

Resistance Profile of *Helicoverpa armigera* (Hubner) to Commonly Used Synthetic Molecules on Cotton

R. K. SHARMA, SATNAM SINGH*, RAJINDER KUMAR, V. K. GUPTA AND V. K. DILAWARI

Department of Entomology, Punjab Agricultural University
 Ludhiana 141004, India
 E-mail : drsatnamsingh@yahoo.co.in
 *Correspondence

Abstract

The studies were made on the LD₅₀ values of American bollworm, *Helicoverpa armigera* (Hubner) to six commonly used insecticides on cotton during *kharif* of 2005-06. The population of *H. armigera* from district of Abohar exhibited highest and lowest LD₅₀ values that were 0.722 µg/larva for fenvalerate and 0.035 µg/larva for quinalphos. Among all the insecticides highest LD₅₀ value was observed for fenvalerate in Bathinda (0.817 µg/larva) and lowest LD₅₀ value was (0.012 µg/larva) for quinalphos in Hoshiarpur district. LD₅₀ values for quinalphos remain almost same throughout all locations. LD₅₀ values for endosulfan varied from 0.034 µg/larva in Hoshiarpur district to 0.122 µg/larva in Mansa district. While for chlorpyrifos, LD₅₀ values were high in Bathinda (0.091 µg/larva), Abohar (0.084 µg/larva), Mansa (0.071 µg/larva), Faridkot (0.069 µg/larva) and low in Ludhiana (0.050 µg/larva), and Hoshiarpur (0.032 µg/larva). This paper reports the extent of variability in LD₅₀ values in *H. armigera* to major group of insecticides like pyrethroids (cypermethrin, deltamethrin and fenvalerate), cyclodiene (endosulfan) and organophosphates (chlorpyrifos and quinalphos) during 2005-06 in six locations of Punjab.

Key words : LD₅₀ values, *Helicoverpa armigera*, Cotton, Pyrethroids.

Cotton (*Gossypium* spp), is a shrub native to the Indian subcontinent and the tropical and subtropical part of Africa and America. In Punjab, during 2006-07, it was grown on 557 thousand hectares with a total production of 2,395 thousand bales, respectively (Anonymous 2007). Cotton is primarily grown in dry tropical and subtropical climates at temperature ranges between 11 and 25 C. Cotton occupies only 5% of total cultivable area in India but consumes more than 55% of the total insecticides used in country (Puri 1995). In North India, jassid, *Amrasca biguttula* (Ishida) and whitefly, *Bemisia tabaci* (Genn.) are serious sucking pests. Among the bollworms, American bollworm, *Helicoverpa armigera* (Hubner), spotted bollworms; *Earias vitella* (Fabricius), *Earias insulana* (Boisduval) and pink bollworm, *Pectinophora gossypiella* (Saunders) are key pests. The cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera : Noctuidae) is a major pest on a wide ranges of crops (Torres-vila et al. 2002). In India, the cotton crop provides ecological niche to 164 insects and mites. Out of these, only 12 are of economic importance as studied by Dhawan (2005a). Cotton boll-

worm, gram pod borer or American bollworm, *H. armigera* occurs in Africa, Asia, southern Europe and Australia. It is a major pest of cotton, maize, sorghum, pigeonpea, chickpea, soyabean, groundnut, sunflower and a range of vegetables (Mc Caffery 1998). Among the various options for their management, the use of insecticides is the dominant tool used in farmer's fields. It has developed insecticide resistance to all the insecticides that have been used for its management (Sparks 1981, Wolfenbarger et al. 1981). Low to moderate levels of resistance were reported in Maharashtra, moderate to high in Tamil Nadu, Uttar Pradesh, Gujarat and Punjab and high in Andhra Pradesh and Madhya Pradesh (Kranthi 2005). Resistance to insecticides in *H. armigera* has been documented throughout the world. Injudicious use of insecticides has also resulted in an increase in environmental pollution and severity of minor pests. To avoid indiscriminate use of insecticides and effective control with insecticides, it is essential to monitor for insecticide resistance. The study reports the result of survey conducted during 2005-2006 to monitor LD₅₀ values in *H. armigera* at various locations of Punjab.

Table 1. Diet ingredients for rearing of *H. armigera*.

Ingredients	Quantity
Part A	
Hot water (60 C)	550 ml
Chickpea flour (kabuli type)	160 g
Ascorbic acid	5.3 g
Wheat germ	60 g
Sorbic acid	1.7 g
Formaldehyde (10%)	13.5 ml
Methyl-4 hydroxyl benzoate	3.3 g
Streptocyclin	2 g
Part B	
Agar-Agar	16 g
Bakers yeast	53 g
DD Water	600 ml

Methods

Insects

Studies were conducted in Insect Molecular Laboratory in the Department of Entomology, Punjab Agricultural University, Ludhiana during 2005-06. Larvae of *H. armigera* were collected from major cotton growing locations of Punjab i.e. Mansa, Bathinda, Abohar, Faridkot, Ludhiana and Hoshiarpur.

Rearing of H. armigera

Larvae were reared singly on artificial diet in 12 cell trays/sample containers. These were provided fresh food until pupation. Freshly formed pupae were transferred to glass jars (15 × 10 cm) having moist sponge and blotting paper at the bottom, covered with muslin, that was fastened with rubber bands.

Adult males and females were paired and released into the earthen pots (27 × 21 cm) lined with blotting paper at bottom and covered with muslin cloth, which were fastened with rubber bands. Two pairs were released per earthen pot. The earthen pots were placed in plastic trays containing water to maintain desired level of humidity (>70%) inside the pot. Temperature of the laboratory was maintained up to 27 ± 2 C. Eggs laid on the muslin cloth were removed daily and kept in glass jars (15 × 10 cm) for hatching. Each glass jar was covered with muslin cloth that was fastened with rubber bands. The neonate larvae were reared and

used for the experimental purpose. Rearing trays, moth chambers, forceps and brushes were to be regularly decontaminated in 5% sodium hypochlorite and rectified spirit.

Larval Diet

For preparation of artificial diet for rearing of *H. armigera*, the diet ingredients were divided into two parts, part A and part B (Table 1). Entire diet ingredients of part A were mixed thoroughly in a mixing bowl to avoid clot formation. Methyl-4-hydroxyl-benzoate being insoluble in water was dissolved in ethyl alcohol. Then it was boiled on a hot plate by adding 150 ml distilled water. For part B, agar-agar was sprinkled in boiling water with continuous stirring following which yeast was sprinkled on it. After removing the ingredients from hot plate, part A and part B were mixed quickly to avoid solidification. The prepared diet was poured in squeeze bottles and then dispensed into rearing trays. When not required immediately, trays with diet were covered with plastic bags after cooling and kept in refrigerator.

The Adult Diet

A liquid mixture of ABDEC (multivitamin) drops, honey and water in the ratio of 0.5 : 1 : 10 was prepared. Cotton swabs dipped this mixture were hung in the pot with pins to provide food for the moths. The food was changed daily. *Insecticides*. Seven serial dilutions of cypermethrin, deltamethrin, fenvalerate, quinalphos, chlorpyrifos and endosulfan were prepared from stock solutions. Technical grade formulations of the different insecticides of known purity were used in the preparation of solutions. Acetone was used as a solvent for the preparation of different concentrations.

Experimental Procedure

Third instars weighing 30—40 mg each were used for testing of different doses. Fifty to seventy five larvae were used for each concentration according to availability. Topical application technique was used for the bioassays as recommended by Entomological Society of America (Anonymous 1970). Hamilton syringe dispenser was used to apply 1 µl of insecticide

solution to the thoracic dorsum of third instar larvae. In control treatments, 1 μ l of acetone was used. It was ensured that acetone does not drip to the lateral sides of the larvae. Once all the larvae in the tray were treated, the lid was closed and labeled.

Mortality assessments were made on the basis of the number of moribund and dead larvae (Armes et al. 1996). Mortality of *H. armigera* in different treatments was recorded up to 72 h after the application.

Data were statistically using log probit technique (Finney 1971) using the computer program PC-POLO software (Robertson et al. 1980). Resistance ratios were calculated by dividing the LD₅₀ values of resistant strains to the LD₅₀ values of susceptible strain.

Results and Discussion

Cypermethrin

The population of *H. armigera* showed highest and lowest LD₅₀ values to cypermethrin in Bathinda and Hoshiarpur districts respectively (Table 2). Populations of *H. armigera* from Mansa and Abohar were nearly the same with LD₅₀ value of 0.694 μ g/larva and 0.672 μ g/larva respectively, that lies between fiducial ranges of 0.412—1.254 and 0.362—1.052 μ g/larva. While population from Ludhiana district showed LD₅₀ of 0.382 μ g/larva with slopes lies between 1.412 \pm 0.322. LD₅₀ was reported low in Hoshiarpur districts which may be due to less numbers of sprays of insecticides done to control insect pests.

The present studies showed high level of resistance to cypermethrin. Resistance of *H. armigera* to insecticide especially to pyrethroids was disastrous to Israeli cotton growers (Horowitz et al. 1993). Higher level of resistance was observed against synthetic pyrethroids in those regions where pyrethroid use was most frequent i.e. 4—8 applications per season (Kranthi et al. 2001). High level of resistance was reported to cypermethrin and monocrotophos while moderate level was reported to endosulfan in the field strains of *H. armigera* collected from Pakistan (Ahmad et al. 1995).

High level of resistance was reported to synthetic pyrethroids (Kapoor et al. 2002). According to Gill and Dhawan (2006) maximum and minimum LD₅₀ value for cypermethrin in three districts of Punjab was 0.863 to 0.143 μ g/larva respectively. High level of resistance was recorded to cypermethrin in strains collected from

cotton growing areas of Guntur and Coimbatore during 1989 (Armes et al. 1992). LD₅₀ of cypermethrin increased with the progression of season for the population of *H. armigera* from Akola region (Ernst and Dittrich 1992). Kranthi (2005) reported high level of resistance to cypermethrin throughout India.

Fenvalerate

Population collected from Abohar and Bathinda districts showed highest LD₅₀ of 0.722 and 0.817 μ g/larva, respectively as compared to the population of *H. armigera* collected from other areas of Punjab. Variation in LD₅₀ values occurs in different areas due to various factors viz. more numbers of sprays, untimely sprays of insecticides and environmental factors that also help to build up more level of resistance in some areas. In Faridkot, Ludhiana and Hoshiarpur, LD₅₀ values were 0.581, 0.360 and 0.072 μ g/larva, respectively. In these areas, LD₅₀ values were less due to less number of sprays used for the management of insect pests. High resistance frequencies were observed to cypermethrin and, fenvalerate in population collected from Central India during 1993-94, 1994-95 and 1995-96 cropping seasons (Kranthi et al. (1997).

Kranthi et al. (2001) reported high resistance ratios to fenvalerate, cypermethrin and cyhalothrin in four strains collected from Central and Southern India.

Endosulfan

It is the one of the safest insecticide used by the farmers for the management of insect pests. Population of *H. armigera* collected from Mansa showed LD₅₀ value of 0.122 μ g/larva while LD₅₀ value of 0.092 μ g/larva was observed in the population collected from Bhatinda district. LD₅₀ value varied in the different areas depends on the numbers of sprays. Low level of LD₅₀ value observed in population collected from Hoshiarpur and Ludhiana that 0.034 and 0.060 μ g/larva respectively, might be due to less numbers of sprays done in these areas for control of insect pests. LD₅₀ values in Abohar and Faridkot districts were almost same as 0.081 and 0.078 μ g/larva, respectively, with different values of heterogeneity.

Kranthi (2005) reported low level of resistance in North India but moderate in Central and South India.

Table 2. Variability of *H. armigera* population to different insecticides in Punjab.

Location	LD ₅₀	Fiducial range (95%)	Heterogeneity	Slope
Cypermethrin				
Abohar	0.672	0.362–1.052	0.17	1.542±0.346
Bathinda	0.702	0.472–1.342	0.05	1.298±0.268
Faridkot	0.547	0.332–0.824	0.18	1.362±0.292
Ludhiana	0.382	0.192–0.720	0.09	1.412±0.322
Mansa	0.694	0.412–1.254	0.07	1.388±0.262
Hoshiarpur	0.09	0.054–0.130	0.13	1.312±0.240
Fenvalerate				
Abohar	0.722	0.412–1.284	0.05	1.291±0.218
Bathinda	0.817	0.502–1.368	0.11	1.460±0.312
Faridkot	0.581	0.312–0.841	0.20	1.517±0.284
Ludhiana	0.360	0.172–0.712	0.09	1.422±0.375
Mansa	0.625	0.392–0.985	0.02	1.622±0.356
Hoshiarpur	0.072	0.038–0.118	0.04	1.318±0.292
Endosulfan				
Abohar	0.081	0.041–0.134	0.02	1.502±0.282
Bathinda	0.092	0.054–0.112	0.07	1.656±0.268
Faridkot	0.078	0.042–0.113	0.05	1.502±0.240
Ludhiana	0.060	0.032–0.104	0.04	1.224±0.312
Mansa	0.122	0.068–0.140	0.10	1.682±0.238
Hoshiarpur	0.034	0.012–0.054	0.12	1.224±0.398
Quinalphos				
Abohar	0.035	0.022–0.060	0.09	1.220±0.212
Bathinda	0.050	0.018–0.086	0.12	1.356±0.232
Faridkot	0.039	0.014–0.042	0.09	1.404±0.292
Ludhiana	0.028	0.011–0.038	0.14	1.312±0.350
Mansa	0.042	0.022–0.068	0.16	1.384±0.259
Hoshiarpur	0.012	0.004–0.104	0.20	1.157±0.412
Chlorpyrifos				
Abohar	0.084	0.501–0.129	0.05	1.472±0.252
Bathinda	0.091	0.522–0.135	0.07	1.622±0.270
Faridkot	0.069	0.037–0.105	0.12	1.222±0.372
Ludhiana	0.050	0.021–0.072	0.14	1.322±0.279
Mansa	0.071	0.050–0.112	0.09	1.506±0.320
Hoshiarpur	0.032	0.018–0.054	0.11	1.220±0.298
Deltamethrin				
Abohar	0.512	0.220–0.834	0.20	1.362±0.412
Bathinda	0.622	0.392–0.912	0.18	1.622±0.356
Faridkot	0.448	0.241–0.612	0.12	1.512±0.318
Ludhiana	0.243	0.120–0.341	0.14	1.388±0.271
Mansa	0.587	0.315–0.872	0.01	1.324±0.300
Hoshiarpur	0.071	0.032–0.116	0.14	1.348±0.298

Moderate level of resistance was observed to endosulfan in the field strains collected from Pakistan (Ahmad et al. 1995, 1997). Mc Caffery et al. (1989) reported that population of *H. armigera* collected in October 1987 from coastal cotton growing districts in Andhra Pradesh were moderately resistant to endosulfan. Low to moderate resistance was observed to endosulfan by Kranthi et al. (2002 a, b). Low levels of resistance i.e. 7 fold were reported in India by Armes et al. (1992, 1996). Low level of resistance to endosulfan was observed due to less amount of selection pressure by this insecticide (Dhawan 2005 b).

Quinalphos

Population of *H. armigera* collected from all locations of Punjab showed low LD₅₀ values. Population collected from Bathinda showed LD₅₀ value of 0.050 µg/larva that lies between fiducial ranges of 0.018–0.086 µg/larva. LD₅₀ of Bathinda area is little bit more than the LD₅₀ values of other locations due to more attack of insects in that area and more sprays were used for the management of insect pests. Population of Faridkot, Abohar, Ludhiana and Hoshiarpur showed LD₅₀ values of 0.039, 0.035, 0.028 and 0.012 µg/larva, respectively. Population of Mansa showed LD₅₀ value of 0.042 µg/larva.

In present studies, low LD₅₀ values were reported to quinalphos. Low level of resistance was observed in North and Central India but moderate in South India (Kranthi 2005). Population of *H. armigera* collected from Mansa district showed 2.30, 3.40 and 6.50 fold resistance to quinalphos during August, September and October, respectively, which is quite low (Gill and Dhawan 2006). Resistance to quinalphos was reported as 45% during 1994-95 (Sekhar et al. 1996). No resistance was observed to quinalphos during 1989 from cotton growing areas of Guntur and Coimbatore (Armes et al. 1992). Consistence level of resistance was reported to quinalphos i.e. 23–27% (Kranthi et al. 2002a).

Chlorpyrifos

Population of *H. armigera* collected from Abohar and Bathinda districts showed high LD₅₀ values i.e. 0.084 and 0.091 µg/larva, respectively. While LD₅₀ values remain almost same in Faridkot, Hoshiarpur

and Ludhiana areas of Punjab state, the LD₅₀ values were low in these areas due to less number of insecticides sprays. Population of *H. armigera* collected from Mansa district showed little bit high LD₅₀ value i.e. 0.071 µg/larva as compared to LD₅₀ value of Faridkot, Hoshiarpur and Ludhiana areas.

The insecticide resistance to chlorpyrifos was low to moderate in the majority of strains (Chaturvedi 2004). Low to moderate level of resistance was reported in India (Kranthi et al. 2002a). The population of *H. armigera* collected from Bathinda district showed 3.55, 4.15 and 8.15 fold resistance in August, September and October to chlorpyrifos (Gill and dhawan 2006). The level of resistance to chlorpyrifos ranged from 1 to 3 fold as compared to susceptible Hoshiarpur strain (Kapoor et al. 2000). Recently, moderate level of resistance was observed throughout India by Kranthi (2005). In present studies also, LD₅₀ values were in accordance with the findings of these workers.

Deltamethrin

Bathinda district showed high level of LD₅₀ value (0.622 µg/larva) as compared to other locations of Punjab area. In Faridkot, Hoshiarpur and Ludhiana areas variability in LD₅₀ value was almost same may be due to less numbers of sprays that were used by farmers for the management of insect pests. LD₅₀ value of population collected from Abohar lies between fiducial ranges of 0.220—0.834 µg/larva. While in Mansa district LD₅₀ values were reported to be almost same in the population of *H. armigera* that were collected from Abohar.

Population collected from Spain showed high resistance values to deltamethrin and cypermethrin (Torres-Vila et al. 2002). Kranthi et al. (2001) reported high resistance ratios of 13,570 and 27,160 to deltamethrin in two strains collected during February 1998 in Central India.

Population of *H. armigera* collected from Bathinda district showed high level of LD₅₀ to insecticides like cypermethrin, fenvalerate, chlorpyrifos, quinalphos and deltamethrin as compared to the population collected from other locations of Punjab. Bathinda area showed high resistance values due to the fact that Bathinda is the main cotton area of Punjab and different types of insecticides were used

in this area to control insect pests.

In Punjab, one of the reason for high resistance was indiscriminate use of synthetic pyrethroids particularly, cypermethrin and deltamethrin for the management of cotton bollworms. In most of the sprays, farmers used synthetic pyrethroids alone or in combinations. On the other side, population collected from Mansa showed high LD₅₀ values to quinalphos than the population collected from other areas. In present studies, LD₅₀ values to quinalphos were low as the insecticide was used mostly in early season.

While on the other side, Hoshiarpur is one of the area in which LD₅₀ values were low against all the insecticides that were used for bioassay studies. Because in Hoshiarpur, more area is under vegetables, so farmers spray insecticides judiciously that leads to less development of insecticide resistance.

Population collected from Faridkot area showed little bit high LD₅₀ values than the population of Ludhiana and Hoshiarpur area against chlorpyrifos, cypermethrin, fenvalerate, endosulfan, quinalphos and deltamethrin. Because Ludhiana and Hoshiarpur are vegetable growing area of Punjab.

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