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Magnitude, Mechanism and Management of Pyrethroid Resistance in *Helicoverpa armigera* Hubner in India

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Abstract: The magnitude of pyrethroid resistance was very high throughout India irrespective of the *Helicoverpa armigera* strains. The mechanism of pyrethroid resistance in *H. armigera* varies across the different regions of India, but PBO suppressible resistance (MFO mediated) appeared to be the major mechanism and the role played by carboxyl esterase was only marginal. Nerve insensitivity mechanism was observed in strains from areas where, the selection pressure from pyrethroids was intense over the past one decade. Since the pyrethroid resistance is more aggressive in India, several resistant management strategies were discussed in this review.

Key words: *Helicoverpa armigera,* pyrethroid resistance, mechanism, management

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 monetary value of damage was more than $450 million[2]. To combat the unprecedented pressure from *H. armigera*, farmers in South India had applied over 30 sprays as against the recommended 6-10 sprays[1]. More than 75% of insecticides sprayed over the cotton crop are being targeted towards *H. armigera*[3] of which, synthetic pyrethroids constituted 50-70%[4]. Frequent outbreaks of this national pest on cotton crop led to severe social disturbances, with several reports of suicide by farmers[5]. The failure to control the *H. armigera* was traced to the development of resistance to insecticides[6]. This pest has developed resistance to all the major group of insecticides (synthetic pyrethroids, organophosphates and organochlorines) used against it[7]. The pyrethroid resistance was peak[7] and ubiquitous across the Indian Sub-continent[8]. The magnitude of resistance, the mechanism involved in pyrethroid resistance and the insecticide resistant management (IRM) strategies recommended to overcome the threat posed by *H. armigera* have been discussed in this review.

Magnitude of pyrethroid resistance in *H. armigera* in India: The compilation of pyrethroid resistance data reported for different strains in India is a complicated task. The methodology followed and subsequent discussion of results obtained on pyrethroid resistance varies widely among the insecticide toxicologists. Some entomologists reported the resistance frequency on the basis of discriminating dose assay and the remainder expressed the resistance ratio on the basis of log-dose-probit-mortality (LDPM) assay. In LDPM assay, the stage of the pest used for bioassay and the susceptibility of the standard strain employed to calculate the resistance ratio vary widely among the reports available in India. So, the compilation and comparison among the strains is not possible for the whole country with the existing data. In this review the median lethal dose (LD_{50} to third instar) reported for different strains were compiled and the resistance ratio was calculated using the susceptible Reading strain as standard (maintained at the University of Reading, UK, for atleast 15 years[9] so as to compare the strains from different regions of India without any ambiguity.

Log dose probit mortality assay: The data compiled in this review clearly indicates that the pyrethroid resistance is ubiquitous throughout India. The Raichur strain exhibited very high level of resistance to cypermethrin (2489 fold) followed by Guntur strain (1213 fold) (Table 1). Raichur, the cotton city of India, is known historically for its high intensity use of insecticides in Asia. The number of sprays recorded was 20-25 in Raichur as against 10-12 in Madurai[1]. This intense selection pressure could be the reason for the development of multifold resistance in Raichur strain. The Madurai strain showed comparatively

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Table 1: Magnitude of cypermethrin resistance in *H. armigera* strains from different regions of India (LDPM assay)

Strains	LD_{50} µg larva^{-1}	References	RR
Reading susceptible	0.00	McCaffery *et al.*[34]	
		Kranthi *et al.*[9]	--
Andhra Pradesh		Fakrudin *et al.*[35]	--
Guntur	10.91		1213
Madhira	3.03		338
Nalgonda	9.98		1109
Karnataka			--
Raichur	22.40		2489
Mysore	4.05		450
Dharwad	4.75		529
Maharashtra			--
Nagpur	1.62		181
Nanded	2.33		259
Tamil Nadu			--
Madurai	0.14		16
Kovilpatti	0.19		21
Hissar (Haryana)	1.98	Tripathy and Singh[36]a	221
Varnasi (Uttar Pradesh)	2.00		222

a: Strains assayed in 1997
RR= LD_{50} of Field Strain / LD_{50} of reading susceptible strain

Table 2: Magnitude of pyrethroid resistance in *H. armigera* strains from different regions of Tamil Nadu (LDPM assay)

Strains	LD_{50} µg larva^{-1}	References	RR
Cypermethrin			
Reading Susceptible	0.009	McCaffery *et al.*[34]	--
		Kranthi *et al.*[9]	
Coimbatore	3.527	Ramasubramanian[10]a	392
Thondamuthur	5.400	Tamilselvi[18]b	600
Kannivadi	5.200		578
Pongalore	4.500		500
Fenvalerate			
Reading Susceptible	0.016	Kranthi *et al.*[9]	--
Coimbatore	3.638	Ramasubramanian[10]	227
Thondamuthur	5.400	Tamilselvi[18]	338
Kannivadi	5.600		350
Pongalore	3.900		244
Deltamethrin			
Reading Susceptible	0.001	Kranthi *et al.*[9]	--
Coimbatore	2.399	Ramasubramanian[10]	2399
Thondamuthur	3.500	Tamilselvi[18]	3500
Kannivadi	1.900		1900
Pongalore	4.300		4300
Lambdacyhalothrin			
Reading Susceptible	0.004	Kranthi *et al.*[9]	--
Coimbatore	1.539	Ramasubramanian[10]	385
Thondamuthur	3.500	Tamilselvi[18]	875
Kannivadi	1.900		475
Pongalore	4.300		1075

a: Strains assayed in 2002; b: Strains assayed in 2001
RR= LD_{50} of Field Strain / LD_{50} of Reading Susceptible Strain

lower level of resistance (16 fold) to cypermethrin in the compiled data.

Among the four synthetic pyrethroids, the extent of resistance was comparatively higher to deltamethrin. The resistance level varied from 1900 to 4300 fold in different strains of Tamil Nadu state (Table 2).

Discriminating dose assay: The pyrethroid resistance was at peak irrespective of the locations in India (Table 3). The resistance frequency was more than 85% to cypermethrin, fenvalerate, deltamethrin and lambdacyhalothrin in all the locations of Tamil Nadu state, India (Table 4). The very high level of resistance to all the synthetic pyrethroids irrespective of the locations indicated the possibility of cross resistance. The laboratory studies conducted at Tamil Nadu Agricultural University, Coimbatore confirmed the positive cross resistance among the pyrethroids. The populations selected for resistance to one pyrethroid exhibited positive cross resistance to all other pyrethroids tested[10,11].

Mechanism of pyrethroid resistance in *H. armigera* in India: Physiological and biochemical mechanisms of pyrethroid resistance can be categorised into three types; delayed penetration, enhanced metabolism and nerve insensitivity[12]. The penetration resistance has generally been considered to be of minor importance[13]. Moreover, the work on this aspect of penetration resistance is almost nil in India.

Enhanced metabolism

Enhanced enzyme activity: The metabolic resistance (oxidases and esterases) was observed to be an important mechanism mediating pyrethroid resistance in *H. armigera* populations in India[14]. The simplest explanation for the greater response of the oxidative over the nerve insensitivity resistance mechanism could be due to differential genetic dominance. The oxidative pyrethroid resistance mechanism in *H. armigera* had shown to be semi dominant, while the nerve insensitive gene is recessive[15-17]. The monitoring survey carried out by Kranthi *et al.*[9] during the 1995-1999 cropping season in seven states of India (Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Punjab, Haryana and Uttar Pradesh-which together account for approximately 80% of the total cotton growing area and 70% of the total insecticide use on cotton in the country) revealed that the activities of mixed function oxidases (MFO) and esterase were significantly higher in approximately 50 and 75% of the strains respectively. Very high level of MFO activity (>300 p mol mg protein^{-1} of the tissue supernatant) was observed in the strains from Karimnagar, Warangal, Rengareddy, Bangalore and Coimbatore districts of South India. The enhanced activity of MFO in Coimbatore strain of *H. armigera* had also been reported by other workers[10,18]. The MFO titre was found to be comparatively higher in almost all the strains assayed when compared to the susceptible Reading strain with the exception of Nagpur and Guntur strains, which were on par with the Reading susceptible strain. However,

Table 3: Magnitude of cypermethrin and fenvalerate resistance in *H. armigera* strains from different regions of India (Discriminating dose assay)

Strains	Cypermethrin 0.1 μg larva^{-1}	Cypermethrin 1.0 μg larva^{-1}	Fenvalerate 0.2 μg larva^{-1}	Reference
Hyderabad (Andhra Pradesh)	86.9	60.6	88.5	Armes and Jadhav[28] a
Guntur (Andhra Pradesh)	98.1	69.4	95.6	Rao *et al.*[37] b
Raichur (Karnataka)	--	65.0	73.8	Lingappa and Basavanagoud[38] b
Dharwad (Karnataka)	--	47.6	54.8	
Nagpur (Maharashtra)	88.4	56.0	84.3	Kranthi *et al.*[14] c
Akola (Maharashtra)	56.3	30.3	66.8	Tikar *et al.*[39] d
Hissar (Haryana)	91.5	79.2	87.5	Tripathy and Singh[36]
Varnasi (Uttar Pradesh)	91.7	83.3	90.1	

a: Strains assayed during 1996 b: Strains assayed during 1995 c: Strains assayed in 1997 d Strains assayed during 1998-99

Table 4: Magnitude of pyrethroid resistance in *H. armigera* strains from different regions of Tamil Nadu (Discriminating dose assay)

Strains	Cypermethrin (0.1 μg larva^{-1})	Fenvalerate (0.2μg larva^{-1})	Deltamethrin (0.0125 μg larva^{-1})	Lambda cyhalothrin (0.025 μg larva^{-1})	Betacyfluthrin (0.2 μg larva^{-1})	Reference
Coimbatore	96.7	95.0	91.9	91.5	81.8	Ramasubramanian and Regupathy[7] a
Madukarai	93.3	93.4	88.9	86.3	81.4	
Poluvampatty	97.4	97.2	95.4	91.9	89.4	
Thondamuthur	96.3	95.7	93.3	95.8	72.0	Tamilselvi[18] b
Kannivadi	94.6	95.8	90.9	94.4	83.3	
Pongalore	93.7	89.7	92.5	95.1	77.1	
Kallapuram	91.4	89.6	-	92.3	-	Niranjankumar[40] c
Vaigai Dam	91.7	90.8	-	92.2	-	
Srivilliputtur	90.2	89.6	-	89.7	-	

a: Strains assayed during 2002-03 b: Strains assayed during 2001 c: Strains assayed during 2001-02

Table 5: Synergistic suppression of pyrethroid resistance in *H. armigera* strains of Tamil Nadu State, India

Pyrethroids	PBO	Pungam oil	Profenofos	Reference
Cypermethrin	47.2-57.5	40.5-52.3	17.0-24.4	Ramasubramanian[10]
Fenvalerate	52.5-64.5	36.5-50.9	20.4-28.1	
Deltamethrin	27.3-42.9	27.2-47.6	15.9-28.6	Tamilselvi[18]
Lambda cyhalothrin	41.8-73.8	35.7-76.2	11.6-28.6	
Betacyfluthrin	68.6-88.9	54.3-80.6	30.5-57.6	

the Nagpur strain exhibited the highest activity of carboxyl esterases (5.30 m mol min^{-1} mg protein^{-1}), indicating the predominance of esterase mediated metabolic resistance.

Synergistic suppression: One of the easiest and fastest ways to gain preliminary information about possible mechanism of resistance is by the use of synergists. Hence, they are frequently utilised as indicators of the possible metabolic pathways and biochemical mechanism involved in resistance[19]. Synergists act by inhibiting specific detoxication enzymes. The synergistic suppression studies with MFO inhibitors (piperonylbutoxide and pungam oil) and carboxyl esterase inhibitor (profenofos) over a decade clearly demonstrated the predominance of MFO mechanism (Table 5) in imparting resistance to *H. armigera* strains from Tamil Nadu state, India[17,18,20-22]. Armes[23] also concluded that the mechanism of pyrethroid resistance in *H. armigera* varies across the different regions of India, but PBO suppressible resistance (MFO mediated) appeared to be the major mechanism and the role played by carboxyl esterase was only marginal.

Enzyme induction: Brattsten[24] pointed out that the maintenance of high levels of microsomal oxidases is an energy expensive task for the insects and argued that induction provides energy economies for activating the detoxication system only when it is needed. This argument had been proved from the studies with *H. armigera* strains of Tamil Nadu State, India. All the five synthetic pyrethroids were found to induce the MFO activity when they were applied topically to the third instar larvae of *H. armigera*. The induction of MFO was found to be higher in cypermethrin selected population and lower in betacyfluthrin selected population[10,18].

Scott and Georghiou[25] had shown that the MFO-mediated mechanism is specific to pyrethroids having phenoxy-benzyl group. Since, all the five synthetic pyrethroids (fenvalerate, cypermethrin, deltamethrin, lambdacyhalothrin and betacyfluthrin) generally used in the Indian subcontinent against *H. armigera* are ester bonded phenoxzy-benzyl alcohols, it is not surprise that MFO-mediated mechanism is the dominant one which dictates the resistance in *H. armigera*. The positive cross resistance observed among all the five synthetic pyrethroids in the laboratory also seems to support the involvement of common MFO-mediated mechanism[10].

Nerve insensitivity mechanism: The MFO and Carboxyl esterase activities of Guntur strain were observed to be low (MFO-183 and 187 p mol mg protein^{-1}; CE-1.24 and 1.33 μ mol min^{-1} mg protein^{-1}) and on par with the susceptible strain of Reading University (MFO 172-212 p mol mg protein^{-1}; CE 1.39-1.67 μ mol min^{-1} mg protein^{-1}); whereas, nerve insensitivity to cypermethrin was the highest in a strain from Guntur[9]. Brattsten[26] suggested that the metabolic resistance is usually selected first as it involves changes in a system that is already designed for defense against toxic chemicals, whereas, target site resistance is more difficult to acquire because it involves changes in a crucially important process that is optimized through evolution. Thus altered target site resistance, such as nerve insensitivity, should evolve later and probably only after prolonged and intense selection pressure[27]. This suggestion had been strengthened by the *H. armigera* strain from Guntur where the selection pressure from pyrethroids was intense over the past one decade.

Management of pyrethroid resistance in *H. armigera* in India: Since the pyrethroid resistance is more aggressive in India, the insecticide resistant management strategies suggested here under are mostly focussing on the pyrethroid resistant management.

1. Regular monitoring should be carried out to detect the extent of resistance to different insecticides used against *H. armigera.*
2. Since, the pyrethroid resistance in *H. armigera* is ubiquitous[28] across the Indian subcontinent, frequent application of synthetic pyrethroids may be discouraged to reduce the selection pressure.
3. Since, the over dependence of synthetic pyrethroids which constituted 50-70% of the insecticides sprayed over the cotton crop[4] for the past one decade resulted in peak level of resistance in *H. armigera*[7], the rotation of insecticides with different modes of action may be preferable than over dependence of a particular chemistry. The reversion of resistance observed due to withdrawal of selection pressure from a particular insecticide group[10,41] also discourages the constant pressure with a single group of insecticides.
4. The new insecticide indoxacarb (the only representative of oxadiazines) may be considered as one of the components in insecticide rotation strategy to manage the resistant cotton bollworm, *H. armigera.* The enhanced level of carboxyl esterases in pyrethroid resistant populations was reported to activate the indoxacarb into its more toxic metabolite DCJW (decarbo methoxyllated JW-64)[29]. This might be the reason for the increased susceptibility of *H. armigera* populations resistant to synthetic pyrethroids. The negative cross resistance noticed in the pyrethroid selected populations of *H. armigera* also seems to support this recommendation. Indoxacarb (100 g a.i ha^{-1}) was found to be effective in managing the pyrethroid resistant field populations of *H. armigera* in Tamil Nadu[10].
5. The pyrethroid resistant population of India was reported to be highly susceptible to spinosad at its discriminating dose of 10 μg larva^{-1}[7]. Hence, careful rotation of spinosad in the IRM strategies of *H. armigera* may be advisable to manage the resistant bollworms. The unique mode of action of spinosad[30], which is different from the modes of action of other available insecticides, may also delay the possibility of cross resistance[10]. The use of new chemistries spinosad and indoxacarb had been proposed as a component of *H. armigera* IRM strategy in Pakistan[31].
6. Since, the MFO activity is directly proportional to the age of the larvae[32], targeting pyrethroids to egg hatch will avoid the detoxification of pyrethroids by MFO.
7. Insecticides possessing both ovicidal and larvicidal actions may be included in the IRM programme.
8. Hand picking and destruction of grownup larvae may be suggested as one of the components in IRM of *H. armigera* in India, where the human resource is abundant.
9. The use of predators and parasitoids may give better management in perennial crop ecosystem and also if the land holdings are large like in developed countries whereas, in developing countries like India wherein more than 75% of the farmers are small or marginal farmers having less than two hectares of land that too not in a continuous stretch, the recommendation of entomophages may not be viable due to dispersal behaviour. Hence, it is essential to go for community approach, but achieving cooperation among the farmers is very difficult since their choice of crop, socio-economic condition and subsequent management aspects vary largely among the Indian farmers. This might be the reason why; the extension functionaries could not convince the Indian farmers even after a prolonged effort hence, it is suggested that instead of going for augmentative release of entomophages, maintaining the natural enemy population by spraying with safer insecticides is preferable.

10. To conserve the natural enemy complex in the cotton eco system, the foliar spray may be replaced with seed treatment chemicals to manage the early season sucking pests. This may avoid the direct contact of natural enemies with insecticides
11. One of the insecticide resistant mechanisms is the absorption of insecticides by the fat bodies because of their lipophilic nature

So that the toxic amount of insecticides cannot reach the target site. It is a known fact that nuclear polyhidrosis viruses multiply mostly in fat bodies. The resistant populations have more fat bodies; this will help in quick and fast multiplication of the virus. Hence, application of NPV at 500 larval equivalents ha^{-1} with jaggery and teepal® as spray adjuvant [33] may be encouraged to manage the pyrethroid resistant populations of *H. armigera*.

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