

LD₅₀ and LD₉₀ HELICOVERPA ARMIGERA ASSOCIATED CROSS RESISTANCE PATTERNS IN CROP ECOSYSTEM

ABSTRACT

Aim: To study the cross resistance patterns associated with Mahaboobnagar, Raichur and 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 LD₅₀ and LD₉₀, 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 LD₅₀ and LD₉₀, respectively. Similarly, the Raichur population has developed 1.435 and 1.037 folds relative resistance at LD₅₀ and LD₉₀, respectively as compared with the Mahaboobnagar population.

The Mahaboobnagar population resistant to indoxacarb at F₃, when subjected to selected insecticides like cypermethrin, methomyl, spinosad 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 to 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 F₁ to F₃. Alternating the new chemistries with old conventional chemicals resulted in no cross resistance development as it was observed in all test populations.

Key words: *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 species. 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 (Reed and Pawar, 1982 and Manjunath, 1990). *H. armigera* has shown wider adaptability, greater capacity to develop resistance to synthetic insecticides used in its management (Armes *et al.* 1996; Kranthi, 1997 and Ramasubramanian and Regupathy, 2004). 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.*, 2000). 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.*, 2003). 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.*, 2003).

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.*, 2004).

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.

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2. MATERIAL 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. armigerain* 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 (2005). Male and female pupae were separated. One pair per jar (σ and φ 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 (1st generation) F₁ with an average weight of 30 mg \pm 0.011 S.E. were treated separately with different concentrations of the test insecticides.



Plate 1: *Helicoverpa armigera* egg



Plate 2: *Helicoverpa armigera* larvae on artificial diet

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°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.

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°C for 1-2 weeks (Kranthi, 2005). 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)

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

S.No	Common name	Formulation	Trade name	Chemical name	Source of supply
1	Methomyl	40 SP	Lannate	S-methyl N- (methyl carbamoxoxyloxy) Thioacetimidate	M/S Dupont Chemical (India) Limited, Mumbai-400076
2	Cypermethrin	10 EC	Cypra	(RS)-a-cyno-3-phenoxybenzyl-(1RS)-cis,trans-3-(2,2 dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate	M/S Hyderabad chemicals supplies Ltd, A-24/25, APIE, Balanagar, Hyderabad-500 037
3	Spinosad	45 SC	Tracer	Mixture of naturally derived fermentation macrolides Spinosyn A and D	M/S De-Nocil Crop Protection Ltd, 1 st floor, Administrative building Vikhroli(E), Mumbai - 79
4	Indoxacarb	14.5 SC	Avaunt	(S)- methyl 7- chloro-2,5- dihydro-2(methoxy-carbonyl)-indeno[1,2-e][1,2,3]oxadiazine-4a(31-1)- carboxylate	M/S Dupont Chemical (India) Limited, Mumbai-400076

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 (1st generation) F₁ 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 LD₅₀ concentration in each test insecticide at F₁ (1st 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 (FAO, 1971).

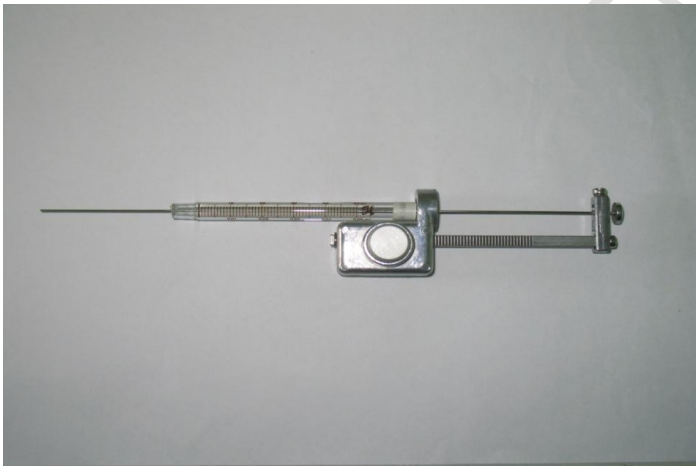


Plate 3: Hamilton Micro applicator

2.3.3 Topical application method

Initially one per cent stock solution of the test insecticide was prepared from the formulated products by dissolving the required quantities after accurate weighing in double distilled water. 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 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 (1992). 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 (LD₅₀)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 (Finney, 1971) using POLO-PC software (Anon, 1987) to calculate LD₅₀, LD₉₀, Heterogeneity (χ^2), intercept (a), slope of the regression line (b), regression equation and fiducial limits. The degree of resistance acquired by *H. armigera* was calculated by dividing the higher LD₅₀ value of a population with the lower LD₅₀ value of population among the three populations for each test insecticide and thus the relative degree of resistance was assessed (Resistance factor = LD₅₀ of the resistant population / LD₅₀ of the susceptible strain).

In resistance studies, LD₅₀ 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 (Dyte, 1970). However reliance on the simple LD₅₀ comparisons may lead to spurious indications of resistance, hence resistance can be detected by using LD₉₀ which is known to kill all susceptible individuals in a population. Therefore LD₉₀ 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 LD₅₀ and LD₉₀ levels. The degree of resistance to indoxacarb was calculated by using the baseline data of Nagpur susceptible strain (Kranthi, 2005)(Table 2).

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, 2005
2	Methomyl	Nagpur susceptible	0.030	0.165	Kranthi, 2005
3	Spinosad	Nagpur susceptible	0.062	0.347	Kranthi, 2005
4	Indoxacarb	Nagpur susceptible	0.00325	0.1189	Kranthi, 2005

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 (Finney, 1971).

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 (1st generation) were reared separately to next generation (F_2) by single pair mating. Third instar larvae from single pair mating (2nd generation) F_2 were again subjected to bioassay and the survivals at LD_{50} of test insecticide treatment (2nd generation) F_2 were reared separately to next generation (F_3). Third instar larvae from single pair mating (3rd 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.

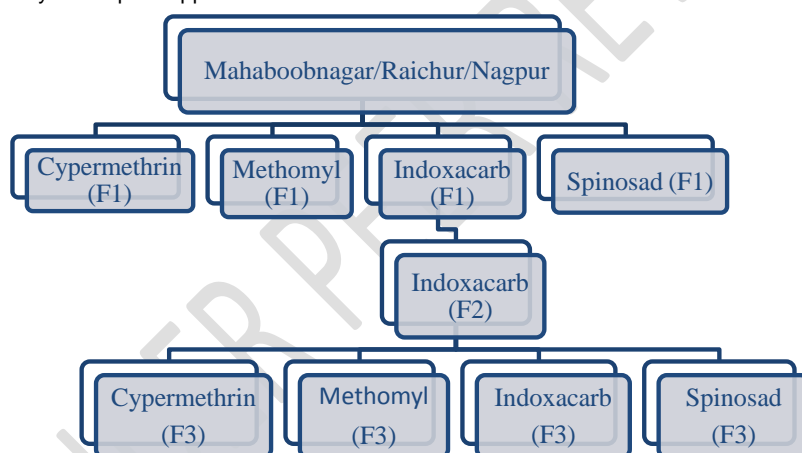


Fig 1. Bio assay procedure for selected population

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 (Anon, 1987). 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 (2004).

$$\text{Cross resistance ratio (CRR)} = \frac{LD_{50} \text{ of } F_n(\text{selected})}{LD_{50} \text{ of } F_1 \text{ (unselected)}}$$

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 LD₅₀ and LD₉₀ values against the test insect with the susceptible population among the above said populations.

Mahaboobnagar population recorded a LD₅₀ of 0.214 µg/larva and which rose sharply to 0.567 µg/larva at LD₉₀ for indoxacarb (Table 3). The corresponding log dose probit (ldp) line had a slope (b) of 3.024. Raichur population displayed still more LD₅₀ and LD₉₀ 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 LD₅₀ and LD₉₀ 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 ($p < 0.05$ %).

Table 3: Relative degree of resistance among the three populations of *H. armigera* to indoxacarb at F₁

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	-	-	-	-

Among the three populations of *H. armigera*, Mahaboobnagar population recorded 1.109 and 0.816 fold resistance at LD₅₀ and LD₉₀, 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 LD₅₀ and LD₉₀, respectively (Table 3). The same Raichur population has developed 1.435 and 1.037 folds relative resistance at LD₅₀ and LD₉₀, 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 (2008) obtained the LC₅₀ values of indoxacarb as 0.21 µg/ larva and LC₉₀ as 1.23 µg/larva in *H. armigera* from cotton fields in Kurnool, Andhra Pradesh. Similarly, Cook et al. (2005) indicated that the LC₅₀ values of indoxacarb for *H. armigera* ranged from 1.05 to 1.33 ppm. Sayyed et al. (2008b) found that the laboratory selection of *H. armigera* (generations G₃ to G₈) 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.* (2009a). Generation-wise selection with indoxacarb revealed the mode of inheritance of resistance. The LC₅₀ 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.* (2009b). 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 (2008), Cook *et al.* (2005) and Ghodki *et al.* (2009a) and Ghodki *et al.* (2009b) 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 (F₁) 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)

F₁ generation third instar larvae when subjected to different concentrations of indoxacarb showed LD₅₀ 0.214 µg/larva and 0.567 µg/larva at LD₉₀. (Table 4). First generation larvae resistant to indoxacarb were taken and reared to F₂, when subjected to different concentrations of indoxacarb F₂ recorded LD₅₀ value 0.218 µg/larva and 0.269 µg/larva at LD₉₀. 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 F₁ and F₂ generation were reared to F₃ 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 LD₅₀ values were 19.716, 2.844, 0.281 and 0.220 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. While, the LD₉₀ 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 F₃.

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 (χ 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

3.2.2 Raichur (Karnataka)

F₁ population showed LD₅₀ value of 0.307 µg/larva and rose sharply to 0.588 µg/larva at LD₉₀ to indoxacarb (Table 5). First generation larvae resistant to indoxacarb were taken and reared to F₂ by single pair mating as described earlier. Same population at F₂ recorded LD₅₀ value 0.318 µg/larva and showed LD₉₀ values (µg/larva) of 0.360 to indoxacarb. The indoxacarb resistant population at F₃ generation subjected to different test insecticides showed the LD₅₀ values were 29.125, 2.245, 0.228 and 0.325 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. The LD₉₀ 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 F₃ generation. Among these the insecticide sequences indoxacarb - indoxacarb - methomyl treatment was the best.

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 (χ 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 - Cyber	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

3.2.3 Nagpur (Maharashtra)

F₁ generation population showed LD₅₀ value of 0.193 µg/larva and 0.695 µg/larva at LD₉₀ to indoxacarb. While, the same population recorded LD₅₀ value of 0.198 µg/larva and recorded 0.227 µg/larva at LD₉₀ in the F₂ 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).

The indoxacarb resistant population selected from F₁ and F₂ generation was reared to F₃ generation by single pair mating and subjected to different test insecticides recorded the LD₅₀ values as 12.121, 2.177, 0.103 and 0.203 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. The LD₉₀ 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 F₃ 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.

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 (χ 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

Comment [DAL3]: Interesting result!

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.*, 2003). 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 (2003) 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 (2004a) 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 to 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 LD₅₀ and LD₉₀, 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 F₁ to F₃. 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.

Data Availability Statement: The data sets generated during and/or analysed during the current study are available with the corresponding author on reasonable request.

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Comment [DAL4]: Old reference! No references in relation with this topic since 2018?

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