Pakistan J. Zool., vol. 44(6), pp. 1677-1682, 2012.

Stability, Cross-resistance and Effect of Synergists, PBO and DEF, on Deltamethrin Resistant Strain of *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan

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Abstract.- *Spodoptera exigua* (Hubner), is a polyphagous pest of vegetables and field crops. A field population of *S. exigua* from Lodhran was found resistant to conventional insecticides. It gave 65, 66, 92, 73, 34 and 29–fold resistance to deltamethrin, cypermethrin, chlorpyrifos, profenofos, abamectin and spinosad respectively compared with Lab-PK susceptible population. Field population was divided into two sub-populations. One was left unselected and the second (Del-SEL) selected for five generations at the dose equal to $LC_{50.}$ Bioassays at G_5 for Del-SEL strain gave resistance ratios of 976, 421, 118, 30, 15 and 17-fold for deltamethrin, cypermethrin, chlorpyrifos, profenofos, abamectin and spinosad, respectively compared with Lab-PK. Resistance was found stable for all the insecticides in the field population but it was more stable in Del-SEL strain for deltamethrin, cypermethrin and chlorpyrifos than profenofos, abamectin and spinosad when reared without exposing to deltamethrin (G_5 - G_{10}). It indicated that cross resistance occurred between deltamethrin, cypermethrin and chlorpyrifos. There was no effect of delta selection on the toxicity of three insecticides *i.e.*, profenofos, abamectin and spinosad, which indicated lack of cross resistance with deltamethrin. Deltamethrin resistance in Del-SEL strain of *S. exigua* was suppressed with the synergists such as piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF), suggesting the involvement of monooxygenase and esterase in the development of resistance in *S. exigua.* Due to the stability and cross resistance with other insecticides deltamethrin should be replaced with insecticides having different mode of action possibly with new chemistries in integration with other techniques like catch and kill method with light traps, altering cropping pattern *i.e.*, rotation of host plants with non host plants etc.

Key words: *Spodoptera exigua*, deltamethrin, synergists, PBO, DEF, cross resistance, stability.

INTRODUCTION

Beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) has become difficult to control with conventional insecticides due to resistance development especially in cotton growing areas of the Punjab. This is attributed to the intensive use of insecticides in the region to control the pest complex. The situation has enabled the insect pests to combat the insecticides in order to survive in an exposed environment. Pyrethroids and organophosphates resistance is a realized fact in lepidopteran insect pests in Pakistani cotton growing areas (Ahmad and Arif, 2009, 2010; Shad *et al*., 2010). *S. exigua* has developed resistance against pyrethroids and organophosphates and has become difficult to control with conventional insecticides in most of the areas of the Southern Punjab in recent years (Ahmad and Arif, 2010; Ishtiaq and Saleem, 2011). Different mechanisms of resistance are adopted by the insects such as biochemical, genetic and sometimes multiple mechanisms to withstand the insecticides.

Resistance to pyrethroids has been identified as stable in populations of *Spodoptera litura* (F.) (Ahmad *et al*., 2007) and *Plutella xylostella* (L.) (Sayyed *et al*., 2005) from the Punjab, which has alarmed the farmers to be careful in the selection of insecticide group or members of a particular group. Microsomal oxidase and esterase-specific inhibitors are the possible mechanisms involved in the deltamethrin resistance in Pakistani population of *S. litura* (Ahmad *et al*., 2007).

The present study was undertaken for a field population of *S. exigua* collected from Lodhran region of the Punjab province, Pakistan in order to check the possible mechanism and cross resistance to other insecticides. Such investigations can provide help to reduce the risk of resistance development in *S. exigua*.

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0030-9923/2012/0006-1677 $ 8.00/0

MATERIALS AND METHODS

Insects

Field collected fourth instar larvae of *S. exigua* from Lodhran, Punjab, Pakistan were reared singly in glass vials at 25±3°C and 65±5% RH on artificial diet. Pupae were collected from the glass vials and shifted to small transparent boxes. Moths were kept in oviposition cages (30cm x 30cm x 24cm) with mesh sides for ventilation and provided with 10 % honey solution for feeding.

Insecticides

Commercially available insecticides used for bioassays were deltamethrin (Decis® 2.5EC; Bayer Crop Sciences), cypermethrin (Arrivo® 10 EC, FMC, Philadelphia, PA), profenofos (Curacron® 50 EC, Syngenta), chlorpyrifos (Lorsban® 40 EC; DowAgroSciences), spinosad (Tracer® 240 SC; DowAgroSciences), abamectin (Cure® 1.8 EC; Hebei Vian Biochemical Company, Shijiazhuang, China) and indoxacarb (Steward® 150 EC; DuPont Agricultural Products).

Bioassays

Bioassays were conducted on F_1 generation of field strain with newly molted 2nd instar larvae of *S. exigua* using standard leaf disc bioassay method (Anonymous, 1990). Leaves of non Bt. Cotton variety CIM-496 grown in pots were cut of the size of a Petri dish *i.e.*, 5 cm^2 diameter. Insecticide solutions were prepared in distilled water. For control distilled water was only used. Four to six serial concentrations were made for each insecticide and replicated for six times. Leaves were dipped in the solution for 10 seconds and air dried on paper towel from both sides. Then these leaves were shifted to Petri dishes containing moistened filter paper and five larvae of *S. exigua* were released in each Petri dish.

Generation of baseline susceptibility

Susceptible strain was maintained in the laboratory for baseline data as described previously by Ishtiaq and Saleem (2011). This strain was named as Lab-PK strain and reared for more than two years in the laboratory.

Selection of Spodoptera exigua *for deltamethrin*

F_1 progeny of field collected population of *S. exigua* was divided into two subpopulations. One subpopulation was left unselected called here as UNSEL. The second was selected with deltamethrin at the dose equal to LC_{50} and named as Del-SEL. Cotton leaves were treated with deltamethrin, air dried on paper towel and shifted to clean glass jars autoclaved at 120°C for 20 minutes. About 800 second instar larvae were released on treated leaves for 48 hours. After two days, survivors were shifted singly to artificial diet in glass bottles for rearing to next generation and selection was made from G_1 to G_5.

Stability of resistance in a Del-SEL and UNSEL strain of Spodoptera exigua

Stability of resistance in *S. exigua* was determined to deltamethrin with and without selection pressure. Larvae from the Del-SEL and UNSEL subpopulations, which had been reared from G_5 to G_{10} in the absence of selection pressure, were bioassayed at G_{10}. The resistance ratios (RR) for both unselected and selected populations were determined by dividing their LC_{50} values by the LC_{50} value of Lab-PK (^{a}RR) and also with LC_{50} of UNSEL at G_5 (^{b}RR).

Synergism test

Effect of two synergists *i.e.*, pipronyl butoxide (PBO; Sigma Ltd, UK), an inhibitor of cytochrome P_{450} monooxigenases (microsomal oxidases) and esterases, and S, S, S-tri-n-butyl phosphorotrithioate (DEF; Sigma Ltd, UK), an esterase-specific inhibitor on the toxicity of deltamethrin were evaluated. The dose of PBO and DEF which showed zero mortality for both the strains were determined using leaf disc bioassay method. To test the effect of PBO on the toxicity of deltamethrin, stock solution of PBO @ 20 mg L^{-1} was prepared in water and serial concentrations of deltamethrin was prepared using the stock solution containing PBO and same procedure was used for DEF @ 10 mg L^{-1} . The data for mortality were taken after 2 days exposure and the synergism ratio (SR) were calculated as LC_{50} of the population treated with deltamethrin/LC_{50} of population treated with deltamethrin + synergist.

Data analysis

Abbott's Formula (Abbott, 1925) was used to correct control mortality data where necessary. The data were analyzed for LC_{50} values and their respective 95% fiducial limits (FL) through Probit analysis using Polo-PC (LeOra, 2003). Resistance ratios (RR) were calculated as LC_{50} of field population or LC_{50} of Del-SEL population / LC_{50} of susceptible population.

RESULTS

Toxicity of different insecticides to Lab-PK and the field population

The results of toxicity to Lab-PK strain showed that this population was susceptible to deltamethrin and other insecticides such as cypermethrin, chlorpyrifos, profenofos, abamectin and spinosad. The slope for deltamethrin was steeper which suggested homogeneous response compared to other insecticides (Table I).

The response of conventional insecticides like deltamethrin, cypermethrin, chlorpyrifos and profenofos to the field population was found significantly higher with resistance ratios (RR) 65, 66, 92 and 73, respectively. Resistance ratios for new chemistry insecticides like abamectin and spinosad were at moderate level, *i.e.* 34 and 29, respectively for field population compared with Lab-PK (Table I).

Selection with deltamethrin and cross resistance with other insecticides

Selection with deltamethrin of field collected population (G_1 to G_5) of *S. exigua* increased the resistance ratio to 976 fold compared to Lab-PK (^{a}RR) and 16 fold (^{b}RR) than that of field population (Table I). A significant increase in the slope ($P<0.05$) for Del-SEL strain was observed compared with field population at G_1 (Table I).

The Del-SEL population showed resistance ratios of 421, 118, 30, 15 and 17 for cypermethrin, chlorpyrifos, profenofos, abamectin and spinosad respectively compared with Lab-Pk and 7, 2, 0.50, and 0.75 compared with field population. The increase in resistance ratio for cypermethrin and chlorpyrifos suggested that the cross resistance mechanism might be involved in Del-SEL population of *S. exigua* for these two insecticides while reduction in RR values for profenofos, abamectin and spinosad showed possible lack of cross resistance in Del-SEL population.

Stability of resistance to Del-SEL and UNSEL populations of Spodoptera exigua

Field population was reared in the laboratory for five generations (G_5) without exposure to deltamethrin and resistance was found almost stable (Table I). Although LC_{50} values were decreased for cypermethrin, chlorpyrifos, profenofos, abamectin and spinosad but non-significant change was observed in their respective slopes (Table I).

Del-SEL strain after selecting up to G_5 and reared for G_{10} without exposure to insecticides showed that resistance was stable for deltamethrin, cypermethrin and to some extent for chlorpyrifos. The toxicities of profenofos, abamectin and spinosad were greatly reduced from (G_1 field population) 73, 34 and 29-fold to 27, 14 and 9 folds for G_{10}. It indicated that there is no effect of selection pressure of deltamethrin on the toxicity of these insecticides. The reduction in toxicity for profenofos, abamectin and spinosad (G_1 to G_{10}) might be due to unstable mechanism of resistance for these insecticides in *S. exigua*.

Effect of synergists on deltamethrin resistance

The efficacy of PBO, a microsomal oxidase specific inhibitor and DEF, an esterase inhibitor, were evaluated against the Lab-PK and Del-SEL strain of *S. exigua* (Table II). Both of these had no effect on susceptible strain (Lab-PK) while the toxicity of deltamethrin was greatly reduced with PBO and DEF with synergistic ratio of 52 and 11-fold respectively. These high values of synergistic ratio indicated that microsomal oxidase and estrase specific inhibitors might be involved in the development of resistance in *S. exigua.*

DISCUSSION

Field collected population of *S. exigua* showed high level of resistance for conventional insecticides *i.e.*, deltamethrin, cypermethrin, chlorpyrifos and profenofos compared with Lab-PK strain. This high level of resistance was due to the

Table I.- **Toxicity of laboratory susceptible (Lab-PK), field, UNSEL and Del-SEL population of *Spodoptera exigua* to different insecticides.**

Population	Insecticides	LC_{50} (95% FL) (µg/ml)	Fit of probit line Slope (±SE)	X^2	df	P	n	RR[a]	RR[b]
Lab-Pk	Deltamethrin	6.19 (4.7 - 8.0)	2.08±0.28	0.97	4		210	---	---
Lab-Pk	Cypermethrin	7.7 (5.8 – 10.0)	1.96±0.26	2.07	4	0.72	210	---	---
Lab-Pk	Chlorpyrifos	5.5 (3.9 - 7.3)	1.77±0.25	1.55	4	0.82	210	---	---
Lab-Pk	Profenofos	11 (7.4 - 13.9)	1.78±0.26	1.30	4	0.86	210	---	---
Lab-Pk	Abamectin	0.064 (0.05 - 0.08)	1.81±0.25	2.96	4	0.57	210	---	---
Lab-Pk	Spinosad	0.034 (0.03 - 0.04)	1.97±0.26	2.6	4	0.63	210	---	---
Field (G_1)	Deltamethrin	440 (315 - 602)	1.96±0.28	0.73	3	0.87	210	65	---
Field (G_1)	Cypermethrin	507 (370 - 676)	1.68±0.24	3.73	4	0.44	210	66	---
Field (G_1)	Chlorpyrifos	505 (380 - 657)	1.90±0.25	2.36	4	0.67	210	92	---
Field (G_1)	Profenofos	802 (591 - 1075)	1.56±0.19	2.73	5	0.74	240	73	---
Field (G_1)	Abamectin	2.19 (1.67 - 2.83)	1.97±0.26	2.63	4	0.62	210	34	---
Field (G_1)	Spinosad	0.99 (0.76 - 1.28)	2.00±0.26	2.02	4	0.73	210	29	---
UNSEL(G_5)	Deltamethrin	427 (327 - 565)	1.87±0.25	2.88	4	0.58	210	63	---
UNSEL(G_5)	Cypermethrin	469 (342 - 623)	1.72±0.24	2.69	4	0.61	210	61	---
UNSEL(G_5)	Chlorpyrifos	347 (259 - 466)	1.70±0.23	2.90	4	0.58	210	63	---
UNSEL(G_5)	Profenofos	682 (498 - 930)	1.57±0.22	1.15	4	0.89	210	62	---
UNSEL(G_5)	Abamectin	1.88 (1.42 - 2.43)	1.96±0.26	1.75	4	0.78	210	29	---
UNSEL(G_5)	Spinosad	0.75 (0.56 - 0.97)	2.05±0.31	1.29	3	0.73	180	22	---
Del-Sel (G_5)	Deltamethrin	6636 (5185 - 8399)	2.27±0.34	2.8	3	0.42	180	976	16
Del-Sel (G_5)	Cypermethrin	3241 (2546 - 4081)	2.45±0.34	2.39	3	0.5	180	421	7
Del-Sel (G_5)	Chlorpyrifos	647 (476 - 839)	2.06±0.32	2.77	3	0.43	180	118	2
Del-Sel (G_5)	Profenofos	333 (256 - 431)	1.98±0.26	3.86	4	0.43	210	30	0.50
Del-Sel (G_5)	Abamectin	0.95 (0.68 - 1.27)	1.61±0.21	1.33	5	0.93	240	15	0.51
Del-Sel (G_5)	Spinosad	0.56 (0.41 - 0.75)	1.68±0.23	1.80	4	0.77	210	17	0.75
Del-Sel (G_{10})	Deltamethrin	5549 (4303 - 7170)	2.03±0.26	3.27	4	0.51	210	816	13
Del-Sel (G_{10})	Cypermethrin	2456 (1698 - 3376)	1.74±0.26	1.79	4	0.77	210	319	5
Del-Sel (G_{10})	Chlorpyrifos	418 (309 – 546)	1.88±0.26	1.99	4	0.74	210	76	1
Del-Sel (G_{10})	Profenofos	295 (207 - 396)	1.98±0.30	2.68	4	0.61	210	27	0.43
Del-Sel (G_{10})	Abamectin	0.92 (0.66 - 1.23)	1.65±0.23	0.98	4	0.91	210	14	0.49
Del-Sel (G_{10})	Spinosad	0.31 (0.23 - 0.42)	1.64±0.23	0.46	4	0.98	210	9	0.41

n, number of larvae used in the bioassay, including controls.
[a]RR, resistance ratio, calculated as (LC_{50} of field, UNSEL or Del-SEL population) / (LC_{50} of Lab-PK).
[b]RR, resistance ratio, calculated as (LC_{50} of Del-SEL population) / (LC_{50} of UNSEL at G_5).

Table II.- **Effect of Synergists on the toxicity of deltamethrin in Lab-PK and Delta-SEL population of *Spodoptera exigua.***

Strain	Treatment	LC_{50} (95% FL) (µg/ml)	Fit of probit line Slope (±SE)		df	P	RR[a]	RR[b]
Lab-PK	Deltamethrin	6.8 (4.8 - 9.0)	1.68±0.24	-	4	0.59	---	-
Lab-PK	Del + PBO	6 (4 - 8)	1.48±0.23	0.88	4	0.91	---	0.88
Lab-PK	Del + DEF	6.6 (4.9 - 8.5)	1.91±0.26	1	4	0.81	---	1
Del-SEL	Deltamethrin	6636 (5185 - 8399)	2.37±0.34	-	3	0.42	976	-
Del-SEL	Del + PBO	128 (96 - 167)	1.87±0.25	52	4	0.58	19	52
Del-SEL	Del + DEF	597 (455 - 777)	1.92±0.25	11	4	0.51	88	11

[a] RR, LC_{50} of Del-SEL or Del-SEL+PBO or Del-SEL+DEF / LC_{50} of lab-PK
[b]SR, Synergism ratio was calculated as LC_{50} of insecticide / LC_{50} of insecticide + PBO or of insecticide + DEF

over reliance on these insecticides for the control of armyworms in the cotton growing areas of Pakistan (Ahmad *et al*., 2007; Ahmad and Arif, 2010). Selection with deltamethrin increases the resistance in a field collected population of *S. exigua* by sixteen fold from an LC_{50} of 440 µg mL^{-1} to 6636 µg mL^{-1} after five generations of selection in the laboratory. There was no significant difference found in the slopes of dose-mortality curves of field population (G_1) and in the Del-SEL population (G_5) of *S. exigua*. It showed low genetic variation in the field population.

The stability of insecticide resistance in the absence of insecticides selection is crucial in insect pest management (Sayyed and Wright, 2001). In our study, UNSEL field strain of *S. exigua* showed stability of resistance to almost all the tested insecticides from G_1 to G_5 as it has been reported in Pakistani population of *S. litura* against synthetic pyrethroids (deltamethrin and cypermethrin) and organophosphates (chlorpyrifos and profenofos) (Ahmad *et al*., 2007). When selected for five generations with deltamethrin, resistance was increased for deltamethrin, cypermethrin and chlorpyrifos indicated the possibility of cross resistance in the tested strain of *S. exigua*. The susceptibility of profenofos, abamectin and spinosad showed decreasing trend in LC_{50} values even in Del-SEL strain and rejected the possibility of cross resistance. Del-SEL strain was then reared for G_5 to G_{10} without exposure to deltamethrin and bioassayed at G_{10} for LC_{50} values. The susceptibility of all the insecticides was decreased but the extent of reduction was high for deltamethrin and cypermethrin. This might be due to the fitness cost of resistance. There are different opinions on the impact of fitness costs on the delay of resistance. Firstly fitness costs due to resistance alleles will have direct impact in the field (Tabashnik *et al*., 1994). Secondly even strong fitness costs have a minimum impact on the evolution of resistance (Rouch, 1997).

The resistance may be due to the involvement of certain enzymes in *S. exigua.* To test this hypothesis we performed synergism tests with PBO and DEF. It showed that cytochrome P_{450} monooxygenases and esterases are responsible for deltamethrin resistance in *S. exigua*. These results were found in accordance with the previous studies on different strains of *S. exigua* which have been shown due to reduction in penetration through cuticle and increased activities of MFO (mixed-function oxidases), esterases and glutathione S-transferases (Delorme *et al*., 1988). It has been proved that changes in metabolism and penetration of insecticides usually cause low level of resistance in insects (Mohan and Gujar, 2003; Kristensen *et al*., 2004; Goff *et al*., 2005; Li *et al*., 2006). If there is a synergistic effect of piperonyl butoxide, it would indicate that decrease in toxicity of deltamethrin occur that can be metabolised by P_{450} oxidative metabolism. Hence here it is proved that the effect of piperonyl butoxide (PBO) on the action of deltamethrin was synergistic in the tested strain of *S. exigua* collected from Lodhran. PBO has different impact on different species of insect pests. It was found synergistic in *S. litura* (Ahmad *et al*., 2007), and *H. armigera* (Young *et al*., 2005), antagonistic in German cockroach, *Blattella germanica* and European corn borer, *Ostrinia nubilalis* (Valles *et al*., 1997; Durham *et al*., 2002) and had no effect in western corn rootworm, *Diabrotica virgifera* (Scharf and Siegfried, 1999).

Short life cycle, large number of egg laying capacity, absence of natural enemies in most of the areas and strong migratory ability are some important characteristics that enable *S. exigua* to develop resistance against insecticides. However, beet armyworm could be managed by IPM (Integrated Pest Management) techniques like altering cropping pattern like growing of lucerne fodder side to the cotton crop helped to attract *S. exigua* and it support the susceptible progeny to develop due to less insecticide applications, rotation of host crops with non-host crops to break the population pressure. Light traps could help in catch and kill technique, to minimize the population development in an area without disturbing the nature. Conventional insecticides should be replaced with newer chemistries like emamectin, abamectin, methoxyfenozide and lufenuron (Ahmad and Arif, 2010). These insecticides should be used judiciously and timely which will protect environment and biological agents also. By utilizing these techniques singly or in integration with each other could be helpful to break the resistance development in *S.*

exigua particularly in Lodhran district of Punjab, Pakistan.

ACKNOWLEDGEMENTS

We are grateful to HEC (Higher Education Commission) Pakistan for providing IRSIP Scholarship to Mr. Ishtiaq for his Ph.D. research in Agricultural Entomology.

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(Received 23 November 2011, revised 23 January 2012)