.44 ^c	1.00	1.380 \pm 0.13 ^a	1.20	19.31 \pm 0.05 ^a	1.13

*Relative activity = Activity of enzyme in respective strain/ Least activity observed in Delhi/Sonepat/Varanasi strain
Values in the same column followed by the same letters are not significantly different at P= 0.05

4. Discussion

The role of detoxification enzymes in insecticide tolerance or resistance is well established. Estimating xenobiotic enzymes in the field strain is one of method to study the pesticide tolerance in insects and it is time and labour saving when compared to conventional bioassays. Over-production of detoxification enzymes coupled with prevalence of target site insensitivity in field strains could be responsible for increase in tolerance of pest against insecticides. Carboxylesterase (CarE), glutathione S-transferases (GST) and cytochrome P450 are the major metabolic enzymes detoxifying pesticides in insects systems [15-18].

The esterases are associated with pyrethroid and organophosphate detoxification in insects and carboxylesterase is an important enzyme under esterase family [19]. In the present study, *S. litura* larvae from Sonepat and Delhi showed enhanced activity of CarE than Varanasi strain. CarE activity in Sonepat strain was 2.81-fold and Delhi strain had 2.67-fold higher towards the substrate α -naphthyl acetate than Varanasi strain. The descending order of carboxylesterase activity in three strains of *S. litura* found as Sonepat > Delhi > Varanasi. The variation in carboxylesterase activity could be prevailing pre-adaptive phenomenon exist in different geographical strains as well as insecticide usage pattern. Enhanced level of carboxylesterase activity in insects would results in decreased susceptibility to OP and pyrethroid insecticides. Elevated CarE activity in Sonepat and Varanasi strain might be due indiscriminate use of organophosphate and pyrethroid insecticides in these locations. Study by Mohan *et al.* [21] reported the elevated CarE activity in endosulfan, oxydemeton methyl, monocrotophos, deltamethrin and fenvalerate tolerant strains of *H. armigera*. Carboxylesterase catalyzes the hydrolysis reactions in insecticide detoxification by introducing the hydrophilic group into apolar molecules, enhancing their water solubility [12]. Likewise, elevated esterase activity was found to be responsible for cross resistance to OPs, carbamates and pyrethroids [22].

Glutathione- S-transferase is specifically involved in organophosphate metabolism *via* conjugation [23, 24]. GST involves in Phase I and Phase II reaction of insecticides detoxification, where the Phase I metabolites are conjugated with endogenous intermediates [12]. In the present study, elevated level of GST was observed in Varanasi strain of *S. litura*. The descending order of GST activity was as Varanasi > Delhi > Sonepat in three strains of *S. litura*. The major role of GSTs in organophosphate insecticide detoxification has been reported earlier by various workers [16, 24, 25, 26]. Over use of organophosphates insecticides might be the case for this enzyme pattern in Varanasi strain.

The metabolic enzymes, cytochrome P450 monooxygenase introduce one or more polar groups into the substrate in Phase I metabolism and make them suitable for conjugation at Phase II reaction of detoxification [12]. Pyrethroid resistance in *S. litura* is associated with the enhanced activity of cytochrome P450 monooxygenase (MFO) [5]. In the present study, elevated cytochrome P450 activity noticed in the *S. litura*

larvae from Sonepat and was 1.31 times higher as compared to larvae from Delhi. Elevated level cytochrome P450 could be attributed to better tolerance against pyrethroid insecticides. The greater tolerance in Sonepat and Varanasi strains of *S. litura* against cypermethrin was due to cytochrome P450 monooxygenase enhanced metabolic detoxification. Delhi strain showed lower titre of cytochrome P450 enzyme as compared to other strains, and correspondingly they were found to be more susceptible to cypermethrin. Similarly, pyrethroid resistance in *S. litura* is associated with the enhanced activity of cytochrome P450 monooxygenase (MFO) and esterase [9, 19]. In Sonepat strain, as like carboxylesterase, enhanced monooxygenase activity attributed that, synthetic pyrethroid might be overused in this location.

5. Conclusion

This study gives handy information of status of major pesticide detoxification enzymes in *S. litura* strains of different geographical origin especially from major cauliflower growing belt of India. This is probably due to pre-adaptive mechanism, prolonged exposure to pesticides for many generations and use of pesticides having similar mode of action to manage the *S. litura* in cauliflower. Role of detoxification enzymes were found be associated with differential susceptibility to insecticide. Although, role of enzymes are specific to certain insecticide groups, the multiple enzymes role might be a reason for this mixed response of *S. litura*. Therefore, level and role of various detoxification enzymes in a strain should be taken into account while framing management strategies against *S. Litura* and it should be location specific. Understanding biochemical mechanism of pesticide detoxification will be helpful to replace the existing conventional molecules with novel molecules which are not affected by these enzymes. Results obtained through this study would give the susceptibility status of *S. litura* to most commonly using conventional insecticides and role of metabolic enzymes. The data can be used for the insecticide resistance monitoring and for other alternative strategy to manage this pest in cauliflower crop.

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