Sarhad J. Agric. Vol. 29, No.4, 2013

STATUS OF INSECTICIDE RESISTANCE IN *HELICOVERPA ARMIGERA* (HÜBNER) IN SOUTHERN PUNJAB, PAKISTAN

*UMAIR FAHEEM[1], TAMSILA NAZIR[2], MUSHTAQ A. SALEEM[2],
MUHAMMAD YASIN[1] and MUHAMMAD BAKHSH[1]

1. *Cotton Research Institute, Rahim Yar Khan - Pakistan*
2 *Department of Entomology, Bahauddin Zakariya University, Multan - Pakistan.*
Corresponding author: umair_faheem_1@hotmail.com

ABSTRACT

Insecticide resistance in Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) was evaluated against nine insecticides, representing conventional group of neuro-toxic insecticides such as endosulfan, profenofos, carbosulfan, and deltamethrin and new chemistry insecticides such as emamectin benzoate, abamectin, spinosad, lufenuron and methoxyfenozide at IPM Sub Station (PARC), Bahauddin Zakariya University, Multan during 2010-11 . Two bioassay techniques i.e. residual method through leaf dip and topical method through micro-applicator were used for comparison. Low to moderate levels of resistance were recorded against these conventional and new chemistry insecticides at different locations of Southern Punjab, Pakistan e. g. in residual method, endosulfan (05-23 folds), profenofos (02-13 folds), carbosulfan (06-64 folds), deltamethrin (07-108 folds), emamectin benzoate (01-42 folds), abamectisn (03-06 folds), spinosad (01-07 folds), lufenuron (02-08 folds), methoxyfenozide (03-14 folds) and in topical method, endosulfan (05-36 folds),profenofos (02-65 folds), carbosulfan (19-105 folds), deltamethrin (13-35 folds), emamectin benzoate (02-06 folds), abamectin (01-04 folds), spinosad (04-61 folds), lufenuron (02-07 folds), methoxyfenozide (02-07 folds .The results indicated the development of multiple resistances in the field populations of H. armigera. Bioassay techniques showed no significant effects on the toxicity of insecticides. Resistance ratios in topical method were found higher as compared to the residual method, which may be attributed to the delayed cuticular penetration and enhanced metabolism of the insecticides. A significantly negative correlation was observed between conventional and new chemistry insecticides. It is suggested that the rotational use of conventional insecticides along with the new chemistry insecticides may be an effective tool in the insecticide resistance management program of H. armigera.

Keywords: Insecticide, resistance, bioassay methods, cross resistance, Helicoverpa armigera.

Citation: Faheem, U., T. Nazir, M. A. Saleem, M. Yasin and M. Bakhsh. 2013. Status of Insecticide Resistance in *Helicoverpa armigera* (Hübner) in Southern Punjab, Pakistan. Sarhad J. Agric 29(4): 563-572

INTRODUCTION

Helicoverpa armigera (Lepidoptera: Noctuidae) is one of the most notorious insect pest of different crops such as maize, tomato, potato, lady finger, citrus, tobacco, carrot, onion, castor, oat, wheat, bajra, barseem, lucern, rice, and sun hemp not only in Pakistan but throughout the world (Fitt, 1989). It is a cosmopolitan pest due to its wide distribution. Use of insecticides is a common practice to control *H. armigera*. The registered insecticides to control this pest in Pakistan are endosulfan, azinphos methyl, flucythrinate, profenofos, thiodicarb, indoxacarb, methomyl, tralomethrin, bifenthrin, lambda-cyhalothrin, beta-cyfluthrin, zeta-cypermethrin and esfenvalerate. Indiscriminate use of these broad spectrum insecticides has resulted in secondary pest outbreaks and development of resistance in this pest (Kranthi *et al.*, 2002; Maumbea and Swintonb, 2003 and Ahmad *et al.*, 2007).

Insecticide resistance in *H. armigera* against insecticides has been reported in different regions of the world, including Pakistan (Gunning *et al.*, 1984; 1991; Forrester *et al.*, 1993; Alaux *et al.*, 1997; Vassal *et al.*, 1997; Ahmad *et al.*, 1995; 1997; 1999; 2001; Torres-Vila *et al.*, 2002; Kranthi *et al.*, 2002; Bue`s and Boudinhon, 2003; Duraimurugan and Regupathy, 2005; Chaturvedi, 2007; Bhosale *et al.*, 2008 and Avilla and Gonza´lez-Zamora, 2009).

New chemistry insecticides were introduced in Pakistan during late 1990s. The use of these new chemistry insecticides has been increased during the current decade. These insecticides were found highly effective in controlling *H. armigera* as compared to conventional insecticides (Memon and Memon, 2005; Razaq *et al.*, 2005). A low level of resistance has also been reported against these new chemistry insecticides in *H. armigera* (Ahmad *et al.*, 2003). In Pakistan, the area under Bt. cotton is also increasing; hence the use of conventional and new chemistry insecticides has been decreasing. In the present study, the degree of resistance in *H. armigera* against both conventional and new chemistry insecticides was evaluated, using residual and topical bioassay techniques. We also determined cross resistance among these insecticides.

MATERIALS AND METHODS

The experiment was conducted at the Integrated Pest Management Sub-Station of Pakistan Agricultural Research Council, at the University College of Agriculture, Bahauddin Zakariya University, Multan.

Study areas

The field populations of *H. armigera* were collected from different locations of the Southern Punjab, Pakistan i.e. Multan, Bahawalpur, Rahim Yar Khan and Dera Ghazi Khan. These populations were collected during the years of 2010 and 2011 from different crops i.e. barseem (*Trifolium alexandrinum*), wheat (*Triticum aesitvum*), maize (*Zea mays*) and cotton (*Gossypium hirsutum*).

Insecticides

The commercial formulations of insecticides used in bioassays were: endosulfan (Thiodan® 350 g/l EC; Bayer CropScience, Germany), profenofos (Curacron® 500 g/l EC; Syngenta, Switzerland), carbosulfan (Advantage® 200 g/l EC; FMC, U.S.A), deltamethrin (Decis Super® 25 g/l EC; Bayer CropScience, Germany), emamectin benzoate (Proclaim® 19 g/l EC; Syngenta, UK), abamectin (Alarm® 18 g/l EC; Syngenta, UK), spinosad (Tracer® 240 g/l SC; Dow Agro Sciences, UK), lufenuron (Match® 50 g/l EC; Syngenta, UK) and methoxyfenozide (Runner® 150 g/l SC; Dow Agro Sciences, UK).

Insect rearing

The field collected larvae of *H. armigera* were brought to the laboratory, kept individually in 5cm diameter Petri dishes and fed on artificial diet as described by Ahmad *et al.*, (2003). After pupation these were transferred into the separate cages. The adults were fed on 10 percent honey solution. The laboratory condition during experiment was maintained at 27±1 ^{0}C with photo-period of 14D: 10L, while the relative humidity was maintained at 65±5%.

Establishment of reference strain

A strain of *H. armigera* collected from Bahawalpur was continuously inbred under the laboratory conditions for more than ten generations. LC_{50} of this strain was used to evaluate the resistance factors of field strains and was referred in this study as Lab-PK strain.

Bioassay

Bioassay was conducted on newly molted second instar (2-3 d old) larvae of *H. armigera* from F_1 laboratory cultures. Stock solution was prepared by adding formulated insecticides in distilled water. From the stock solution 5-7 serial dilutions were prepared in distilled water.

Residual method

Five cm diameter cotton leaf discs were cut, dipped into the insecticide solutions for 10s, and allowed to dry for thirty min. These leaf discs were placed into plastic Petri dishes lined with moistened filter paper to avoid desiccation. Five larvae of *H. armigera* were released on to the leaf discs. Thirty larvae were used in each treatment and each treatment was replicated four times. The Petri dishes were covered with black cloth to minimize cannibalism (Ahmad *et al., 2001*).

Topical application

This experiment was conducted to measure the response of larvae that had direct contact with insecticides as could occur under the field conditions. Larvae were directly treated with insecticides by using an auto-microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) equipped with a 1 ml glass syringe. A droplet of 0.25µl of an insecticide solution was applied to the individual 2nd instar larva. The treated larvae were separately kept in individual 5 cm diameter Petri dishes along with artificial diet. Thirty 2nd instar larvae were treated with each insecticidal concentration and each treatment was replicated four times.

Data analysis and interpretation of resistance levels

Mortality was assessed after 48 h for conventional and 72 h for new chemistry insecticides. Insects were considered dead if they gave no response to stimulation by touch. Results were expressed as percentage mortality. Data were analyzed by probit analysis (Finney, 1971) with POLO-PC (Le Ora Software, 1987). To determine cross-resistance among the insecticides tested, pair wise correlation coefficients of log LC_{50} values of the common populations for each insecticide were calculated by MSTAT statistical computer program (MSTAT-C, 1989).

Resistance factor was calculated as less than 10-fold RF was generally considered as a very low resistance, 11-20-fold as low resistance, 21-50- fold as moderate resistance, 51-100-fold as high resistance, and >100-fold as very high resistance (Ahmad *et al.*, 2007).

RESULTS AND DISCUSSION

Susceptibility of reference strain

In leaf dip bioassay, emmamectin benzoate was found to be the most toxic against *H. armigera*. Toxicity of emmamectin benzoate was significantly higher than all other test insecticides. The toxicity of deltamethrin was found similar to that of methoxyfenozide (overlapping of fudicial limits) but more than profenofos but toxicity of methoxyfenozide was found similar to that of profenofos (overlapping of fudicial limits). The toxicity of profenofos and endosulfan was similar (Table 1). Like leaf dip method in topical method new chemistry insecticides were found more toxic against *H. armigera* than that of conventional insecticides (Table 2).

Comparison of the Bioassays

Table 1 and 2 indicate that there is no significant difference in the toxicities of insecticides either applied topically or fed to the insect along with the food. The LC_{50} values of endosulfan in topical application against *H. armigera* were higher in 4 out of 5 strains, but the difference was non-significant (overlapping of fudicial limits) except for Multan strain (no overlapping of fudicial limits). There was no significant difference (overlapping of fudicial limits) in the topical and leaf dip LC_{50} values of profenofos against *H. armigera.* The LC_{50} values of carbosulfan in topical application against *H. armigera* were higher as compared to the leaf dip method but the difference was non-significant (overlapping of fudicial limits) except for Multan strain, where the difference was significant (no overlapping of fudicial limits). The LC_{50} values of deltamethrin obtained through leaf dip and topical bioassay methods against *H. armigera*, from four different locations showed non-significant difference (overlapping of fudicial limits) except for Multan strain.

The toxicities of new chemistry insecticides were found significantly different in residual method from that of topical method. Emmamectin benzoate and spinosad were significantly more toxic in residual method than that of topical method. Contrarily abamectin, lufenuron and methoxyfenozide were found to be more toxic when applied topically.

Monitoring of Resistance

Resistance to Endosulfan

The four strains tested in this study, showed very low level of resistance in populations from Bahawalpur (2 folds) and Multan (5 folds) and moderate resistance in D. G. Khan (23 folds) and R. Y. Khan (27 folds) against endosulfan in leaf dip method as compared to the Lab-PK (Table 1). The resistance factor measured in topical method against endosulfan showed similar pattern as of leaf dip method. Out of four strains tested, Bahawalpur strain (5 folds) exhibited very low resistance. Multan (16 folds) and D. G. Khan strains (12 folds) exhibited a low resistance but R. Y. Khan strain (36 folds) manifested a moderate resistance as compared to the Lab-PK (Table 2).

Resistance to Profenofos

Out of the four insect strains tested against profenofos in leaf dip method, three strains i.e. Bahawalpur (2 folds), R. Y. Khan (7 folds) and Multan (7 folds) exhibited very low level of resistance. While strain from of D. G. Khan showed (13 folds) low level of resistance as compared to the Lab-PK (Table 1). In topical method, the level of resistance measured against profenofos was higher as compared to the leaf dip method. Very low level of resistance was observed in Bahawalpur (2 folds) and R. Y. Khan strains (9 folds). Moderate level of resistance was exhibited by the Multan strain (32 folds), whereas high level of resistance was exhibited in D. G. Khan strain (65 folds) (Table 2).

Resistance to Carbosulfan

Two strains i.e. Multan (6 folds) and Bahawalpur (9 folds) exhibited very low level of resistance to carbosulfan in leaf dip method. D. G. Khan strain (12 folds) manifested low level of resistance while R. Y. Khan strain (64 folds) showed high level of resistance to carbosulfan in leaf dip method (Table 1). Topical application of carbosulfan revealed that Multan strain (19 folds) exhibited the low level of resistance. In contrast to this the insect populations from Bahawalpur (33 folds) and D. G. Khan (27 folds) manifested the moderate level of resistance, while R. Y. Khan strain (105 folds) showed very high level of carbosulfan resistance (Table 2).

Table 1. ***Residual effects of conventional and new chemistry insecticides against laboratory susceptible and field populations of Helicoverpa armigera***

Insecticide	**Location**	**Year**	**Host**	**LC_{50} ppm (95% FL)**	**Slope ± SE**	**x^2**	**df**	***P***	**RR[a]**	***n*[b]**
endosulfan	Lab-PK			23.30 (15.9-31.0)	2.44±0.38	2.71	5	0.74		210
	D.G. Khan	Oct, 10	Cotton	538.12 (322.0-946.3)	1.06±0.19	0.19	5	0.99	23	210
	Bahawalpur	April, 10	Wheat	58.30 (38.1-83.6)	1.47±0.21	1.53	5	0.91	02	210
	R.Y. Khan	Oct, 10	Maize	625.10 (350.8-1585.1)	0.97±0.20	1.10	5	0.95	27	210
	Multan	April, 11	Berseem	116.25 (88.7-156.5)	1.76±0.21	1.33	5	0.93	05	210
profenofos	Lab-PK			14.21 (9.9-18.5)	2.13±0.33	1.39	5	0.92		210
	D.G. Khan	Oct, 10	Cotton	182.38 (118.3-274.3)	1.32±0.21	0.79	5	0.98	13	210
	Bahawalpur	April, 10	Wheat	27.25 (17.8-38.0)	1.67±0.24	1.55	5	0.91	02	210
	R.Y. Khan	Oct, 10	Maize	94.57 (54.1-149.2)	1.08±0.18	1.85	5	0.87	07	210
	Multan	April, 11	Berseem	94.91 (68.1-126.5)	2.04±0.03	1.38	5	0.93	07	210
carbosulfan	Lab-PK			5.30 (3.3-7.5)	1.59±0.24	3.47	5	0.63		210
	D.G. Khan	Oct, 10	Cotton	61.97 (40.1-85.2)	1.65±0.24	1.81	5	0.87	12	210
	Bahawalpur	April, 10	Wheat	47.94 (24.7-90.7)	0.93±0.19	0.61	5	0.99	09	210
	R.Y. Khan	Oct, 10	Maize	378.85 (263.2-616.3)	1.27±0.19	3.41	5	0.64	64	210
	Multan	April, 11	Berseem	31.66 (21.2-43.6)	1.79±0.25	4.05	5	0.54	06	210
deltamethrin	Lab-PK			4.42 (2.89-6.0)	2.14±0.34	2.54	5	0.77		210
	D.G. Khan	Oct, 10	Cotton	478.60 (284.8-987.4)	1.05±0.20	0.39	5	0.99	108	210
	Bahawalpur	April, 10	Wheat	29.22 (21.0-40.5)	1.38±0.18	1.20	5	0.94	07	210
	R.Y. Khan	Oct, 10	Maize	126.07 (83.5-181.7)	1.41±0.20	0.44	5	0.99	29	210
	Multan	June, 11	Berseem	287.56 (187.2-520.4)	1.39±0.28	1.58	5	0.90	65	210
emamectin benzoate	Lab-PK			0.0062(0.0042-0.0094)	1.37±0.22	4.79	3	0.19		150
	D.G. Khan	Oct, 10	Cotton	0.0089(0.0062-0.0128)	1.55±0.28	0.27	4	0.96	01	180
	Bahawalpur	April, 10	Wheat	0.2583(0.0045-14.8687)	1.20±0.79	5.76	5	0.22	42	210
	R.Y. Khan	Oct, 10	Maize	0.0969(0.0738-0.1271)	1.93±0.26	1.53	5	0.82	16	210
	Multan	June, 11	Berseem	0.2156(0.1485-0.2923)	2.03±0.35	2.26	3	0.52	35	150
abamectin	Lab-PK			20.27(15.21-27.03)	1.91±0.27	0.74	3	0.94		150
	D.G. Khan	Oct, 10	Cotton	98.81(70.44-138.61)	1.49±0.23	1.49	3	0.84	05	150
	Bahawalpur	April, 10	Wheat	114.47(85.54-153.19)	2.14±0.27	2.26	3	0.69	06	150
	R.Y. Khan	Oct, 10	Maize	69.32(54.48-88.19)	2.14±0.27	2.26	3	0.69	03	150
	Multan	June, 11	Berseem	60.76(49.60-74.93)	2.87±0.35	2.38	5	0.79	03	210
spinosad	Lab-PK			0.4035(0.3086-0.5276)	1.94±0.26	1.24	4	0.87		180
	D.G. Khan	Oct, 10	Cotton	0.8352(0.6128-1.1383)	1.62±0.23	2.85	4	2.85	02	180
	Bahawalpur	April, 10	Wheat	1.325(0.991-1.771)	1.86±0.26	1.32	4	0.86	03	180
	R.Y. Khan	Oct, 10	Maize	0.4929(0.3872-0.6274)	2.16±0.27	2.60	4	0.63	01	180
	Multan	June, 11	Berseem	2.99(1.42-4.81)	1.70±0.32	1.26	4	0.87	07	180
lufenuron	Lab-PK			1.363(0.955-1.945)	1.46±0.23	2.95	4	0.57		180
	D.G. Khan	Oct, 10	Cotton	6.586(4.998-8.678)	1.87±0.25	1.55	4	0.82	08	180
	Bahawalpur	April, 10	Wheat	2.275(1.741-2.975)	1.84±0.24	3.32	4	0.51	03	180
	R.Y. Khan	Oct, 10	Maize	0.8562(0.6460-1.1349)	1.80±0.25	1.90	4	0.75	02	180
	Multan	June, 11	Berseem	6.81(5.05-9.95)	1.70±0.23	1.07	6	0.98	05	240
methoxyfenozide	Lab-PK			7.668(5.376-10.937)	1.68±0.26	0.94	4	0.92		180
	D.G. Khan	Oct, 10	Cotton	19.44(14.05-26.90)	1.68±0.27	1.38	4	0.85	03	180
	Bahawalpur	April, 10	Wheat	50.50(37.05-68.82)	1.70±0.24	0.74	4	0.95	07	180
	R.Y. Khan	Oct, 10	Maize	26.08(19.71-34.49)	1.84±0.25	0.94	4	0.92	04	180
	Multan	June, 11	Berseem	110.59(84.17-155.48)	1.91±0.26	2.47	5	0.78	14	210

RR[a] = Resistance ratio was calculated by dividing LC_{50} of field population with that of LC_{50} of Lab-PK.

n[b] = Total number of insects used.

Table 2 ***Topical effects of conventional and new chemistry insecticides against laboratory susceptible and field populations of Helicoverpa armigera***

Insecticide	**Location**	**Year**	**Host**	**LC$_{50}$ ppm (95% FL)**	**Slope ± SE**	**x^2**	**df**	***P***	**RR[a]**	***n*[b]**
Endosulfan	Lab-PK			17.66 (11.8-23.7)	2.37±0.39	1.34	5	0.93		210
	D.G. Khan	Oct, 10	Cotton	215.07 (110.8-370.2)	1.13±0.23	0.84	5	0.97	12	210
	Bahawalpur	April, 10	Wheat	95.93 (66.6-130.4)	1.96±0.30	0.39	5	0.99	05	210
	R.Y. Khan	Oct, 10	Maize	640.49 (409.5-1298.3)	1.17±0.19	1.89	5	0.86	36	210
	Multan	April, 11	Berseem	285.25 (213.5-442.5)	1.90±0.29	1.21	5	0.94	16	210
Profenofos	Lab-PK			6.13 (3.9-8.4)	2.07±0.35	1.72	5	0.89		210
	D.G. Khan	Oct, 10	Cotton	398.88 (222.6-853.9)	0.98±0.19	2.10	5	0.83	65	210
	Bahawalpur	April, 10	Wheat	13.28 (07.1-19.8)	1.61±0.27	2.96	5	0.71	02	210
	R.Y. Khan	Oct, 10	Maize	52.63 (28.8-81.3)	1.17±0.19	4.99	5	0.42	09	210
	Multan	April, 11	Berseem	196.18 (119.6-350.6)	1.21±0.23	1.21	5	0.94	32	210
Carbosulfan	Lab-PK			4.40 (3.1-5.8)	1.78±0.27	1.53	5	0.91		210
	D.G. Khan	Oct, 10	Cotton	117.10 (64.5-179.4)	1.38±0.22	2.20	5	0.82	27	210
	Bahawalpur	April, 10	Wheat	145.39 (89.1-289.8)	1.23±0.25	1.40	5	0.92	33	210
	R.Y. Khan	Oct, 10	Maize	461.91 (306.6-835.9)	1.17±0.18	1.23	5	0.94	105	210
	Multan	April, 11	Berseem	84.22 (62.2-103.6)	3.39±0.56	0.81	5	0.98	19	210
Deltamethrin	Lab-PK			6.12 (4.1-8.0)	1.88±0.27	3.40	5	0.64		210
	D.G. Khan	Oct, 10	Cotton	214.71 (143.9-325.7)	1.29±0.19	1.54	5	0.91	35	210
	Bahawalpur	April, 10	Wheat	129.66 (76.7-279.6)	1.03±0.20	1.04	5	0.96	21	210
	R.Y. Khan	Oct, 10	Maize	199.47 (123.5-317.3)	1.18±0.20	1.52	5	0.91	33	210
	Multan	June, 11	Berseem	80.79 (53.2-119.1)	1.36±0.20	1.21	5	0.94	13	210
emamectin benzoate	Lab-PK			0.041(0.028-0.054)	2.80±0.48	0.75	5	0.98		210
	D.G. Khan	Oct, 10	Cotton	0.067(0.042-0.097)	1.50±0.22	3.68	5	0.60	02	210
	Bahawalpur	April, 10	Wheat	0.107(0.075-0.145)	1.83±0.25	2.78	5	0.73	03	210
	R.Y. Khan	Oct, 10	Maize	0.154(0.113-0.210)	1.43±0.20	1.55	5	0.91	04	210
	Multan	June, 11	Berseem	0.188(0.129-0.274)	1.41±0.21	1.07	5	0.96	06	210
Abamectin	Lab-PK			9.19(6.48-12.08)	2.22±0.33	2.93	5	0.71		210
	D.G. Khan	Oct, 10	Cotton	13.64(9.23-18.81)	1.42±0.19	2.54	5	0.77	01	210
	Bahawalpur	April, 10	Wheat	34.81(20.11-57.58)	1.01±0.18	1.41	5	0.92	04	210
	R.Y. Khan	Oct, 10	Maize	21.02(13.38-30.27)	1.46±0.21	2.97	5	0.70	02	210
	Multan	June, 11	Berseem	17.77(10.71-25.18)	1.91±0.31	4.92	5	0.43	02	210
Spinosad	Lab-PK			0.123(0.079-0.173)	1.95±0.30	3.83	5	0.57		210
	D.G. Khan	Oct, 10	Cotton	0.54(0.290-0.86)	1.22±0.19	4.75	5	0.45	04	210
	Bahawalpur	April, 10	Wheat	3.46(2.33-5.93)	1.23±0.18	2.84	5	0.72	28	210
	R.Y. Khan	Oct, 10	Maize	5.20(3.32-10.46)	1.18±0.20	0.82	5	0.98	42	210
	Multan	June, 11	Berseem	7.54(4.63-17.54)	1.25±0.23	1.52	5	0.91	61	210
Lufenuron	Lab-PK			0.25(0.118-0.413)	1.30±0.21	4.63	5	0.46		210
	D.G. Khan	Oct, 10	Cotton	1.79(1.027-2.528)	1.94±0.38	3.03	5	0.69	07	210
	Bahawalpur	April, 10	Wheat	0.477(0.254-0.735)	1.38±0.21	4.80	5	0.44	02	210
	R.Y. Khan	Oct, 10	Maize	1.56(0.827-2.423)	1.29±0.23	2.46	5	0.78	06	210
	Multan	June, 11	Berseem	1.15(0.544-1.803)	1.48±0.28	2.40	5	0.79	05	210
methoxyfenozide	Lab-PK			0.137(0.070-0.219)	1.26±0.19	3.77	5	0.58		210
	D.G. Khan	Oct, 10	Cotton	0.295(0.164-0.459)	1.27±0.18	4.34	5	0.50	02	210
	Bahawalpur	April, 10	Wheat	0.904(0.467-1.437)	1.25±0.22	3.56	5	0.61	07	210
	R.Y. Khan	Oct, 10	Maize	0.391(0.172-0.677)	1.13±0.19	4.90	5	0.43	03	210
	Multan	June, 11	Berseem	1.025(0.622-1.548)	1.30±0.20	2.19	5	0.82	07	210

RR[a] = Resistance ratio was calculated by dividing LC$_{50}$ of field population with that of LC$_{50}$ of Lab-PK.

n[b] = Total number of insects used

Table 3. ***Pair wise correlation coefficient comparisons between LC_{50} values in leaf dip and topical application of the insecticides on field strains of Helicoverpa armigera (Hübner)***

	endosulfan		**profenophos**		**carbosulfan**		**deltamethrin**		**emmamectin benzoate**		**abamectin**		**spinosad**		**Lufenuron**	
	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical	Leaf Dip	Topical
profenofos	$0.76^{0.05}$	0.06^{ns}														
carbosulfan	$0.74^{0.05}$	$0.92^{0.01}$	0.19^{ns}	-0.16^{ns}												
deltamethrin	$0.54^{0.05}$	$0.62^{0.05}$	$0.96^{0.01}$	$0.51^{0.05}$	$-0.80^{0.05}$	$0.67^{0.05}$										
emmamectin benzoate	$-0.91^{0.01}$	0.47^{ns}	$-0.90^{0.01}$	-0.43^{ns}	-0.33^{ns}	0.22^{ns}	$-0.71^{0.05}$	$-0.65^{0.05}$								
abamectin	-0.20^{ns}	-0.34^{ns}	-0.17^{ns}	$-0.82^{0.05}$	-0.39^{ns}	0.05^{ns}	-0.15^{ns}	-0.32^{ns}	0.06^{ns}	-0.02^{ns}						
spinosad	$-0.74^{0.05}$	0.36^{ns}	-0.21^{ns}	-0.49^{ns}	$-0.61^{0.05}$	0.14^{ns}	0.09^{ns}	$-0.73^{0.05}$	$0.59^{0.05}$	$0.99^{0.01}$	-0.41^{ns}	0.07^{ns}				
lufenuron	-0.18^{ns}	$0.54^{0.05}$	$0.59^{0.05}$	$0.68^{0.05}$	$-0.72^{0.05}$	0.30^{ns}	$0.83^{0.05}$	$0.68^{0.05}$	-0.19^{ns}	-0.16^{ns}	-0.15^{ns}	$-0.90^{0.01}$	$0.60^{0.05}$	-0.28^{ns}		
methoxyfenozide	$-0.74^{0.05}$	-0.45^{ns}	-0.34^{ns}	-0.42^{ns}	-0.48^{ns}	$-0.50^{0.05}$	-0.07^{ns}	$-0.98^{0.01}$	$0.68^{0.05}$	$0.55^{0.05}$	-0.48^{ns}	0.49^{ns}	$0.98^{0.01}$	$0.65^{0.05}$	0.44^{ns}	$-0.80^{0.05}$

(Superscripts denote significance of the regression)

Resistance to Deltamethrin

Of four strains tested for resistance against deltamethrin through leaf dip method, Bahawalpur strain (7 folds) exhibited very low level of resistance as compared to lab-PK. Multan strain (65 folds) displayed high level of resistance, while D.G. Khan strain (108 folds) demonstrated very high level of resistance (Table 1). In topical method, the *H. armigera* populations from D. G. Khan (35 folds), Bahawalpur (21 folds) and R. Y. Khan (33 folds) exhibited moderate level of resistance against deltamethrin, while Multan strain (13 folds) demonstrated low level of resistance (Table 2).

Resistance to Emmamectin Benzoate

Among different populations of *H. armigera*, Bahawalpur and Multan populations exhibited a moderate level of resistance against emamectin benzoate by 42 folds and 35 folds, respectively. R.Y. Khan population had low level of resistance (16 folds). D.G. Khan population showed no resistance against emamectin benzoate (Table 1). In topical application very low level of resistance was observed and was within the range 02-06 folds (Table 2).

Resistance to Abamectin

H. armigera exhibited very low level of resistance in all the populations treated by both leaf dip and topical application methods against abamectin (Table 1). In leaf dip method the resistance was in the range of 03-06 folds while in topical method the resistance was recorded in the range of 01-04 folds (Table 2).

Resistance to spinosad

The strains also showed very low level of resistance against spinosad. In leaf dip method the resistance recorded was in the range of 01-07 folds among different strains (Table 1). In contrast to this, in topical method Multan strain exhibited 64 folds of resistance followed by moderate resistance in R.Y. Khan (42 folds) and Bahawalpur (28 folds) strains (Table 2).

Resistance to lufenuron

Our results revealed that in leaf dip method the populations of different localities from Southern Punjab exhibited very low level of resistance against lufenuron the resistance levels observed was in the range of 02-08 folds (Table 1). A very low level of resistance was observed among all the populations treated by topical and leaf dip method (Table 2)

Resistance to Methoxyfenozide

Methoxyfenozide is an ecdysone agonist. Among different strains, Multan strain showed a low level of resistance against methoxyfenozide (14 folds), while all other strains exhibited a very low level of resistance (Table 1).

Pair wise Correlation

Table 3 shows that endosulfan had a significant ($P<0.05$) correlation with all conventional chemicals and significantly ($P<0.05$) negative correlation with emmamectin benzoate, spinosad and methoxyfenozide in leaf dip bioassay. No correlation was observed between endosulfan and profenofos when applied topically. A highly significant ($P<0.01$) correlation between endosulfan and carbosulfan, while significant ($P<0.05$) correlation of endosulfan with deltamethrin and lufenuron was observed in topical application method. Profenofos showed highly significant ($P<0.01$) correlation with deltamethrin in leaf dip method and significant ($P<0.05$) in topical method. Highly significant ($P<0.01$) negative correlation with emmamectin benzoate, was observed with lufenuron both in residual and topical method. A significantly ($P<0.05$) negative correlation was observed between profenofos and abamectin in topical method. It can be seen that carbosulfan exhibited significantly ($P<0.05$) negative correlation with deltamethrin in leaf dip method and in contrast to this in topical method it had significantly ($P<0.05$) positive correlation. Carbosulfan also had significantly ($P<0.05$) negative correlation with spinosad and lufenuron in leaf dip method and with methoxyfenozide in topical method. Deltamethrin showed significantly ($P<0.05$) negative correlation with emmamectin benzoate both in leaf dip and topical methods. It manifested significantly ($P<0.05$) negative correlation with spinosad and highly significant ($P<0.01$) negative correlation with methoxyfenozide in topical method. Deltamethrin showed significant ($P<0.05$) correlation with lufenuron both in leaf dip and topical method (Table 3).

The present study was conducted to monitor the current scenario of insecticide resistance in *H. armigera* against conventional and new chemistry insecticides, by comparing the residual and topical bioassay techniques.

The results of the present study revealed that insecticide resistance against the conventional insecticides in *H. armigera* mostly prevailed at a low to moderate levels e.g. in residual method, endosulfan (05-23 folds), profenofos (02-13 folds), carbosulfan (06-64 folds), deltamethrin (07-108 folds) and in topical method endosulfan (05-36 folds), profenofos (02-65 folds), carbosulfan (19-105 folds), deltamethrin (13-35 folds) at almost all the studied locations of Southern Punjab, Pakistan. Resistance levels were mostly found below than those previously reported by Ahmad *et al.*, 1995; 1997; 1999; 2001; showing that the absence of selection pressure can dramatically reduce the resistance levels and the effectiveness of insecticides can be reverted (Sayyed *et al.,* 2005; Razaq *et al.,* 2007). The resistance level increases due to the increased use of pyrethroids and decreased as their use decreases (Forrester, 1990). The reason behind this decrease in resistance level is attributed to increasing awareness of farming communities due to intensive extension work of Government and non Governmental Organizations throughout Pakistan including use of insecticides bearing novel modes of action, which have been toxic to target insects but safer for non-target organisms. Our data suggest that the increased use of new chemistry insecticides has increased the level of resistance as previously reported by Ahmad *et al.,* (2003) to moderate level against lufenuron and spinosad which are now the most commonly used insecticides against *H. armigera* in Pakistan. Similarly, moderate to high level of resistance was observed in *Spodoptera litura* against the new chemistry insecticides in Pakistan (Ahmad *et al.,* 2008). In India, resistance to endosulfan was low to moderate in *H. armigera* (Kranthi *et al.,* 2002). Our data suggests that multiple resistances also prevailed in the field populations of *H. armigera*.

The results also exhibited that the toxicity of the conventional insecticides in residual method was not significantly different from that of topical method. Similarly, non-significant differences were observed between the comparison of toxicities of insecticides in residual and topical methods against *Chrysoperla carnea,* (Stephens) (Pathan *et al.,* 2008). But in some strains, significant differences in toxicity between residual and topical methods were observed, which may be due to the multiple resistance in the test strains. Resistance ratios observed in topical method were found higher as compared to the residual method, which may be attributed to the delayed cuticular penetration and enhanced metabolism of the insecticides (Ahmed *et al.,* 2006). Delayed penetration can give detoxifying enzymes more time to metabolize the pesticide before it reaches its target site (Plapp and Hoyer, 1968). The resistance in *H. armigera* could be due to the combined effect of decreased sensitivity to AChE, higher levels of esterases, phosphatases and the expression of P-glycoprotein (Srinivas *et al.,* 2004).

In the present experimentation significant cross resistance was observed between endosulfan-carbosulfan, endosulfan-deltamethrin and profenofos-deltamethrin. Similar cross resistance was observed for the same insecticides in *Spodoptera litura* (Saleem *et al.,* 2008). Ramasubramanian and Regupathy (2004) suggested that populations selected for resistance to one pyrethroid showed positive cross resistance to all other pyrethroids, but no cross resistance to endosulfan. The results confirmed that the applications should be discouraged to manage insecticide resistance. Interestingly, in this study no cross resistance was observed between carbosulfan and deltamethrin in residual method but there was a significant cross resistance in topical method. Lack of cross resistance between carbosulfan and deltamethrin in residual method may be due to different mode of action of carbamates and pyrethroids. Cross resistance between carbosulfan and deltamethrin may be attributed to the involvement of cuticular resistance, which can be the reason of development of resistance to both insecticides. Ahmad *et al.,* (2007) observed no cross resistance between endosulfan and profenofos but in our study, a significant cross resistance was found between these insecticides. The results clearly indicate that there is a significantly negative correlation among the conventional and new chemistry insecticides. This negative correlation among the insecticides can be a very effective tool in the insecticide resistance management programs. Yamamoto *et al.,* (1993) effectively used *N*-propylcarbamate and *N*-methylcarbamate for the control *Nephotettix cincticeps* populations containing mutant and wild-type acetylcholinesterases. Their results enabled to shift the resistance level back and forth by alternating between using the two aforementioned carbamates. It was found that some mite field strains were resistant to organophosphates but hyper-sensitive to the synthetic pyrethroids (Chapman and Penman, 1979).

Emamectin benzoate was found to be most toxic against (overlapping of fudicial limits) *H. armigera* when applied by both topical and residual methods, while endosulfan was found to be least effective. In a laboratory study Ahmad *et al.,* (2003) observed emmamectin benzoate as least effective insecticide against *H. armigera* as compared to other new chemistry insecticides. In contrast to this, Razaq *et al.,* (2005) found emamectin benzoate as the most toxic insecticide against *H. armigera* in a field experiment on cotton. Another study proved emamectin benzoate as the most toxic insecticide among to all other new chemistry insecticides against *Spodoptera litura* (Ahmad *et al.,* 2008).

In Pakistan, many insecticides with novel modes of actions are available but farmers are still habitual to buy the conventional ones although these are highly toxic against *H. armigera*.

CONCLUSIONS AND RECOMMENDATIONS

It can be inferred from the above study that the conventional insecticides can help in the insecticide resistance management if used in rotation with the new chemistry insecticides, at least or until the farmers get much awareness about the insecticide resistance management strategies.

Acknowledgements

First author would like thank Pakistan Agricultural Research Council for providing the research facilities.

REFERENCES

Ahmad, M., A. H. Sayyed, M. A. Saleem and M. Ahmad. 2008. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. Crop Prot. 22: 1367– 1372.

Ahmad, M., I. Denholm and R. H. Bromilow. 2006. Delayed cuticular penetration and enhanced metabolism of deltamethrin in pyrethroid-resistant strains of *Helicoverpa armigera* from China and Pakistan. Pest Manag. Sci. 62: 805-810.

Ahmad, M., M. I. Arif and A. Zahoor. 2003. Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to new chemistries in Pakistan. Crop Prot. 22: 539-544.

Ahmad, M., M. I. Arif and M. Ahmad. 2007. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 26: 809-817.

Ahmad, M., M. I. Arif and M. R. Attique. 1997. Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Bull. Entomol. Res. 87: 343-347.

Ahmad, M., M. I. Arif and Z. Ahmad. 1995. Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. J. Econ. Entomol. 88: 771-776.

Ahmad, M., M. I. Arif and Z. Ahmad. 1999. Patterns of resistance to organophosphate insecticides in field populations of *Helicoverpa armigera* in Pakistan. Pestic. Sci. 55: 626-632.

Ahmad, M., M. I. Arif and Z. Ahmad. 2001. Resistance to carbamate insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 20: 427-432.

Alaux, T., J. M. Vassal and M. Vaissayre. 1997. Suivi de la sensibilite´ aux pyrethrinoıdes chez *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) en Coˆ te d'Ivoire. J. Afr. Zool. 111: 63-69.

Avilla, C. and J. E. Gonza´lez-Zamora. 2009. Monitoring resistance of *Helicoverpa armigera* to different insecticides used in cotton in Spain. Crop Prot. 29: 100-103.

Bhosale, S. V., D. S. Suryawanshi and B.V. Bhede. 2008. Insecticide resistance in field population of American bollworms, *Helicoverpa armigera* (Hübner). (Lepidoptera: Noctuidae). Pestol. 32: 19-22.

Bue` S. R and L. Boudinhon. 2003. Resistance aux insecticides de *Helicoverpa armigera* (Lepidoptera: Noctuidae) dans le sud de la France. Cah. Agric. 12: 1-7 (In French).

Chapman, R. B and D. R. Penman. 1979. Negatively correlated cross-resistance to a synthetic pyrethroid in organophosphorus-resistant *Tetranychus urticae*. Nature (London) 218: 298-299.

Chaturvedi, I. 2007. Status of insecticide resistance in the cotton bollworm, *Helicoverpa armigera* (Hübner). J. Cent. Eur. Agri. 8: 171-182.

Duraimurugan, P and A. Regupathy. 2005. Synthetic pyrethroid resistance in field strains of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Tamil Nadu, South India. Amer. J. Appl. Sci. 2: 1146-1149.

Finney, D. J. (Ed.) 1971. Probit Analysis. 3rd Edition. Cambridge University Press, UK.

Fitt, G. P. 1989. The ecology of Heliothis in relation to agroecosystems. Ann. Rev. Entomol. 34: 17-52.

Forrester, N. W. 1990. Designing, implementing and servicing an insecticide resistance management strategy. Pestic. Sci. 28:167-179.

Forrester, N. W., M. Cahill, L. J. Bird and J. K. Layland. 1993. Pyrethroid resistance: synergists. Bull. Entomol. Res., 1 (Supp.). 62-100.

Gunning, R. V., C. S. Easton, L. R. Greenup and V. E. Edge. 1984. Pyrethroid resistance in *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. J. Econ. Entomol. 77: 1283-1287.

Gunning, R.V., C. S. Easton, M. E. Balfe and I. Ferris. 1991. Pyrethroid resistance mechanisms in Australian *Helicoverpa armigera.* Pestic. Sci. 33**:** 473-490.

Kranthi, K. R., D. R. Jadhavb, S. Kranthia, R. R. Wanjaria, S. S. Alia and D. A. Russell. 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 21: 449-460.

Le Ora Software. 1987. POLO-PC, A User Guide to Probit or Logit Analysis, Le Ora Software, Berkeley, CA, USA.

Maumbea, B. M and S. M. Swintonb. 2003. Costs of pesticide use in Zimbabwe's smallholder cotton growers. Soc. Sci. Medic. 57: 1559-1571.

MStat-C. 1989. UserÕs guide to MSTAT-C-A microcomputer program for the design, management, and analysis of agronomic research experiments. Michigan State University, East Lansing, MI.

Plapp, F. W and R. F. Hoyer. 1968. Insecticide resistance in the house fly: decreased rate of absorption as the mechanism of action of a gene that acts as an intensifier of resistance. J. Econ. Entomol. 61: 1298–1303.

Pathan, A. K., A. H. Sayyed, M. Aslam, M. Razaq, G. Jilani and M. A. Saleem. 2008. Evidence of field-evolved resistance to organophosphates and pyrethroids in *Chrysoperla carnea* (Neuroptera: Chrysopidae). J. Econ. Entomol. 101: 1676-1684.

Ramasubramanian, T and A. Regupathy. 2004. Pattern of cross-resistance in pyrethroid-selected populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from India. J. Appl. Entomol. 128: 583-587.

Razaq, M., A. Suhail, M. Aslam, M. J. Arif, M. A. Saleem and M. H. A. Khan. 2005. Evaluation of new chemistry and conventional insecticides against *Helicoverpa armigera* (Hubner) on cotton at Multan (Pakistan). Pakistan Entomol. 27: 71-73.

Razzaq, M., A. Suhail, M. J. Arif, M. Aslam and A. H. Sayyed. 2007. Effect of rotational use of insecticides on pyrethroids resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Appl. Entomol. 131: 460-465.

Saleem, M. A., M. Ahmad, M. Ahmad, M. Aslam and A. H. Sayyed. 2008. Resistance to selected organochlorine, organophosphate, carbamate and pyrethroid in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. 101:1667-1675.

Sayyed, A. H., M. N. R. Attique and A. Khaliq. 2005. Stability of field selected resistance to insecticides in *Plutella xylostella* (Lepidoptera: Plutellidae) from Pakistan. J. Appl. Entomol. 129: 542-547.

Srinivasa, R., S. S. Udikerib, S. K. Jayalakshmib and K. Sreeramulu. 2004. Identification of factors responsible for insecticide resistance in *Helicoverpa armigera.* Comp. Biochem. Physiol. Part C. 137: 261-269.

Torres-Vilaa, L. M., M. C. Rodriguez-Molinaa, A. Lacasa-Plasenciab and P. Bielza-Linoc. 2002. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. Crop Prot. 21: 1003-1013.

Vassal, J. M., M. Vaissayre and T. Martin. 1997. Decrease in the susceptibility of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to pyrethroid insecticides in Coˆ te d'Ivoire. Res. Pestic. Manag. 9: 14-15.

Yamamoto, I., N. Kyomura and Y. Takahashi. 1993. Negatively correlated cross resistance: combinations of *N*-methylcarbamate with *N*-propylcarbamate or oxidiazolone for green rice leafhopper. Arch. Insect Biochem. Physiol. 22: 227-288.