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Status of insecticide resistance in field populations of tomato fruit borer (*Helicoverpa armigera*) (Lepidoptera: Noctuidae) in Punjab, India

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ABSTRACT

Laboratory bioassays were conducted during 2013-14 to observe the level of insecticide resistance against tomato fruit borer [*Helicoverpa armigera* (Hubner)]. Populations collected from major tomato growing districts (Amritsar, ASR; Kapurthala, KPT; Patiala, PTA) of Punjab, India. Among different populations, Amritsar populations showed least susceptibility against synthetic pyrethroids, organophosphate as well as diamide followed by Patiala and Kapurthala. In the present study, fenvalerate was observed to be the least effective and have acquired high level of resistance (480-1270 fold) in *H. armigera.* As per the organophosphate and diamide groups of insecticides, Amritsar, Kapurthala and Patiala populations showed more susceptibility to profenofos and flubendiamide as compared to other insecticides tested. The susceptibility level against profenofos was almost same for all the tested populations. Flubendiamide was most effective insecticides against all populations. Pearson's correlation coefficient (r) showed significant relationship among fenvalerate and profenophos, deltamethrin and profenophos, flubendiamide and profenophos which suggest that resistance to profenophos, deltamethrin and flubendiamide might be due to possible cross-resistance mechanisms. However, although there was positive relationship among other insecticides, but none showed their statistical significance on log LC_{50} values of tested insecticides on field population of *H. armigera.* A strong positive correlation between monooxygenase activity and pyrethroid resistance indicated that the elevated cytochrome P_{450} monooxygenase activity is associated with pyrethroid resistance in different strains of *H. armigera.*

Key words: Bioassay, *Helicoverpa armigera,* Insecticides, Monooxygenase, Resistance, Tomato

Tomato fruit borer [*Helicoverpa armigera* (Hubner)] is one of the important and dominant insect pest in agriculture (Raheja 1996) and has become serious pest in India, Pakistan and China (Reghupathy and Ayyasamy 2003). The ability of *Helicoverpa* to thrive on diverse host plants is an adaptive advantage for its better survival in the ecosystem. *H. armigera* is also characterized by its high mobility and fecundity. The versatility of this species may be due to the presence of a strong genetic variability governing the behavior of *H. armigera* making it a serious pest on several crops (Zhou *et al.* 2000). These pests cause damage to the aerial parts of the tomato plant from the very early growth till to the fruit maturation stage (Tripathy and Singh 1999) and results in yield loss ranged from 12.9 to 54.82% (Kaur *et al*. 2011). The loss incurred to growing tomato crop is insurmountable and may extend up to 63.20% in Punjab (Kaur 2001). Apart from tomato, the pest is reported to infest cotton, maize, chickpea, pigeon pea, sorghum, sunflower, soybean, and groundnut and hence has attained the national importance as key pest (Yaqoob and Arora 2005).

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Indiscriminate and extensive use of chemical insecticides in past few decades has led to development of resistance and hence the management of *Helicoverpa* has become increasingly difficult due to development of resistance to most commonly used chemical class of insecticides were reported earlier by various workers (Armes *et al.* 1996, Kranthi 1997, Ramasubramaniam and Regupathy 2004). Various insecticides belonging to different classes are being used for the management of this pest all over the world. Higher inclination is also observed towards use of newer molecules available in the market for the management of the tomato fruit borer.

So far, no systematic, comprehensive studies have been done on the status of insecticide resistance in *H. armigera* population in Punjab. Keeping in view the losses, failure of management, problem against recommended insecticides and prognosis of *H. armigera,* the present study was undertaken to detect the susceptibility status of *H. armigera* in diverse tomato growing areas of Punjab.

MATERIALS AND METHODS

The populations comprising of egg masses and larvae of *H. armigera* were collected from infested plants in various tomato growing areas of Punjab, viz. Amritsar (31°37 ′N, 74°51′ E), Kapurthala (31° 22' 45" N, 75° 23' 5" E) and

Patiala (30°19′ N, 76°24′ E) which are approximately 120 km apart from Punjab Agricultural University, Ludhiana, during the period from March to April of 2013 and 2014 and brought to the laboratory in plastic jars covered with muslin. Each larva was kept in separate vial along with food. Each collection of larva was made by hand picking at the very first morning from tomato field, the collected larva were brought to the laboratory and were reared on chickpea based semi synthetic diet given by Armes *et al*. (1992). First they were reared in group of 100-200 in wide plastic vials containing 2-5 mm thick layer of artificial diet. After 4-5 days, when the larva become cannibalistic in nature, they were transferred to 50 ml cup separately containing artificial diet and were allowed to feed until the pupation. After emergence, ten pairs of moths were released in jars for rearing. Before releasing into the jar, ensured that the jar is covered by black paper to maintain darkness as well as the upper orifice is closed with muslin. Cotton swab dipped in 10% honey solution was provided as a food for the moths. Muslin of each jar was examined daily for egg layings, if found was removed and placed in a separate jar.

Different dilutions of the selected recommended insecticides such as fenvalerate (25 EC), cypermethrin (10 EC), deltamethrin (2.8 EC), profenophos (40 EC) and flubendiamide (480 SC) were prepared according to field recommended doses of respective insecticides. A serial dilution from the stock solutions of the formulated test insecticides was prepared. The third instar larva of body weight ranging from 30 to 50 mg were subjected to leaf disc dip method of bioassay recommended by IRAC (Anonymous 1990). The aqueous dilutions of various insecticides were prepared and fresh leaves of cucurbits were dipped for 10 sec and air dried at room temperature for 30 min. The dried leaves were transferred to plastic petri plates with the three replications. Ten third instar larvae of *H. armigera* were exposed to the treated leaves in control insects were released on petri plates containing leaves treated with water alone. The larvae were allowed to feed and mortality was recorded after 24 and 48 hr. A larva was considered dead if it failed to move in coordinated manner. The data were analyzed by probit analysis using the POLO programme based on calculations given by Finney (1971). The values of LC_{50}, LC_{90}, heterogeneity (χ^2), slope of the regression line (b) and fiducial limits were calculated. The degree of resistance acquired by *H. armigera* (Resistance ratio) was calculated by dividing the higher LC_{50} value of a population with a lower LC_{50} value of another population for each insecticide.

RESULTS AND DISCUSSION

Susceptibility level of H. armigera *populations from different locations of Punjab*

The commercial formulations of recommended insecticides, viz. fenvalerate (Sumicidin 20 EC), cypermethrin (Super fighter 10 EC), deltamethrin (Decis 2.8 EC), profenofos (Profex 40 EC) and flubendiamide (Fame 480 SC), with different concentrations were tested against Amritsar, Kapurthala and Patiala populations of *H. armigera.* The LC_{50} values and related parameters of insecticides and graphical presentation are given in Table 1. The LC_{50} values for fenvalerate, cypermethrin, deltamethrin, profenofos and flubendiamide were 0.127, 0.090, 0.100, 0.007, 0.001% against pest populations collected from Amritsar; 0.048, 0.043, 0.059, 0.002, 0.0004% for Kapurthala pest populations and 0.076, 0.084, 0.081, 0.004, 0.0005% for

Table 1 Toxicity and resistance ratio of various insecticides against *Helicoverpa armigera* in major tomato growing districts of Punjab

Insecticide	Locations	LC_{50} (%)	LC_{90} (%)	FL at 95% CL	Slope	χ^2 (df)	RR
Fenvalerate*	ASR	0.127	0.306	0.105-0.154	3.362 ± 0.440	0.74 (5)	1270
	KPT	0.048	0.194	0.037-0.061	2.125 ± 0.244	0.82 (5)	480
	PTA	0.076	0.250	0.055-0.103	2.470 ± 0.286	1.12 (5)	760
Cypermethrin**	ASR	0.090	0.239	0.073-0.109	3.020 ± 0.370	0.88 (5)	90.00
	KPT	0.043	0.184	0.022-0.075	2.020 ± 0.238	2.65 (5)	43.00
	PTA	0.084	0.353	0.046-0.158	2.064 ± 0.241	2.84 (5)	84.00
Deltamethrin***	ASR	0.100	0.263	0.072-0.140	3.060 ± 0.381	1.45 (5)	50.00
	KPT	0.059	0.186	0.040-0.086	2.574 ± 0.298	1.61 (5)	29.50
	PTA	0.081	0.219	0.057-0.118	2.985 ± 0.365	1.70 (5)	40.50
Profenofos****	ASR	0.007	0.041	0.005-0.009	1.694 ± 0.207	0.75 (5)	16.66
	KPT	0.002	0.012	0.001-0.003	1.894 ± 0.228	0.44 (6)	6.19
	PTA	0.004	0.023	0.002-0.006	1.785 ± 0.228	1.07 (5)	9.52
Flubendiamide*****	ASR	0.001	0.006	0.0009-0.0017	2.020 ± 0.217	1.06 (6)	25.00
	KPT	0.0004	0.001	0.0003-0.0005	2.443 ± 0.304	0.22 (6)	10.00
	PTA	0.0005	0.002	0.0004-0.0006	2.043 ± 0.245	0.41 (6)	12.50

Base line values (%): 0.0001*, 0.001**, 0.002***, 0.00042****, 0.00004 *****. Source: Madende and Brettell (1976)*, Madhusudan *et al.* (2011)**, Kapoor *et al.* (2002)***, Faheem *et al.* (2013)****, Basavanneppa and Balikai (2014)*****.

the populations collected from Patiala district of Punjab. The extent of resistance in terms of fold found to be in the range of 480-1270-fold for fenvalerate followed by cypermethrin (43-90-fold) and 29-50-fold for deltamethrin. In the survey on insecticide resistance monitoring, it was observed that the field populations of *H. armigera* from Amritsar showed a very high level of resistance to synthetic pyrethroids followed by Patiala and Kapurthala as compared to organophosphate and diamide groups of insecticides. Chaturvedi (2013) reported that *H. armigera* has lost the susceptibility to commonly used insecticides especially pyrethroids, as well as they have exhibited low to high levels of resistance in the range of 10 to 255- fold against fenvalerate from main cotton-growing districts of Central and South India. Similarly, Kapoor *et al.* (2002) reported high level of resistance in *H. armigera* larvae from cotton growing areas of Punjab to pyrethroids and worked out 8.64, 13.94, 34.20 and 111.8- fold resistance to fenvalerate, deltamethrin, cypermethrin and alphamethrin, respectively. Many other workers reported the high level of resistance to fenvalerate acquired by this pest from India as well as from other parts of the world (Gunning and Easton 1994, Armes *et al.* 1992, Kranthi 1997, 2001, Chaturvedi 2013). Kranthi *et al.* (2001) also observed in field populations of *H. armigera* high level of resistance to deltamethrin (24.21 to 69 fold) from Amravathi and Akola region of Maharashtra. They also reported that despite a reduction in the use of pyrethroids, the resistance levels increased to 64-207 folds. As per as the organophosphate and diamide groups of insecticides, Amritsar, Kapurthala and Patiala populations showed more susceptibility to profenophos and flubendiamide as compared to other insecticide tested and showed low to medium level of resistance (6.19 to 25 fold) against the tested populations (Table 1). Faheem *et al.* (2013) reported 2, 9 and 32 fold resistance to profenophos in *H. armigera* from three districts of Pakistan. Armes *et al.* (1996) reported that highly resistant population of *H. armigera* were generally found in the Central and Southern region of India (Armes *et al.* 1994) and other parts of the world (Martin *et al.* 2000). Resistance in *H. armigera* against different group of insecticides including new insecticides is still prevalent even after the introduction of *Bt* cotton. Earlier, Qaim and Ziberman (2003) have reported that though the amount of insecticides applied had reduced about 1/3rd after the introduction of *Bt* cotton, yet the development of resistance level in *H. armigera* was there and this could be due to polyphagous nature of *H. armigera.*

Relative toxicity of different insecticides to H. armigera *populations from different locations*

The relative toxicity of different insecticides against *H. armigera* was calculated and presented in Table 2. Flubendiamide with LC_{50} value of 0.001% was 127 times more toxic than the fenvalerate against Amritsar population, while profenofos, cypermethrin and deltamethrin showed 18.14, 1.41 and 1.27 times more toxicity than fenvalerate, respectively. In Kapurthala populations, the toxicity of flubendiamide, profenofos, fenvalerate and cypermethrin were 147.50, 22.69, 1.22 and 1.37 times more toxic than deltamethrin, respectively. The *H. armigera* populations collected from Patiala when treated with different insecticides by leaf dip technique, the LC_{50} values were 0.0005, 0.004, 0.076, 0.081 and 0.084 for flubendiamide, profenofos, fenvalerate, deltamethrin and cypermethrin, respectively, thereby showing 168, 21.00, 1.10 and 1.03 times toxicity than cypermethrin. Based on LC_{50} values obtained, the order of toxicity of recommended insecticides was flubendiamide > profenofos > cypermethrin > deltamethrin > fenvalerate for the Amritsar populations. However, for *H. armigera* populations from Kapurthala, the trend was flubendiamide > profenofos > cypermethrin > fenvalerate > deltamethrin, wherein the trend of toxicity for Patiala populations was flubendiamide > profenofos > fenvalerate > deltamethrin > cypermethrin. Nauen (2006) reported that flubendiamide is an excellent option for insecticide resistance management (IRM) strategies as an additional class of chemistry and mode of action as well as due its effectiveness against several lepidopteron insects.

Pair wise correlations between log LC_{50} values of different insecticides

A cross resistance mechanism was determined among the tested insecticides by pair wise correlation coefficients of log LC_{50} values of the population by the Pearson correlation with the help of computer program CPCS1. Pearson's correlation coefficient (r) showed significant (P<0.05) relationship among fenvalerate and profenophos, deltamethrin and profenophos, flubendiamide and profenophos which suggest that resistance to profenophos, deltamethrin and flubendiamide might be due to possible cross-resistance mechanisms (Table 3). However, although there was positive relationship among other insecticide, but none showed their statistical significance on log LC_{50} values of tested insecticides on field population of *H. armigera.* Campanahola and Plapp (1987) reported

Table 2 Comparative toxicity of recommended insecticides against *Helicoverpa armigera* from different locations

Location	LC_{50} (%) insecticides				
	Flubendiamide	Profenophos	Deltamethrin	Fenvalerate	Cypermethrin
Amritsar	0.001 (127)	0.007 (18.14)	0.100 (1.27)	0.127 (1.00)	0.090 (1.41)
Kapurthala	0.0004 (147.5)	0.0026 (22.69)	0.059 (1.00)	0.048 (1.22)	0.043 (1.37)
Patiala	0.0005 (168)	0.004 (21.00)	0.081 (1.03)	0.076 (1.10)	0.084 (1.00)

Figures in parentheses are the toxicity ratios

Table 3 Pair-wise correlation coefficient comparison between log LC_{50} values of tested insecticides on field population of *H. armigera*

	Fenvalerate	Cypermethrin	Deltamethrin	Profenophos	Flubendiamide
Fenvalerate	1				
Cypermethrin	0.884	1			
Deltamethrin	0.778	0.962	1		
Profenophos	0.999*	0.864	0.969*	1	
Flubendiamide	0.908	0.773	0.917	0.987*	1

*Correlation significant *LSD*< 0.05 (2-tailed)

the spectrum of cross resistance in pyrethroid resistant strain to organophosphorus insecticide in *Heliothis virescens*.

Correlation between mixed function oxidase (MFO) activities and LC_{50} values

H. armigera populations were collected from Amritsar (ASR) along with the susceptible strain procured from (NBAIR), Bengaluru (SUS) and fenvalerate selected strain was developed in the laboratory by giving selection pressure with fenvalerate up to 10 generations (ASR-Sel). The specific activity of the enzyme was highest in ASR-Sel (471.47 nmole/min/mg) and ASR (240.83 nmole/min/mg) compared to that of susceptible strain (SUS) which exhibited specific activity of (117.17 nmoles/min/mg protein), which was significantly lower than all other populations. MFO activity of ASR-Sel and ASR population of *H. armigera* was 4.02 and 2.05- fold, respectively as compared with susceptible population (Fig 1). It is likely that enhanced detoxification by MFOs is the major mechanism of pyrethroid resistance in *H. armigera* populations of Punjab. The study was in conformity with Yang *et al* (2004) who highlighted that P450 monooxygenases are a major metabolic mechanism responsible for pyrethroid resistance in *H. armigera* from India, China and Pakistan with combined evidence from synergism experiments, monooxygenases activity assays with multiple substrate and *in vitro* metabolism studies. In the present study significant positive correlation (r= 0.99) was observed between MFO and log LC_{50} value of fenvalerate in different strains of *H. armigera*. The coefficient of determination R^2 was also calculated and found to be 0.989, hence clear that MFO was major contributing factors in imparting metabolic resistance. These results were in accordance with the studies conducted by Faheem *et al.* (2013) who also observed the highly significant ($P<0.01$) correlation with synthetic pyrethroid (deltamethrin) in leaf dip method as well as in topical application method against *H. armigera* populations collected from Pakistan. Similar studies conducted for pyrethroid resistance in *H. armigera* also showed higher MFO activity in the selected strain and strong positive correlation between MFO activity and pyrethroid resistance (Bues *et al.* 2005, Chen *et al.* 2005).

The present study, thus, presents a comprehensive resistance profile of *H. armigera* populations collected from diverse tomato growing areas of Punjab. The information generated for five insecticides can be used for monitoring insecticide resistance in future work. The results revealed that use of pyrethroids should be discouraged and in order to protect other insecticides and to postpone the development of resistance, a resistance management strategy of decreased selection pressure could be achieved by alternating these insecticides on the basis of proper pest scouting and pest status for decision of control application.

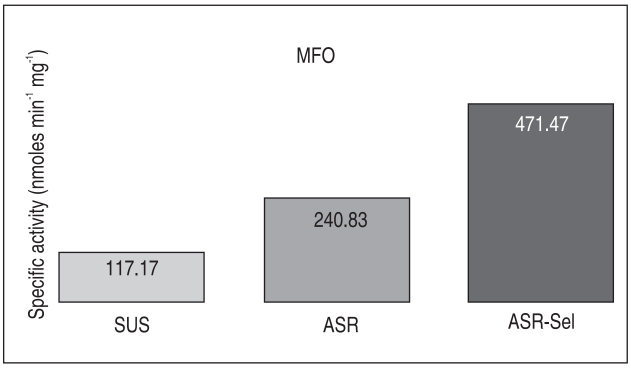

Fig 1 Specific activity of MFO in different populations of *H. armigera.* MFO- Mixed function oxidase, SUS- Susceptible population, ASR- Amritsar resistant population, ASR-Sel fenvalerate selected Amritsar population.

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Table 4 Specific activity of MFO in different populations of *H. armigera*

Strain	Mixed function oxidase (nmoles of *p*- nitrophenol formed/ min/mg of protein)	Enzymatic activity ratio (Fold increase in mixed function oxidase activity)
SUS	117.17±7.61	1
ASR	240.83 ± 9.15	2.05
ASR-Sel	471.47 ±11.32	4.02
LSD (P<0.05)	33.78	

Mean of three replications ± SE. SUS- Susceptible population, ASR- Amritsar resistant population, ASR-Sel Fenvalerate selected Amritsar population, MFO- Mixed function oxidase.

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