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DETECTION OF INSECTICIDES RESISTANCE IN *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE) DEPENDS UPON INSECT COLLECTION METHODS

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ABSTRACT

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest with high migratory potential, which could contribute significantly to the population outbreaks. Due to indiscriminate use of insecticides the pest has developed resistance to several groups of insecticides. We were interested to establish if the fourth instar larvae of *S. exigua* collected from potato and cabbage would be more resistant than the adults collected using light traps. The results of bioassays suggest that the toxicity of pyrethroids to *S. exigua* was significantly lower for field populations ($P < 0.01$) compared with laboratory susceptible population (Lab-Pak). Levels of resistance to pyrethroids in *S. exigua* collected from potato field were in excess of 20–fold for 8 populations out of 10 populations. The population was significantly more resistant to organophosphates tested {average LC_{50} = 306. 20 (237.39-395.18)} than pyrethroids {average LC_{50} = 146.25 (114.44-186.98)}. The observed resistance in the population collected from cabbage was significantly ($P < 0.01$) higher for spinosad, indoxacarb and chlorfenopyr with a resistance ratio of 702-fold, 1219- and 436-fold respectively compared with Lab-Pak. A possible explanation for variation in resistance to insecticides on different host plants is that different secondary metabolites in cabbage or potato plants have elicited different metabolic changes in *S. exigua*, such as activation or inhibition of metabolic ability, detoxification enzymes or a general increase in esterase activities. The results are discussed in relation to role of different host plants in insecticides resistance management.

Keywords: *Spodoptera exigua*, host plants, organophosphates, pyrethroids, spinosad, indoxacarn, resistance

INTRODUCTION

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) is a cosmopolitan polyphagous insect pest with a worldwide distribution. The *S. exigua* has a wide host range and is considered a serious pest of vegetables, field and flower crops (Suenaga and Tanaka, 1997) and is known as one of the migrant species of the genus *Spodoptera*, including *S. frugiperda, S. litura*, and *S. exempta* (Mitchell, 1979). It undertakes long distance migration in Asia, Europe, and North America (Feng *et al.,* 2003). Its migratory capacity contributes significantly to the population outbreaks and facilitates the geographic expansion of populations (Adamczyk *et al*., 2003). The worthseeing pest problem of *S. exigua* on various crops in many regions of the world including Pakistan is at least partially attributed to its strong migratory capacity (Han *et al.,* 2005) and resistance to insecticides. In Pakistan cotton and cabbage are the two main host plants for the *S. exigua* (Syed and Abro, 2003). In addition to these crops, the pest could also attack peas, wheat and other cruciferous vegetables crops. To determine whether a host plant can contribute in development of resistance to insecticides in the field could be a first step in formulating a resistance management strategy. For example, if a particular host plants delays in development of resistance to insecticides then the plant could be used in crop rotation to keep insect pest population under control.

Spodoptera exigua has a long history of insecticides usage and thus has resulted in the development of resistance to diverse chemical classes, including chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids

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and benzoylphenylureas (Delorme *et al*., 1988; Brewer and Trumble, 1989; Yu *et al*., 2003). The introduction of Bollgard cotton has reduced the numbers of insecticide applications applied for *Heliothis virescence* (Williams, 2001). However, Bollgard cotton is typically treated multiple times annually with pyrethroids to control sucking pests. *Spodoptera exigua* are inherently tolerant to many insecticides and have a high propensity for developing resistance to insecticides (Wolfenbarger, 2002). Resistance is a genetic ability of insect population to increase its tolerance to the toxic effects of an insecticide and requires genetic changes within populations, yet most of the documented resistance relies on measurement of phenotype, which is influenced by environmental as well as genetic factors (Omer *et al*., 1993). Environmental factors that could influence resistance phenotype include host plant, temperature and previous exposure to sub-lethal amounts of insecticides or other toxins (Martinson *et al*., 1991). It has been previously shown that responses to pyrethroid insecticides could significantly be different between various immature stages of an insect species fed on different host plants (Riley and Tan, 2003). We were interested to establish if the fourth instar larvae of *S. exigua* collected from host plants would be more resistant than the adults collected using light traps. Since the fourth instar larvae are expected to have been direct exposure to insecticides, whereas the adults collected from light trap could be migrated from unsprayed fields. Furthermore, we also compared if the larvae collected from cabbage will be significantly more resistant than collected from potato. Due to high commercial value, growers spray large quantity of insecticides on cruciferous vegetables than potato to control various lepidopteran pests. We were more interested to compare the level of insecticides resistance on vegetables since these crops harbor the insect when main cash crop (cotton) is not present in the field. If we can formulate a resistance management strategy for vegetables then it would be very easy to manage on cotton crop.

MATERIALS AND METHODS

Insect rearing and handling

Spodoptera exigua is present throughout the year. The numbers of *S. exigua* are always the lowest in the month January and February but with a peak in the 3rd week of March and whole month of April before cotton season. During cotton season maximum moths can be collected in October and then the population is gradually declined (Fig. 1). When cotton crop is harvested, then it is moved to cruciferous crops/vegetables. The pest is continuously exposed to insecticides and it receives sprays first on cotton crop then on vegetables (cauliflower, arum, okra and to lesser extent on potato). Growers usually carry out 1–2 sprays per week on cotton crop (Saleem *et al*., 2008) using a recommended field rate of an organophosphate (chlorpyrifos or profenofos), a pyrethroid (deltamethrin, cypermethrin or alpha-cyahlothrin) and/or one of the several newer insecticides (spinosad, fipronil or indoxacarb).

By walking through the crops in each field from Multan, approximately 500 larvae were collected. The area is under multiple cropping systems with several cultivated crops such as cotton, maize, sorghum, millet, rice, sugarcane, wheat, potato, vegetables and fodder crops. These crops are grown side by side, depending on the season. Larvae were reared on cabbage leaves in the laboratory at 25 ± 2 ◦C and 60–65% relative humidity with a 14: 10 h light: dark photoperiod. The leaves were replaced after 24 h and pupae were collected on alternate days. The adults that emerged were kept in perspex oviposition cages (30×30×30 cm) with two sides sealed with muslin to maintain ventilation and fed on a solution containing sucrose (100 mg/ml), vitamin solution (20l/ml) and methyl 4-hydroxybenzoate (2mg/ml) presented on a soaked cotton wool ball. Populations were reared in the laboratory for one generation to obtain sufficient numbers of insects for bioassays. To compare the resistance level in population collected using light trap, the adults of *S. exigua* were collected using mercury bulb light trap in cabbage field. The adults were brought into the laboratory and reared as described above to obtain larvae for bioassays.

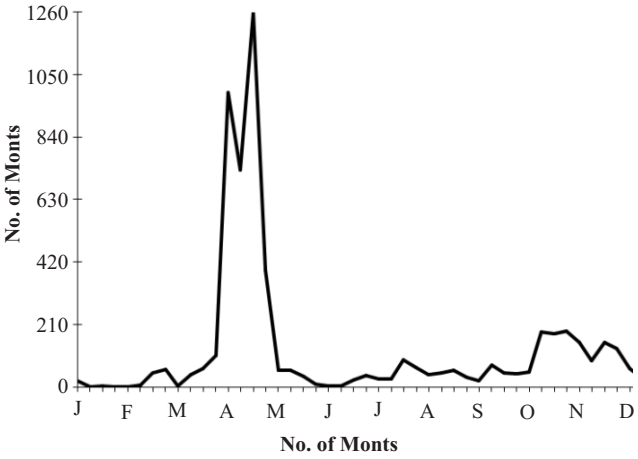

Fig. 1
Outbreak of *Spodoptera exigua* in Multan, Pakistan.

Insecticides

The commercial formulations of different insecticides used for bioassays were: cypermethrin (Prevail 10% a.i. w/v EC FMC Asia Pacific Inc., Hong Kong), deltamethrin (Decis 10% a.i. w/v EC; Syngenta), bifenthrin (Talstar 10% a.i. w/v EC FMC Asia Pacific Inc., Hong Kong), lambdacyhalothrin (Karate 2.5% a.i. w/v EC Novartis, UK), chlorpyriphos (Dursban 40% a.i. w/v EC Dow Agrosciences, USA), triazophos (Hostothion1 40% a.i. w/v EC Novartis, UK), profenophos (Chempro 501 50% a.i. w/v EC Novartis, UK), indoxacarb (Steward, 35% a.i. w/v EC DuPont, Wilmington, Delaware, USA), spinosad (Tracer, 240 g lx1 SC; Dow AgroSciences, Indianapolis, Indiana, USA) and emamectin benzoate (Strategy, 19 g lx1 EC; Syngenta). Stapple (Sygenta Pakistan), a non-ionic surfactant, was used at 5ppm to enhance the adhesiveness of insecticides.

Bioassays

Bioassays were conducted using newly moulted second-instar larvae (3–6 h old) of *S. exigua* from F1 laboratory cultures using a standard leaf disc bioassay method (Sayyed *et al.*, 2000). The discs of 5 cm diameter were cut either from

cabbage or potato leaves collected from unsprayed field crop. These were washed, dried, immersed in a test solution for 10 s and allowed to dry on corrugated kitchen foil at ambient temperature for 1–1.5 h. Test solutions of insecticides were freshly prepared in distilled water containing 5 ppm surfactant (Stapple®; Dupont, Pakistan). Leaf discs immersed in distilled water and stapple only comprised the control treatments. On drying, the leaf discs were placed in individual Petri dishes (5 cm diameter) containing moistened filter paper. Each treatment (concentration) was replicated 8 times, including controls. Five second-instar larvae were placed on each leaf disc (replication), and thus the total number of tested larvae per concentration was 40. The bioassays were kept at a temperature 25 ± 2 ◦C and 65% relative humidity with a 14: 10 h light: dark photoperiod. Mortality was assessed after 48 h for old generation insecticides and 72 h for new chemistry, exposure to the insecticides.

Data analysis

Where necessary, bioassay data were corrected for control mortality (Abbott, 1925). Data from the replicates were pooled and estimates of LC_{50} values and their 95% fiducial limits (FL) were obtained by maximum-likelihood logit regression analysis in GLIM using generalized modeling techniques, from which differences between sets were extracted by analysis of deviance (Crawley, 1993). Because of the inherent variability of bioassays, pair-wise comparisons of LC50 values were at the 1% significance level (where individual 95% FL for two treatments do not overlap; (Litchfield and Wilcoxon, 1949). The resistance ratio for each product was estimated by dividing the LC_{50} value for field populations by the LC_{50} value for the susceptible population.

RESULTS

Response of standard laboratory population to insecticides

The toxicities of spinosad and chlorfenapyr to Lab-Pak were similar but significantly greater ($P < 0.01$) than all other compounds tested (Table 1). Among the pyrethroid insecticides tested, the cypermethrin was the least toxic, while the betacypermethrin was the most toxic. The toxicity of betacypermethrin, zetacypermethrin, lambdacyhalothrin and tralomethrin was similar (Table 1). The toxicity of all organophosphate insecticides tested against the Lab-Pak population was similar (Table 1). The slopes of regression lines of spinosad were significantly shallow ($P < 0.05$; Table

Table 1
Response of susceptible population of *Spodoptera exigua* to pyrethroids, organosphosphates and new insecticides.

Insecticide	LC_{50} (95% FL) (ppm)	Slope ±SE	No. Tested [a]
Endosulfan	14.0 (11.5 - 17.0)	2.54±0.25	280
Cypermethrin	10.9 (8.84 - 13.4)	1.99 ± 0.18	320
Betacypermethrin	2.11 (1.76 - 2.53)	2.60 ± 0.25	280
Deltamethrin	7.9 (5.8 - 12.1)	2.17 ± 0.21	280
Zetacypermethrin	4.7 (1.9 - 6.5)	2.00 ± 0.20	280
Bifenthrin	5.11 (3.17 - 8.6)	1.56 ± 0.13	360
Lambdaeyhalothrin	6.7 (2.0 - 8.5)	2.34 ± 0.25	240
Fenpropathrin	5.55 (4.62 - 6.68)	2.57 ± 0.27	240
Cyfluthrin	3.41 (2.69 - 4.31)	1.61 ± 0.14	360
Tralomethrin	3.0 (1.2 - 4.1)	1.72 ± 0.16	320
Esfenvalerate	4.54 (3.68 - 5.59)	2.00 ± 0.18	320
Ethophenprox	1.7 (1.2 – 2.33)	3.09 ± 0.33	240
Chlorpyrifos	3.2 (1.4 - 6.7)	1.65 ± 0.15	320
Profenofos	3.43 (2.67 – 4.40)	1.43 ± 0.12	400
Quinalphos	2.58 (1.73 – 4.04)	1.69±0.14	360
Phoxim	1.81 (1.2 – 3.14)	2.73 ± 0.28	240
Spinosad	0.21 (0.10-0.56)	1.12±0.10	360
Indoxacarb	1.22 (0.88-2.93)	1.62±0.16	320
Chlorfenapyr	0.68 (0.56-0.83)	2.31±0.23	280

[a]Number of larvae exposed in bioassays including controls

Response of *S. exigua* collected from potato to insecticides

The toxicity of pyrethroids tested against *S. exigua* was significantly lower for field populations ($P < 0.01$) compared with Lab-Pak (Table 2). Levels of resistance to pyrethroids in *S. exigua* collected from potato field was generally high, with resistance ratios in excess of 20–fold for 8 population out of 10 populations tested compared with Lab-Pak (Table 2). The insecticides tested, i.e. cypermethrin, betacypermethrin, tralomethrin, lambdacyhalothrin and esfenvarate, had similar toxicity to *S. exigua.* The zetacypermethrin and bifenthrin are the most toxic pyrethroids tested with toxicity ratio of 5-fold and 6-fold while betacypermethrin and cyfluthrin were the least toxic with resistance ration of 61-fold and 55-fold compared with Lab-Pak (Table 2). Generally, populations of *S. exigua* showed significantly high level of resistance to organophosphates tested {average LC_{50} = 306. 20 (237.39-395.18)} compared with the level of resistance to pyrethroids {average LC_{50} = 146.25 (114.44-186.98)}. Quinalphos was no longer used for the last few years in Pakistan; yet, a high level of resistance (63-fold) similar to chlorpyrifos (31-fold) compared with Lab-Pak was found in the present studies. Farmer used profenofos and ethophenprox on all crops including vegetables (S. A. Shad personal communication) therefore significantly high level of resistance 183-fold and 360-fold respectively compared with Lab-Pak was identified. Phoxim was introduced recently in Pakistan to use against cotton pests but its use on vegetable crops like potato was very limited. A low level of resistance to phoxim (18-fold) compared with Lab-Pak was found in the population collected from potato (Table 2).

Spinosad and chlorfenapyr was introduced into Pakistan at the beginning of this decade and due to their efficacy, farmers start used against pests of several crops. Both insecticides had significantly higher toxicity ($P < 0.01$) compared with pyrethroids and organophosphates tested in populations collected from potato field and light traps. The toxicity of spinosad and chlorfenapyr was similar to the toxicity of the compounds against Lab-PaK (Table 1& 2).

Response of *S. exigua* collected from cabbage to insecticides

The LC_{50}s of population collected from cabbage was generally higher for pyrethroids {average 196.36 (161.73-242.91) and organophosphates {average 412.12 (326.14-524.22)} than the LC_{50}s of pyrethroids {average 146.25 (114.44-186.98)} and organophosphates {average 306.20 (237.39-395.18)} for the population collected from potato (Table 2 & 3). For example, LC_{50}s of betacypermethrin, zetacypermethrin, bifenthrin and fenpropathrin for population collected from cabbage were significantly ($P < 0.01$) greater than the population collected from potato (Table 2 & 3).

Table 2

Response of *Spodoptera exigua* collected from potato plants to pyrethroids, organophosphates and new chemistry insecticides.

Insecticide	Hosts	LC_{50} (95 % FL) (ppm)	Slope ±SE	No.Tested[a]	RR[b]
Endosulfan	Potato	61.49 (50.63-74.69)	2.30±0.22	280	4
Cypermethrin	Potato	195.7 (157.6-242.9)	1.95±0.19	280	18
Betacypermethrin	Potato	149.3 (116.7-191.0)	1.60±0.17	280	61
Deltamethrin	Potato	274.3 (213.6-352.4)	1.46±0.13	360	35
Zetacypermethrin	Potato	28.21 (22.85-34.83)	2.01±0.20	280	6
Bifenthrin	Potato	25.34 (19.35-33.19)	1.31±0.12	360	5
Lambda-cyhalothrin	Potato	138.4 (106.3-180.2)	1.35±0.12	360	21
Fenpropathrin	Potato	86.49 (66.79-112.0)	1.41±0.13	360	16
Cyfluthrin	Potato	188.7 (147.1-242.1)	1.51±0.14	320	55
Tralomethrin	Potato	135.7 (107.5-171.3)	1.69±0.16	320	45
Esfenvalerate	Potato	240.4 (186.6-309.9)	1.50±0.14	320	53
Ethophenprox	Potato	610.4 (483-771.6)	1.72±0.17	280	360
Chlorpyrifos	Potato	98.15 (74.4-129.4)	1.27±0.12	360	31
Profenofos	Potato	628.4 (477.2-827.6)	1.37±0.14	320	183
Quinalphos	Potato	161.8 (127.2-205.9)	1.59±0.15	320	63
Phoxim	Potato	32.27 (25.14-41.42)	1.57±0.16	280	18
Spinosad	Potato	0.41 (0.30-0.56)	1.12±0.10	360	2
Indoxacarb	Potato	6.22 (4.88-7.93)	1.62±0.16	320	5
Chlorfenapyr	Potato	0.683 (0.562-0.830)	2.31±0.23	280	1

[a]Number of larvae exposed in bioassays including controls

[b]RR = resistance ratio, calculated as (LC_{50} of field population)/(LC_{50} of Lab-Pak)

Table 3
Response of *Spodoptera exigua* collected from cabbage plants to pyrethroids, organophosphates and new chemistry insecticides.

Insecticide	Host	LC_{50} (95% FL) (ppm)	Slope ± S.E.	No.Tested[a]	RR[b]
Endosulfan	cabbage	82.84 (65.73 - 102.7)	2.20±0.23	320	6
Cypermethrin	cabbage	102.1 (86.0 - 121.4)	3.00±0.32	240	9
Betacypermethrin	cabbage	287.5 (236.5 - 350.3)	2.32±0.23	280	136
Deltamethrin	cabbage	133.9 (117.14 - 174.67)	2.55±0.33	200	17
Zetacypermethrin	cabbage	233.9 (177.0 - 299.0)	2.04±0.24	280	50
Bifenthrin	cabbage	313.4 (258.0 - 382.0)	2.34±0.23	280	61
Lambda -cyhalothrin	cabbage	214.3 (175.0 - 261.0)	2.57±0.2 8	240	32
Fenpropathrin	cabbage	177.7 (130.0 - 240.0)	2.20±0.24	280	32
Cyfluthrin	cabbage	141.4 (121.6 - 181.95)	2.40±0.32	240	41
Tralomethrin	cabbage	162.6 (140.4 - 197.15)	2.26±0.23	280	54
Esfenvalerate	cabbage	196.8 (175.7 - 221.6)	2.18±0.26	240	43
Ethophen prox	cabbage	301.5 (232.3 - 393.9)	1.57±0.16	320	177
Chlorpyrifos	cabbage	919.0 (758.0 - 1114)	2.41±0.24	280	287
Profenofos	cabbage	112.4 (92.5 - 136.6)	2.35±0.23	280	33
Quinalphos	cabbage	110.9 (89.9 - 136.6)	2.09±0.21	280	43
Phoxim	cabbage	616.8 (458.0 - 840. 0)	2.10±0.21	280	341
Spinosad	cabbage	147.5 (128.4 - 179.3)	2.01±0.20	280	702
Indoxacarb	cabbage	1487 (1226 - 1815)	2.36±0.24	280	1219
Chlorfenapyr	cabbage	296.7 (240 - 363)	2.44±0.26	280	436

[a]Number of larvae exposed in bioassays including controls
[b]RR = resistance ratio, calculated as (LC_{50} of field population)/(LC_{50} of Lab -Pak)

In contrast to population collected from potato, the population from cabbage showed significantly ($P < 0.01$; non over lapping 95% FL) high level of resistance to spinosad, indoxacarb and chlorfenopyr with a resistance ratio of 702-, 1219- and 436-fold, respectively (Table 2 & 3) compared with Lab-Pak.

Response of *S. exigua* collected using light traps to insecticides

The LC_{50}s of population collected using light traps was significantly lower ($P < 0.01$) than the populations collected from potato and cabbage (Table 4). On average the LC_{50}s of pyrethroids 44.94 (36.56.57-2) and organophosphates 44.68 (35.02-57.8) for the population were significantly lower than the LC_{50}s of pyrethroids or organophosphates for populations collected from potato and cabbage. Similarly the LC_{50}s of new chemistry insecticides for the population collected using light traps were significantly lower ($P < 0.01$) than the LC_{50}s of the population collected from cabbage but the LC_{50}s of spinosad and indoxacarb was significantly higher ($P < 0.01$) than the LC_{50}s for population collected from potato (Table 2 & 4).

Pairwise correlations between insecticides in different zones

Significant positive correlation ($P < 0.05$) between the toxicities of each pair of insecticides from new chemistry tested on population collected from potato with the toxicity to population from cabbage (Table 5). However significantly negative relationship between toxicity of pyrethroids to populations collected from potato and the cabbage was observed but the relationship between cabbage and potato and light was not significant ($P > 0.05$) (Table 5).

DISCUSSION

Bioassays were conducted to investigate the insecticidal activities of representative pyrethroids, organophosphates and new chemistry compounds against two populations collected at larval stage from cabbage or potato and a third population at adult stage from cabbage field using light traps. The data suggest that the population collected at larval stage from cabbage was generally highly resistant against pyrethroids, organophosphates and new chemistry insecticides than the populations collected either from potato at larval stage or from cabbage at adult stage. The resistance in population collected at larval stage from cabbage was high,

Table 4
Response of *Spodoptera exigua* collected using light trap to pyrethroids, organophosphates and new chemistry insecticides.

Insecticide	Host	LC_{50} (95% FL) (ppm)	Slope ± S.E.	No.Tested[a]	RR[b]
Endosulfan	Light trap	69.25 (55.3 - 86.7)	1.82±0.18	280	5
Cypermethrin	Light trap	103.7 (84.5 - 127.2)	2.07±0.19	320	9
Betacypermethrin	Light trap	12.69 (10.4 - 15.5)	2.25±0.22	280	6
Deltamethrin	Light trap	41.46 (34.5 - 50.00)	2.62±0.27	240	5
Zetacypermethrin	Light trap	47.65 (39.0 - 58.2)	2.25±0.24	240	10
Bifenthrin	Light trap	40.06 (33.2 - 48.3)	2.51±0.26	240	8
Lambda - cyhalothrin	Light trap	27.37 (22.5 - 33.4)	2.23±0.22	280	4
Fenpropathrin	Light trap	73.75 (60.8 - 89.5)	2.3 2±0.22	280	13
Cyfluthrin	Light trap	51.3 (38-88)	2.39±0.23	280	15
Tralomethrin	Light trap	29.87 (24.9 - 35.9)	2.53±0.25	280	10
Esfenvalerate	Light trap	21.52 (17.8 - 26.0)	2.40±0.24	280	5
Ethophenprox	Light trap	18.30 (14.6-22.9)	1.78±0.16	320	11
Chlorpyr ifos	Light trap	56.21 (45.1-70.1)	1.87±0.19	280	18
Profenofos	Light trap	32.65 (27.2-39.3)	2.53±0.25	280	9
Quinalphos	Light trap	45.1 (28.2 - 72.4)	1.90±0.17	320	17
Phoxim	Light trap	71.12 (60.0 - 84.3)	3.03±0.35	200	39
Spinosad	Light trap	11.07 (8.8-13.9)	1.73±0.16	320	53
Indoxacarb	Light trap	4.74 (3.8 - 5.9)	1.94±0.18	320	4
Chlorfenapyr	Light trap	19.04 (15.9-22.9)	2.54±0.25	280	28

[a]Number of larvae exposed in bioassays including controls

[b]RR = resistance ratio, calculated as (LC_{50} of field population)/(LC_{50} of Lab - Pak)

Table 5
Pairwise correlation coefficient comparisons between log LC_{50}'s of pyrethroids, organophosphate and new chemistry insecticides for *Spodoptera exigua* collected from three host plants.

Host	Cabbage	Potato	Light
Pyrethroids			
Cabbage	1		
Potato	$-0.634^{P<0.05}$	1	
Light	$-0.578^{P<0.1}$	$-0.036^{P>0.1}$	1
Organophosphates			
Cabbage	1		
Potato	$-0.611^{P>0.05}$	1	
Light	$0.616^{P>0.1}$	$-0.912^{P<0.05}$	1
New Chemistry			
Cabbage	1		
Potato	$0.998^{P<0.05}$	1	
Light	$-0.770^{P>0.1}$	$-0.807^{P>0.1}$	1

generally 10 or 10-fold greater than the susceptible strain. It has been suggested that insects should not be considered resistant until a resistance ratio of 10 is exhibited (Ahmad *et al*., 2008). Accordingly, we would consider that less than 10-fold toxicity to spinosad, indoxacarb and chlorfenapyr in a population collected from potato field or from cabbage at adult stage, was tolerance rather than resistance. It implies that these compounds might be less severely compromised by resistance mechanism(s) present in *S. exigua* population. Our field experience suggest no control failure of an insecticide happen when an insect species develop tolerance less than 10-fold compared with a standard laboratory population. Similarly poor control was also observed for *Helicoverpa armigera* in Australia when it showed resistance of 12-fold to fenvalerate or 15-fold to cypermethrin (Gunning *et al*., 1984). Analysis of resistant populations generally relies on comparisons with standard susceptible laboratory populations and these can be hypersensitive to some compounds (Gonzalez-Cabrera *et al*., 2001). The Lab-Pak population was less susceptible to spinosad, phoxim and esfenvalerate than that of the laboratory population of *S. exigua* used by (Wang *et al*., 2006). We could not find the toxicity of other insecticides we tested, to susceptibl.

population from literature to have a comparison with our susceptible strain. Since the susceptible strain we used was collected from the field and most likely the population still had resistance allele(s) for the insecticides. The presence of resistance allele(s) in susceptible strain suggests that the resistance might be under estimated. Nevertheless, resistance to insecticides is high enough to describe the populations as a resistant in comparison with a population of *S. exigua* from China (Wang *et al*., 2006).

This high level of resistance observed in the population collected from cabbage at larval stage could be due to intensive use of pyrethroids and organophosphates in response to severe outbreak of major cruciferous pest *P. xylostella* on cabbage (Khaliq *et al*., 2007). Since *S. exigua* is not a direct target of insecticides spray on cabbage or other vegetable crops but it receives indirect insecticides application due to occurrence of other insect pests. High levels of resistance found in a population collected from cabbage at larval stage might be a consequence of multiple resistance mechanisms. However, significantly ($P < 0.01$) lower resistance to cypermethrin found in the population is intriguing. It suggests that an independent mechanism of resistance may be involved. Similarly low to high level of resistance in another population collected from potato also indicates the same patter of development of resistance to pyrethroids or organophosphate in *S. exigua.* The low resistance could also reflect low usage of these compounds in potato. Organophosphorus resistance is much more complicated than pyrethroid, since organophosphorus insecticides have very diverse molecular structures which have diverse modes of toxic action and metabolism, and thus induce different resistance responses in insects (Cheng, 1986).

These results also suggest that the host plants could have an important effect on *S. exigua* resistance to pyrethroids, organophosphates or new chemistry insecticides. Previously it has been shown that different host plants could influence the response of *Heliothis armigera* to pyrethroids due to change in the activities of esterase and aldrin epoxidase in metabolic process (Tan and Guo, 1996). Similarly (Hunter *et al*., 1994) found that a dominant allelochemical, dihydrochalcone glycoside phloridzin, in foliage of apple could manipulate the tolerance of susceptible and resistant populations of *Platynota idaeusalis* to organophosphates. Although we did not determine the mechanism of host plant effects on variation in response to the insecticides, but the data demonstrate a significant variation in resistance phenotype of *S. exigua*. A possible explanation is that different secondary metabolites in cabbage or potato plants have elicited different metabolic changes in *S. exigua*, such as activation or inhibition of metabolic ability, detoxification enzymes or a general increase in esterase activities (Bush *et al*., 1993). These metabolic processes could cause the observed differences in expression of resistance phenotype to pyrethroids, organophosphates and new insecticides on different host plants.

Pairwise comparisons of the log LC_{50} values of new chemistry insecticides tested for the populations collected from potato and cabbage showed significantly positive correlations among new chemistry insecticides (spinosad, indoxacarb and chlorfenapyr) (Table 3) suggesting a cross-resistance mechanism. However the correlation between LC_{50}s of pyrethroids and organophosphates for populations collected from potato and cabbage was negative suggesting that the observed resistance in the populations was independently evolved. Due to commercial value, the growers usually spray larger amount of insecticides on cabbage than on potato (see above) as several insect pests including *S. litura* and *P. xylostella* attack cabbage (Syed and Abro, 2003). The divergent (negative and positive) correlation between potato, cabbage and light traps collected populations for new chemistry suggest the occurrence of more than one mechanism of resistance exists for imparting resistance to new chemistry insecticides. However the positive correlation of organophosphates for the population collected using light trap suggest that the adults might have migrated from adjacent areas where only new chemistry insecticides were in use as resistance ratio for the new chemistry insecticides were higher than the population collected from potato field. The positive correlation ($r = 0.236$; $P > 0.05$) between pyrethroids and new chemistry or pyrethroids and organophosphate ($r = 0.112$; $P > 0.05$) suggest that cross-resistance between the insecticides could occur. Pyrethroid resistance can be due to modifications to the target site of these insecticides or due to enhanced activity of detoxifying enzymes (Morin *et al*., 2002). Similarly the predominant mechanism of resistance to organophosphate could also be due to enhanced activity of detoxifying enzymes (Gunning *et al*., 2005) or a modification of the enzyme acetylcholinesterase, which is the target site of organophosphate and carbamate insecticides (Hama and Hosoda, 1983). Resistance to spinosad and indoxacarb has been shown to be esterases mediated in *P. xylostella* and *Helicoverpa armigera* (Sayyed and Wright, 2006: Wang *et al*., 2009). Similarly resistance to spinosad in *S. exigua* has been reported to be associated with monooxygenases (Wang *et al*., 2006). Previously we also have reported cross-resistance between pyrethroids and indoxacarb in *S.litura* and *P. xylostella* (Sayyed and Wright, 2006). The monooxygenase system is composed of many isoenzymes (Ishaaya and Casida, 1980) and if an insecticide selects specific isoenzymes, which can act on different insecticides, cross-resistance might be possible. The pyrethroids and organophosphates are heavily being used against several insect pests in Pakistan since 1980 (Ahmad *et al.*, 2007a); however, in the late 1990s, new chemistry insecticides spinosad, and indoxacarb were introduced to use against cotton pests. The positive correlation between new chemistry and pyrethroids imply either a common resistance mechanism, which is affecting these insecticides or genetically linked independent mechanisms for indoxacarb, spinosad or chlorfenapyr and pyrethroids or organophosphates in *S. exigua*.

In Pakistan, *S. exigua* is a polyphagous pest therefore it could also receive an indirect insecticide exposure on crops where it is not a major pest such as vegetables. If the use of pyrethroids and organophosphates can be reduced/ replaced, problems associated with pyrethroid and organophosphate resistance in *S. exigua* found in the present studies are likely to decline. Many of the novel chemicals available for the control of *S. exigua* and other lepidopteran pests are considered less

harmful to natural enemies than pyrethroids or organophospates. Use of such novel products, for example emamectin and *Bacillus thuringiensis* toxins, should also help to conserve natural enemies. Establishing a resistance management programme in developing countries poses a tremendous challenge and will require an integrated struggle against several decades of primary dependence on insecticides for pest management. What can be done to implement a resistance management programme? The scientific literature offers several theoretical models and recommendations to manage resistance with multiple tactics (Tabashnik and Croft, 1982). However, use of other components along with chemical insecticides, of a resistance management programme, must be increased. Such as use of resistant natural enemies *Chrysoperla carnea* along with chemical insecticides will make better use of various insect pest management practices (Pathan *et al*., 2008). Additionally, further studies are required to obtain a better understanding of resistance mechanisms affecting two or more insecticides within a single pest species. The latter may help to fine-tune the pesticide registration regulations and design a resistance management strategy.

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