International Journal of Environment and Climate Change

Volume 13, Issue 11, Page 1317-1330, 2023; Article no.IJECC.107682
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Helicoverpa armigera (Hubner) Associated Cross Resistance Patterns in South Indian Crop Ecosystem

Upendhar S. [a*], Satyanarayana J. [a] and Vani Sree K. [a]

[a] Professor Jayashankar Telangana State Agricultural University, Hyderabad-500030, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i113284

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107682

Original Research Article

Received: 12/07/2023
Accepted: 05/09/2023
Published: 20/10/2023

ABSTRACT

Aim: To study the cross resistance patterns associated with Mahaboobnagar, Raichur, Nagpur populations of *Helicoverpa armigera* (Hubner).
Study design: Bioassay
Place and Duration of Study: The experiment was carried out from February 2010 to May 2011 at Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Telangana.
Methodology: *Helicoverpa armigera* was selected for indoxacarb in F1 and F2 continuously then the population subjected to different selected insecticides to know the cross resistance patterns associated.
Results: Mahaboobnagar population recorded 1.109 and 0.816 fold resistance at LD_{50} and LD_{90}, respectively, while Raichur population has developed still higher levels of relative resistance by 1.591 and 0.846 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively. Similarly, the Raichur population has developed 1.435 and 1.037 folds relative resistance at LD_{50} and LD_{90}, respectively as compared with the Mahaboobnagar population.
The Mahaboobnagar population resistant to indoxacarb at F_3, when subjected to selected insecticides like cypermethrin, methomyl, spinosad showed a negative cross resistance ratio of

**Corresponding author: E-mail: supendhar@gmail.com;*

0.665, 0.830, 0.916 to cypermethrin, methomyl, spinosad respectively, and a positive cross resistance ratio of 1.019 to indoxacarb, while similar trend was displayed by Raichur population showing a negative cross resistance ratio of 0.932, 0.565, 0.803 to cypermethrin, methomyl, spinosad respectively and positive cross resistance of 1.036 indoxacarb further, same trend was shown by Nagpur population by displaying a negative cross resistance ratio of 0.610, 0.735, 0.519 to cypermethrin, methomyl, spinosad and positive cross resistance ratio of 1.026 to indoxacarb.
Conclusion: Continuous application of single insecticide belonging to a specific group across the generations increases the resistance from F_1 to F_3. Alternating the new chemistries with old conventional chemicals resulted in no cross resistance development as it was observed in all test populations.

Keywords: Helicoverpa armigera; cross resistance; indoxacarb resistance and South India.

1. INTRODUCTION

Helicoverpa armigera is known as the cotton bollworm, corn earworm, old World (African) bollworm. The larvae feed on a wide range of plants, including many important cultivated crops. It is a major pest in cotton and one of the most polyphagous and cosmopolitan pest specie s. The cotton bollworm is a highly polyphagous species. The most important crop hosts are tomato, cotton, pigeon pea, chickpea, rice, sorghum, and cowpea. Other hosts include groundnut, okra, peas, field beans, soybeans, lucerne tobacco, potatoes, maize, a number of fruit trees, forest trees, and a range of vegetable crops.

It is causing a substantial crop losses every year [1,2]. *H. armigera* has shown wider adaptability, greater capacity to develop resistance to synthetic insecticides used in its management Armes et al. [3]; [4,5]. The strong genetic variability of this species may be a governing factor for the behavior of *H. armigera* making it a serious pest on several crops Zhou et al. [6]. Understanding the variations among the *H. armigera* populations occurring on different host plants has become essential to understand the variations in their susceptibility to different insecticides. The adaptive advantage of insect species to thrive on different host plants is an ability for their better survival in the ecosystem. In Pakistan majority of field collected populations of *H. armigera* showed greater susceptibility close to the baselines for indoxacarb and spinosad, which are having novel modes of action. However, signs of resistance development to the new chemistries may be due to a cross-resistance mechanism from already selected against older chemistries Ahmad et al. [7]. By reducing the selection pressure and using alternate insecticides with novel mode of action the occurrence of insecticide resistant strains can be reduced or delayed. The pyrethroids and organophosphorus combination insecticides were found to be effective against the resistant insect pest population of *H. armigera* and *S. litura etc.* Martin et al. [8].

Frequent outbreaks and evolving resistance to insecticides at a faster rate in the cotton ecosystem may be attributed to genetic variation within and between geographical populations of *H. armigera The field collected populations of H. armigera* from the South Indian cotton ecosystem were analyzed using RAPD markers and 12 populations were classified into two distinct groups Fakrudin et al. [9].

To understand the structure, population dynamics, behavior and response to various selection pressures can be very useful for a better understanding of the genetic differences of polyphagous pest like *H. armigera.* In depth studies of molecular characterization useful for insecticide resistance in *H. armigera* in understanding the phenomenon and management of the problem. In the Indian subcontinent, a systematic and concerted effort to view the problem of insecticide resistance and cross resistance from this perspective is very important.

In the light of the above, the present study was undertaken to determine the cross resistance pattern associated with *H. armigera* to Spinosad, cypermethrin, carbaryl and indoxacarb.

2. MATERIALS AND METHODS

The present investigation on cross resistance patterns was carried from February 2010 to May 2011 at the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Telangana.

2.1 Collection of *H. armigera* (Hub.)

H. armigera (Hub.) larvae were collected from Mahaboobnagar, Raichur and Nagpur on red gram, cotton and bengal gram crops during February 2010 to May 2011.

2.2 Mass Rearing of *H. armigera* in the Laboratory

The population collected from Mahaboobnagar, Raichur and Nagpur were reared on artificial diet (Plate 1 and 2) in the laboratory as per the procedure given by Kranthi [10]. Male and female pupae were separated. One pair per jar (♂ and ♀ pupae) was kept for adult emergence, mating and oviposition. The eggs obtained from single pair were reared to get first generation larvae. Third instar *H.armigera* larvae from (1^{st} generation) F_1 with an average weight of 30 mg ± 0.011 S.E. were treated separately with different concentrations of the test insecticides.

2.2.1 Artificial diet preparation for *H. armigera*

- Measured quantities of chick pea flour (160 g), wheat germ (60 g), sorbic acid (1.7 g), ascorbic acid (5.3 g), methyl parabenzoate (3.3 g) and aureomycin (2.5 g) were added into a large bowl. Then 500 ml of pre boiled warm water was added and stirred thoroughly to mix well.
- Fifty three grams of active dried yeast was dissolved in 350 ml water and boiled for 5 min.
- Sixteen grams of agar was added to 350 ml water and boiled for 5 min after complete dispersion.
- Then, both yeast and agar solutions were mixed and again boiled for 5 min and added to the bowl containing other diet ingredients. All the ingredients were mixed well using electrical blender.
- Formaldehyde (10 per cent) 13.5 ml and 2 ml anti mould solution were added during blending.
- After thorough blending, the hot diet was transferred into soft plastic squeeze bottles having lids with spouts trimmed to 1 cm and dispensed the diet into wells of multicell trays.
- The trays were allowed to cool in a laminar air flow under UV lamp for 2-3 hours to sterilize the diet surface.
- After sterilization, the diet trays were stored in refrigerator at 4.0-8.0^{0}C and used whenever necessary upto one week.

Neonate larvae were transferred to multiwell (25 wells) rearing trays containing artificial diet. The larvae were offered with fresh diet for every 2 days until pupation and the pupae were kept for adult emergence in plastic containers.

Plate 1. *Helicoverpa armigera* egg

Plate 2. *Helicoverpa armigera* larvae on artificial diet

2.2.2 Adult maintenance

One pair of adults (♂ and ♀) were kept in plastic containers for mating and egg laying and were allowed to feed on adult diet after emergence. For adult diet, 5 gm each of sucrose and honey was dissolved in 90 ml of sterile water and boiled for 5 minutes. After proper cooling, 0.2 g each of ascorbic acid and methyl hydroxy para benzoate were added and stored at 4.0^0C for 1-2 weeks [10]. Sterile absorbent cotton swabs were soaked in the solution and placed in jars for adult feeding which were changed on alternate days. The entire setup was covered with a fine muslin cloth. The eggs laid on muslin cloth and cotton swab were removed with camel hair brush and dipped in surface sterile solution. The eggs were placed in small plastic jars for hatching, the neonates were gently transferred to multiwell (25 wells) rearing trays containing artificial diet.

2.3 Determination of the Insecticide Resistance in *H. armigera*

The three different populations of *H. armigera* were tested against indoxacarb for acquired degree of resistance (Table 1)

2.3.1 Test insect population

The field collected larvae from Mahaboobnagar, Raichur and Nagpur were reared separately in the laboratory to obtain pupae. Male and female pupae were separated and kept for single pair mating. The eggs obtained from single pair were reared to get first generation larvae. Third instar *H. armigera* larvae from (1^{st} generation) F_1 with an average weight of 30 mg ± 0.011 S.E. of Mahaboobnagar, Raichur and Nagpur populations were subjected separately to different concentrations of the test insecticide. The survivals at LD_{50} concentration in each test insecticide at F_1 (1^{st} generation) were further used.

2.3.2 Bioassay

Bioassay was done by topical application method using Hamilton micro applicator (Plate 3) to evaluate the toxicity of all the test insecticides [11].

2.3.3 Topical application method

Initially, one percent stock solution of the test insecticide was generated from the designed goods by dissolving the requisite quantities in double distilled water following correct weighting. The stock solution thus prepared was preserved in refrigerator for further use. Individual working concentrations test insecticide was prepared from the one per cent stock solution through serial dilution technique using double distilled water as solvent. One micro litre of the respective insecticidal solution was applied on the dorsum of second thoracic segment by micro

Table 1. Insecticides used for the determination of insecticide resistance in *H. armigera*

S.No	Common name	Formulation	Chemical name
1	Methomyl	40 SP	S-methyl N- (methyl carbamoxyloxy) Thioacetimidate
2	Cypermethrin	10 EC	(RS)-a-cyno-3-phenoxybenzlyl-(1RS)-cis,trans-3-(2,2 dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate
3	Spinosad	45 SC	Mixture of naturally derived fermentation macrolides Spinosyn A and D
4	Indoxacarb	14.5 SC	(S)- methyl 7- chloro-2,5- dihydro-2(methoxy-carbonyl]-indeno[1,2-e][1,2,3]oxadiazine-4a(31-1)- carboxylate

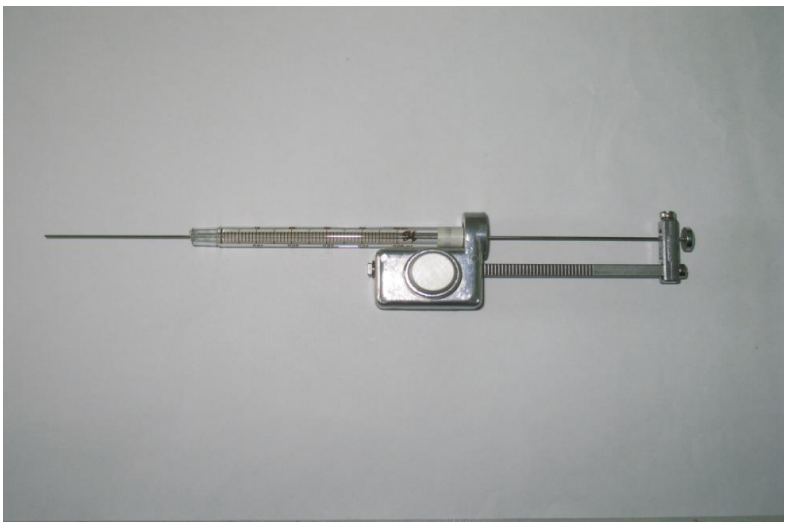

Plate 3. Hamilton micro applicator

applicator. Three replications were maintained for each insecticidal concentration with ten larvae in each replication.

2.3.4 Data collection

Mortality of the larvae was recorded at 24, 48 and 72 hours after treatment. The mortality at 72 hours after treatment was considered as end point for the assessment of toxicity of test insecticides as reported by Fisk and Wright [12]. Thus, concentrations of wide range initially and narrow range subsequently were tested so as to get mortality data in the range of 5-90 %. The moribund larvae also were considered as dead while recording the mortality data. The amount of insecticide present in one micro litre of test concentration was calculated and expressed as (LD_{50}) dose in µg/µl.

2.3.5 Assessment of the degree of resistance acquired by *H. armigera*

The mortality data obtained on third instar larvae of Mahaboobnagar, Raichur and Nagpur populations to different test insecticides were subjected to probit analysis [13] using POLO-PC software [14] to calculate LD_{50}, LD_{90}, Heterogeneity (χ^2), intercept (a), slope of the regression line (b), regression equation and fudicial limits. The degree of resistance acquired by *H. armigera* was calculated by dividing the higher LD_{50} value of a population with the lower LD_{50} value of population among the three populations for each test insecticide and thus the relative degree of resistance was assessed (Resistance factor = LD_{50} of the resistant population / LD_{50} of the susceptible strain).

In resistance studies, LD_{50} level comparison was most useful and appropriate when the slope of the log concentration probit mortality lines for the three populations happened to be parallel [15]. However reliance on the simple LD_{50} comparisons may lead to spurious indications of resistance, hence resistance can be detected by using LD_{90} which is known to kill all susceptible individuals in a population. Therefore LD_{90} values were also calculated. The degree of resistance

acquired by all the three populations was also calculated by comparing the present data with the available baseline data at LD_{50} and LD_{90} levels. The degree of resistance to indoxacarb was calculated by using the baseline data of Nagpur susceptible strain [10] (Table 2).

Resistance factor = LD_{50} of the F_1 resistant population / LD_{50} of the Nagpur susceptible strain The log concentration probit (lcp) lines were drawn by plotting log concentration (x) on X-axis and probits of the respective concentrations on Y-axis [13].

2.4 Determination of Cross Resistance Pattern in *H. armigera*

Cross resistance pattern in *H. armigera* was studied by using the test insecticides *viz.,* methomyl representing carbamates, cypermethrin representing synthetic pyrethroids and spinosad belongs to spinosyns. The larvae collected from Mahaboobnagar, Raichur and Nagpur locations were reared as described earlier and were subjected separately to test insecticide. The survivals at LD_{50} concentration of test insecticide at F_1 (1^{st} generation) were reared separately to next generation (F_2) by single pair mating. Third instar larvae from single pair mating (2^{nd} generation) F_2 were again subjected to bioassay and the survivals at LD_{50} of test insecticide treatment (2^{nd} generation) F_2 were reared separately to next generation (F_3). Third instar larvae from single pair mating (3^{nd} generation) F_3 were subjected to different doses of all the test insecticides for assessing the cross resistance pattern. The insecticidal treatments were given here under in the flow chart (Fig.1). The same procedure was followed for all the three locations as stated in the flow chart. The procedure followed for bioassay was topical application and data collected was same as described earlier.

2.4.2 Assessment of the cross resistance pattern in *H. armigera*

The mortality data obtained from Mahaboobnagar, Raichur and Nagpur populations were subjected to probit analysis using POLO – PC software [14]. The degree of cross resistance acquired by *H. armigera* was calculated by dividing LD_{50} value of $F_n{}^{th}$ generation with the LD_{50} value of F_1 generation test insecticide and thus the relative degree of cross resistance was assessed by using the formula suggested by Ramasubramanian and Regupathy [5].

Cross resistance ratio (CRR) = LD_{50} of F_n (selected) / LD_{50} of F_1 (unselected)

If the CRR ratio is

<1 – Negative cross resistance
>1 – Positive cross resistance

3. RESULTS AND DISCUSSION

The results pertaining to the present study were presented here under in different headings.

3.1 Determination of the Degree of Resistance

The degree of resistance developed in *H. armigera* third instar larvae of Mahaboobnagar (Andhra Pradesh), Raichur (Karnataka) and Nagpur (Maharashtra) to the test insecticide indoxacarb was studied through bioassay. The resistance acquired was expressed by comparing the LD_{50} and LD_{90} values against the test insect with the susceptible population among the above said populations.

Mahaboobnagar population recorded a LD_{50} of 0.214 µg/larva and which rose sharply to 0.567 µg/larva at LD_{90} for indoxacarb (Table 3). The corresponding log dose probit (ldp) line had a slope (b) of 3.024 . Raichur population displayed still more LD_{50} and LD_{90} values for indoxacarb as 0.307 and 0.588 µg/larva, respectively (Table 4) with a slope (b) of 4.533. Nagpur population showed very less LD_{50} and LD_{90} values of indoxacarb as 0.193 and 0.695 µg/larva, respectively (Table 5) with a shallow slope (b) of 2.299. The chi-square test revealed that all the three populations used in the study were homogenous ($p < 0.05$ %).

Among the three populations of *H. armigera,* Mahaboobnagar population recorded 1.109 and 0.816 fold resistance at LD_{50} and LD_{90}, respectively, while Raichur population has developed the higher levels of relative resistance by 1.591 and 0.846 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively (Table 3).The same Raichur population has developed 1.435 and 1.037 folds relative resistance at LD_{50} and LD_{90}, respectively as compared with the Mahaboobnagar population. The present findings were in accordance with the earlier reports available regarding the efficacy of indoxacarb against *H.armigera*. where, Rao ([16) obtained the LC_{50} values of indoxacarb as 0.21 µg/ larva and LC_{90} as1.23 µg/larva in *H.armigera* from cotton fields in Kurnool, Andhra Pradesh.

Similarly, Cook et al. [17] indicated that the LC_{50}values of indoxacarb for *H.armigera* ranged from 1.05 to 1.33 ppm. Sayyed et al. [18] found that the laboratory selection of *H. armigera* (generations G_3 to G_8) increased the resistance ratio by only one fold for indoxacarb. Further, maximum tolerance level for indoxacarb was shown by Amaravati strain (5.09 ppm) and the minimum tolerance level by Fatehbad strain (0.22 ppm) the seasonal monitoring ranged from 1.62 ppm to 17.14 ppm from July-2005 to March-2007 reported by Ghodki et al. [19]. Generation-wise selection with indoxacarb revealed the mode of inheritance of resistance. The LC_{50} of indoxacarb was 2.81 ppm for the first selected generation and it increased to 272.55 ppm after eight selected generations, which is a 1238.86-fold resistance compared to the susceptible strain reported by Ghodki et al. [20]. From the present findings it is evident that there was a slight increase in the level of resistance to indoxacarb in *H. armigera* compared to latest reports of Rao [16], Cook et al. [17]; Ghodki et al. [19]; Ghodki et al. [20] which may be probably due to significant increase in the use of indoxacarb against all the larval instars management in almost all the crops.

3.2 Evaluation of Cross Resistance Pattern

H. armigera larvae from Mahaboobnagar, Raichur and Nagpur population first generation (F_1) with an average weight of 30 mg ± 0.011 S.E. were subjected separately to different concentrations of the test insecticide indoxacarb and studied cross resistance pattern.

3.2.1 Mahaboobnagar (Andhra Pradesh)

F_1 generation third instar larvae when subjected to different concentrations of indoxacarb showed LD_{50} 0.214 µg/larva and 0.567 µg/larva at LD_{90}. (Table 4).First generation larvae resistant to indoxacarb were taken and reared to F_2, when subjected to different concentrations of indoxacarb at F2 recorded LD_{50} value 0.218 µg/larva and 0.269 µg/larva at LD_{90}. Population resistant to indoxacarb showed a positive cross resistance ratio of 1.019 to indoxacarb. The chi-square test revealed that the population used in the study was homogenous (P<0.05%).

The indoxacarb resistant population from F_1 and F_2 generation were reared to F_3 generation by single pair mating and the resulting third instar larvae subjected to different test insecticides, the results depicted were presented in Table 4. The LD_{50} values were 19.716, 2.844, 0.281 and 0.220 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. While, the LD_{90} values (µg/larva) of cypermethrin, methomyl, spinosad and indoxacarb were 27.571, 3.716, 0.308 and 0.252, respectively.

Larvae resistant to indoxacarb showed a negative cross resistance ratio of 0.677 to cypermethrin, 0.779 to methomyl, 0.912 to spinosad and a positive cross resistance ratio of 1.028 to indoxacarb at F_3.

3.2.2 Raichur (Karnataka)

F_1 population showed LD_{50} value of 0.307 µg/larva and rose sharply to 0.588 µg/larva at LD_{90} to indoxacarb (Table 5). First generation larvae resistant to indoxacarb were taken and reared to F_2 by single pair mating as described earlier. Same population at F2 recorded LD_{50} value 0.318 µg/larva and showed LD_{90} values (µg/larva) of 0.360 to indoxacarb. The indoxacarb resistant population at F_3 generation subjected to different test insecticides showed the LD_{50} values were 29.125, 2.245, 0.228 and 0.325 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. The LD_{90} values (µg/larva) of cypermethrin, methomyl, spinosad and indoxacarb were as follows *i.e.* 59.609, 2.896, 0.297 and 0.388, respectively. (Table 5). Further, larvae resistant to indoxacarb showed a positive cross resistance ratio of 1.059 to indoxacarb and a negative cross resistance of 0.897 to cypermethrin, 0.618 to methomyl, and 0.803 to spinosad at F_3 generation. Among these the insecticide sequences indoxacarb - indoxacarb -methomyl treatment was the best.

3.2.3 Nagpur (Maharashtra)

F_1 generation population showed LD_{50} value of 0.193 µg/larva and 0.695 µg/larva at LD_{90} to indoxacarb.

While, the same population recorded LD_{50} value of 0.198 µg/larva and recorded 0.227 µg/larva at LD_{90} in the F_2 generation. Further, indoxacarb resistant population showed a positive cross resistance ratio of 1.026 to indoxacarb. The chi-square test revealed that the population used in the study was homogenous (P<0.05%) (Table 6).

Table 2. Particulars of base line data used to calculate the degree of insecticide resistance in the larvae of *H. armigera*

S. No	Insecticide	Name of strain	LD_{50} µg/larva	LD_{90} µg/larva	Reference
1	Cypermethrin	Nagpur susceptible	0.007	0.028	Kranthi, [10]
2	Methomyl	Nagpur susceptible	0.030	0.165	Kranthi, [10]
3	Spinosad	Nagpur susceptible	0.062	0.347	Kranthi, [10]
4	Indoxacarb	Nagpur susceptible	0.00325	0.1189	Kranthi, [10]

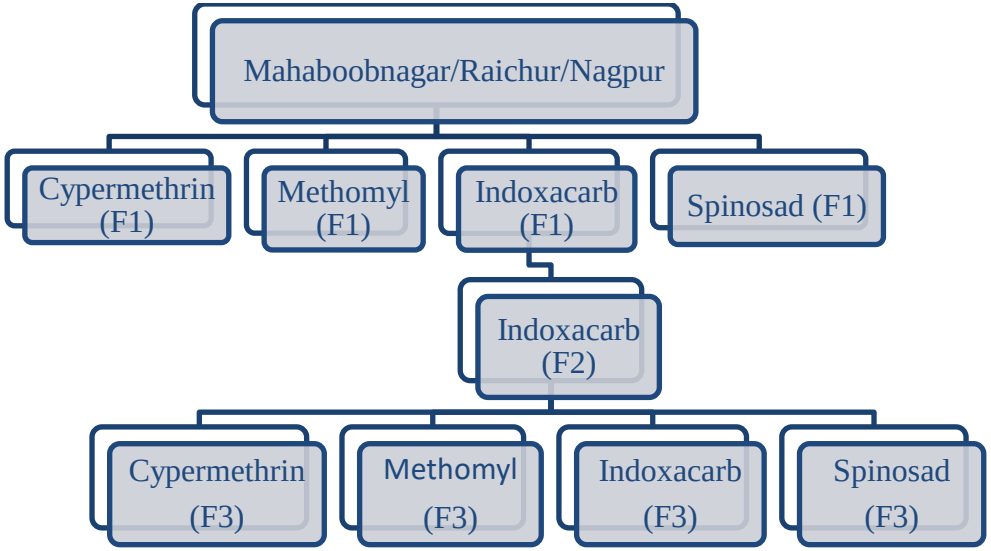

Fig 1. Bio assay procedure for selected population

Table 3. Relative degree of resistance among the three populations of *H. armigera* to indoxacarb at F_1

Population	LD_{50} µg/larva	LD_{90} µg/larva	Resistance factor in comparison with			
			Mahaboobnagar population (folds)		Nagpur population (folds)	
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Raichur	0.307	0.588	1.435	1.037	1.591	0.846
Mahaboobnagar	0.214	0.567	-	-	1.109	0.816
Nagpur	0.193	0.695	-	-	-	-

Table 4. Cross resistance pattern in indoxacarb-indoxacarb selected Mahaboobnagar population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity ($\chi 2$)	Regression equation	CRR
1	Indoxacarb	F_1	0.214 (0.165-0.255)	0.567 (0.457-0.810)	3.024 + 0.496	2.039	Y = 7.028 + 3.024 X	--
2	Indo – Indo	F_2	0.218 (0.099 – 0.249)	0.269 (0.236 – 0.627)	13.900 ± 6.195	0.508	Y = 14.203 + 13.900 X	1.019
3	Indo – Indo - Cyper	F_3	19.716 (8.104 – 23.677)	27.571 (22.951 – 65.506)	8.801 ± 3.687	0.926	Y = -6.396 + 8.801 X	0.677
4	Indo – Indo - Metho	F_3	2.844 (2.083 – 3.199)	3.716 (3.292 – 5.682)	11.032 ± 3.906	0.953	Y = -0.007 + 11.032 X	0.779
5	Indo – Indo- Spino	F_3	0.281 (0.253 – 0.294)	0.308 (0.294 – 0.355)	31.857 ± 10.978	0.703	Y = 22.572 + 31.857 X	0.912
6	Indo – Indo - Indo	F_3	0.220 (0.134 – 0.239)	0.252 (0.232 – 0.426)	22.280 ± 9.935	0.445	Y = 19.635 + 22.280 X	1.028

**CRR- Cross Resistance Ratio*

Table 5. Cross resistance pattern in indoxacarb –indoxacarb selected Raichur population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity ($\chi 2$)	Regression equation	CRR
1	Indoxacarb	F_1	0.307 (0.259 - 0.346)	0.588 (0.512 - 0.731)	4.533 + 0.694	2.193	Y = 7.327 + 4.533 X	---
2	Indo - Indo	F_2	0.318 (0.275 – 0.338)	0.360 (0.339 - 0.450)	23.601 ± 8.489	0.629	Y = 16.747 + 23.601 X	1.036
3	Indo – Indo - Cyper	F_3	29.125 (23.867 – 33.325)	59.609 (51.126-76.562)	4.120 ± 0.669	0.809	Y = -1.033 + 4.120 X	0.897
4	Indo – Indo - Metho	F_3	2.245 (1.638 – 2.534)	2.896 (2.562 –4.565)	11.578 ± 4.210	1.511	Y = 0.934 + 11.578 X	0.618
5	Indo – Indo- Spino	F_3	0.228 (0.167 – 0.256)	0.297 (0.263 – 0.455)	11.032 ± 3.906	0.953	Y = 12.094 + 11.032 X	0.803
6	Indo – Indo - Indo	F_3	0.325 (0.264 – 0.354)	0.388 (0.356 – 0.528)	16.646 ± 5.962	1.404	Y = 13.122 + 16.646 X	1.059

**CRR- Cross Resistance Ratio*

Table 6. Cross resistance pattern in indoxacarb - indoxacarb selected Nagpur population of *H. armigera*

S. No.	Strain	Generation	LD_{50} µg/larva (95%FL)	LD_{90} µg/larva (95%FL)	Slope ± S.E (b)	Heterogeneity ($\chi 2$)	Regression equation	CRR
1	Indoxacarb	F_1	0.193 (0.138 - 0.240)	0.695 (0.515 – 1.219)	2.299 + 0.420	0.895	Y = 6.645 + 2.299 X	---
2	Indo - Indo	F_2	0.198 (0.171 – 0.211)	0.227 (0.212 – 0.276)	21.480 ± 7.236	0.636	Y = 20.131 + 21.480 X	1.026
3	Indo – Indo - Cyper	F_3	12.121 (1.731 – 15.804)	19.905 (15.299 – 190.231)	5.949 ± 2.694	1.919	Y = -1.446 + 5.949 X	0.604
4	Indo – Indo - Metho	F_3	2.177 (0.990 – 2.489)	2.692 (2.359 – 6.272)	13.900 ± 6.195	0.507	Y = 0.303 + 13.900 X	0.821
5	Indo – Indo-Spino	F_3	0.103 (0.007 – 0.131)	0.151 (0.118 – 1.589)	7.647 ± 3.611	0.283	Y = 12.551 + 7.647 X	0.563
6	Indo – Indo - Indo	F_3	0.203 (0.172 – 0.217)	0.234 (0.218 – 0.299)	20.683 ± 7.368	1.356	Y = 19.335 + 20.683 X	1.052

**CRR- Cross Resistance Ratio*

The indoxacarb resistant population selected from F_1 and F_2 generation was reared to F_3 generation by single pair mating and subjected to different test insecticides recorded the LD_{50} values as 12.121, 2.177, 0.103 and 0.203 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. The LD_{90} values (µg/larva) of cypermethrin, methomyl, spinosad and indoxacarb were as follows *i.e.* 19.905, 2.692, 0.151 and 0.234, respectively. (Table 6). Larvae resistant to indoxacarb showed a negative cross resistance ratio of 0.604 to cypermethrin, 0.821 to methomyl, 0.563 to spinosad and a positive cross resistance ratio of 1.052 to indoxacarb in F_3 generation.

From the results it is evident that CRR increased among all the locations when same chemical repeated. Similar trend was followed in Raichur and Nagpur populations. indoxacarb - indoxacarb rotation of Mahaboobnagar population recorded a CRR of 1.019 and indoxacarb - indoxacarb - indoxacarb rotation recorded a CRR of 1.028.

The results obtained during the present investigations revealed that the continuous application of indoxacarb insecticide across the generations increased the resistance from F_1 to F_3. Alternating the new chemicals with old conventional chemicals results in no cross resistance development. From the literature it is noticed that Pakistan field populations of *H. armigera* were highly resistant to conventional insecticides with well-known mode of action. However, majority of the populations exhibited susceptibility close to the baselines to indoxacarb, showing signs of resistance development to the new chemistries as demonstrated by a low level of tolerance in many populations. This might be due to a cross resistance from the metabolic resistance mechanisms of already selected to older chemistries Ahmad et al. [7]. Further, Gunning and Devonshire (2002) concluded that indoxacarb requires biological activation to become a toxic metabolite. Results showed that *H. armigera* activates indoxacarb using esterase involved in pyrethroid resistance. Increased esterase activity leads to increased activation and, therefore, increased susceptibility to indoxacarb indicating the negative cross resistance of *H. armigera* between pyrethroids and indoxacarb. Similarly, Gunning and Devonshire [21] reported that pyrethroid resistant *H. armigera* has overproduced esterases and the results showed greater indoxacarb conversion compared to susceptible strains. Indoxacarb had significantly better efficacy against more highly pyrethroid resistant strains of *H. armigera*. Negative cross resistance between indoxacarb and pyrethroid resistance should be a valuable tool for the management of pyrethroid resistance in *H. armigera*. Finally, Ramasubramanian and Regupathy [22] found that the induction of carboxyl esterases in pyrethroid selected populations might have resulted in the activation of indoxacarb, thereby accounting for the observed negative cross resistance.

The results of present study were in accordance with the earlier workers showing negative cross resistance with cypermethrin. Resistance to indoxacarb is a critical task emerging in near future, hence pest management module should incorporate all the available chemicals like pyrethroids

4. SUMMARY AND CONCLUSIONS

The experiment was carried out from February 2010 to May 2011 in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh to determine the level of resistance acquired by third instar larvae of *H. armigera* from Mahaboobnagar, Raichur and Nagpur to indoxacarb and the associated cross resistance patterns.

The Mahaboobnagar population resistant to indoxacarb showed a negative cross resistance ratio of 0.665, 0.830, 0.916 to cypermethrin, methomyl, spinosad respectively, and a positive cross resistance ratio of 1.019 to indoxacarb while similar trend was displayed by Raichur population showing a negative cross resistance ratio of 0.932, 0.565, 0.803 to cypermethrin, methomyl, spinosad respectively and positive cross resistance of 1.036 indoxacarb and almost same trend was followed by Nagpur population by displaying a negative cross resistance ratio of 0.610, 0.735, 0.519 to cypermethrin, methomyl, spinosad and positive cross resistance ratio of 1.026 to indoxacarb.

Mahaboobnagar population recorded 1.109 and 0.816 fold resistance at LD_{50} and LD_{90}, respectively, while, Raichur population has developed the higher levels of relative resistance by 1.591 and 0.846 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, The Raichur population has developed 1.435 and 1.037 folds relative resistance at LD_{50} and LD_{90}, respectively as

compared with the Mahaboobnagar population. The similar type of results obtained during the present investigations revealed that the continuous application of single insecticide over generations increases the resistance from F_1 to F_3. Alternating the new chemistries with old conventional chemicals results in no cross resistance development as it was observed for all the three populations.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

ACKNOWLEDGEMENTS

The authors are grateful to the Acharya N.G. Ranga Agricultural University (ANGRAU) and faculty members of Department of Entomology, College of Agriculture, PJTSAU Rajendranagar, Hyderabad for their support extended during the conduct of the experiment and publishing the article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reed W, Pawar CS. Heliothis, a global problem. In: Proceedings of International Workshop on Heliothis Management, ICRISAT, Patancheru, Andhra Pradesh, India. 1982;9-14.
2. Manjunath TM. Controlling cotton pests. Deccan Herald, January 4,1990 Science and society. 1990;1-3.
3. Armes NJ, Jadhav DR, Desouza KR. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. Bulletin of Entomological Research. 1996;86:499-514.
4. Kranthi KR, Armes NJ, Rao NGV, Raj S, Sundaramurthy VT. Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India. Pesticide Science. 1997;50:91-98.
5. Ramasubramanian T, Regupathy A. Laboratory and field evaluation of spinosad against pyrethroid resistant population of *Helicoverpa armigera* (Hub). Journal of Biological Sciences. 2004;4(2):142-145.
6. Zhou X, Fartor O, Applebaum SW, Coll M. Population structure of the pestiferous moth *Helicoverpa armigera* in the Eastern Mediterranean using RAPD analysis. Heredity. 2000;85:251-256.
7. Ahmad M, Arif MI, Zahoor Ahmad. Susceptibility of *Helicoverpa armigera* (*Lepidoptera*: *Noctuidae*) to new chemistries in Pakistan. Crop Protection. 2003;22(3):539-544.
8. Martin T, Ochou GO, Vaissayre M, Fournier D. Monitoring of insecticide resistance in *Helicoverpa armigera* (Hubner) from 1998 to 2002 in Cote d'Ivoire, West Africa. Resistant Pest Management Newsletter. 2003;12(2):51-55.
9. Fakrudin B, Prakash SH, Krishnareddy KB, Vijaykumar, Prasad PRB, Patil BV, Kuruvinashetti MS. Genetic variation of cotton bollworm, *Helicoverpa armigera* (Hubner) of South Indian cotton ecosystem using RAPD markers. Current Science. 2004;87(12):1654-1657.
10. Kranthi KR. Insecticide resistance monitoring, mechanisms and management manual. Central Institute for Cotton Research, Nagpur. 2005;80-94.
11. FAO (Food and Agricultural Organization). Recommended methods for the detection and measurement of pest resistance to pesticides. Tentative method for larvae of Egyptian cotton leafworm (*Spodoptera littoralis* Biosd.) F.A.O method No.8 Food and Agricultural Organization. Plant Protection Bulletin. 1971;19:32-35.
12. Fisk T, Wright DJ. Speed of action and toxicity of acylurea insect growth regulators against *Spodoptera exempta* (Walk.) and *Spodoptera littoralis* (Biosd.) larvae: effect of inter moult age. Pesticide Science 1992;35:331-337.
13. Finney DJ. Probit analysis, Cambridge University, London. 1971;333.
14. Anon. POLO-PC - a user's guide to Probit or Logit analysis. California, LeOra Software, California. 1987;22.
15. Dyte CE. Insecticide resistance in stored product insects with special reference to *Tribolium castaneum.* Tropical Stored Products Information. 1970;20:13-18.
16. Rao GMVP. Status of insecticide resistance in Kurnool population of *Spodoptera litura.* Indian Journal of Plant Protection. 2008;36(2):173-175.
17. Cook DR, Leonard BR, Gore J, Temple JH. Baseline responses of bollworm,

Helicoverpa zea (Boddie) and tobacco budworm, *Heliothis virescens* (F.) to indoxacarb and pyridalyl. Journal of Agricultural and Urban Entomology. 2005;22(2):99-109.

18. Sayyed AH, Ahmad M, Crickmore N. Fitness costs limit the development of resistance to indoxacarb and deltamethrin in *Heliothis virescens* (*Lepidoptera*: *Noctuidae*). Journal of Economic Entomology. 2008;101(6):1927-1933.
19. Ghodki BS, Thakare SM, Moharil MP, Rao NGV. Seasonal and geographical toxicity of indoxacarb against *Helicoverpa armigera* and influence of different host plants against indoxacarb in India. Entomological Research. 2009;39(1):43-49.
20. Ghodki BS, Thakare SM, Moharil MP, Rao NGV. Genetics of indoxacarb resistance in *Helicoverpa armigera* (Hubner). Entomological Research. 2009;39(1):50-54.
21. Gunning RV, Devonshire AL. Negative cross resistance between indoxacarb and pyrethroids in the cotton bollworm, *Helicoverpa armigera* in Australia: A tool for resistance management. The BCPC International Congress: Crop Science and Technology, Volumes 1 and 2. Proceedings of an international congress held at the SECC, Glasgow, Scotland, UK, 10th to 12th November. 2003; 789-794.
22. Ramasubramanian T, Regupathy A. Magnitude and mechanism of insecticide resistance in *Helicoverpa armigera* (Hub.) population of Tamil Nadu, India. Asian Journal of Plant Sciences. 2004;3(1):94-100.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107682