

# Insecticide Resistance in Field Populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)<sup>1</sup>

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**Abstract** *Helicoverpa armigera* (Hübner) is a notorious pest of various field crops. A contributing factor in its pest status is the development of resistance to insecticides, making the insect difficult to control. The objective of this study was to assess the toxicity of selected insecticides against natural populations of *H. armigera* in Pakistan and thus identify possible levels of insecticide resistance. Insects were collected from three locations in the province of Punjab, Pakistan, in three consecutive years. The median lethal concentration (LC<sub>50</sub>) of selected insecticides was determined for each field population as well as a susceptible lab strain designated as Lab-PK. Resistance ratios (RRs) for each insecticide were calculated as the ratios of the LC<sub>50</sub> for each field population relative to that of the Lab-PK strain. Based on the calculated RRs, the field populations tested were highly resistant to bifenthrin (RR = 34.1 to 48.0), moderately to highly resistant to lambda-cyhalothrin (RR = 19.6 to 68.2) and deltamethrin (RR = 19.3 to 37.2), and minimally to moderately resistant to profenofos (RR = 9.80 to 12.11), methoxyfenozide (RR = 6.0 to 11.8) and thiodicarb (RR = 5.6 to 11.5). Resistance was low for emamectin benzoate (RR = 1.7 to 5.2), chlorpyrifos (RR = 3.5 to 9.6), and lufenuron (RR = 1.0 to 2.2). Pairwise comparison of the log LC<sub>50</sub> of the insecticides against all populations showed a correlation among the various insecticides, suggesting possible development of cross-resistance.

**Key Words** insecticide resistance, *Helicoverpa armigera*, pyrethroids, organophosphates

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*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), also known as the cotton bollworm, is a cosmopolitan pest that is characterized as having multiple generations each year with high fecundity rates and an ability to migrate long distances. Cotton, chickpea, tomato, sunflower, okra, pea, tobacco, potato, and eggplant are significantly damaged by *H. armigera*. In Pakistan, insecticides are routinely used as the control strategy for this pest (Basit et al. 2013). However, indiscriminate use of insecticides has resulted in development of resistance in many *H. armigera* populations (Ferre and Van 2002, Sayyed and Wright 2006).

Resistance to a range of insecticides has been reported worldwide, including in Pakistan (McCaffery et al. 1998). Moderate to high levels of resistance to chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, neonicotinoids, and insect growth regulators (IGRs) has been reported in field populations (Ahmad et al. 1995, Nauen and Bretschneider 2002). Moderate to high levels of

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resistance to neonicotinoids and other conventional chemical insecticides also have been reported from Punjab Province in Pakistan (Aheer et al. 2008).

Monitoring the development of insecticide resistance is crucial to devising a successful insecticide resistance management (IRM) plan (Forrester 1990). The IRM plan helps to document geographical occurrence of resistance to insecticides and variability of resistance within insect populations. It also provides an early warning for developing resistance issues and problems and identifies which pesticides are no longer effective due to resistance (Brent 1986). Resistance to commonly used conventional insecticides in Pakistan in the late 1990s led to the adoption and use of insecticides with new and differing modes of action to control chewing and sucking insect pests of cotton (Ahmad et al. 2007b). Reports of failure of these insecticides in the field led to the study reported herein with its objectives of measuring possible changes in the susceptibility to insecticides in three agricultural regions of Punjab, Pakistan (Multan, Bahawalpur, and Faisalabad).

## Materials and Methods

**Insects.** Fifth- and sixth instars of *H. armigera* larvae were collected from cotton growing in the Faisalabad, Multan, and Bahawalpur districts in Punjab Province of Pakistan in 2009, 2010, and 2011. At each site/date, 1,000 larvae were collected from 2.2-ha fields and transported to the laboratory at the Entomological Research Institute (Ayub Agricultural Research Institute, Faisalabad). A susceptible strain of *H. armigera* maintained in the laboratory for 5 yr without exposure to pesticides (identified as the “Lab-PK” strain) was used for toxicity comparisons as per Ahmad et al. (2007b).

Larvae were fed a semisynthetic wheatgerm-based diet and maintained at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity with a 14:10-h light:dark photoperiod (Sayyed and Wright 2006). Diet was replenished each day, and pupae were collected and placed in emergence cages. Adults were kept in Perspex oviposition cages ( $30 \times 30 \times 30$  cm), two sides of which were covered with muslin cloth to allow ventilation, and fed on a solution containing sucrose (100 g), vitamins (20 ml), and methyl 4-hydroxybenzoate with a cotton ball soaked in the solution (Ahmad et al. 2007b).

**Insecticides.** Commercial formulations of insecticides tested in this study were profenofos (Curacron® 500 EC, Syngenta, Basel, Switzerland), emamectin benzoate (Proclaim® 1.9 EC, Syngenta), lambda-cyhalothrin (Karate® 2.5 EC, Syngenta), chlorpyrifos (Lorsban® 40 EC, Dow AgroSciences, Indianapolis, IN, USA), bifenthrin (Talstar® 10 EC, FMC Professional Solutions, Philadelphia, PA, USA), lufenuron (Match® 50 EC, Syngenta), deltamethrin (Decis® 2.5 EC, Bayer CropScience, Leverkusen, Germany), thiodicarb (Larvin® 80 DF, Bayer CropScience), methoxyfenozide (Dow AgroSciences).

**Bioassays.** Newly molted second and third instars (30 to 40 mg each) from the F<sub>2</sub> laboratory colonies were bioassayed using a leaf-dip technique recommended by the Insecticide Resistance Action Committee (Anonymous 1990). Technical-grade preparations of the insecticides were serially diluted. Leaf discs (5-cm diameter) were cut from unsprayed fresh cotton leaves, immersed in the test solution for 10 s (Sayyed et al. 2000), air-dried on paper, toweling, and then transferred to moistened filter paper in plastic petri dishes (5-cm diameter). Five newly

molted .larvae were placed on each dried leaf disc and dishes were covered. Seven concentrations plus an untreated control were replicated eight times for each insecticide tested. Test containers with larvae were covered with black paper to reduce the risks of cannibalism.and were maintained at  $25 \pm 2^{\circ}\text{C}$  for 48 h, when mortality was assessed. Larvae were regarded as dead when they did not move when probed with a blunt probe or brush.

**Analysis.** Mortality data were corrected using Abbott's formula (Abbott 1925) and subjected to probit.analysis (Finney 1971) on pooled data by using POLO-PC (LeOra Software, Polo-PC 2003). Median lethal concentration ( $\text{LC}_{50}$ ) values and their 95% fiducial. limits (FL) were estimated by probit analysis using POLO. Due to the inherent variability of bioassays, pairwise comparison to  $\text{LC}_{50}$  values was made at the 1% significance level, where individual 95% FL for two treatments.did not overlap (Litchfield and Wilcoxon 1949). Resistance ratios (RRs) were calculated by dividing the  $\text{LC}_{50}$  values of each of the field populations by the  $\text{LC}_{50}$  of the Lab-PK population. The level of insecticide resistance was determined using the methods described by Ahmad et al. (2007a) and Torres-Vila et al. (2002), where levels of resistance were defined as follows: susceptible ( $\text{RR} = 0$  to  $1$ ), tolerance to low level of resistance ( $\text{RR} = 2$  to  $10$ ), moderate resistance ( $\text{RR} = 11$  to  $30$ ), high. resistance ( $\text{RR} = 31$  to  $100$ ), and very high resistance ( $\text{RR} > 100$ ).

## Results and Discussion

**Mortality response of susceptible Lab-PK strain to insecticides.** Emamectin benzoate was significantly ( $P < 0.01$ ) more toxic to *H. armigera* larvae than all other insecticides tested ( $\text{LC}_{50} = 0.11 \mu\text{g/ml}$ ; 95% FL =  $0.08\text{--}0.18$ ), while deltamethrin was least toxic ( $\text{LC}_{50} = 67.23 \mu\text{g/ml}$ ; 95% FL =  $59.45\text{--}78.49$ ) (Table 1). The slopes of the regression lines of bifenthrin, thiodicarb, lambda-cyhalothrin, profenofos, deltamethrin, and chlorpyrifos were similar (overlapping of 95% FL,  $P > 0.05$ ). Lufenuron (an IGR) was notably more toxic ( $P < 0.01$ ) than methoxyfenozide, the other IGR tested (Table 1).

**Mortality response of field populations to insecticides.** Emamectin benzoate was also the most toxic of the insecticides tested against the field populations of *H. armigera*, with  $\text{LC}_{50}$  valuess of  $0.22 \mu\text{g/ml}$  at Bahawalpur,  $0.13 \mu\text{g/ml}$  at Multan, and  $0.52 \mu\text{g/ml}$  at Faisalabad (Table 1). Lufenuron also exhibited low  $\text{LC}_{50}$  values against the field populations with values of  $0.53 \mu\text{g/ml}$  at Bahawalpur,  $0.68 \mu\text{g/ml}$  at Multan, and  $0.63 \mu\text{g/ml}$  at Faisalbad. The highest  $\text{LC}_{50}$  values were with deltamethrin at Multan ( $241.04 \mu\text{g/ml}$ ) and Faisalbad ( $195.34 \mu\text{g/ml}$ ).

**Resistance assessments.** Valles et al. (1997) proposed that insect populations should not be regarded as resistant to an insecticide unless the RR is  $\geq 10.0$ . Therefore, field populations of *H. armigera* were highly resistant ( $\text{RR} = 31$  to  $100$ ) to bifenthrin at all three locations (Multan, 48.0; Faisalabad, 40.1; Bahawalpur, 34.1), lambda-cyhalothrin at two locations (Bahawalpur, 68.2; Faisalabad, 48.5), methoxyfenozide at one location (Multan, 64.4), and deltamethrin at one location (Multan, 37.2) (Table 2). Field populations were moderately resistant ( $\text{RR} = 11$  to  $30$ ) to methoxyfenozide at two locations (Bahawalpur, 29.2; Faisalabad, 14.5), deltamethrin at two locations (Bahawalpur, 28.2; Faisalabad, 19.3), lambda-cyhalothrin at one location (Multan, 19.6), thiodicarb at one location (Multan,

**Table 1. Mortality response of *Helicoverpa armigera* populations from Punjab Province of Pakistan to selected insecticides, 2009–2011.**

Insecticide	Colony	LC <sub>50</sub> (95% FL)* (µg/ml)	Fit of Probit Analysis			
			Slope ± SE	χ <sup>2</sup>	P	df
Methoxy-fenozide	Lab-PK**	1.05 (0.86–1.13)	2.34 ± 0.22	0.22	0.96	6
	Bahawalpur	29.29 (23.1–36.4)	2.24 ± 0.22	7.0	0.30	6
	Multan	32.18 (26.3–39.4)	1.46 ± 0.29	3.48	0.32	6
	Faisalabad	39.41 (34.11–47.77)	1.71 ± 0.17	3.76	0.41	6
Profenofos	Lab-PK**	11.23 (8.67–13.21)	2.13 ± 0.67	0.58	0.71	5
	Bahawalpur	21.16 (17.22–27.9)	1.06 ± 0.19	2.32	0.65	5
	Multan	27.93 (21.22–35.32)	1.04 ± 0.23	2.15	0.92	5
	Faisalabad	34.89 (28.75–41.21)	1.56 ± 0.91	3.11	0.95	5
Emamectin benzoate	Lab-PK**	0.11 (0.08–0.18)	1.89 ± 0.52	0.13	0.78	6
	Bahawalpur	0.22 (0.11–0.28)	1.42 ± 0.13	8.05	0.11	6
	Multan	0.13 (0.12–0.19)	1.80 ± 0.22	6.17	0.83	6
	Faisalabad	0.52 (0.44–0.61)	2.74 ± 0.17	5.79	0.92	6
Lambda-cyhalothrin	Lab-PK**	8.43 (4.16–13.75)	1.98 ± 0.21	1.2	0.99	6
	Bahawalpur	55.02 (51.23–61.89)	1.63 ± 0.23	2.71	0.67	6
	Multan	15.68 (12.56–18.76)	1.72 ± 0.16	3.18	0.56	6
	Faisalabad	37.54 (33.54–42.11)	1.53 ± 0.15	5.87	0.57	6
Chlorpyrifos	Lab-PK**	2.45 (0.56–4.19)	2.11 ± 0.11	2.10	0.45	6
	Bahawalpur	4.56 (3.11–4.19)	1.63 ± 0.18	4.02	0.94	6
	Multan	4.75 (3.01–5.39)	1.70 ± 0.17	4.49	0.84	6
	Faisalabad	9.63 (7.9–13.28)	1.35 ± 0.16	6.17	0.78	6
Bifenthrin	Lab-PK**	16.12 (11.56–21.63)	1.98 ± 0.11	1.22	0.96	6
	Bahawalpur	22.86 (18.79–25.23)	1.81 ± 0.18	5.78	0.49	6
	Multan	31.40 (28.12–36.87)	1.28 ± 0.16	5.23	0.31	6
	Faisalabad	32.18 (28.93–37.23)	1.56 ± 0.16	6.01	0.98	6
Lufenuron	Lab-PK**	0.10 (0.05–1.23)	1.25 ± 0.41	1.32	0.54	5
	Bahawalpur	0.53 (0.43–0.61)	1.47 ± 0.12	2.67	0.94	5
	Multan	0.68 (0.62–0.71)	1.35 ± 0.18	2.93	0.89	5
	Faisalabad	0.63 (0.55–0.67)	1.55 ± 0.19	3.19	0.66	5

Table 1. Continued.

Insecticide	Colony	LC <sub>50</sub> (95% FL)* (µg/ml)	Fit of Probit Analysis			
			Slope ± SE	χ <sup>2</sup>	P	df
Deltamethrin	Lab-PK**	67.23 (59.45–78.49)	2.23 ± 0.71	1.89	0.91	6
	Bahawalpur	96.46 (87.56–104.9)	1.92 ± 0.18	1.77	0.44	6
	Multan	241.04 (222.53–257.11)	1.84 ± 0.16	5.03	0.71	6
	Faisalabad	195.34 (186.53–211.34)	1.97 ± 0.13	4.11	0.40	6
Thiodicarb	Lab-PK**	41.23 (36.9–51.3)	2.11 ± 0.26	2.76	0.36	6
	Bahawalpur	70.91 (63.11–74.89)	1.50 ± 0.14	5.43	0.74	6
	Multan	52.45 (47.23–56.74)	1.71 ± 0.16	4.91	0.76	6
	Faisalabad	65.71 (59.3–71.25)	2.09 ± 0.22	5.85	0.63	6

\* Median lethal concentration (LC<sub>50</sub>) and their 95% fiducial limits (FL) were calculated by probit analysis for each population ( $n = 280$ ).

\*\* Lab-PK = nonresistant laboratory colony.

11.5), and profenofos at one location (Multan, 12.1). RRs for other insecticides and/or other locations were  $<10$ , where the resistance level is described as tolerance or a low level of resistance.

Ahmad et al. (2007a) and Armes et al. (1997) previously reported resistance to pyrethroids, carbamates, and organophosphates in *H. armigera* populations on the Indo-Pakistan subcontinent. Our results demonstrated *H. armigera* resistance to the three pyrethroids tested (bifenthrin, deltamethrin, lambda-cyhalothrin). Resistance to the carbamate (thiodicarb) was observed at only one of the three locations, whereas resistance to the organophosphate profenofos was detected at two of the three locations. Populations from all three locations were resistant to methoxyfenozide, an IGR.

Pairwise correlations of the log LC<sub>50</sub> values of the insecticides assayed in our study yielded a significant inverse correlation of profenofos values with thiodicarb values ( $r = -0.66$ ;  $P = 0.05$ ) and significant positive correlations of methoxyfenozide values with profenofos values ( $r = 0.61$ ;  $P = 0.05$ ) and of lufenuran values with emamectin benzoate values ( $r = 0.93$ ;  $P = 0.01$ ) (Table 3). These significant positive correlations indicate the potential of *H. armigera* acquiring resistance due to cross-resistance mechanisms such as those that have been reported with resistance to pyrethroids and organophosphates (Ahmad et al. 2008).

Furthermore, resistance to insecticides with different modes of action has developed in these *H. armigera* populations. Bifenthrin, deltamethrin, and lambda-cyhalothrin are pyrethroids that disrupt cell membrane integrity, which interferes with normal muscle cell contractions. Profenofos is an organophosphate and thiodicarb is a carbamate; both are acetylcholinesterase inhibitors. Methoxyfenozide is an IGR juvenile hormone mimic. Development of resistance to these chemicals with different modes of action may be the result of application history

**Table 2. Resistance of *Helicoverpa armigera* populations in Punjab Province of Pakistan to selected insecticides, 2009–2011.**

Insecticide	Population	RR*	Level of Resistance**
Methoxyfenozone	Bahawalpur	29.15	Moderate
	Multan	64.36	High
	Faisalabad	14.50	Moderate
Profenofos	Bahawalpur	10.23	Moderate
	Multan	12.11	Moderate
	Faisalabad	9.80	Tolerant; low
Emamectin benzoate	Bahawalpur	1.69	Tolerant; low
	Multan	5.22	Tolerant; low
	Faisalabad	4.00	Tolerant; low
Lambda-cyhalothrin	Bahawalpur	68.17	High
	Multan	19.60	Moderate
	Faisalabad	48.52	High
Chlorpyrifos	Bahawalpur	3.48	Tolerant; low
	Multan	9.62	Tolerant; low
	Faisalabad	6.56	Tolerant; low
Bifenthrin	Bahawalpur	34.10	High
	Multan	48.0	High
	Faisalabad	40.08	High
Lufenuron	Bahawalpur	1.70	Tolerant; low
	Multan	2.19	Tolerant; low
	Faisalabad	1.01	Tolerant; low
Deltamethrin	Bahawalpur	28.21	Moderate
	Multan	37.17	High
	Faisalabad	19.33	Moderate
Thiodicarb	Bahawalpur	7.21	Tolerant; low
	Multan	11.50	Moderate
	Faisalabad	5.60	Tolerant; low

\* Resistance ratio (RR) calculated as ratio of median lethal concentration (LC<sub>50</sub>) of respective field population to LC<sub>50</sub> of nonresistant laboratory colony.

\*\* Level of resistance based on calculated RR as per Valles et al. (1997).

Table 3. Correlation coefficients (*r*) of pairwise comparisons of log median lethal concentration values of selected insecticides in assays of populations of *Helicoverpa armigera* in Punjab Province of Pakistan, 2009–2011.

	Bifenthrin	Emamectin Benzoate	Lufenuran	Methoxy-fenozide	Profenofos	Thiodicarb
Bifenthrin		0.14	0.05	0.48	0.09	0.07
Emamectin benzoate	0.14		0.93**	–0.44	–0.05	0.43
Lufenuran	0.05	0.93**		–0.22	0.03	0.47
Methoxy-fenozide	0.48	–0.44	–0.22		0.61*	–0.33
Profenofos	0.09	–0.05	0.03	0.61*		–0.66*
Thiodicarb	–0.07	0.43	0.47	–0.33	–0.66**	

\* Significantly different, *P* = 0.05.

\*\* Significantly different, *P* = 0.01.

(Shen and Wu 1995). Mixing new insecticides with more frequently used insecticides occurs in many areas of the world (Yu et al. 2003), including Pakistan, where it is a common practice, and could be a plausible explanation for development of resistance to insecticides with different modes of action. Indeed, monooxygenases are reportedly involved in cross-resistance between thiodicarb and lambda-cyhalothrin, bifenthrin, and emamectin benzoate (Sayyed et al. 2008a, 2008b). Monooxygenase systems are comprised of many isozymes (IShaaya and Casida 1980); thus, if insecticide exposure selects for a specific isozyme, then this may also select for other insecticides impacted by the isozyme.

High levels of resistance to insecticides have been reported to rapidly decline in selected fields or laboratory populations (Carriere et al. 2001). To study the stability of resistance to the insecticides we observed in our study, each field population was reared for six generations without exposure to the insecticide. When the insecticide-resistant populations were reared for six generations and then challenged with the insecticide, the resultant RR decreased with a reversion rate of  $-0.27$  for thiodicarb,  $-0.26$  for profenofos,  $-0.23$  for bifenthrin,  $-0.22$  for methoxyfenozide,  $-0.21$  for emamectin benzoate,  $-0.19$  for chlorpyrifos,  $-0.18$  for lufenuron,  $-0.17$  for lambda-cyhalothrin, and  $-0.16$  for deltamethrin. Rapid retrogression of resistance to the tested insecticides in the field-collected populations suggests that high fitness costs may occur with resistance. The decline in resistance may also be due to the presence of heterozygotes in the population.

Recently, control failures of commonly used insecticides followed by subsequent outbreaks of *H. armigera* in Pakistan could be attributed to development of resistance to insecticides. In Pakistan, *H. armigera* is an important pest of several field crops, including cotton and vegetables (Ahmad et al. 2007a). Hence, it is frequently exposed to insecticides used in management programs. Exposure of this pest to diverse groups of insecticides throughout the year is an important factor in the rapid development of resistance to insecticides. This is an impediment to developing and implementing efficacious integrated pest management (IPM) programs for this pest. An effective means of reducing, slowing, or managing resistance development in the region must be pursued. Moreover, effective monitoring of pest populations (pheromones or light trapping) and use of genetically modified crop varieties (e.g., *Bacillus thuringiensis* Berliner toxins) could enhance IPM programs.

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