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Cross Resistance Patterns Associated with Spinosad Resistant *Helicoverpa armigera* (Hubner) in South India

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Authors' contributions

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

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ABSTRACT

Aim: To study the cross resistance patterns associated with *Helicoverpa armigera* (Hubner) in south India.
Study Design: Bioassay.
Place and Duration of Study: The experiment was carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad,Telangana from February 2010 to May 2011.
Methodology: Spinosad resistant *Helicoverpa armigera* population in F1 and F2 subjected to different insecticides to know the cross resistance patterns associated.
Results: American bollworm population of Mahaboobnagar has developed 0.308 and 0.646 folds and 0.284 and 0.624 folds in Raichur population as compared with the Nagpur baseline population at F_1. Mahaboobnagar population displayed a negative cross resistance ratio of 0.677 fold to cypermethrin, 0.806 fold to methomyl, 0.935 fold to indoxacarb and positive cross resistance of 1.039 fold to spinosad, similar trend was followed in Raichur population with a negative cross resistance ratio of 0.918 fold to cypermethrin, 0.543 fold to methomyl, 0.642 fold to indoxacarb and 1.060 fold to spinosad. Further, the Nagpur population exihibited a similar trend with a negative

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cross resistance ratio of 0.604 fold to cypermethrin, 0.690 fold to methomyl, 0.570 fold to indoxacarb and positive cross resistance ratio of 1.077 fold to spinosad at F_3.
Conclusion: The present study revealed that the continuous application of same insecticide across the 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 in all three populations studied.

Keywords: Helicoverpa armigera; spinosad resistance; cross resistance patterns; South India.

1. INTRODUCTION

The bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide occurrence inflicting annual crop global economic losses of over 3 billion US dollars annually [1]. In India this insect occurs as a major pest in many economically important crops, including cotton, pigeonpea, chickpea, tomato, okra, blackgram, maize, sorghum and many other crops, inflicting substantial crop losses every year [2,3]. *H. armigera* is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management [4,5,6]. The versatility of this species may be due to the presence of a strong genetic variability governing the behavior of *H. armigera* making it a serious pest on several crops [7].

Understanding the genetic variation among the *H. armigera* populations occurring on host plants has become essential to understand the variation in their susceptibility to different insecticides. The ability of insect species to thrive on diverse host plants is an adaptive advantage for their better survival in the ecosystem.

Majority of field populations of *H. armigera* in Pakistan exhibited susceptibility close to the baselines for indoxacarb and spinosad having novel modes of action, there were, nevertheless, signs of resistance development to the new chemistries as demonstrated by a low level of tolerance in many populations. This may be due to a cross-resistance from the resistance mechanisms, particularly metabolic, already selected against older chemistries [8].

With the use of alternate chemistries and more chemicals of different modes of action environmental impacts will be more, even on the beneficial fauna. Globally changing environmental conditions and concerns are the prime criteria for selecting different chemicals against American bollworm. The occurrence of insecticide resistant strains can be reduced or delayed by reducing the selection pressure, by using alternate insecticides with novel mode of action. The pyrethroids and organophosphorus combination insecticides were found to be effective against the resistant insect pest population of *H. armigera* and *S. litura etc* [9].

Understanding of genetic variation within and between geographical populations of *H. armigera* in the cotton ecosystem and genome-fluxing patterns, coupled with estimating resistance folds to each insecticide can expectedly help in pinning down the exact causes for such frequent outbreaks and versatility in evolving resistance to insecticides at a faster rate. The genetic variation among geographic populations of *H. armigera* collected from the South Indian cotton ecosystem was analyzed using RAPD markers and 12 populations could be classified into two distinct groups [10]. In this regard a better understanding of the genetic differences of polyphagous pest like *H. armigera* can be very useful to understand the structure and population dynamics, their behavior and response to various selection pressures. Elucidation of gene statements responsible for insecticide resistance in *H. armigera* would bring more light in understanding the phenomenon and management of the problem. In the Indian context, a systematic and concerted effort to view the problem of insecticide resistance and cross resistance from this perspective is important.

In India development of resistance by *H. armigera* to chemical insecticides, the high cost of insect control, environmental concerns, legal restrictions on the use of chemicals and frequent outburst of American bollworm suggest that efforts are now needed to understand the basis of insecticide resistance, cross resistance patterns and molecular diversity to formulate the best management strategies accordingly. In the light of the above, the present study was done to determine the insecticide cross resistance pattern associated in spinosad selected *H. armigera* with reference to Spinosyns, pyrethroids, carbamates and oxadiazines.

2. MATERIALS AND METHODS

The present investigation on cross resistance patterns was carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Telangana situated at $17^0 31'$N latitude and $78^0 39'$E longitude from February 2010 to May 2011.

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

At least hundred larvae of *H. armigera* (Hub.) were collected on red gram, cotton and bengal gram crops during February 2010 to May 2011 from Mahaboobnagar, Raichur and Nagpur (Fig. 1).

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

The larvae collected from different locations (100/location) were reared on artificial diet (Plates 1 and 2) in the laboratory as per the procedure outlined by Kranthi, 2005 [11]. 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 with 10larvae/concentration.

Fig. 1. Map showing the *Helicoverpa armigera* collection sites

Plate 1. *Helicoverpa armigera* egg

Plate 2. *Helicoverpa armigera* larvae on artificial diet

2.2.1 Artificial diet preparation for *H. armigera*

The detailed procedure followed Kranthi, 2005 [11].

- 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 3).

2.2.2 Adult maintenance

The adults were allowed to feed on adult diet after emergence and one pair of adults (♂ and ♀) were kept in plastic containers for mating and egg laying. 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^{0}C for 1-2 weeks (Kranthi, 2005) [11]. 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 (Plate 4).

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. 20 adult single pairs were maintained per site as per the procedure explained.

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

The degree of resistance acquired by *H. armigera* of different populations were tested against spinosad (Table 1).

Plate 3. *H. armigera* rearing in the incubator

Plate 4. Adult maintenance

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 carbamoxyloxy) Thioacetimidate	M/S Dupont Chemical (India) Limited, Mumbai-400076
2	Cypermethrin	10 EC	Cypra	(RS)-a-cyno-3-phenoxybenzlyl-(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, Ist 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

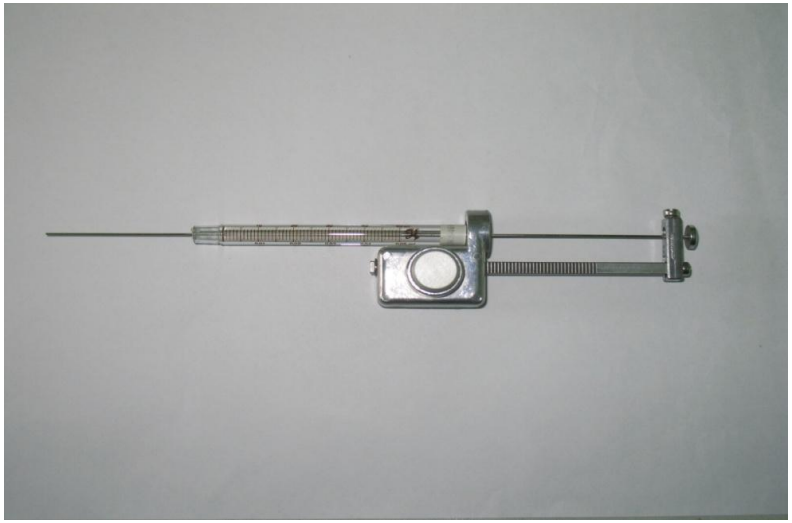

Plate 5. Hamilton Micro applicator

2.3.1 Test insect population

The larvae collected 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 strains 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 5) to evaluate the toxicity of all the test insecticides (FAO, 1971) [12].

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 weighment 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 (Plate 6). 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 [13]). 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 of third instar *H. armigera* larvae of Mahaboobnagar, Raichur and Nagpur populations to the test insecticide was subjected to probit analysis [14] using POLO-PC software [15] 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 strains happened to be parallel [16]. 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 of the strain. Therefore LD_{90} values were also calculated. The degree of resistance acquired by all the three strains was also calculated by comparing the present data with the available baseline data at LD_{50} and LD_{90} levels. The degree of resistance spinosad was calculated by using the baseline data of Nagpur susceptible strain [11] (Table 2).

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 [14].

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, spinosad belongs to spinosyns and indoxacarb belonging to oxadiazine group of insecticides (Table 1).

Plate 6. Application of insecticide on the thorasic segment of third instar larvae

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 [11]
2	Methomyl	Nagpur susceptible	0.030	0.165	Kranthi, 2005 [11]
3	Spinosad	Nagpur susceptible	0.062	0.347	Kranthi, 2005 [11]
4	Indoxacarb	Nagpur susceptible	0.00325	0.1189	Kranthi, 2005 [11]

Resistance factor = LD_{50} of the F_1 resistant population / LD_{50} of the Nagpur susceptible strain

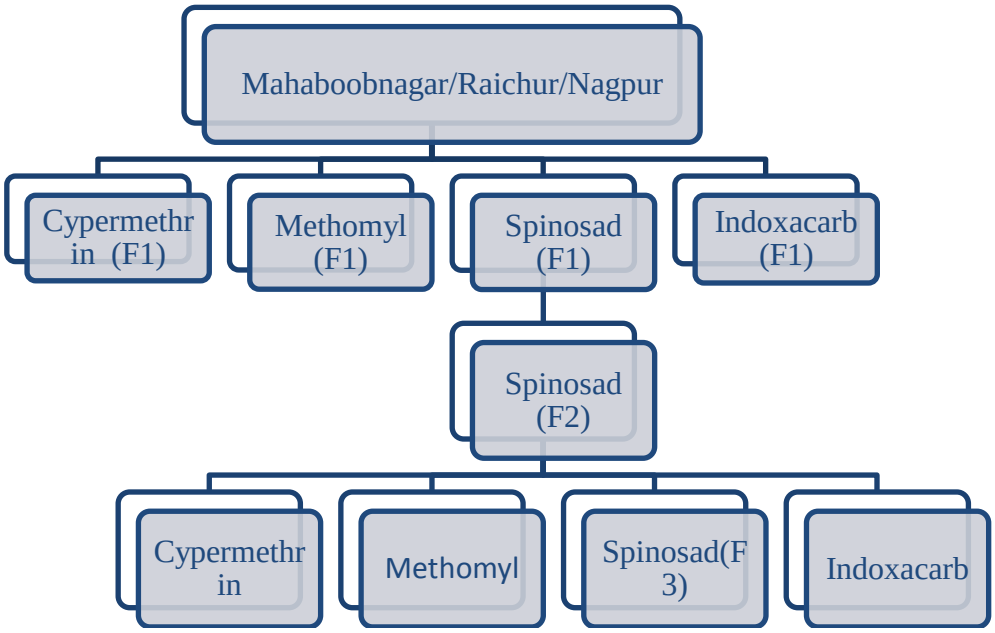

Fig. 2. Bio assay procedure for selected population

2.4.1 Test insect population

The larvae collected from three locations were reared as indicated earlier and were subjected separately to test insecticide spinosad. The survivals at LD_{50} concentration of test insecticide at F_1 (1^{st} generation) were reared separately to next generation (F_2). Male and female pupae were separated and allowed for single pair mating as described earlier. Third instar larvae from single pair mating (2^{nd} generation) F_2 were again subjected to different doses of test insecticide. The survivals at LD_{50} of test insecticide treatment (2^{nd} generation) F_2 were reared separately to next generation (F_3). The survivals in F_2 were reared upto F_3 generation in the same manner of as earlier. 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. The insecticidal treatments were given here under in the flow chart (Fig. 2). The same procedure was followed for all the locations as stated in the flow chart.

The procedure followed for bioassay, topical application and data collection was same as that described in earlier paragraph.

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

The mortality data of *H. armigera* larvae of Mahaboobnagar, Raichur and Nagpur were subjected to probit analysis using POLO – PC software [15].

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 [17].

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

If the CRR ratio is <1 – Negative cross resistance
>1 – Positive cross resistance

3. RESULTS AND DISCUSSION

The results of the present investigation are presented here under in different sections.

3.1 Determination of the Degree of Resistance Acquired by Third Instar Larvae of *H. armigera*

The development of resistance in the third instar larvae of *H. armigera* of Mahaboobnagar (Andhra Pradesh), Raichur (Karnataka) and Nagpur (Maharashtra) to the test insecticide spinosad belongs to spinosyns 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.

3.1.1 Mahaboobnagar (Andhra Pradesh)

The *H. armigera* larvae of the Mahaboobnagar displayed a LD_{50} of 0.308 µg/larva and 0.646 µg/larva at LD_{90} for spinosad (Table 3). The corresponding log dose probit (ldp) line had a slope (b) of 3.976 (Fig. 3). The chi-square test revealed that the population used in the study was homogenous ($p < 0.05$ %).

3.1.2 Raichur (Karnataka)

Raichur population of *H armigera* showed a LD_{50} and LD_{90} values of spinosad as 0.284 and 0.624 µg/larva, respectively (Table 3) with a slope (b) of 3.754 (Fig. 3). The chi-square test revealed that the population used in the study was homogenous ($p < 0.05$ %).

3.1.3 Nagpur (Maharashtra)

Toxicity of spinosad to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 0.183 and 0.497 µg/larva, respectively (Table 3) with a shallow slope (b) of 2.949 (Fig. 3). The chi-square test revealed that the population used in the study was homogenous ($p < 0.05$ %).

Amongst the three populations of *H. armigera*, the population of Mahaboobnagar has developed 1.085 and 1.035 fold relative resistance at LD_{50} and LD_{90}, respectively as compared with the Raichur population. The same Mahaboobnagar population has developed the higher levels of relative resistance by 1.683 and 1.300 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, while Raichur population recorded 1.552 and 1.256 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur population (Table 3).

The present study showed that the results are in conformity with Kranthi et al. [18] who reported that spinosad LD50 as 0.023-0.24 µg/larva and LD90 as 0.27-4.33 µg/larva against H.armigera with a baseline value for spinosad was 0.058 µg/larva. Dayakar and Venkateswarlu [19] indicated high resistance frequencies of 6.28 per cent to 28.03 per cent in the population of H. armigera during the crop season against spinosad. Resistance frequencies recorded with 1.5 µg of spinosad in Prakasam district of Andhra Pradesh ranged between 4.00 to 30.67 per cent. While, Singh and Mahal (2005) reported that the LC50 values for spinosad was 0.40 µg /ml and Suryawanshi et al. [20] reported that the LD50 value of spinosad was 0.0641 µg/larva. Stanley et al. [21] found that the median lethal concentrations (LC50) of spinosad were found to 2.94 ppm.

From the present investigations it is evident that there was a slight increase in the level of resistance to spinosad in H. armigera compared to reports of Stanley et al. [21], Suryawanshi et al. [19] and Singh and Mahal [22], which may be due to significant increase in the use of spinosad in managing the pest in all crop ecosystems.After introduction of spinosad in the market, it is used extensively in all the crop ecosystems. Hence, the present study shown that increased levels of resistance against spinosad.

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

Population	LD_{50} µg/larva	LD_{90} µg/larva	Resistance factor in comparison with				Resistance factor in comparison with Baseline data	
			Raichur population (folds)		Nagpur population (folds)			
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Mahaboobnagar	0.308	0.646	1.085	1.035	1.683	1.300	4.968	1.862
Raichur	0.284	0.624	-	-	1.552	1.256	4.581	1.798
Nagpur	0.183	0.497	-	-	-	-	2.952	1.432
Baseline data (Kranthi, 2005)	0.062	0.347	-	-	-	-	-	-

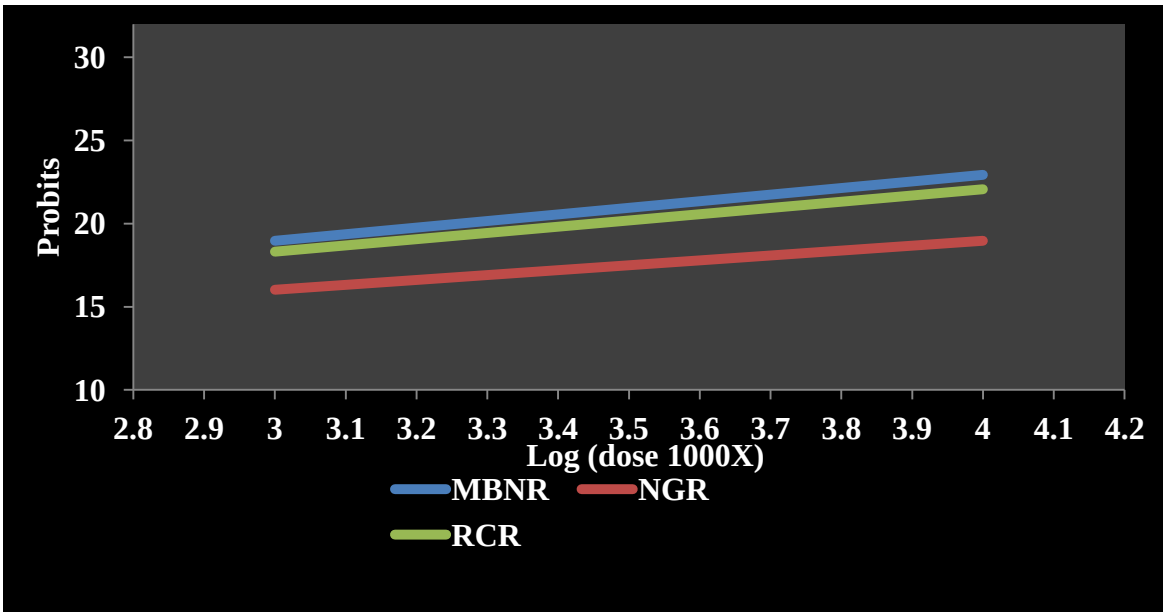

Fig. 3. Log dose probit lines of spinosad against the three populations of *H. armigera* (MBNR – Mahaboobnagar, NGR – Nagpur, RCR – Raichur)

Table 4. Cross resistance pattern in spinosad – spinosad 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	Spinosad	F_1	0.308 (0.249-0.355)	0.646 (0.528 - 0.988)	3.976+ 0.822	0.391	Y = 7.035 + 3.976 X	--
2	Spino - Spino	F_2	0.311 (0.218 – 0.329)	0.336 (0.322 - 0.577)	19.512 ± 18.495	0.492	Y = 25.019 + 19.512 X	1.010
3	Spino – Spino - 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	Spino – Spino - Metho	F_3	2.944 (2.607 – 3.088)	3.279 (3.122 – 3.900)	17.373 ± 9.661	0.805	Y = -7.836 + 17.373 X	0.806
5	Spino – Spino - Spino	F_3	0.320 (0.272 – 0.340)	0.361 (0.339 - 0.454)	14.481 ± 9.059	0.611	Y = 17.118 + 14.481 X	1.039
6	Spino – Spino - Indo	F_3	0.200 (0.149 – 0.215)	0.229 (0.212 – 0.315)	11.307 ± 8.576	0.734	Y = 19.916 + 11.307 X	0.935

**CRR- Cross Resistance Ratio*

Table 5. Cross resistance pattern in spinosad – spinosad 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	Spinosad	F_1	0.284 (0.227 - 0.329)	0.624 (0.525 – 0.837)	3.754 + 0.643	2.105	Y = 7.051 + 3.754 X	---
2	Spino - Spino	F_2	0.293 (0.229 – 0.325)	0.367 (0.330 – 0.508)	13.004 ± 4.398	0.698	Y = 11.938 + 13.004 X	1.032
3	Spino – Spino - Cyper	F_3	29.802 (17.695 – 33.747)	37.402 (33.041 – 64.253)	12.992 ± 5.310	0.818	Y = -14.153 + 12.992 X	0.918
4	Spino – Spino - Metho	F_3	1.972 (0.810 – 2.368)	2.757 (2.295 – 6.551)	8.801 ± 3.687	0.926	Y = 2.405 + 8.801 X	0.543
5	Spino – Spino - Spino	F_3	0.301 (0.232 – 0.334)	0.372 (0.335 – 0.517)	13.973 ± 4.916	0.870	Y = 12.288 + 13.973 X	1.060
6	Spino – Spino - Indo	F_3	0.197 (0.081 – 0.237)	0.276 (0.230 – 0.655)	8.801 ± 3.687	0.926	Y = 11.206 + 8.801 X	0.642

**CRR- Cross Resistance Ratio*

Table 6. Cross resistance pattern in spinosad- spinosad 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	Spinosad	F_1	0.183 (0.141 – 0.219)	0.497 (0.405 - 0.685)	2.949+ 0.449	6.215	Y = 7.176 + 2.949 X	---
2	Spino - Spino	F_2	0.191 (0.149 – 0.210)	0.233 (0.211 – 0.318)	14.720 ± 5.167	0.854	Y = 15.597 + 14.720 X	1.044
3	Spino – Spino - 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	Spino – Spino - Metho	F_3	1.830 (1.129 – 2.183)	2.736 (2.283 – 5.174)	7.337 ± 2.611	1.081	Y = 3.075 + 7.337 X	0.690
5	Spino – Spino - Spino	F_3	0.197 (0.081 – 0.237)	0.276 (0.230 – 0.655)	8.801 ± 3.687	0.926	Y = 11.206 + 8.801 X	1.077
6	Spino – Spino - Indo	F_3	0.110 (0.000 – 0.149)	0.191 (0.141 – 7798.363)	5.326 ± 2.700	1.825	Y = 10.112 + 5.326 X	0.570

**CRR- Cross Resistance Ratio*

3.2 Evaluation of Cross Resistance Pattern in *H. armigera* to Certain Insecticide Molecules

Third instar *H. armigera* larvae from first generation (F_1) with an average weight of 30 mg ± 0.011 S.E. of Mahaboobnagar, Raichur and Nagpur population were subjected separately to different concentrations of the test insecticide and taken to study cross resistance pattern in the population. The results are presented here under.

3.2.1 Mahaboobnagar (Andhra Pradesh)

F_1 generation third instar larvae when subjected to different concentrations of spinosad showed LD_{50} value of 0.308 µg/larva. However the values at LD_{90} rose sharply 0.646 µg/larva to spinosad. The chi-square test revealed that the population used in the study was homogenous ($p<0.05$) (Table 3).

First generation larvae resistant to spinosad were taken and reared to F_2, when subjected to different concentrations of spinosad F_2 recorded LD_{50} values of 0.311 µg/larva. The resistant population of F_2 generation showed LD_{90} values (µg/larva) 0.336 to spinosad. Population resistant to spinosad showed a positive cross resistance ratio of 1.010 to spinosad. The chi-square test revealed that the population used in the study was homogenous (P<0.05%).(Table 4).

The spinosad resistant population selected 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 are presented in Table 4. The LD50 values were 19.716, 2.944, 0.320 and 0.200 µg/larva to cypermethrin, methomyl, spinosad and indoxacarb, respectively. The LD90 values (µg/larva) of cypermethrin, methomyl, spinosad and indoxacarb were 27.571, 3.279, 0.361 and 0.229, respectively.

Larvae resistant to spinosad in F1 and F2 generations when subjected to different test insecticides showed a negative cross resistance ratio of 0.667 to cypermethrin, 0.806 to methomyl, 0.935 to indoxacarb and a positive cross resistance ratio of 1.039 to spinosad at F3.

3.2.2 Raichur (Karnataka)

F_1 generation population when subjected to different concentrations of spinosad showed LD_{50} value of 3.630 µg/larva spinosad. However the values at LD_{90} rose sharply to 0.624 µg/larva to spinosad. The chi-square test revealed that the population used in the study was homogenous ($p<0.05$) (Table 5).

First generation larvae resistant to spinosad were taken and reared to F_2 by single pair mating and the larvae obtained in F_2 were when subjected to different concentrations of spinosad F2 recorded LD_{50} value 0.293 µg/larva. Resistant population of spinosad in the F_2 generation showed LD_{90} values (µg/larva) of 0.367to spinosad. spinosad resistant population showed a positive cross resistance ratio of 1.032 to spinosad .The chi-square test revealed that the population used in the study was homogenous (P<0.05%).

The spinosad 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. The results are depicted in Table 5. The LD_{50} values were 29.802, 1.972, 0.301 and 0.197 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.* 37.402, 2.757, 0.372 and 0.276, respectively.

Larvae resistant to spinosad in F_1 and F_2 generations, when subjected to different insecticides showed a positive cross resistance ratio of 1.060 to spinosad and a negative cross resistance of 0.918 to cypermethrin, 0.543 to methomyl, and 0.642 to indoxacarb at F_3 generation. Among these the insecticide sequences Spinosad-Spinosad-Methomyl was the best.

3.2.3 Nagpur (Maharashtra)

F_1 generation population subjected to different concentrations of spinosad showed LD_{50} value of 0.183 µg/larva. However, the values at LD_{90} were 0.497 µg/larva. The chi-square test revealed that the population used in the study were homogenous (P<0.05%) (Table 6).

First generation larvae resistant to spinosad were taken and reared up to F_2 and the resulting larvae were subjected to different different concentrations of spinosad recorded LD_{50} values of 0.191 µg/larva. Resistant population of spinosad in the F_2 generation showed LD_{90} values (µg/larva) of 0.233. spinosad resistant population showed a positive cross resistance ratio of 1.044 to spinosad . The chi-square test

revealed that the population used in the study was homogenous (P<0.05%).

The spinosad resistant population selected from F_1 and F_2 generation was reared to F_3 generation by single pair mating and subjected to test insecticides, the pattern of cross resistance was explained in Table 6. The LD_{50} values were 12.121, 1.830, 0.197 and 0.110 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, 2.736, 0.276 and 0.191, respectively.

Larvae resistant to spinosad in F_1 and F_2 generations when subjected to different insecticides showed a negative cross resistance ratio of 0.604 to cypermethrin, 0.690 to methomyl, 0.570 to indoxacarb and a positive cross resistance ratio of 1.077 to spinosad in F_3 generation.

It is evident that CRR increased among all the locations when same chemical repeated. Similar trend was followed in Raichur and Nagpur populations. Spinosad-Spinosad rotation of Mahaboobnagar population recorded a CRR of 1.010 and Spinosad-Spinosad-Spinosad rotation recorded a CRR of 1.039.

The *H. armigera* larvae of the Mahaboobnagar recorded a LD_{50} of 0.308 µg/larva and 0.646 µg/larva at LD_{90} for spinosad (Table 3) at F_1. Further they were increased to 0.311 and 0.336 µg/larva at LD_{50} and $LD_{90,}$ respectively at F_2 (Table4). Further, the values were increased to 0.320 and 0.361 µg/larva at LD_{50} and $LD_{90,}$ respectively at F_3 (Table 4). The Raichur population of *H armigera* recorded a LD_{50} and LD_{90} values of spinosad were 0.284 and 0.624 µg/larva, respectively (Table 3) at F_1. Further they were increased to 0.293 and 0.367 µg/larva at LD_{50} and $LD_{90,}$ respectively at F_2 (Table 5). Further they were recorded as 0.301 and 0.372µg/larva at LD_{50} and $LD_{90,}$ respectively at F_3 (Table 5). Toxicity of spinosad to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 0.183 and 0.497 µg/larva, respectively (Table 3) at F_1. Further they were increased to 0.191 and 0.233 µg/larva at LD_{50} and $LD_{90,}$ respectively at F_2 (Table 6). Similarly, they were further increased to 0.197 and 0.276 µg/larva at LD_{50} and $LD_{90,}$ respectively at F_3 (Table 6).

Spinosad resistant population of Mahaboobnagar showed a negative cross resistance ratio of 0.720, 0.824, 0.949 to cypermethrin, methomyl, indoxacarb, respectively and a positive cross resistance of 1.010 to spinosad (Table 4). Similar trend was followed by Raichur showing a negative cross resistance ratio of 0.902, 0.543, 0.642 to cypermethrin, methomyl, indoxacarb, respectively and positive cross resistance ratio of 1.032 to spinosad (Table 5). Further, same trend exihibited by Nagpur population showing a negative cross resistance ratio of 0.610, 0.730, 0.990 to cypermethrin, methomyl, indoxacarb, respectively and positive cross resistance ratio of 1.044 spinosad at F_2 (Table 6).

The results obtained during the present study revealed that continuous application of spinosad insecticide across the generations increases the resistance from F_1 to F_3, However, alternating the old chemicals with new chemicals decreased the development of cross resistance even for the older chemicals like cypermethrin and methomyl. Spinosad followed by cypermethrin or methomyl or indoxacarb indicated negative cross resitance ratio, this implies the efficacy of the molecules intact, Interestingly, spinosad followed by new chemistries like spinosad and indoxacarb showed the cross resistance ratio nearly one indicating future threat of resistance. The sequence of insecticide is very important in future for designing of pest control modules.

It is evident from the literature that the present findings were in accordance with the earlier workers. The findings are in conformity with Ahmad et al. [8], who reported that field populations of *H. armigera* in Pakistan exhibited susceptibility close to the baseline to the new molecule spinosad. This might be due to a cross resistance from the resistance mechanisms, particularly metabolic, already selected against older chemistries. Wang Dong et al. [23] reported that *H. armigera* of Shandong province strain developed more than 20 fold resistance to spinosad after 15 generations of selection in the laboratory. At LD_{50} level, no significant cross resistance was found between spinosad and chlorpyrifos, methomyl, avermectin and chlorfenapyr except for fenvalerate with a low cross resistance of 2.4 fold. The results indicated that resistance to spinosad in the cotton bollworm might be associated with an increase in cytochrome P_{450} monooxygenase.

From the present study it is evident that there is possibility of cross resistance development even for the new chemistries, hence alternating the chemicals in pest control programmes is one of

the best stratagies to postpone the development of cross resistance in the natural population.

4. SUMMARY AND CONCLUSIONS

The experiments were carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh from February 2010 to May 2011 to determine the level of resistance acquired by third instar larvae of *H. armigera* (weighing 30 mg ± 0.011 S.E) from Mahaboobnagar, Raichur and Nagpur to spinosad and the associated cross resistance patterns of insecticide resistant *H. armigera*.

The Mahaboobnagar larvae resistant to spinosad in F_1 and F_2 generations reared to F_3 when subjected to different insecticides showed a negative cross resistance ratio of 0.667 fold to cypermethrin, 0.806 fold to methomyl, 0.935 fold to indoxacarb and positive cross resistance of 1.039 fold to spinosad (Table 4), similar trend was followed in Raichur population displaying a negative cross resistance ratio of 0.918 fold to cypermethrin, 0.543 fold to methomyl, 0.642 fold to indoxacarb and 1.060 fold to spinosad (Table 5). Further the Nagpur population exihibits a similar trend with a negative cross resistance ratio of 0.604 fold to cypermethrin, 0.690 fold to methomyl, 0.570 fold to indoxacarb and positive cross resistance ratio of 1.077 fold to spinosad at F_3 (Table 6).

Mahaboobnagar population has developed 1.085 and 1.035 fold resistance at LD_{50} and LD_{90}, respectively as compared with the Raichur population for spinosad. The same Mahaboobnagar population has developed still higher levels of relative resistance by 1.683 and 1.300 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, while Raichur population recorded 1.552 and 1.256 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur.

The results obtained during the present investigations revealed that the continuous application of same 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 almost all populations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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