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Pattern of Cross Resistance in Lambdacyhalothrin and Betacyfluthrin Selected Populations of *Helicoverpa armigera* Hub.

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Abstract: In *Helicoverpa armigera*, withdrawal of selection pressure for fourteen consecutive generations resulted in 2.58 and 3.01 fold increase in the susceptibility to lambdacyhalothrin and betacyfluthrin, respectively. Similarly, continuous selection enhanced the resistance level to the extent of 6.77 and 7.14 fold to the respective pyrethroids. Populations selected for resistance to lambdacyhalothrin and betacyfluthrin showed positive cross resistance to all other pyrethroids tested and no cross resistance to endosulfan. The increased level of mixed function oxidases with advancement of generation favoured the positive cross resistance among the pyrethroids.

Key words: *Helicoverpa armigera,* cross resistance, lambdacyhalothrin, betacyfluthrin

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) which was never traced as a major bollworm of cotton in any part of the country before 1986 has become number one agricultural pest in India. The crop loss due to this pest was estimated at 47–90%[1], the monitory value of which was more than Rs 2000 crores ($ 450 m)[2]. To combat the unprecedented pressure from *H.armigera,* farmers in southern peninsular region of India had applied over 30 sprays as against the recommended 8–10 sprays[1]. More than 75% of the insecticides used in cotton are being targeted towards *H. armigera*[3] of which, synthetic pyrethroids constitute 50-70%[4]. This high selection pressure for the past one decade slowly builds up the resistance in *H.armigera* and now it has climbed to a peak of more than 80% to synthetic pyrethroids in the Tamil Nadu State, India[5]. Low to high level of resistance had been detected in *H. armigera* to insecticides for which it had never been exposed earlier. This is due to the phenomenon of cross resistance. So, cross resistance in *H.armigera* is a major threat to present day intensive agriculture and is likely to remain an important aspect in the management strategies for the foreseeable future. The cross resistance pattern observed in lambdacyhalothrin and betacyfluthrin selected populations of *H.armigera* is discussed in this study.

MATERIALS AND METHODS

Development of resistant strains: *H. armigera* larvae collected from the Tamil Nadu State, India were reared on standard chickpea based semi synthetic diet[6] for fourteen consecutive generations. One set of laboratory-reared population was subjected to selection pressure with the synthetic pyrethroids to develop the respective pyrethroid resistant strains. The other set was maintained without exposure to any of the insecticide to serve as susceptible strain. The selection pressure was created by two different methods

I Discriminating doses (lambdacyhalothrin 0.025 μg and betacyfluthrin 0.2 μg-LD_{99} calibrated for susceptible strains in Australia[7]) were applied topically to create selection pressure in the first generation and the doses were gradually increased at the rate of 0.1 μg for each generation up to third.

II The LD_{50} value arrived for F_3 (unselected) generation was subsequently used to create selection in respective resistant populations from F_4 onwards.

The resistant strains were developed by retaining the survivors of the respective synthetic pyrethroids.

Insecticide bioassay: The pyrethroid resistant strains were topically treated with other insecticides of similar and different modes of action to assess the level of cross resistance in F_9 and F_{14} generations.

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Third instar larvae (30-40 mg) were used for bioassay. The required concentrations/discriminating doses prepared from technical grade insecticides were applied on the thoracic dorsum of each insect @ 1.0 µl using Hamilton repeating dispenser. The larvae were allowed to feed on the artificial diet. Minimum of 48 larvae were used per concentration. The treated larvae were kept at 25±2°C for 48 h when mortality was recorded. Larvae were considered dead if they were unable to move in a coordinated manner when prodded.

Enzyme activity in pyrethroid selected populations: The activity of mixed function oxidases (MFO) and carboxyl esterases (CE) was estimated in F_2 and F_{12} generations. MFO assay was conducted following the method of Hansen and Hodgson[8]. Carboxyl esterase was assayed by following the method of Devonshire[9] and the protein estimation was done by the method of Bradford[10].

RESULTS

The LD_{50} of the insecticides to F_1 generation of *H.armigera* was 3.63, 3.52, 2.39, 1.53, 1.02 and 6.36 µg larva^{-1} to fenvalerate, cypermethrin, deltamethrin, lambdacyhalothrin, betacyfluthrin and endosulfan, respectively. Continuous culturing of the population in laboratory without exposure to any insecticide resulted in decline of LD_{50} values to the extent of 2.58 and 3.01 fold to lambdacyhalothrin and betacyfluthrin, respectively when the population advanced to F_{14} (Table 1 and 2). Selection by pyrethroids initially with discriminating doses and subsequently with respective median lethal doses of F_3 unselected population (1.37 and 0.76 µg to lambdacyhalothrin and betacyfluthrin) enhanced the level of resistance to 6.77 fold to lambdacyhalothrin and 7.14 fold to betacyfluthrin by the end of fourteenth generation (Table 1 and 2).

Population selected for resistance to one pyrethroid showed positive cross resistance to all other pyrethroids tested. The extent of cross resistance was 3.98, 3.14, 2.96 and 4.80 fold to fenvalerate, cypermethrin, deltamethrin and betacyfluthrin, respectively in F_{14} population selected by lambdacyhalothrin (Table 3). There was no cross resistance was observed to endosulfan (1.11 fold both in F_9 and F_{14} generations). Betacyfluthrin selected population showed 2.47, 2.69, 3.42, 4.34 and 0.95 fold cross resistance to fenvalerate, cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan, respectively (Table 4).

The MFO activity in lambdacyhalothrin selected second generation was 52.5 n mol min^{-1} mg of protein^{-1} and it was increased to 71.8 n mol min^{-1} mg of protein^{-1} when the generation advanced to F_{12}. Similarly, the MFO activity increased to 68.5 n mol min^{-1} mg of protein^{-1} in F_{12} from 45.3 n mol min^{-1} mg of protein^{-1} in F_2 in the population selected for resistance to betacyfluthrin. Carboxyl esterase activity in the F_2 population selected for resistance to lambdacyhalothrin and betacyfluthrin was 385.3 and 323.5 n mol min^{-1} mg of protein^{-1}, respectively. Successive selection with respective pyrethroids increased the carboxyl esterase activity with the

Table 1: Acute toxicity of lambdacyhalothrin to third instar *H. armigera* over generations

Generation	Regression equation	χ^2	LD_{50}	Fiducial limits	LD_{95}	Fiducial limits	SI / RR
Unselected population							
F_1	0.85+1.30x	0.12	1.53	1.12-2.11	28.35	20.66-38.922	-
F_3	0.85+1.32x	0.96	1.37	1.01-1.85	24.12	17.80-32.693	1.23
F_9	0.25+1.61x	0.02	0.86	0.67-1.10	9.011	7.03-11.551	1.78
F_{14}	0.34+1.92x	0.11	0.59	0.48-0.74	4.270	3.44-5.301	2.58
Selected population							
F_3	1.02+1.82x	0.15	1.99	1.59-2.48	15.84	12.70-19.753	1.29
F_9	1.42+1.69x	1.61	6.26	4.98-7.86	58.76	46.77-73.828	4.07
F_{14}	3.28+2.06x	9.73	10.41	8.52-12.71	65.28	53.44-79.748	6.77

SI: Susceptibility index; RR: Resistance ratio

Table 2: Acute toxicity of betacyfluthrin to third instar *H. armigera* over generations

Generation	Regression equation	χ^2	LD_{50}	Fiducial limits	LD_{95}	Fiducial limits	SI / RR
Unselected population							
F_1	0.14+1.613x	0.58	1.02	0.79-1.30	10.69	8.35-13.69	-
F_3	0.01+1.731x	0.82	0.76	0.60-0.95	6.80	5.43-8.53	1.34
F_9	0.69+1.628x	0.53	0.43	0.34-0.56	4.49	3.52-5.73	2.33
F_{14}	0.24+1.879x	3.24	0.33	0.27-0.42	2.54	2.04-3.16	3.01
Selected population							
F_3	0.92+1.287x	0.19	1.47	1.07-2.03	28.03	20.38-38.54	1.44
F_9	1.43+1.773x	9.82	4.27	3.39-5.38	36.18	28.70-45.61	4.18
F_{14}	2.08+1.833x	0.57	7.29	5.85-9.08	57.56	46.20-71.72	7.14

SI: Susceptibility index; RR: Resistance ratio

Table 3: Cross resistance pattern in lambdacyhalothrin selected population of *H. armigera*

Insecticides	Generation	Regression equation	χ^2	LD_{50}	Fiducial limits	LD_{95}	Fiducial limits	Cross resistance
Fenvalerate	F_9	1.32+1.65x	1.70	6.57	5.20-8.31	64.75	51.23-81.84	1.81
	F_{14}	0.57+1.34x	0.21	14.48	10.74-19.51	244.73	181.58-329.85	3.98
Cypermethrin	F_9	1.56+1.68x	2.44	7.98	6.31-10.08	75.80	59.96-95.83	2.26
	F_{14}	2.06+1.74x	1.65	11.07	8.79-13.93	96.91	76.99-121.98	3.14
Deltamethrin	F_9	0.55+1.49x	0.96	5.24	3.99-6.90	66.46	50.54-87.39	2.19
	F_{14}	0.97+1.55x	0.20	7.10	5.50-9.16	81.46	63.12-105.12	2.96
Beta cyfluthrin	F_9	0.68+1.70x	0.29	2.10	1.66-2.66	19.27	15.23-24.39	2.06
	F_{14}	0.19+1.40x	1.39	4.90	3.70-6.49	72.34	54.63-95.81	4.80
Endosulfan	F_9	0.93+1.54x	0.25	7.06	5.49-9.08	82.29	64.03-105.76	1.11
	F_{14}	2.06+1.83x	0.36	7.05	5.65-8.78	55.44	44.48-69.11	1.11

Table 4: Cross resistance pattern in betacyfluthrin selected population of *H. armigera*

Insecticides	Generation	Regression equation	χ^2	LD_{50}	Fiducial limits	LD_{95}	Fiducial limits	Cross resistance
Fenvalerate	F_9	2.30+1.90x	1.43	7.01	5.69-8.62	51.43	41.80-63.27	1.93
	F_{14}	3.38+2.12x	1.46	9.00	7.46-10.85	53.68	44.53-64.73	2.47
Cypermethrin	F_9	1.38+1.67x	2.34	6.46	5.12-8.15	62.01	49.17-78.21	1.83
	F_{14}	1.93+1.74x	0.56	9.49	7.58-11.87	83.33	66.60-104.27	2.69
Deltamethrin	F_9	0.49+1.46x	1.78	5.56	4.19-7.39	73.60	55.40-97.78	2.32
	F_{14}	1.63+1.69x	2.71	8.19	6.51-10.30	76.63	60.91-96.40	3.42
Lambda cyhalothrin	F_9	1.14+1.75x	2.29	3.18	2.53-4.00	27.62	21.97-34.73	2.07
	F_{14}	2.70+2.01x	2.78	6.67	5.42-8.21	43.73	35.52-53.83	4.34
Endosulfan	F_9	1.34+1.66x	0.66	6.49	5.13-8.21	63.20	49.97-79.93	1.02
	F_{14}	3.09+2.13x	1.13	6.05	4.97-7.37	35.55	29.21-43.28	0.95

advancement of generation (492.5 and 473.5 n mol min^{-1} mg of $protein^{-1}$ in F_{12}).

DISCUSSION

Cross resistance is a potential problem that could limit the effectiveness of any insecticide. Information on development of cross resistance is important in formulating resistant management strategies. Furthermore, cross resistance would reveal information on the mechanism of action and metabolic pathways of insecticides. In the present investigation, the pattern of cross resistance studied in F_9 and F_{14} generations revealed that the population selected for resistance to one pyrethroid extended cross resistance to other four pyrethroids tested.

Of the several types of reactions affecting the primary metabolism of pyrethroids, oxidation by MFO is of considerable importance and often plays a dominant role in determining the toxicity[11]. The trans and cis- methyl positions of acid moiety and 4 - phenyl position are the major sites in pyrethroids susceptible to oxidative metabolism[12]. The results of the monitoring studies conducted since 1993 indicated that the predominant mechanism of pyrethroid resistance in *H. armigera* populations from Tamil Nadu was by the induction of MFO as evidenced by the effective suppression of resistance by MFO inhibitors, piperonylbutoxide, propargyloxypthalimide and pungam oil[5,13-15]. Scott and Georghiou[16] had shown that MFO-mediated resistance is specific to pyrethroids having phenoxy-benzyl group. Since all the five synthetic pyrethroids detected for the level of cross resistance in the current investigation are ester bonded phenoxy-benzyl alcohols, the common MFO-mediated mechanism could be the reason for positive cross resistance observed among the pyrethroids. The enhanced level of MFO activity with the advancement of generation due to pyrethroids selection under laboratory condition also seems to support the above points. The present investigation clearly indicates that the enhanced metabolic degradation of synthetic pyrethroids due to MFO and to certain extent by carboxyl esterases favoured the cross resistance under laboratory selection. The development of cross resistance might be the reason for very high level of resistance to all synthetic pyrethroids observed[5] in *H.armigera* populations of Tamil Nadu State, India.

No cross resistance was observed to endosulfan in both the pyrethroid selected populations. The synthetic pyrethroids act principally on the voltage sensitive sodium channels[17-19] whereas the cyclodienes including endosulfan specifically attacks the picrotoxinin receptor site[20,21]. Thus, theoretically at least there should be no cross resistance between pyrethroids and endosulfan due to differential site of action. The present study in the laboratory also seems to support this.

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