Resistance Status of *Helicoverpa armigera* (Hub.)
Against Mixture of *Profenofos* and *Indoxacarb* (1:1)
Insecticides at Faisalabad, Pakistan

Abdul Ghafoor, Muhammad Saleem Khan, Ansar Mahmood, Fawad Ahmad, Muhammad Hassan and Abdul Rehman Anees

Department of Zoology, GC University Faisalabad, Pakistan

**Abstract:** The resistance status of *Helicoverpa armigera* (Hub.), collected from cotton fields at Faisalabad, was assessed against different concentrations of insecticides (Profenofos and Indoxacarb). Profenofos 6, 8, 10 and 12 ppm and indoxacarb 1, 1.75, 2.00 and 2.25 ppm were applied as combined concentrations from T1 to T4 respectively. Resistance status (% Survival rates) of *H. armigera* (Hub.) were recorded after 24, 48 and 72 hours of insecticidal application. Decreasing patterns of survival rates (%) were observed from T1-T4 at 24, 48 and 72 hours respectively. Maximum survival rates (%) were recorded as 72.00±4.12, 62.67±3.28, 49.43±2.32 and 13.33±1.42 from T1 to T4 after 24 hours. It is concluded from the study that with the increased combined concentrations of both insecticidal applications were indirectly proportional to the survival rates (%) of *Helicoverpa armigera* (Hub.).

**Key words:** Insecticide resistance • Helicoverpa armigera • Profenofos • Indoxacarb

**INTRODUCTION**

Cotton is the main cash crop in Pakistan and is known as “Silver fiber” which accounts for 11.7% of value added in agriculture and about 2.9% of GDP [1]. It is a major source of foreign exchange earning. Cotton is, therefore, rightly called as backbone of Pakistan’s economy. Pakistan is the third largest cotton lint producer in the world after China and U.S.A. [2]. The gram caterpillar or American bollworm *Helicoverpa armigera* (Hub.) is a polyphagous and multigeneration noctuid pest. *H. armigera* has a wide host range of over 200 weed plants, 25 crop plants including cotton, maize, sorghum, sunflower, tomato, okra and legumes like pigeon pea, chickpea etc. This wide host range allows the pest to breed throughout the year by causing extensive damage [3]. American bollworm, *Helicoverpa armigera* (Hub.) mainly feed on fruiting parts of cotton reduces in considerable quantity [4], 20-60% damage and market value of fiber [5]. American bollworms tunnels into small squares, terminal buds [6] and large bolls from the base by leaving posterior half portion of the body outside the bolls [7]. This may causes non-reproductive growth [6].

The bollworm is extremely well adapted to agroecosystem and can exhibit up to 11 generations a year under good conditions [8]. The bollworm has evolved 2 major strategies for adapting to adverse conditions. First, it has excellent migratory abilities and can fly up to 1.55 miles (250 Km) in search of viable food source [9]. Secondly, it has the ability to enter into facultative diapause when conditions become too hot or cold [10]. This allows the bollworm to survive until environmental conditions improved.

Presently, the development of resistance in *H. armigera* stands well documented [11, 9, 12] to carbamates and organophosphates [13] and pyrethroids [14]. Even the phenomenon of insecticide resistance mechanisms has been clearly understood [15, 16]. To overcome the losses and to increase the yield, pesticide application is utmost important. In Pakistan during 2000-2001, about 16402 tons of pesticides were imported [17] and about 70-80% of these were sprayed for controlling the cotton pests (Mehryuddin et al. 1997). Previous investigation about the evaluation of different insecticide to the control of cotton bollworms were conducted by Sharma et al. [18] and Allen et al. [19]. Keeping in view the importance of Cotton, Vegetables and Cereal crops in

**Corresponding Author:** Dr. Abdul Ghafoor, Department of Zoology GC University, Faisalabad, Pakistan
E-mail: dragondal@yahoo.com, Tel: +92-0345-7704127.
Pakistan, the present project has been designed to know the efficacy of insecticides Profenofos and Indoxacarb against *H. armigera* and to reduce the indiscriminate use of these insecticides. The present study was also initiated to find out the one of the most effective insecticide and its appropriate dose for the control of *H. armigera*.

**MATERIALS AND METHODS**

Larvae of American bollworm, *H. armigera* (Hub.) were collected from the September to December 2008 from cotton fields, at Ayub Agriculture Research Center, 15 Risala and Chabba village Faisalabad. The specimens were brought to the Arachnology laboratory, Zoology Department, GC University, Faisalabad and kept in glass cages under the controlled laboratory conditions at 26±2°C. Resistance was assessed after 24, 48 and 72 hours of insecticide application.

Larvae were collected in plastic vials. Each vial was capped with a finely perforated mesh to allow the exchange of air. Collected larvae were transferred to the rearing trays (30cm x 15cm x 4.5cm). Each rearing tray was divided into 24 squared chambers. The lid of the tray had 24 holes (diameter: 2cm) covered with brass screen for ventilation. The larvae were placed in the separate chambers (one larva in each) to avoid cannibalism. The chambers were cleaned daily to get rid of the feces of larvae. The larvae were offered with natural diet i.e. Okra (*Hibiscus esculentus*) and *Trifolium* leaves. The pupae were transferred to the pupal chamber (18cm x 18cm x 20cm) having sand covered floor. Pupae were sorted sex-wise and placed in separate pupal chambers. Moths collected from these chambers were kept in glass chimneys having muslin cloth on both openings. A Petri-dish was placed in the bottom of the chimney. Ten to fifteen pairs of moth were present in single chimney and provided with ten percent sugar solution as feed present in plastic vial having cotton pad for the mass production of the different stages of larvae.

Sugar solution soaked the cotton pads by capillary action and moths sucked the solution from cotton pads. Eggs laid by the female moths were collected daily from muslin cloth present on the openings of the chimney and were transferred in plastic bags. Bags were labeled with date and kept for incubation and maintained at 26±2°C, 70± 5% RH and a 12:12 (L:D) Photoperiod, respectively. Insecticides used in experiment were Profenofos and Indoxacarb.

Their recommended field doses were as follows: Profenofos 800ml/100lit water and Indoxacarb 175ml/100lit water Stock solution of Profenofos and Indoxacarb prepared in 1000ml distilled water. In first treatment the following quantities used which was measured in ppm. Profenofos 6ppm, 8ppm, 10ppm and 12ppm Indoxacarb, 1ppm, 1.75ppm, 2.00ppm and 2.25ppm from treatment T, to treatment T. Four treatments including a control tested against *H. armigera*. Third instars larvae were treated with insecticides such as Profenofos and Indoxacarb. Both insecticides were used in combined ratio (1:1). For this purpose leaf dip method [20] was used. Formulations of test compounds were prepared in distilled water as parts per million of active ingredient i.e. 1000 ppm. Cotton leaf disks (5cm diameter) were cut and dipped into the test solutions for 15 seconds with gentle agitation. They were allowed to surface-dry on a paper towel and then placed into Petri dishes containing moistened filter papers to avoid desiccation of leaves. Larvae were transferred to the leaf disk by tapping lightly to disperse 5 larvae per Petri dish per replicate. Each treatment was replicated 3 times along with an untreated control under complete randomized design. The whole experiment was run under controlled laboratory conditions at 26±2°C. Resistance was assessed after 24, 48 and 72 hours of insecticides application. Insects were considered dead if they gave no response to stimulation by touch and No. of alive insects will be the indicator of resistance. Data was analyzed by analysis of variance (ANOVA) to see the difference between treatments and hours. In case of significant difference, Duncan Multiple Range test (DMR) [21] test was applied.

**RESULTS**

The present study was conducted to determine the resistance of American bollworm, *H. armigera* against Profenofos and Indoxacarb in various combined concentration levels viz. 6ppm + 1ppm, 8ppm + 1.75ppm, 10ppm + 2.00ppm, 12ppm + 2.25ppm from T, to T, against 3rd larval instars of *Helicoverpa armigera* (Hub.) under laboratory conditions. The data regarding resistance percentage of larval instar of the *H. armigera* was recorded 24, 48 and 72 hours after treatments.

**Treatment (T):** The results revealed that variations were significant among various concentration levels after 24, 48 and 72 hours of treatment. In treatment T, observations recorded after insecticidal treatment showed 68.00 percent
Table 1: Analysis of variance (ANOVA) for the data of testing the effect of Profenofos and Indoxacarb on the survival rate of American bollworm, *Helicoverpa armigera* (Hub.)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>1791.333</td>
<td>597.111</td>
<td>134.99**</td>
</tr>
<tr>
<td>Hours</td>
<td>2</td>
<td>422.232</td>
<td>211.111</td>
<td>4.90*</td>
</tr>
<tr>
<td>Treatments X Hours</td>
<td>6</td>
<td>38.667</td>
<td>3.111</td>
<td>0.67**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1034.667</td>
<td>43.111</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>5371.387</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01)

Table 2: Mean % survival rate in American bollworm, *Helicoverpa armigera* (Hub.) treated with Profenofos and Indoxacarb as tested through Duncan's Multiple Range (DMR) test.

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>Ranked ordered means</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>72.00±4.12</td>
<td>60.00±3.21</td>
<td>64.00±2.91</td>
<td>64.00±2.19 A</td>
</tr>
<tr>
<td>T2</td>
<td>62.67±3.38</td>
<td>58.67±4.02</td>
<td>58.67±5.94</td>
<td>58.67±4.96 B</td>
</tr>
<tr>
<td>T3</td>
<td>45.33±2.32</td>
<td>41.33±1.42</td>
<td>41.33±1.51</td>
<td>45.33±1.59 C</td>
</tr>
<tr>
<td>T4</td>
<td>13.33±1.42</td>
<td>12.00±1.01</td>
<td>4.00±0.01</td>
<td>9.7±0.98 D</td>
</tr>
<tr>
<td>Control</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant. DMR test at 5% level of significance.

Treatment (T1): It was evident from results that resistance of American bollworm, *H. armigera* differed significantly among various concentration levels in T2. The observations recorded after 24, 48 and 72 hours of treatment showed 58.67 percent resistance of American bollworm, *H. armigera*. At this insecticidal dose of 8ppm + 1.75ppm Profenofos and Indoxacarb respectively showed intermediate resistance of American bollworm, *H. armigera*. (Table 2).

Treatment (T2): In treatment T3 at this insecticidal dose of 10ppm + 2.00ppm proifenofos and Indoxacarb respectively showed 45.33 percent resistance of American bollworm, *H. armigera*. This treatment also showed intermediate resistance, in treatment T2 and T3 the resistant values were recorded between the maximum and minimum values of resistance of American bollworm, *H. armigera*. (Table 2).

Treatment (T3): The results showed significantly different resistant values of American bollworm, *H. armigera*. This insecticidal dose 12ppm + 2.25ppm of Profenofos and Indoxacarb respectively showed minimum resistance of American bollworm, *H. armigera*. (Table 2).

Resistant Values Decreases from T1 to T2. It shows effect of mean survival rate of American bollworm was statistically highly significantly affected treatment-wise. Effect of Profenofos and Indoxacarb was statistically significant on the treatment-wise mean survival rate of American bollworm (P<0.01) (Table 1).

Fig. 2: Leaf Dip Method for Insecticidal Treatments.
Hour-wise administration of both the insecticides showed statistically significant effect on the survival rate of American bollworm. Similar trend is found in the interaction of treatments and hours (P<0.05) (Table: 1)

In Table (2) Mean % survival rate in American bollworm treated with Profenofos and Indoxacarb as tested through Duncan's Multiple Range (DMR) test. It shows treatment-wise survival rate of American bollworm was statistically similar but was decreasing at 24 hours. Similar trend in decrease with survival rate of American bollworm was noted at 48 and 72 hours. This trend was statistically significant different when the hours data was combined. There was gradual decrease in the survival rate of American bollworm from T1 to T4 (Table: 2). Hour-wise effect of both insecticides did not show any statistical variation (Table: 3). So concluded that the present study shows the resistance of American bollworm maximum in treatment (T1) and minimum resistance in treatment (T4).

These results are also showed Graphically, X-axis shows treatments and Y-axis shows resistance (%).

**DISCUSSION**

Ranganathan and Govindan [22] performed a field experiment during 1992-93 for evaluating the efficacy of new insecticidal mixtures Polytrin C 44 EC (cypermethrin 4 g a.i. + profenofos 40 g a.i.) in comparison with a few other conventional insecticides, viz. cypermethrin 10 EC, profenofos 50 EC and endosulfan 35 EC against the American bollworm, *Helicoverpa armigera* (Hubn) in cotton. The study revealed the superiority of the mixture Polytrin C in controlling the bollworm infestation and producing more seed cotton yield over other insecticides which were applied individually.

Present findings correspond to Dhawan and Simwat [23] they evaluated Indoxacarb (Avaunt 15 SC) at variable dosages in four different experiments during 1998 and 1999 crop season along with chlorpyrifos as standard. Indoxacarb @ 60 g a.i./ha was effective against spotted bollworms. However, for the control of American bollworm higher dose of 80 g a.i./ha proved effective. Indoxacarb at this dose gave better bollworm control and higher seed cotton yield than chlorpyrifos. Indoxacarb was relatively safe to beneficial arthropods.

Similar studies were conducted by Khan and Hamed [24]. They performed an experiment in which they combined Profenofos with three other commercial insecticide and find out their toxicities under lab conditions against *Helicoverpa armigera* (Hub.) larvae of 1st, 2nd and 3rd instars. Results showed that curacon 500 EC and somialfa 110 EC were highly effective by causing 100 and 80% mortality after 24 hours exposure which reached to 100% each after 72 hours exposure. Both insecticides caused 100% mortality after 24, 48 and 72 hours exposure of 2nd instar larvae. Only curacon was effective against 3rd instar larvae by causing 25, 100 and 100% mortality after an exposure of 24, 48 and 72 hours. It was concluded from the results that curacon was highly effective against 1st, 2nd and 3rd instar larvae followed by somialfa. The results of recent study were in line with the finding of Hamed et al. [25] who studied the toxicity of different insecticide mixtures belonging to different groups. They grouped Profenofos (Curacon) with different insecticide and showed results the results are also matched my results. They combined profenofos as decis 2.5EC + thiadan 35EC, decis 2.5EC + curacon 500EC, decis 2.5EC + somialfa 110EC, decis 2.5EC + advantage 20EC, thiadan 35EC + curacon 500EC and thiadan 35EC + somialfa 110EC was recorded using their field recommended doses against *Helicoverpa armigera* (Hubner) by leaf-dip method. Percent mortality was determined. The results concluded that significantly high toxicity was shown by decis 2.5EC + curacon 500EC which caused 100 per cent mortality to the insect followed by thiadan 35EC + somialfaTM110EC causing 83.3 per cent mortality after 72hrs of exposure. These findings on insecticide mixtures could serve as useful tool in the management of insecticide resistance.

Similarly, same results were also obtained by Hamed et al. [26] they studied the various concentrations of Steward 150 SC indoxacarb viz. 50, 100, 200, 300 and 500 ppm were tested against different larval instars of cotton bollworm, *Helicoverpa armigera* (Hub.) to determine its toxicity range under controlled laboratory conditions. Percent mortality of the insect was recorded after 12, 24 and 48 hours. Results showed that mortality or second instars varied significantly in between, treatments after 12 hours but non-significantly with cent per cent mortality after 24 and 48 hours. Third and fourth instars exhibited variable mortality range after 12.24 and 48 hours. It was 46-73%, 47-80% and 13-1 00% in third instar and 33%, 13-40% and 20-100% in fourth instar after 12.24 and 48 hours respectively.

**REFERENCES**


