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SUSCEPTIBILITY OF *SPODOPTERA LITURA* (FABRICIUS) FROM TARAI REGION TO INSECTICIDES AND ITS ESTERASE PROFILE

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ABSTRACT

Studies were carried out to monitor the level of esterase activity at different age intervals of *Spodoptera litura* and to determine the relative susceptibility of seven day old larvae against six different synthetic insecticides. The esterase activities at different stages of *S. litura* showed that it keeps on increasing. The activity slightly increased from 2nd to 4th day *i.e* 0.120- 0.158 ì moles /mg /min. and from the 5th day, there was drastic increase. Indoxacarb followed by emamectin benzoate were the most effective. The data on relative toxicity to cypermethrin showed that except fenvalerate all other insecticides *viz.*, lambda cyhalothrin, profenophos, indoxacarb and emamectin benzoate were 1.21, 2.45, 7.20 and 2.87 fold toxic to *S. litura*, respectively.

Key words: Insecticide resistance, *Spodoptera litura* , esterase, relative susceptibility

Esterases are the important enzymes involved in the detoxification of various insecticides. Enhanced esterase activity with insecticide resistance has been established in several insect species. The involvement of esterase in insecticide resistance can be detected by insecticide hydrolysis products in metabolic studies, synergistic study of insecticide toxicity by non toxic esterase inhibitors and detection of general esterase activity by electrophoretic and / or spectrophotometric studies towards simple substrates (Cho *et al.*, 1999). Pyrethroid resistance in field populations of Australian *Helicoverpa armigera* is a consequence of the over production of esterase isozymes which metabolize and sequester pyethroid insecticides (Young *et al.*, 2005). Huang and Han (2007) suggested that esterases and mixed function oxidases play an important role in the detoxification of organophosphorus (OP) and pyrethroid insecticides, respectively in *Spodoptera litura* (F.). Therefore, the aim of the present study was to investigate the level of esterases at different stages of *S. litura* and the relative susceptibility of the third instar larvae to commonly used insecticides for monitoring the insecticide resistance, and to identify the possible alternative insecticides or insecticide combinations for controlling *S. litura*.

MATERIALS AND METHODS

Commercial formulations of profenophos (Curacron® 50 % EC, M/s. Novartis India Limited), lamda cyhalothrin (Karate® 5 % EC, M/s Syngenta Crop Protection Limited), cypermethrin (Lacer® 10 %EC) and fenvalerate (Fenval® 20% EC, M/s Searle Agrochemicals India Limited), indoxacarb 14.5 % SC (Avaunt® E I Dupont India Private Limited), emamectin benzoate (Proclaim® 5 % SG, Syngenta Crop Protection Limited) were obtained. Different emulsifiable concentrations were prepared using distilled water following serial dilution technique. For each insecticide, preliminary screening was done by taking broad range of concentrations.

The egg masses of *S. litura* were collected from the cauliflower fields of Pantnagar, Udham Singh Nagar District, Uttarakhand. Thereafter, the insects were reared in laboratory on tender castor leaves under controlled conditions at 27±1°C and 60±5% RH. The larvae of *S. litura*, 7±1 day old, weighing about 40–50 mg from F_1 generation were sorted out from the rearing jar and kept separately for preconditioning for one hour before the treatment. The larvae were exposed to insecticidal residues on castor leaves. The leaf-discs of approximately 6 cm diameter were cut from well-grown castor leaves and thoroughly washed with water and later dipped in the required concentrations of emulsions for 20 seconds and then dried. Treated leaf-discs were then transferred to clean jars (15cm x 10cm). In each jar ten larvae were placed and each treatment including control was replicated thrice. Mortality counts were taken 24 hours after the treatment. The moribund insects were also counted

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as dead. Five to seven concentrations of each insecticide were tested, and data were subjected to probit analysis (Finney, 1971).

Enzyme preparation

Batches of 30- larvae each from different age were sorted out and mass homogenized in 2 ml homogenization buffer (100mM phosphate buffer, pH 7.0 containing 1mM each of EDTA, PTU, PMSF and 20% glycerol), and the homogenates were subjected to centrifugation at 10,000 rpm for 20 minutes. The volume of the supernatant obtained was made up to 2ml using phosphate buffer (100mM, pH7.0). The aliquot (100 ml) was taken from the supernatant in a 1.5ml microcentrifuge tube and the volume was made up to one ml. This solution was used to assay the esterase activity and named as enzyme assay solution.

Determination of esterase activity

Esterase activity was determined according to the method described by Kranthi (2005). Fifty microlitres of enzyme assay solution was taken in a 10 ml test tube and the volume made up to one ml with 950 µl phosphate buffer (40 mM, pH 6.8) and then five ml of substrate solution (1 ml of 30 mM α- naphthyl acetate in 99 ml of phosphate buffer, 40 mM, pH 6.8) was added. One ml of 40 mM phosphate buffer with 5 ml of substrate solution without the enzyme assay solution was kept as control. The whole set was maintained in dark for 20 min at 30^0 C with occasional shaking. After incubation, one ml of staining solution (2 parts of 1 % fast blue BB solution in 5 parts of 5 % SDS) was added to each tube including control and the tubes were kept in dark for 20 min at room temperature. The 1 – naphthol was produced as a product during the esterase action on the substrate (α-napthyl acetate). This 1-napthol was coupled with fast blue BB salt (Sigma, USA). A strong blue colour was produced, which was measured at its absorbance maxima of 590 nm, on a double beam spectrophotometer. For the calibration of the 1- naphthol produced, the procedure of Van Asperen (1962) was followed. Enzyme inhibition was expressed as the mean percentage of activity remaining (with respect to an un-inhibited control) for dihydrodillapiole. Three individual assays of esterase activity were made for each treatment (time interval).

Electrophoretic separation of esterases

Haemolymph was removed from fourth instar larvae (0-24 h after ecdysis) by puncturing proleg and drawing the exuded haemolymph into a 40µl pipette, and placed in 1.5 ml ice cooled microcentrifuge tube that contained a few crystals of 1 phenyl-2- thio urea (PTU) to prevent melanization of haemolymph. The haemolymph was centrifuged at 10,000 rpm for 10 minutes at 4°C and the clear plasma supernatant was used as the enzyme source.

Electrophoresis

Non denaturing polyacrylamide gel electrophoresis (PAGE) was done in a vertical electrophoresis unit using 7.0% acrylamide separating gel and 4 % spacer gel with Tris - glycine buffer system (Davis, 1964). Haemolymph samples were diluted at 1:1 ratio with sample buffer (40% sucrose, 0.0372 % EDTA and 0.2% Triton x 100 in glycine buffer system, pH 8.3). Fifteen µl of the sample (10µg protein) was loaded on each lane. Gels were run until the tracking dye migrated to the end of the gel at 4°C under a constant current of 2.5mA/sample well. After the run that lasted for 6 hr, the gel for esterase activity was first pre-incubated in 500 ml of phosphate buffer (40 mM, pH 6.5)) containing 0.02 % αnaphthyl acetate. After pre-incubation, gel was transferred to 500 ml phosphate buffer (40mM, pH 6.5) containing 0.02% αnaphthyl acetate and 0.1 % fast blue BB salt in dark room for 20-30 min at room temperature for incubation with occasional shaking. After the incubation the gels were washed thoroughly with distilled water (pH 6.5 with glacial acetic acid). The gel was then transferred into fixing solution (glacial acetic acid: methanol: water:1: 2: 7) for 1 hour. The protein gels were stained with coomassie brilliant blue over night and destained in 7% acetic acid. Standard marker proteins were run simultaneously and the molecular weight of esterases and protein fractions were calculated from the relative mobility values using standard curve drawn between Rf value and molecular weight protein.

RESULTS AND DISCUSSION

Activity of esterase at different stages of *S.litura*

The role of carboxyl esterases in detoxification has been exploited for imparting selectivity to some insecticides making them highly toxic to insects and relatively harmless to mammals. The titre of carboxyl esterases is important in conferring resistance to insect species against pesticides. The esterase activity of different ages of *S. litura* showed that it kept on increasing from 2^{nd} to 4^{th} day *i.e.,* 0.120- 0.158 µ moles /mg /min. From the 5^{th} day onwards drastic

Table 1. Relative susceptibility of 7-day-old *Spodoptera litura* (Pant Nagar population) to different insecticides

Insecticides	Heterogenity at 4 df. X^2	Regression Equation (Y)	LC_{50} (%)	Fiducial limits	Relative toxicity	Relative resistanc**	
						LC_{50} (%) Delhi population*	LC_{50} (%) Pantnagar population
Cypermethrin	0 .3082	1.3179x + 6.9750	0.0317	0.0199-0.0504	1.00	0.0112	2.83
Fenvalerate	2.1062	1.3399x + 1.3636	0.0960	0.0612-0.1506	0.33	0.0314	3.05
Lambda cyhalothrin	1.2116	1.0087x + 6.5930	0.0263	0.0150-0.0461	1.21	0.0110	2.40
Profenophos	1.1463	0.7602 + 6.4423	0.0127	0.0057-0.0282	2.45	0.0662	0.20
Indoxacarb	0.4517	1.0822x + 7.5505	0.0044	0.0027-0.0073	7.20	0.0051	0.86
Emamectin benzoate	5.1394	0.6648x + 6.2928	0.0114	0.0051-0.0255	2.87	0.0035	3.25

*Shankarganesh *et al.*, 2007

**Relative resistance = $\frac{LC_{50} \text{ of } \textit{Spodoptera litura} \text{ larvae from Pantnagar}}{LC_{50} \text{ of } \textit{Spodoptera litura} \text{ larvae from Delhi}}$

increase was observed (Fig. 1). Mehrotra and Phokela (1986) found high carboxyl esterase activity in *H. armigera* populations collected from Hyderabad followed by Pantnagar and least activity in the populations collected from Hisar. Sahgal *et al.* (1994) found the involvement of ester hydrolysis of pyrethroid in resistant larvae of *Culex quinquefaciatus* by esterases and these larvae had elevated esterases as compared to susceptible larvae.

The esterase profile of 7-day old larvae showed large number of medium size molecular weight bands *i.e*., 20, 23, 28, 29 and 37 kDa (Fig. 2).This is in accordance with the findings of Cho *et al.* (1999) who characterized the specific esterase isozymes related to OP and pyrethroid resistant tobacco cut worm *S. litura*. The laboratory selected strains with decamethrin and chlorpyriphos methyl had higher larval esterase than wild strains. The resistant strains had decreased number and increased intensity of esterase bands. These suggested that the decrease in band is associated with insecticide resistance, refluxing from successive selections with insecticides for several generations.

Toxicity bioassay

Toxicity bioassay was carried out to see the response of seven day old larvae to the selected insecticides by leaf disc residue bioassay technique because third instar larvae used in the biochemical study were found to be very high as compared to other stages tested. The results showed that indoxacarb followed by emamectin benzoate was most effective against this pest. The descending order of toxicity was indoxacarb (0.0044 %), emamectin benzoate (0.0114%), profenophos (0.0127%), lambda cyhalothrin (0.0263 %), cypermethrin (0.0317%) and fenvalerate (0.0960 %).

The data on relative toxicity to cypermethrin showed that except fenvalerate all other insecticides

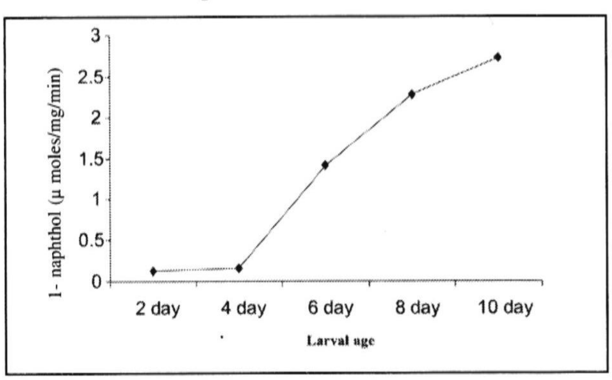

Fig. 1 Esterase activity of *spodoptera litura*

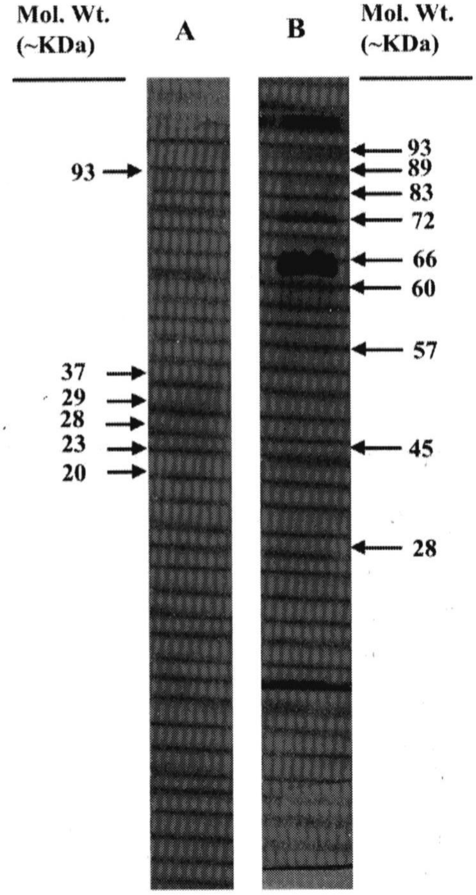

Fig.2 Esterase (A) and protein (B) profile of Pantnagar population of *Spodoptera litura*

viz., lambda cyhalothrin, profenophos, indoxacarb and emamectin benzoate were 1.21, 2.45, 7.20 and 2.87 fold toxic to *S.litura,* respectively. The relative resistance of Pantnagar *S.litura* when compared with Delhi *S.litura* (Shankarganesh *et al*., 2007) showed that the Pantnagar population started to develop resistance against all synthetic pyrethroids and emamectin benzoate. The relative resistance value showed that Pantnagar population of *S.litura* was 2.83, 3.05, 2.40 and 3.25 fold resistant against cypermethrin, fenvalerate, lambda cyhalothrin and emamectin benzoate, respectively. However this population was found to be susceptible to indoxacarb and profenophos. The results are in accordance with the findings of Kranthi *et al.* (2002) who reported the presence of a high level of resistance to cypermethrin ranging from 67 to 148 -fold in the strains of *S. litura* collected from both north and south India. This range of variations in resistance developed by *S. litura* against different insecticides may be attributed to several factors including the type and frequency of insecticides

sprayed on a particular location, cropping pattern, other management practices adapted towards reducing the infestation of *S. litura*.

In the present study, indoxacarb and emamectin benzoate demonstrated higher toxicity against both the populations. The indoxacarb, a member of oxadiazine class of insecticide that acts by inhibiting sodium ion entry into the nerve cell was found highly toxic against *S. litura* (Wing *et al.,* 1998). Emamectin benzoate, a semi synthetic derivative of a natural product, abamectin, effective against *S. litura*, was also found toxic next to indoxacarb (Gupta *et al.,* 2004). They also reported that emamectin benzoate was 9.0- times more toxic than cypermethrin to *S. litura*. Recent reports on other insects *Trichoplusia ni*, *Plutella xylostella,* and *H. armigera* tested against indoxacarb also indicated high susceptibility to new molecules (Liu-Tongxion *et al.*, 1999; Gupta *et al.,* 2004; Loganathan and Dhingra 2005 and Shankarganesh *et al.*, 2007). The present findings are in accordance with the findings of above. Since most of the damage is caused by the seven day old larvae, we can control this stage effectively by using these new insecticides.

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