



Research Article

Insecticide Resistance Studies Against *Helicoverpa armigera* from Faisalabad, Pakistan

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Abstract | *Helicoverpa armigera* is considered one of the economically important polyphagous and cosmopolitan insect in Pakistan. It causes serious damages to cotton crops and has negative impact on economy of the country. At the present study, the effects of different new generation insecticides and insect growth regulators were evaluated against 3rd instar larvae of *H. armigera*. Three insecticides viz., pyriproxyfen 10.8%EC, chlorantraniliprole 20SC, emamectin benzoate 1.9EC, and one insect growth regulator (IGR), lufenuron, 50EC was tested against *H. armigera* larvae by using leaf dip bioassay method under laboratory conditions at Entomological Research Institute, Faisalabad. On the basis of LC₅₀ values chlorantraniliprole and emamectin benzoate was proved most effective insecticide to control this polyphagous pest as compared to pyriproxyfen and lufenuron which showed high resistance against *H. armigera*. Estimated LC₅₀ values of emamectin benzoate on these larvae after 3, 6, 12, 24 and 48 hours were 1457.354, 1268.327, 390.215, 300.947 and 122.711 respectively.

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Introduction

Helicoverpa armigera belongs to family Noctuidae and a subgroup of polyphagous heliothine moths, some of which are regarded as most significant insect pests of field and horticultural crops Cunningham and Zalucki (2014). Management of *H. armigera* (Hubner) (Lepidoptera; Noctuidae) is facing significant challenges all over the world due

to its polyphagous and cosmopolitan nature (Li *et al.*, 2018). This destructive pest is distributed all over the middle East (UAE, Behreen and Muskat), Africa, central and southeast Asia including Pakistan, many Pacific Islands, eastern and northern Australia, New Zealand and southern Europe which have 29 families invading many host plants like chickpea (Fite *et al.*, 2018) tomato, cabbage, tobacco and maize in Pakistan Sarwar (2012) but the chickpea is most af-

fectured crop due to *H. armigera* having 10.17 million ha cultivated area with 9.93 million tones production (Zahid *et al.*, 2008). The major chickpea producing states where more than 95% production occur are Rajasthan, Uttar Pradesh, Maharashtra, Karnataka, Chhattisgarh, Bihar and Jharkhand. A huge amount of crop destruction occurs in Ethiopia while 30% crop yield loss occurs in India due to this pest Dangi (2017). As we know that population of the world has been increased enormously now a days so it is very necessary to increase the demand of crop production to fulfill the needs of the people (Thudi *et al.*, (2016). Due to pod borer 10-60 % crop damage occurs in normal weather conditions Jat and Ameta (2013), while 50-100 % loss happens in optimum climatic conditions, mostly in the territories where frequent rains and gloomy weather are dominant throughout the crop season (Ahmad *et al.*, 2013). Due to its migratory behavior, high fecundity and high adaptation it is responsible over Rs. 35000 million losses annually in spite of heavy pesticides exposure (Chatar *et al.*, (2010), while it outbreaks chickpea all over the crop growth, significant yield loss occurs through pod formation and flowering stages (Narayanamma *et al.*, 2007).

Resistance to insecticide may be described as "the reduced sensitivity of an insect population to an insecticide that was effective in the past to control the particular insect pest. It has developed resistance to some traditional insecticides i.e., carbamates, organophosphates (Ops), organochlorines and pyrethroids etc. but nevertheless the most effective tool in IPM, by using these chemicals and their effectiveness and its importance cannot be neglected. The effective, careful and timely use of these insecticides is the basic requirement to control this pest (Ahmad *et al.*, 2013). Spinosad is most effectively used insecticide against this pest. It is derived from *Saccharopolyspora spinosa* which is a naturally occurring actinomycete and it is a combination of spinosyns A and D (Meng *et al.*, 2009). It proves strongly effective against Lepidoptera due to its exceptional action mechanism as well as it shows comparatively minute toxicity to non-targeted insects and low poisonousness to mammals (Zhang *et al.*, 2009). According to perception of organic evolution almost all insecticides can obtain resistance to target insects as well rising resistance to this insecticide can be shown in field population (Qayyum *et al.*, 2015). Another study showed low level of resistance against spinosad. In spite of low resistance level in

field population there is need for the better resistance management about spinosad and for this purpose laboratory strain of the *H. armigera* with the application of spinosad can be chosen Sharma (2005). Another most important and useful insecticides against this pest are synthetic insecticides which have been primarily applied for the management of *H. armigera*. Due to the broad range of activity the synthetic insecticides have the ability to eliminate almost all the pests from the crop growing area. To reduce the damage of important crops it is necessary the exposure of these insecticides which has the ability to penetrate into the nearby grazing environments and its performance is highly destructive not only for the invertebrates but also for the vertebrates. As we know that synthetic insecticides accumulate in the environment and retained it for a sustained period of time because of long half-life so, accumulation of synthetic insecticides can be seen in different trophic levels of food net (Shen *et al.*, 2020). The aims of current study were to find out the effective insecticide against *H. armigera* and to investigate the effectiveness of different insecticides against *H. armigera*

Materials and Methods

Insect collection

H. armigera larvae of fifth and sixth instars were collected from cotton filed in the Faisalabad district, Punjab Province of Pakistan. The larvae were collected from two-hectare areas and transferred to laboratory at Entomological Research Institute (ERI) Ayub Agricultural Research Institute, Faisalabad (AARI). Susceptible strain of *H. armigera* were kept in laboratory for five years devoid of introduction to insecticides (identified as the "Lab-PK" strain) was utilized for toxicity assessments as per (Ahmad *et al.*, 2007). The Larvae were nursed a semi-synthetic wheat-germ-based diet and retained at the temperature of $26 \pm 1^\circ\text{C}$ and relative humidity $60 \pm 5\%$ with a 14:10-h light: dark photoperiod (Sayyed and Wright 2006). Diet was replaced on a daily basis, and the pupae were stored and retained in emergence cages. Adults were placed in Perspex oviposition cages, two sides of which were wrapped with the muslin cloth to permit 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.*, 2007).

Insecticides

Commercial formulations of insecticides tested in this study were used: Lufenuron (Match 10% EC, Syngenta Pakistan Limited) Pyriproxyfen (Priority 10.8% EC, Kanzo AG, Multan, Pakistan), Emamectin benzoate (Hammer 5% WDG, Kanzo AG, Multan, Pakistan), Chlorantraniliprole (Coragen 20% SC Syngenta Pakistan Limited).

Bioassays

Newly hatched third instars larvae (30 to 40 mg each) from the F2 generations were bioassayed by using a leaf-dip method suggested by the Insecticide Resistance Action Committee (IRAC) (Anonymous 1990). Technical grade formulations of the insecticides were serially diluted. Leaf discs having diameter of five centimeter were cut from the unsprayed fresh cotton leaves, immersed in the test solution for 10 seconds (Sayyed *et al.*, 2000), air-dried on paper towel, and then shifted to wet filter paper in plastic petri dishes (5-cm diameter). Molted larvae were put on each dried leaf disc and dishes were covered. Four concentrations of each insecticide and an untreated control were replicated four times for each insecticide tested. Petri dishes with larvae were covered with black paper to decrease the risks of cannibalism. Mortality data was recorded after 3,6,12,24 and 48 hours. Larvae were considered 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 (Pollo-PC 2003). Median lethal concentration (LC50) values and their 95% fiducial limits (FL) were estimated by probit analysis using POLO PC software.

Results and Discussion

Emamectin benzoate was also the most toxic of the insecticides used against the field populations of *H. armigera*, with the LC50 value after 3 hours was 1457.354 mgL⁻¹ along its slop value i.e. 1.275. The fiducial limit was 905.557-3865.360 for that period. LC₅₀ value was decreased to 1268.327 mgL⁻¹ after 6 hours with its fiducial limit 645.361-5918.661. The slop value was decreased to 0.977 after 6 hours exposure. LC₅₀ values were 390.215, 300.947, 122.71 at 12, 24 and 48 hours respectively. Their slop values were decreased to 0.601, 0.671, 0.725 after that pe-

riod of time. Fiducial limits were 5.017-1594.167, 4.256-836.353, 0.048-327.467 after 12, 24 and 48 hours correspondingly (Table 1).

Table 1: LC₅₀ values of Emamectin Benzoate against *H. armigera*.

Time (h)	LC50 (mgL ⁻¹)	Fiducial Limit Lower-Upper	Fit of probit analysis			
			Slope	S. E	χ ²	df
3	1457.354	(905.557-3865.360)	1.275	0.309	1.459	4
6	1268.327	(645.361-5918.661)	0.977	0.316	0.026	4
12	390.215	(5.017-1594.167)	0.601	0.281	0.050	4
24	300.947	(4.256-836.353)	0.671	0.294	0.052	3
48	122.711	(0.048-327.467)	0.725	0.312	0.058	3

The results regarding LC₅₀ values for lufenuron against *H. armigera* was given in table 2. After 3 hours LC₅₀ value was 9948.201 along its slop value i.e. 0.942. The fiducial limit was 4780.202-129288.773 for that period of time. LC₅₀ value was decreased to 5943.035 after 6 hours with its fiducial limit 3188.125-35468.578. The slop value was decreased to 0.916 after 6 hours exposure. LC₅₀ values were 3733.760, 2390.671, 2337.7 at 12, 24 and 48 hours respectively. Their slop values were decreased to 1.019, 1.071 and 1.247 after that period of time. Fiducial limits were 1968.264-10850.625, 1052.187-5125.541, 1159.649-4329.694 after 12, 24 and 48 hours correspondingly (Table 2).

Table 2: LC₅₀ values of Lufenuron against *H. armigera*.

Time (h)	LC50 (mgL ⁻¹)	Fiducial Limit Lower-Upper	Fit of probit analysis			
			Slope	S. E	χ ²	df
3	9948.201	(4780.202-129288.773)	0.942	0.301	0.499	3
6	5943.035	(3188.125-35468.578)	0.916	0.286	0.720	4
12	3733.760	(1968.264-10850.625)	1.019	0.308	0.149	4
24	2390.671	(1052.187-5125.541)	1.071	0.321	0.089	4
48	2337.7	(1159.649-4329.694)	1.247	0.334	0.162	4

The results regarding LC₅₀ values for pyriproxyfen against *H. armigera* was presented Table 3. After 3 hours LC50 value was 10566.474 along its slop value i.e. 2.009. The fiducial limit was 5714.432-136927.094 for that period of time. LC₅₀ value was decreased to 9264.138 after 6 hours with its fiducial limit 4863.741-65600.969. The slop value was decreased to 1.519 after 6 hours exposure. LC₅₀ values were 8886.68, 5533.021, 4355.814 at 12, 24 and 48 hours respectively. Their slop values were decreased to 1.143, 1.251 and 1.057 after that period of time. Fiducial limits were 4204.935-106014.172, 3130.173-

23114.016 and 2226.173-26256.461 after 12, 24 and 48 hours correspondingly. [Table 3](#).

Table 3: LC_{50} values of Pyriproxyfen against *H. armigera*.

Time (h)	LC50 (mgL ⁻¹)	Fiducial Limit Lower-Upper	Fit of probit analysis			
			Slope	S. E	χ^2	df
3	10566.474	(5714.432-136927.094)	2.009	0.671	0.365	4
6	9264.138	(4863.741- 65600.969)	1.519	0.431	0.598	4
12	8886.68	(4204.935-106014.172)	1.143	0.343	0.426	4
24	5533.021	(3130.173-23114.016)	1.251	0.328	0.535	4
48	4355.814	(2226.173-26256.461)	1.057	0.342	0.318	4

The results regarding LC_{50} values for chlorantraniliprole against *H. armigera* was given in table 4. After 3 hours LC_{50} value was 347.659 along its slop value 0.803. The fiducial limit was 176.087-3521.792 for that period of time. LC_{50} value was decreased to 142.455 after 6 hours with its fiducial limit 13.260-72953266. The slop value was decreased to 0.541 after 6 hours exposure. LC_{50} values were 116.776, 36.667, 20.120 after 12, 24 and 48 hours respectively. Their slop values were decreased to 0.747, 0.577 and 0.821 after that period of time. Fiducial limits were 29.789-330.108, 0.000-108.231 and 0.087-52.341 correspondingly. [Table 4](#).

Table 4: LC_{50} values of Chlorantraniliprole against *H. armigera*.

Time (h)	LC50 (mgL ⁻¹)	Fiducial Limit Lower-Upper	Fit of probit analysis			
			Slope	S. E	χ^2	df
3	347.659	(176.087 -3521.792)	0.803	0.279	0.033	3
6	142.455	(13.260 -72953.266)	0.541	0.267	0.203	3
12	116.776	(29.789-330.108)	0.747	0.285	0.466	4
24	36.667	(0.000-108.231)	0.577	0.293	0.044	4
48	20.120	(0.087-52.341)	0.821	0.315	0.025	4

The current study showed that *Helicoverpa armigera* has developed resistance to various insecticides such as pyriproxyfen, emamectin benzoate, lufenuron, and chlorantraniliprole. Insecticides which have less LC_{50} values were considered the most effective against *H. armigera* regarding less resistance to the pest.

Very low to moderate level of resistance was observed to chlorantraniliprole, emamectin benzoate and lufenuron while high resistance was demonstrated to pyriproxyfen. The results showed that chlorantraniliprole was proved enormously toxic insecticide

against *H. armigera* which caused high mortality after 24 hours. Similar results were reported by Bird (2015) and ([Zhang et al., 2009](#)) who also observed very low level of resistance to this insecticide. ([Butter et al., 2003](#)) finds out toxicity of lufenuron and proved the highly noxious insecticide to control *H. armigera* because at higher concentrations it showed high mortality at different time intervals and it disturbed the growth and physical structure of the pest as well as their LC_{50} values were 5.63, 7.89, 8.03, 11.39 and 14.76 mg /L respectively. These results were quite similar to present findings. The level of resistance to emamectin benzoate was proved to be very low for all populations of *H. armigera* in current study. Similar results were demonstrated by ([Ahmad et al., 2003](#); [Qayyum et al., 2015](#); [Bird, 2015](#); [Sattar et al., 2017](#) and [Hussain et al., 2014](#)) who proved that LC_{50} value for emamectin benzoate was greater at initial stage which was decreased after 24 and 48 hours and high mortality caused in the *H. armigera* species. Pyriproxyfen which is also very important insecticide showed high resistance against *H. armigera*

Conclusions and Recommendations

The present study concluded that *Helicoverpa armigera* is the cosmopolitan and polyphagous insect pest which is the cause of destruction of many important crops. According to recent study chlorantraniliprole and emamectin benzoate was proved most effective insecticide to control this polyphagous pest as compared to pyriproxyfen and lufenuron which showed high resistance against *H. armigera*. The presented results will be helpful to make decision on the proper usage of insecticides against *Helicoverpa armigera* control and to reduce the development of insecticide resistance

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Novelty Statement

Emamectin benzoate and chlorantraniliprole were the most effective insecticides for managing this

polyphagous pest, in contrast to pyriproxyfen and lufenuron, which shown a significant level of resistance against *H. armigera*.

Author's Contribution

Muhammad Saleem and Dilbar Hussain: Performed the experiment and collected data

Saeed Ahmad and Muhammad Ihsan Ullah: Write first manuscript and Managed overall crop

Muhammad Luqman and Muhammad Shafqat: Helped in paper write up

Fiaz Hussain, Tamseela Mumtaz and Kaynat Shahzadi: Performed the statistical analysis

Muhammad Hasnain, Muhammad Sajjad and Muhammad Jawad Saleem: Collected the literature and supervised the study

Conflict of interest

The authors declared absence of conflict of interest.

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