Insecticide Resistance Mechanisms in Indian *Helicoverpa armigera* (Hübner)

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ABSTRACT

The cotton bollworm Helicoverpa armigera (Hübner), has developed resistance to almost all groups of insecticides used for its management in India. Studies using biochemical assays and synergists over the past five years on laboratory and field populations indicate that Helicoverpa displays a range of enzymes/isozymes, some of which are unique to the resistant strains. Neurophysiological studies indicate that nerve insensitivity contributes to resistance in individuals assayed in most field strains tested. Pyrethroid resistance was found to be mediated through increased activity of mixed function oxidases or esterases and also to some extent, by nerve insensitivity. Resistance to organophosphates was mainly due to high esterase activity, while preliminary evidence indicates that certain specific esterase isozymes also contribute to resistance against endosulfan.

Introduction

The cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is the major pest of cotton and legumes in India. In recent years, management of this pest has become increasingly difficult due to its development of resistance to most chemical classes of insecticides commonly used in the country (Armes *et al.*, 1992, 1994 and 1996). Tactics used to manage resistance will largely depend on the extent of information available on the development of resistance, cross-resistance patterns; physiological and biochemical mechanisms and genetics, in the species concerned. Studies to understand heritability, stability, and mechanisms of resistance are, therefore, fundamental to any research programme that aims to provide strong leads for an effective resistance management strategy. Moreover, from studies on insecticide resistance, it is also understood that *H. armigera* adapts to toxins through complex mechanisms (Kranthi *et al.*, 1997). Resistance mechanisms in Indian strains of *H. armigera* are not yet fully understood. Information available so far from biochemical and synergist studies suggests that in the majority of cases, metabolic resistance is the major mechanism (Phokela and Mehrotra., 1989, Kranthi *et al.*, 1997), while nerve insensitivity (West and McCaffery, 1992) and reduced penetration (Kumari *et al.*, 1995) were also found to be present to varying degrees in our laboratory over the past five years.

Materials and Methods

Insects

Helicoverpa armigera larvae were collected from different cotton growing regions in India from various crops during the cropping seasons of 1994-98. The larvae were reared individually on a chickpea based semisynthetic diet in 75 ml cells of 12 well tissue culture plates. Laboratory cultures were established for each strain from 200-400 moths. Alan McCaffery, (The University of Reading, UK) through Nigel Armes, (ICRISAT, India), kindly provided an insecticide susceptible strain. This strain, originally collected in Southern Africa had been maintained at Reading for at least 15 years. Endosulfan and originating from Nanjing Agricultural University, China and organophosphate susceptible strains from Pokhara, Nepal were provided by Nigel Armes. Resistance levels in some field strains collected from Nagpur and Wardha in Central India were found to be low. These strains were maintained at CICR, Nagpur for at least six generations. The Nagpur and Wardha strains were subjected to increasing selection pressure in the laboratory with a range of insecticides for at least eight generations to obtain resistant strains.

Insecticides and chemicals

The following technical grade insecticides were used for bioassays: cis:trans (c. 50:50 ratio) cypermethrin (900g /kg ; Zeneca Agrochemicals, UK); endosulfan (940 g/kg; Excel Industries India); quinalphos (720g/kg: Sandoz, India); profenofos (940 g/kg ; Ciba Geigy, Switzerland). Piperonyl butoxide (Pbo) (900 g/kg), was obtained from Goodeed Chemical Co., UK. All other chemicals were of high purity and obtained from either Sigma Chemicals, USA or Hi-media Chemicals, Bombay.

Discriminating dose assays

Once the larvae attained 30-40mg (third-fourth-instar), they were randomly assigned to the following topical application treatments following methods in Kranthi *et al.*, (1997): cypermethrin 0.1 µg per larva (approximations to the LD_{99} values for pyrethroid susceptible *H. armigera*,). In addition the following synergists were applied to larvae in combination with

cypermethrin: 0.1µg Pbo 50.0 µg per larva (to determine the extent of Pbo suppressible oxidase mediated pyrethroid resistance), profenofos 0.1 µg per larva (to determine the extent of profenofos-suppressible esterase mediated pyrethroid resistance) and diethyl maleate (DEM) 50.0 µg per larva (to estimate the role of glutathion-s-transferases (GST). Pbo and profenofos were applied as pre-mixes with cypermethrin. DEM, was applied 15-20 min prior to the cypermethrin. When applied alone, none of the synergists caused mortality (Kranthi, unpubl. data). At least 100 (usually 120-250) larvae were treated topically on the dorsal thorax with 1.0µl of one of the insecticide treatments. Larval mortality was assessed at six days after treatment.

Log dose probit assays

Once established, the field strains were maintained for at least 3-4 generations and used for bioassays. Serial dilutions of technical insecticides or synergists in acetone were applied as 1.0 µl drops (by Hamilton repeating dispenser), to the thoracic dorsum of at least 40 larvae at each of five or more concentrations. Larvae were held individually in 12-well tissue culture plates containing semi-synthetic diet for six days when mortality assessments were made. Larvae were considered dead if they were unable to move in a co-ordinated manner when prodded. All rearing and bioassay operations were carried out at 25 $(+2)_0$ C under a 12:12h light: dark regime**.**

Enzyme assays

Three replicates each of at least twenty 80-100 mg, fourth instar larvae were dissected and their midguts removed. Dissections were carried out in ice-cold sodium phosphate buffer (100mM pH 7.6) containing potassium chloride (11.5 g per litre). Fat bodies and food particles were removed from the midguts, which were then homogenized individually in fresh sodium phosphate buffer containing 1mM each of EDTA, phenyl thiourea and phenyl methyl sulfonyl flouride and glycerol (200 g per litre). The homogenate was centrifuged at 10,000g for 15 min at 0_0 C and the resultant post-mitochondrial supernatant used as the enzyme source. Protein was estimated according to Lowry *et al*. (1951) using BSA (typeV) as standard.

Cytochrome p450 was determined from the dithionite reduced CO difference spectrum method described by Omura and Sato (1964) using a molar extinction coefficient of 91/mM /cm.

For esterase determination, five millilitres of 0.3mM naphthyl acetate in 40mM sodium phosphate buffer (pH 7.0) was incubated with 1.0ml of gut homogenate supernatant containing 5µg of protein, at 30_0 C for 10 min. The reaction was stopped by addition of 1.0 ml of a freshly prepared aqueous solution containing fast blue BB salt (2.8 g /litre). Absorbance at 590 nm was read against blanks and the activity quantified using naphthol as the standard.

For Glutathione-S-transferase determination, the assay mixture consisted of 50 µl of 50 mM 1-chloro-2,4-dinitrobenzene (CDNB) (in 95% ethanol), 150 µl of 50 mM reduced glutathione (GSH) and an aliquot (3.0ml) of enzyme source containing protein (80 µg) in sodium phosphate buffer (100mM ; pH 7.6) and phenyl thiourea (0.1 mM). The assay was conducted at $25(+1)_0$ C. Change in absorbance at 340 nm was recorded and the amount of GSH conjugate formed was calculated using an extinction coefficient equal to 9.6 /mM / min.

A double beam UV spectrophotometer (HitachiU-2000) was used for protein estimation and all enzyme assays. Enzyme activity is expressed as per mg protein.

Neurophysiological assays were carried out according to McCaffery *et al.* (1997). The effect of *cis*-cypermethrin on the spontaneous multi-unit activity of nerves from 30-35 third –instar larvae of each strain was measured, using the cumulative dose response assay. Third-instar larvae (30-40 mg) were decapitated, opened dorso-medially and pinned out under saline on a layer of Sylgard resin (Dow Corning, UK) to expose the nervous system. A peripheral nerve was picked up with a 27-guage stainless steel, suction recording electrode with an insulated outer coating. A stainless steel entomological pin grounded the preparation and served as a reference electrode. Extra-cellular neuronal activity was amplified and filtered with a high gain low noise front end amplifier and conditioning system (Neurolog Digitimer, UK) before relay to an "AxoScope version 1.1" on Windows '95 for data recording and analysis. Neural activity was monitored on an oscilloscope. Simultaneously occurring action potentials were discriminated from background noise above a visually adjusted threshold and were counted and recorded by computer in 15-s epochs in blocks of 5-min periods. Nerve preparations were first bathed for 5-min in saline, followed by successive 5-min perfusions of saline containing step–wise increasing doses of *cis*-cypermethrin. Technical cypermethrin dissolved in analytical grade acetone at 1 mM was diluted in lepidopteran saline to get a final range of concentrations of 10^{-9} to 10^{-6} M. Saline containing 0.1% acetone was also tested periodically as a control.

The end point of the assay was defined as the lowest concentration of *cis*-cypermethrin at which the frequency of action potentials was over five times greater than the mean value during the pre-treatment control period (typically 5-40 Hz).

For each set of assays for each strain, about 25-40 individual larvae were tested and EC_{50} for the effect of cypermethrin on nerve sensitivity was determined by probit analysis using Polo-PC.

Statistical analysis

Weekly pooled binomial standard error for discriminating dose assay data was calculated as follows:

$$\text{Weekly pooled binomial standard error} = \sqrt{\frac{P(1-p)}{n-1}}$$

Where p= proportion of surviving larvae

n = total number of larvae dosed

Dose-mortality regressions were computed by probit analysis (Finney, 1971). When required, corrections for control mortality were made using Abbott's formula (Snedecor and Cochran, 1989). Control mortality never exceeded 2%. Where appropriate, significance of differences between treatments were determined by the Student's t-test (Snedecor and Cochran, 1989)

Results and Discussion

Laboratory selection

Pyrethroids

Cypermethrin selection pressure for nine generations resulted in an elevated level of 90 fold resistance in the laboratory strain (Table 1). Pbo and profenofos enhanced toxicity of cypermethrin by about five and eight fold, respectively, indicating a significant role of metabolic mechanisms in resistance. The glutathione transferase inhibitor DEM did not have much impact on pyrethroid toxicity. *In vitro* assays for detoxification enzymes (Figure 1) showed that the selected strains had higher levels of cytochrome P450 and esterases compared to the susceptible strains.

Endosulfan

Cyclodiene bioassay results and effect of synergists on toxicity in endosulfan selected and field strains, are summarized in Table 4. Endosulfan selection pressure for nine generations resulted in enhanced resistance levels of 27 fold, as compared to the Nagpur susceptible strain. All field strains assayed were resistant to endosulfan (2.5-16 fold). In all cases slopes were lower than that recorded for the susceptible, indicating heterogeneity of endosulfan resistance in field populations. In the synergist assays, pbo and DEM were found to reduce toxicity of endosulfan in the selected strain by 2-3.5 times and profenofos synergized endosulfan by 6.6 times. In the field strains, Pbo lowered the toxicity of endosulfan by a factor of about 1.3 to 2.8 times, whereas synergism with profenofos ranged between 2.5 to 9 fold. Pbo was also reported to be antagonistic to endosulfan toxicity in *Spodoptera littoralis* (Boisd) (Guirguis *et al.,* 1980). The endosulfan resistance selected strain was found to have significantly reduced cytochrome P450 and elevated esterase activities as compared to the susceptible strain. The evidence obtained points to the possibility of an *in vivo* activating role for mixed function oxidases in endosulfan toxicity and a detoxification role for esterases. Although a less sensitive target site on the GABA receptor ionophore complex has been shown to be the major mechanism of cyclodiene resistance (French–Constant *et al.,* 1993), with results of Pbo antagonism and profenofos synergism of endosulfan, it appears likely that metabolic enzymes also contribute to resistance, albeit less significant compared to target site insensitivity. However, in the endosulfan resistance selected strain, none of the synergists provided high levels of suppression of resistance, suggesting that metabolic mechanisms are probably secondary to one or more other resistance mechanisms, presumably target site cyclodiene resistance. It has been suggested (Kern *et al.,* 1990) that GST's play a significant role in endosulfan resistance in *H. armigera*. However, in our tests we found a negative correlation between GST activity and endosulfan resistance and DEM was a potent inhibitor of endosulfan toxicity. GST levels were in fact higher in our endosulfan susceptible strains than the resistant strain.

The results however have a significant bearing on the strong likelihood of cross resistance between cyclodienes and organophosphates in *H. armigera*, as has been reported in other insect species (Bauernfiend and Chapman, 1985, Hoy and Cave, 1989). In a discriminating dose resistance monitoring programme carried out in India during 1993-98, it has been shown that weekly quinalphos resistance profiles consistently follow the same trends as that of endosulfan (Kranthi unpubl. data), strongly supporting the likelihood of cross resistance. Further, as phosphorothionate organophosphates are activated by oxidative transformation to more toxic oxons, it is likely that oxidative metabolism of endosulfan and subsequent hydrolytic conversion to non-toxic conjugates could utilize the same metabolic resistance mechanism pathways as organophosphate detoxification.

Organophosphate

Laboratory selection for seven generations with quinalphos (Table 1) did not result in significantly high resistance levels, compared to the original Nagpur strain.. Pbo application resulted in a reduction of quinalphos toxicity by 10 to 30 fold, whereas profenofos enhanced toxicity by about 2.5 fold in all strains assayed (Table 1). Comparison of detoxification enzyme assays of the quinalphos susceptible and the resistance selected strain (Figure 1) indicated an enhanced activity of esterases and a decrease in cytochrome P450 content in the selected strain. Antagonism of quinalphos toxicity with Pbo indicates oxidase mediated bioactivation of quinalphos. The phosphorothionate group of organophosphates, to which quinalphos belongs, are known to be activated by oxidases to more toxic metabolic intermediates, hence the antagonism with oxidase inhibitors such as Pbo, is not surprising. Resistance due to elevated esterase activity, as found in our studies, has been reported in *H. armigera* recently (Zhao *et al.,* 1996). The elevated esterase

activity was shown to be responsible for cross resistance to OPs, carbamates and pyrethroids (Ibid.). Elevated esterase activity in field strains of *Heliothis virescens* (Fab) collected in Lousiana were implicated in resistance to profenophos (Harold and Ottea, 1997). Organophosphate insensitive acetyl cholinesterases was reported as one of the major mechanisms of resistance in *Heliothis virescens* (Brown *et al.,* 1996, Harold and Ottea., 1997) but was not been found to be important in these studies with *Helicoverpa armigera* (unreported).

The glutathione transferase inhibitor DEM did not exhibit strong synergism with quinalphos. Moreover, the activity of glutathione transferases was almost the same in the resistant and susceptible strains. Resistance to profenophos in *Heliothis virescens* could not be attributed to GST (Konno *et al.*, 1989), while a moderate correlation of resistance with GST activity, only with the substrate CDNB (1-chloro-2-4-dinitrobenzene) was reported recently (Harold and Ottea, 1997). Insensitive ACHE mechanisms and GST were considered to be of minor importance in OP resistant *H. armigera* in India (Armes *et al.*, 1996).

Field Studies

High levels of resistance to pyrethroids were recorded in all the field collected strains (Figure 2). Survival to the discriminating dose of cypermethrin 0.1 µg/larva ranged from 55.86 to 100 per cent. Resistance was clearly highest in regions where insecticide use (ave. 16 spray applications), specifically pyrethroid use was high, while resistance was moderate to high in regions which were subjected to moderate (ave. 10 spray applications) use of insecticides (Table 2). Suppression of cypermethrin resistance by Pbo ranged from 1 to 46 per cent with maximum synergism in regions where pyrethroid use was low to moderate. Profenofos synergism ranged between 1 and 57 percent, with higher synergism in strains collected from Nagpur, Wardha, Akola, Amaravati and Yavatmal of Central India. It was also observed that resistance mechanisms were not usually consistent temporally. Pbo synergism was inconsistent over a period of time in any particular region e.g.. Pbo synergism decreased towards the end of cropping season in the Hyderabad region (Armes *et al.*, 1996) and Central India (Kranthi *et al.*, 1997). Circumstantial evidence indicates that regions with highest use of pyrethroids have resistance strains with nerve insensitivity as the most important mechanism. Reports from Australia (Forrester *et al.*, 1993) point out that a significant reduction in pyrethroid selection pressure resulted in a shift in pyrethroid resistance mechanisms from nerve insensitivity to oxidative metabolism. Enhanced synergism by Pbo and profenofos was also associated with high levels of cytochrome P450 and esterases respectively. Pbo and profenofos synergism was consistently the lowest in Guntur district in all the strains collected during 1993-98. The Guntur strains had the lowest levels of detoxification enzymes. Neurophysiological assays indicated the highest levels of nerve insensitivity in strains collected from Guntur. Clearly, this serves as a good example for nerve insensitivity as a prominent resistance mechanism in the absence of synergism by either esterase or oxidase inhibitors in high pyrethroid use areas. High levels of nerve insensitivity were also observed in strains collected from Central India at Akola and Amaravati. In the majority of the regions surveyed, resistance was mediated through metabolic mechanisms. Oxidases and esterases were found to be important mechanisms mediating pyrethroid resistance in *H. armigera* in India (Kranthi *et al.*, 1997) and Australia (Gunning, 1994).

Andhra Pradesh alone consumes more than 33 percent of the insecticides used in the country, with over 60 per cent of this on cotton. The use of insecticides, especially pyrethroids, has been particularly high in the coastal belt of Andhra pradesh (Guntur and Prakasam) over the past 14 years. During the past five or six years, insecticide use has increased in the Telangana region (Khammam, Karimnagar and Warangal) of Andhra Pradesh. Expectedly, pyrethroid resistance was high in the state, with nerve insensitivity as a major mechanism in Guntur district. Nerve insensitivity in *H. armigera* was demonstrated earlier in strains from India (West and McCaffery, 1992), China (McCaffery *et al.*, 1997) and Australia (Gunning *et al.*, 1995).

Conclusion

Studies carried out through biochemical estimation of detoxification activity and the use of synergists in laboratory selected resistant strains and field strains collected from various parts in India, indicate that metabolic detoxification through enhanced esterase activity is an important mechanism for OPs and pyrethroids. Preliminary evidence also indicates that esterases could also play a role in resistance to endosulfan. Enhanced cytochrome P450 content was found be a major mechanism of pyrethroid resistance in most of the strains collected. Interestingly pyrethroid resistance was found to switch predominant mechanisms (esterases or oxidases) during different parts of the season at the same locations. Nerve insensitivity was also found to be a major mechanism in some parts of the country where pyrethroids use was very high in recent years.

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Table 1. Effect of synergists on toxicity of cypermethrin in cypermethrin selected *H. armigera.*

Product/Strain	n	LD50 (95% F.L) µl/larva	LD90	Slope (± S.E.)	RF	Impact of synergists
Cypermethrin						
Reading susceptible	330	0.0048 (0.003-0.007)	0.027	1.72 (0.19)		
Wardha susceptible	160	0.053 (0.015-0.09)	0.28	1.75 (0.44)		
Resistance selected strain						
Cypermethrin	328	4.8 (2.89-13.56)	37.42	1.44 (0.21)	90	
Cypermethrin + Pbo	282	0.99 (0.65-1.95)	5.22	1.77 (0.24)		4.8
Cypermethrin + Profenofos	290	0.58 (0.4-0.87)	2.38	2.1 (0.24)		8.3
Cypermethrin + DEM	282	3.48 (2.24-9.16)	17.6	1.82 (0.38)		1.4
Endosulfan						
Reading susceptible	230	2.55(0.96-3.98)	8.8	2.37 (0.33)	-	
Nanjing susceptible	819	2.2 (1.9-2.6)	7.0	2.56 (0.17)	-	
Nagpur susceptible	236	3.34(2.33-4.29)	15.6	1.9 (0.26)	-	
Resistance selected strain						
Endosulfan	265	90.86 (49.97-321.72)	1076	1.194 (0.25)	27	-
Endosulfan + Pbo	240	321.11 (104.99-17800)	4616	1.107 (0.33)		0.28
Endosulfan + Profenofos	220	13.7 (10.0-17.5)	71	1.74 (0.30)		6.6
Endosulfan + DEM	230	168.48 (70.64-1719)	3138	1.009 (0.25)		0.54
Quinalphos						
Reading susceptible	246	0.08 (0.047-0.116)	0.20	3.26 (0.39)		
Nepal susceptible	250	0.12 (0.075-0.18)	0.28	3.67 (0.37)		
Nagpur susceptible	342	0.17 (0.15-0.20)	0.42	3.38 (0.32)		
Resistance selected strain						
Quinalphos	232	0.66 (0.56-0.78)	2.15	2.52 (0.33)	3.9	
Quinalphos + Pbo	258	0.59 (1.6-2474)	133	0.95 (0.37)		0.1
Quinalphos + Profenophos	240	0.24 (0.16-0.28)	0.69	2.75 (0.34)		2.7
Quinalphos + DEM	272	0.57 (0.44-0.72)	3.5	1.62 (0.23)		1.2

Table 2. Effect of synergists on toxicity of cypermethrin in field strains of *H. armigera* collected from regions where insecticide use was high.

Collection site	Map Ref	Collectn date	(Discriminating dose assay) % survival ± SE Cypermethrin 0.1µg plus			% Suppression of resistance by		Nerve insensty Cyperm. EC_{50} nM (95% FL)
				pbo 50µg	prof 0.1µg	pbo	profenofos	
High insecticide use region								
Guntur		Feb '98	97.72 ± 1.3	96.43 ± 1.6	95.84 ± 1.8	1.29	1.88	93.36
Karimnagar	9	Feb '98	90.84 ± 2.6	57.50 ± 4.5	90.00 ± 2.8	33.34	0.84	29.74
Khammam	10	Feb '98	100.0 ± 0.0	65.00 ± 4.4	82.58 ± 3.3	35.00	17.42	
Warangal	6	Feb '98	97.22 ± 2.5	70.00 ± 4.2	84.88 ± 3.3	27.22	12.34	20.72
Akola	4	Sept '97	93.34 ± 2.3	72.23 ± 3.7	45.84 ± 4.2	21.11	47.5	
		Feb '98	100.0 ± 0.0	96.66 ± 1.6	60.42 ± 5.0	3.34	39.58	66.17
Amaravati	3	Feb '98	88.89 ± 2.6	82.29 ± 3.9	47.50 ± 4.6	6.60	41.39	37.43
Yavatmal	5	Feb '98	88.60 ± 2.9	80.71 ± 3.7	35.00 ± 4.4	7.89	53.60	
Low insecticide use region								
Varanasi	16	Oct '93	75.00 ± 3.9	38.80 ± 4.5	49.16 ± 4.6	36.2	25.84	
		Dec '94	81.90 ± 3.6	76.05 ± 4.4	40.00 ± 4.5	5.85	41.9	
Bangalore	13	Apr. '94	83.17 ± 2.7	42.71 ± 5.0	73.96 ± 4.5	40.46	9.21	
		Dec '95	75.00 ± 3.6	52.78 ± 4.2	40.82 ± 4.2	22.22	34.18	
Nagpur	1	Feb '98	90.91 ± 2.9	73.68 ± 4.5	34.35 ± 4.2	17.23	56.56	23.25
Wardha	2	Sept '93	93.75 ± 2.5	52.88 ± 4.2	89.58 ± 2.5	40.87	4.17	
		Nov '93	77.77 ± 3.5	57.50 ± 4.5	41.13 ± 4.4	20.27	36.64	
		Oct '94	71.22 ± 3.9	64.58 ± 4.9	58.34 ± 5.1	6.64	12.88	
		Nov '95	88.34 ± 2.4	69.45 ± 3.8	65.21 ± 4.1	18.89	23.13	
		Feb '98	55.84 ± 4.5	37.50 ± 4.2	28.79 ± 3.9	18.34	27.05	
Sirsa	15	Nov '93	91.53 ± 2.6	45.46 ± 4.7	81.90 ± 3.6	46.07	9.63	
Coimbatore	14	Apr. '94	80.11 ± 2.8	55.24 ± 3.4	72.83 ± 3.3	24.87	7.28	
		Nov '94	70.76 ± 4.4	49.31 ± 4.2	44.45 ± 4.1	21.45	26.31	
		Sept '95	80.21 ± 4.1	59.38 ± 4.3	66.94 ± 4.2	20.83	13.27	
		Nov '96	75.97 ± 4.2	55.21 ± 5.1	38.30 ± 5.0	20.76	37.67	
Prakasam	12	Feb '98	84.10 ± 3.2	65.63 ± 4.8	50.00 ± 5.1	18.47	34.1	
Siddipet	7	Feb '98	88.88 ± 2.6	86.36 ± 2.9	78.34 ± 3.8	2.52	10.54	
Rangareddy	8	Oct '93	73.33 ± 4.0	31.95 ± 3.8	65.00 ± 4.4	41.38	8.33	
		Dec '93	66.38 ± 4.4	49.26 ± 4.3	40.16 ± 4.3	17.12	26.22	

Figure 1. Detoxification enzymes in strains selected for insecticide resistance.

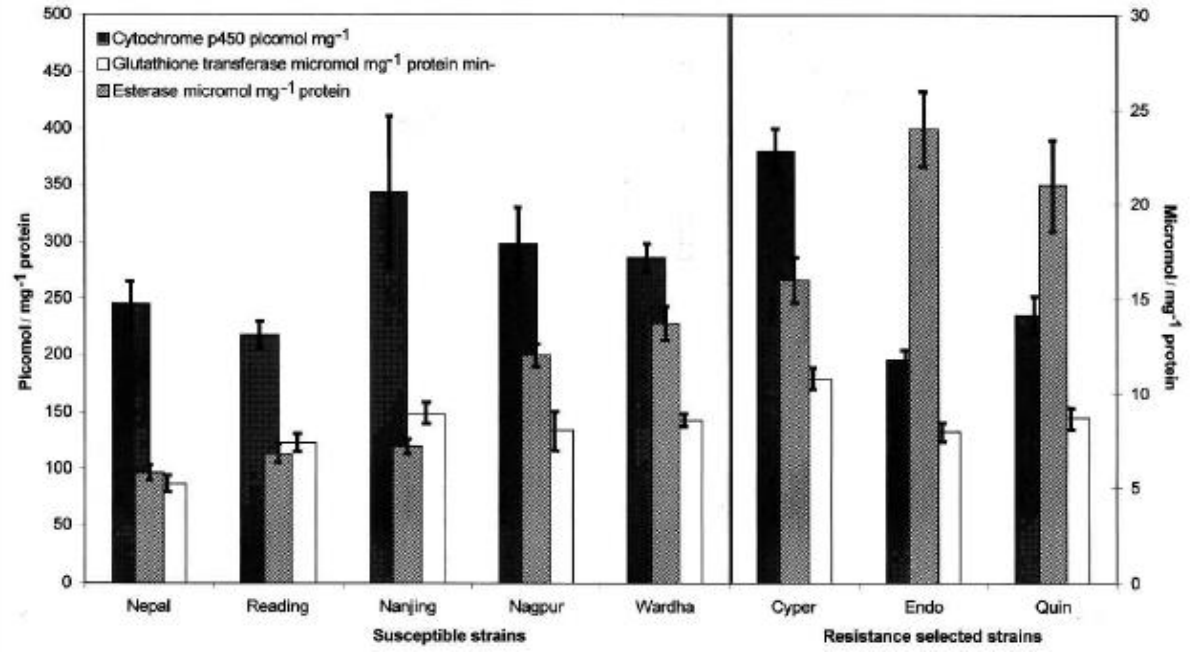
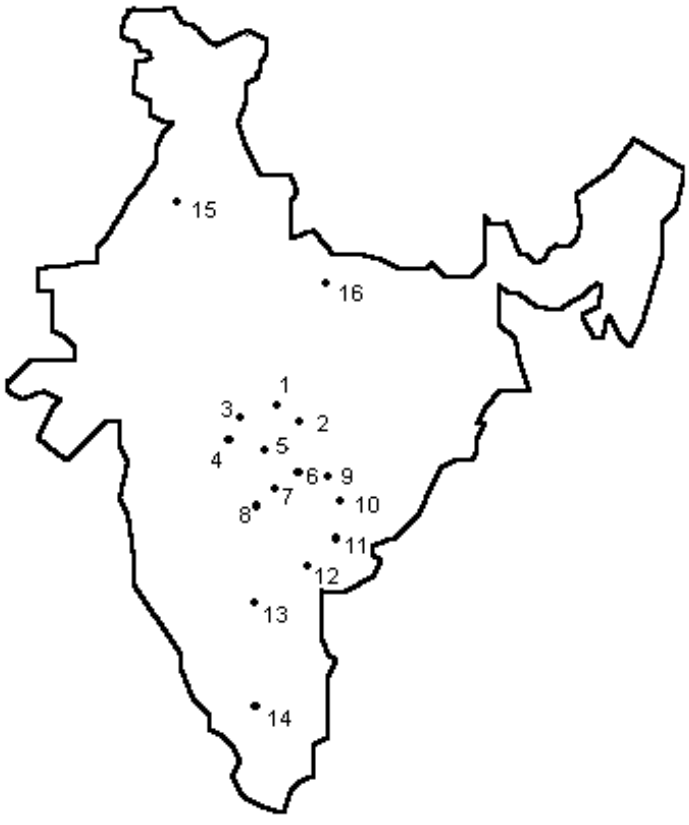


Figure 2. H. armigera Sampling locations in India.



Sampling locations where *Helicoverpa armigera* were collected

Ref	Location	Ref	Location	Ref	Location	Ref	Location
1	Nagpur	5	Yavatmal	9	Karimnagar	13	Bangalore
2	Wardha	6	Warangal	10	Khammam	14	Coimbatore
3	Amaravati	7	Siddipet	11	Guntur	15	Sirsa
4	Akola	8	Rangareddy	12	Prakasam	16	Varanasi