Cypermethrin and Methomyl Resistance in *Helicoverpa armigera* (Hubner)

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Abstract

The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 29.125 µg larva^{-1} and 59.609 µg larva^{-1} at LD_{90} for cypermethrin. The LD_{50} and LD_{90} values of cypermethrin for Raichur population of *H armigera* was 32.481 and 38.172 µg larva^{-1}, respectively. Toxicity of cypermethrin to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 20.069 and 54.708 µg larva^{-1}, respectively, The chi-square test revealed that the population used in the study was homogenous ($^{*}p<0.05$%). The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 3.651 µg larva^{-1} and 10.287 µg larva^{-1} at LD_{90} for methomyl. The LD_{50} and LD_{90} values of methomyl for Raichur population of *H armigera* was 3.630 and 10.417 µg larva^{-1}, respectively, while Toxicity of methomyl to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 2.652 and 7.214 µg larva^{-1}, respectively, when the chi-square test revealed that the population used in the study was homogenous ($^{*}p<0.05$%). From, the results obtained we can conclude that the continuous application of same chemistries across the generations increases the resistance from F_1 to F_3. Alternating with the new chemistries results in no cross resistance development as it was observed in all the three populations.

Keywords: *Helicoverpa armigera*, resistance, cypermethrin, methomyl

1. Introduction

The bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide occurrence inflicting annual crop damage in India worth US $1 billion. 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 (Reed and Pawar, 1982; Manjunath, 1990). 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. *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 (Armes et al., 1996; Kranthi et al., 1997; Ramasubramaniam and Regupathy, 2004b). 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 (Zhou et al., 2000).

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* (Martin et al., 2003). To study the resistance levels in the field population present study with cypermethrin and methomyl was conducted.

2. Materials and Methods

2.1. Determination of the insecticide resistance in H. armigera

2.1.1. Collection of H. armigera

The larvae of *H. armigera* were collected on red gram, cotton and bengal gram crops during October, 2006 from Raichur, December-2006 and January, 2007 from Mahaboobnagar and February2010 from Nagpur for conducting the investigation. The work was carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh

2.1.2. Test insecticides

The degree of resistance acquired by *H. armigera* to test insecticides viz., cypermethrin representing synthetic pyrethroids and methomyl representing carbamates group of insecticides (Table 1) were tested.

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

Common name	Formulation	Trade name	Chemical name	Source of supply
Methomyl	40 SP	Lannate	S-methylN-(methyl carbamoxyloxy) Thioacetimidate	M/S Dupont Chemical (India) Ltd, Mumbai-400076
Cyper methrin	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

2.1.3. Test insect population

The larvae collected from Mahaboobnagar, Raichur and Nagpur were reared separately in the laboratory to obtain pupae. Male, 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) 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 insecticides. The survivals at LD_{50} concentration in each test insecticide at F_1 (1st generation) were further used for resistance studies.

2.1.4. Bioassay

Bioassay was done by topical application method using Hamilton micro applicator to evaluate the toxicity of all the test insecticides (FAO, 1971).

2.1.4.1. Topical application method

Initially 1.0% 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 for each of the test insecticides were prepared from the 1.0% 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 10 larvae in each replication.

2.1.5. 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 (LD_{50}) dose in µg μl^{-1}.

2.2. Assessment of the degree of resistance acquired by H. armigera to the test insecticides

The mortality data of third instar *H. armigera* larvae of Mahaboobnagar, Raichur and Nagpur populations of all the test insecticides were subjected to probit analysis (Finney, 1971) using POLO-PC software (Ross, 1987) 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 strain with the lower LD_{50} value of another strain among the three populations for each test insecticide and thus the relative degree of resistance was assessed (Resistance factor = LD_{50} of the resistant strain ÷ LD_{50} of the susceptible strain).

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 to methomyl and cypermethrin were calculated by using the baseline data of Nagpur susceptible strain (Kranthi, 2005) (Table 2).

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

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

Name of the insecticide	Name of strain	LD50 µg larva^{-1}	LD90 µg larva^{-1}	Reference
Cypermethrin	Nagpur susceptible	0.007	0.028	Kranthi, 2005
Methomyl	Nagpur susceptible	0.030	0.165	Kranthi, 2005

3. Results and Discussion

The larvae of *H.armigera* collected from Mahaboobnagar, Raichr and Nagpur selected for cypermethrin and methomyl insecticides at F_1 generation and they were reared to F_2 by single pair mating further proceeded to F_3 by the same single pair method, larvae subjected to these insecticides of different doses at F_2 and F_3, the results were discussed here under.

The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 29.125 µg larva^{-1} which rose sharply to 59.609 µg larva^{-1} at LD_{90} for cypermethrin at F_1, which later still increased to 32.123 and 37.109 µg larva^{-1} at LD_{50} and LD_{90} respectively at F_2 Resistance increased to 35.115 and 42.124 at LD_{50} and LD_{90} respectively at F_3 (Table 3). The LD_{50} and LD_{90}

values of cypermethrin for Raichur population of *H armigera* was 32.481 and 38.172 µg larva^{-1}, respectively at F_1, which were rosed to 35.467 and 41.875 µg larva^{-1} at LD_{50} and LD_{90}, respectively at F_2, further they were increased to 38.906 and 74.202 at LD_{50} and LD_{90}, respectively at F_3 (Table 4). Toxicity of cypermethrin to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 20.069 and 54.708 µg larva^{-1}, respectively at F_1, further they were sharply increased to 23.383 and 32.233 µg larva^{-1} at LD_{50} and LD_{90}, respectively at F_2, later they were increased to 25.054 and 32.224 µg larva^{-1} at LD_{50} and LD_{90}, respectively (Table 5) at F_3

Among the three populations of *H. armigera*, the population of Raichur has developed 1.115 and 0.640 fold relative resistance at LD_{50} and LD_{90}, respectively as compared with the Mahaboobnagar population. The same Raichur population has developed still more higher levels of relative resistance by 1.168 and 0.698 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, while Mahaboobnagar population recorded 1.451 and 1.090 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur. In comparison with base line data of Nagpur susceptible population reported by Kranthi (2005) the three populations of Raichur, Mahaboobnagar and Nagpur acquired 4640.143, 4160.714, 2867.000 folds at LD_{50} and 1363.286; 2128.893 and 1953.857 fold resistance to cypermethrin at LD_{90} levels, respectively (Table 6).

The *H. armigera* larvae of the Mahaboobnagar district recorded a LD_{50} of 3.651 µg larva^{-1} which rose sharply to 10.287 µg larva^{-1} at LD_{90} for methomyl at F_1. Further the values rosed to 3.872 and 5.829 µg larva^{-1} at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 3.934 and 4.668 at LD_{50} and LD_{90}, respectively at F_3 (Table 3). The LD_{50} and LD_{90} values of methomyl for Raichur population of *H armigera* was 3.630 and 10.417 µg larva^{-1}, respectively at F_1.

Table 3: Resistance pattern in Mahaboobnagar population of *H. armigera*

Sl. No.	Strain	Generation	LD50 µg larva^{-1} (95% FL)	LD_{90} µg larva^{-1} (95% FL)	Slope±S.E (b)	Heterogeneity (χ^2)	Regression equation
1.	Cypermethrin	F_1	29.125 (23.867 – 33.325)	59.609 (51.126-76.562)	4.120± 0.669	0.809	Y=-1.033+4.120 X
2.	Cyper-Cyper	F_2	32.123 (12.508 – 35.456)	37.109 (33.641 –97.573)	20.452± 9.654	0.777	Y=-25.818+20.452 X
3.	Cyper-Cyper-Cyper	F_3	35.115 (28.231 – 38.382)	42.124 (38.525–55.876)	16.213± 5.669	0.828	Y=-20.058+16.213 X
4.	Methomyl	F_1	3.651 (2.693 – 4.460)	10.287 (8.259-14.714)	2.849± 0.471	3.333	Y=3.398+2.849 X
5.	Metho-Metho	F_2	3.872 (0.569 – 4.824)	5.829 (4.667–29.441)	7.215± 3.274	0.190	Y=0.758 + 7.215 X
6.	Metho-Metho-Metho	F_3	3.934 (3.285 – 4.261)	4.668 (4.305–5.971)	17.250± 5.818	0.658	Y=-5.261+17.250 X

Table 4: Resistance pattern in Raichur population of *H. armigera*

Sl. No.	Strain	Generation	LD_{50} µg larva^{-1} (95% FL)	LD_{90} µg larva^{-1} (95% FL)	Slope±S.E (b)	Heterogeneity (χ^2)	Regression equation
1.	Cypermethrin	F_1	32.481 (15.858 – 35.904)	38.172 (34.615 – 87.453)	18.279± 8.376	1.091	Y=-22.630+18.279 X
2.	Cyper-Cyper	F_2	35.467 (23.520 – 38.971)	41.875 (38.106 – 62.869)	17.767± 7.332	0.611	Y=-22.537+17.767 X
3.	Cyper-Cyper-Cyper	F_3	38.906 (32.472 – 43.748)	74.202 (64.183-96.176)	4.571± 0.811	0.911	Y=-2.267+4.571 X
4.	Methomyl	F_1	3.630 (2.719 – 4.408)	10.417 (8.357-14.905)	2.799± 0.450	3.321	Y=3.433+2.799 X
5.	Metho-Metho	F_2	3.659 (2.259 – 4.365)	5.471 (4.566 – 10.348)	7.337± 2.611	1.081	Y=0.866 + 7.337 X
6.	Metho-Metho-Metho	F_3	3.851 (3.058 – 4.208)	4.708 (4.297 – 6.485)	14.684± 5.189	0.891	Y=-3.598 + 14.684 X

Table 5 : Resistance pattern in Nagpur population of *H. armigera*

Sl. No.	Strain	Generation	LD_{50} µg $larva^{-1}$ (95% FL)	LD_{90} µg $larva^{-1}$ (95% FL)	Slope±S.E (b)	Heterogeneity (χ^2)	Regression equation
1.	Cypermethrin	F_1	20.069 (15.312–24.138)	54.708 (44.089–78.011)	2.943± 0.477	2.602	Y=1.167+2.943 X
2.	Cyper-Cyper	F_2	23.383 (16.024–26.927)	32.233 (27.885–53.609)	9.193± 3.261	1.004	Y=-7.585+9.193 X
3.	Cyper-Cyper-Cyper	F_3	25.054 (18.153–28.330)	32.224 (28.485–47.966)	11.725± 4.165	0.931	Y=-11.401+11.725 X
4.	Methomyl	F_1	2.652 (2.051–3.174)	7.214 (5.583–11.827)	2.949± 0.555	1.675	Y=3.751+2.949 X
5.	Metho-Metho	F_2	2.844 (2.083–3.199)	3.716 (3.292–5.682)	11.032± 3.906	0.953	Y=-0.007 + 11.032 X
6.	Metho-Metho-Metho	F_3	2.944 (2.607–3.088)	3.279 (3.122–3.900)	27.373± 9.661	0.805	Y=-7.836+27.373 X

Table 6 : Relative degree of resistance among the three populations of *H. armigera* to cypermethrin at F_1

Population	LD_{50} µg $larva^{-1}$	LD_{90} µg $larva^{-1}$	Resistance factor in comparison with				Resistance factor in comparison with Baseline Data	
			Mahaboobnagar population (folds)		Nagpur population (folds)			
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Raichur	32.481	38.172	1.115	0.640	1.618	0.698	4640.143	1363.286
Mahaboobnagar	29.125	59.609	-	-	1.451	1.090	4160.714	2128.893
Nagpur	20.069	54.708	-	-	-	-	2867.000	1953.857
Baseline data (Kranthi, 2005)	0.007	0.028	-	-	-	-	-	-

Further the values rosed to 3.659 and 5.471 µg $larva^{-1}$ at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 3.851 and 4.708 at LD_{50} and LD_{90} ,respectively at F_3 (Table 4). Toxicity of methomyl to Nagpur population of *H. armigera* showed that the LD_{50} and LD_{90} values were 2.652 and 7.214%, respectively at F_1. Further the values rosed to 2.844 and 3.716 µg $larva^{-1}$ at LD_{50} and LD_{90}, respectively at F_2. Interestingly at F_3 the resistance recoded was 2.944 and 3.279 at LD_{50} and LD_{90}, respectively at F_3 (Table 5).

Amongst the three populations of *H. armigera*, the population of Mahaboobnagar has developed 1.006 and 0.988 fold relative resistance at LD_{50} and LD_{90}, respectively as compared with the Raichur population. The same Mahaboobnagar population has developed still more higher levels of relative resistance by 1.377 and 1.426 fold when compared with the Nagpur population at LD_{50} and LD_{90}, respectively, while Raichur population recorded 1.369 and 1.444 fold resistance at LD_{50} and LD_{90}, respectively in comparison with Nagpur. In comparison with base line data of Nagpur susceptible population reported by Kranthi (2005) the three populations of Mahaboobnagar, Raichur and Nagpur acquired 121.700, 121.000, 88.400 folds at LD_{50} and 62.345, 63.133, 43.721 folds resistance to methomyl at LD_{90} levels, respectively (Table 7).

The present results of cypermethrin resistance are in

Table 7: Relative degree of resistance among the three populations of *H. armigera* to methomyl at F_1

Population	LD_{50} µg $larva^{-1}$	LD_{90} µg $larva^{-1}$	Resistance factor in comparison with				Resistance factor in comparison with Baseline Data	
			Mahaboobnagar population(folds)		Nagpur population (folds)			
			at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}	at LD_{50}	at LD_{90}
Raichur	3.651	10.287	1.006	0.988	1.377	1.426	121.700	62.345
Mahaboobnagar	3.630	10.417	-	-	1.369	1.444	121.000	63.133
Nagpur	2.652	7.214	-	-	-	-	88.400	43.721
Baseline data (Kranthi, 2005)	0.03	0.165	-	-	-	-	-	-

approximity with (Kranthi et al., 2001) who reported >1000 folds resistance to cypermethrin in four strains of *H. armigera* of Central India. Singh and Mahal (2005) reported that 2087 fold resistance to cypermethrin. Rao et al., (2005) reported that the synthetic pyrethroids showed high resistance frequencies (>80%) with a LD_{50} value of 8.51 µg $larva^{-1}$ and it had developed highest resistance (946 folds) against cypermethrin. Chaturvedi (2007) was reported that the *H. armigera* exhibited widespread resistance (RF=48-919) to cypermethrin. Suryawanshi et al. (2008) revealed that the synthetic pyrethroid cypermethrin showed high resistance frequencies (84-88%) with a LD_{50} values of 1.399 g $larva^{-1}$ and it had developed higher resistance (279.80 fold) against cypermethrin. Nimbalkar et al. (2009) were reported that the LD_{50} value for cypermethrin was highest, during October (1.459 mg $larva^{-1}$ in Aurangabad) and lowest during August in Parbhani (0.157 µg $larva^{-1}$) during 2008, which might be due to continuous use of cypermethrin to manage this pest. Achaleke et al. (2009) indicated high resistance to cypermethrin (RF=67-1771) among *H. armigera* field populations and laboratory-selected strains. From the present findings the resistance ratio appears to be high because of the comparison with most susceptible strain as base line (LD_{50}=0.007 µg $larva^{-1}$), but in reality it is indicated that there is decrease in the levels of resistance to cypermethrin in *H. armigera* compared to latest reports of Suryawanshi et al. (2008); Nimbalkar et al. (2009) which may be probably due to decreased selection pressure with significant decrease in the use of cypermethrin and increasing the area under *Bt* cotton.

The present results of methomyl resistance are in approximity with Jhansi et al., 2004 according to whom, during early part of the season on cotton the per cent resistance was found almost constant (31.34 to 64.00) and 6-30 folds of resistance to methomyl from Guntur region. Resistance level against methomyl in Khandwa district of Madhya Pradesh was found quite low (2.50%) in the first week of October and then varied from 2.50% to 18.06% during the remaining period of the month. However, the highest resistance level (44.52 %) was recorded during the third week of November (Choudhary et al., 2004). From the present investigations it is evident that there is decrease in the levels of resistance to methomyl in *H. armigera* compared to latest reports of Suryawanshi et al. (2008) which may be probably due to significant decrease in the use of methomyl in managing the pest due to reduced bollworm incidence and due to increased area under *Bt* cotton.

4. Conclusion

The results obtained during 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 for almost all populations.

5. Further Research

Insecticide sequences like Cypermethrin-Cypermethrin-Cypermethrin, Methomyl-Methomyl-Methomyl, Cypermethrin-Methomyl-Methomyl, showed the CRR equal and above 1.0 showing future cross resistance threat and the insecticides like, Indoxacarb, Spinosad rotations may prove to be the better combinations for further study. Future studies on insecticide sequences with technical grade insecticides may give more conclusive results.

Molecular characterization of insecticide resistant *H.armigera* may through light on the nucleotide base level differences of different locations of the resistant individuals through which the resistance is governed.

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