STATUS OF INSECTICIDE RESISTANCE IN THE COTTON BOLLWORM, HELICOVERPA ARMIGERA (HUBNER)

INDIRA CHATURVEDI

BEHIND MEHTA BUILDING, JARHABHATA, SINDHI COLONY, BILASPUR 495001, CHHATTISGARH, INDIA
Email: Ind_Chaturvedi@yahoo.com

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ABSTRACT

The status of insecticide resistance in some field populations of Helicoverpa armigera (Hubner) from the main cotton growing regions of central and south India was determined during the cropping seasons of 2001-2005. Seven insecticides viz. endosulfan, methomyl, monocrotophos, quinalphos, chlorpyriphos, fenvalerate and cypermethrin were tested against second-, third- and fifth-instar Helicoverpa armigera larvae. Dose-mortality regressions, LD_{50}s and their fiducial limits were computed by probit analysis. Resistance factors (RF) were estimated at the LD_{50} level as RF=LD_{50} field strain/LD_{50} susceptible strain. The Helicoverpa armigera (Hubner) exhibited widespread resistance (RF=48-919) to cypermethrin. Insecticide resistance to chlorpyriphos was low to moderate in the majority of the strains tested. A substantial inter-strain variation in insecticide resistance was evident.

KEY WORDS: Cyclodiene, Helicoverpa armigera, insecticide resistance, organophosphate, pyrethroid.

INTRODUCTION

Cotton occupies only 5% of the total cultivable area in India but consumes more than 55% of the total insecticides used in the country [39]. The cotton bollworm, Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a major pest on a wide range of crops in Europe, Africa, Asia and Australia. H. armigera is able to adapt to various cropping systems: high polyphagy, wide geographical range, mobility, migratory potential, facultative diapause, high fecundity and propensity to develop resistance to insecticides are physiological, ethological and ecological factors that have strongly contributed to its pest status [17, 19, 30]. This pest has been recorded feeding on 182 plant species across 47 families in the Indian subcontinent, of which 56 are heavily damaged and 126 are rarely affected [36]. In India, crop losses due to H. armigera are commonly more than half the yield, and annual losses to cotton and pulses alone have been estimated at US $ 300-500 million [24]. Insecticides had been found very effective for the control of chewing and sucking insect pests in the early 1980s. However, with their extensive use, a widespread resistance to insecticides occurred in H. armigera in India in 1990s. Existence of resistance to pyrethroids, organophosphates, carbamates and cyclodienes as also reported by Dhingra et al. [15], McCaffery et al. [31], Armes et al. [9, 10] and Kranthi et al. [26]. In India, the first case of control failure after spraying synthetic pyrethroids from suspected insecticide resistance in H. armigera (Hubner) was from Guntur in Andhra Pradesh [40]. The pest management difficulties in the coastal belt of Andhra Pradesh in 1987 were shown to involve pyrethroid resistance in H. armigera [15]. The first outbreak of H. armigera was seen in the cotton belt of Guntur, Prakasham and parts of Krishna districts in Andhra Pradesh. This population showed high level of resistance to various insecticides [31]. Frequent outbreaks of Helicoverpa armigera in India on cotton crops have led to severe social disturbances, with several reports of suicide by farmers [35]. During 1992-1997, crop failure in many states of the South Indian cotton ecosystem, particularly Andhra Pradesh and Karnataka, was followed by the suicide of several farmers, which has been traced to insecticide resistance in H. armigera [25]. The pyrethroids which were considered most potent insecticides for its control lost their efficacy [8]. Before the probable existence of the pesticides resistance was reported in India by large-scale crop failures in Andhra Pradesh, it has been suggested that H. armigera obtained from various regions of the country differ significantly with respect to susceptibility/resistance to pesticides [41, 37].

Plant protection continues to rely heavily on chemical pesticides, a not very viable, long-term strategy if one looks at recent failures against cotton bollworms and several other crop pests. Strategies on insecticides resistance were followed on the rational use of insecticides, restriction of treatments and alternation with compounds of different modes of action in order to prevent selection for resistance [42]. Over-dependence of a particular group of chemical is one of the important reasons for rapid development of resistance. This is evidenced by very high level of resistance to synthetic pyrethroids, which occupied 50-70 percent of the insecticides sprayed over the cotton in India [23]. The number of insecticides being used to control bollworm varied across locations in India. Cypermethrin, endosulfan and chlorpyriphos, as representative of the pyrethroid, cyclodiene and organophosphate insecticides respectively, rank amongst the most commonly used insecticides on cotton in India and account for at least 40% of all insecticides used on cotton [28]. In the present investigation we monitored the insecticide resistance levels in H. armigera from 2001-2005 in the main cotton-growing regions of Central and South India.

MATERIALS AND METHODS

Insects

Larvae of H. armigera (second-, third- and fifth instar) were collected from different cotton growing regions in Central and South India during the cropping seasons of 2001-2005 (Table 1). Collections comprised a variable number of larvae per location. Larvae of H. armigera were reared on a semi-synthetic diet described by Ahmad & McCaffery [3], which consisted of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin (1.5 g), corn oil (12 ml), yeast (48 g), agar (17.3 g) and distilled water (1300 ml) with a vitamin mixture. Adults were fed on a sucrose solution with the addition of vitamins and methyl-4-hydroxybenzoate.

Laboratory reared susceptible strain of H. armigera

Some field populations of H. armigera, collected from traditionally unsprayed regions of Madurai and Akola exhibited low levels of resistance to almost all the groups of insecticides tested. These were established in the laboratory on semi-synthetic diet without selection pressure of insecticides for seven generations. The second-, third- and fifth instar larvae of seven-generation population were exposed to different insecticides to determine the LD_{50} value. The values of median lethal dose (LD_{50}) were compared with the field-collected population for monitoring the prevalent level of

Table 1. Sampling sites of *Helicoverpa armigera* in Central and South India (2001-2005)

Location/strain	Origin*	Collection date
Madurai	Cotton	Jan. 99, Dec. 02, Sep. 03
Akola	Cotton	[2]Mar. 99, Dec. 01, Dec. 2000, Oct. 01, Jan. 03
Nagpur	Pigeonpea	Sep. 2000, Jan. 02, Dec. 03
Wareham	Cotton	Dec. 99, Sep. 01, Mar. 02, Jan. 03
Amaravati	Cotton	5Mar. 2000, Dec. 02, Feb. 03
Nanded	Cotton	Feb. 99, Dec. 2000, Sep. 01, Feb. 02, Mar. 03
Yavatmal	Cotton	Sep. 01, Jan. 02, Dec. 03
Raichur	Tomato	Mar. 99, Dec. 01, Oct. 02, Jan. 03
Dharwad	Cotton	Jan. 99, Feb. 02, Sep. 03
Guntur	Cotton	Sep. 99, Jan. 01, Jan. 02, Dec. 03
Medak	Chickpea	Sep. 99, Feb. 01, Dec. 02, Dec. 03
Khammam	Sunflower	Mar. 2000, Jan. 02, Feb. 03
Nalgonda	Cotton	Oct. 01, Feb. 02, Sep. 03
Coimbatore	Potato	Sep. 01, Dec. 02, Sep. 03

* Range of host plants

Table 2. Response of field strains of *Helicoverpa armigera* for cypermethrin bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Madurai susceptible	55	0.31	0.18-0.26	1.18 ± 0.21	
Akola					
Madurai	65	15.21	11.3-19.3	1.32 ± 0.21	49
Nagpur	47	136.3	107-145	2.11 ± 0.11	439
Wardha	50	22.01	17.0-21.3	2.07 ± 0.20	71
Amaravati	55	285.3	119-359	2.32 ± 0.12	919
Nanded	75	15.01	12.2-22.5	2.01 ± 0.31	48
Yavatmal	60	77.07	60.9-89.1	1.72 ± 0.12	248
Raichur	65	148.2	110-160	2.41 ± 0.11	479
Dharwad	75	18.23	14.1-23.5	1.09 ± 0.23	58
Guntur	95	112.2	91.2-141	1.21 ± 0.13	361
Medak	80	39.11	31.3-47.1	2.03 ± 0.11	126
Khammam	85	41.21	34.4-49.1	2.21 ± 0.13	133
Nalgonda	63	165.6	122-179	1.85 ± 0.14	532
Coimbatore	68	220.4	35.7-260	1.78± 0.14	712

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

insecticide resistance in H. armigera.

Survey areas

Insects were collected from four cotton-growing states (Maharashtra, Andhra Pradesh, Tamilnadu and Karnataka) in India.

Central zone

Insects were collected from cotton fields in the Nagpur, Wardha, Amaravati, Akola, Nanded and Yavatmal districts of Maharashtra.

South zone

Insects were collected from cotton fields in the Guntur, Medak, Khammam, and Nalgonda districts of Andhra Pradesh. In Tamilnadu, the collections were made from the Madurai and Coimbatore districts. The survey areas also included the Raichur and Dharwad district of Karnataka.

In all of the regions, cypermethrin, fenvalerate and quinalphos were the primary choices, by more than 35 percent, of insecticides for use in controlling bollworm. In all locations the usage of insecticides was erratic and indiscriminate. Overall, 60-70% of the farmers applied the insecticides in an interval of 2-3 days during the critical period. This resulted in over 30 sprays (against the 8-10 recommended) during the season but growers were unable to achieve effective control with any of the available insecticides. Armes et al. [9] reported similar insecticide usage patterns in Karnataka and Andhra Pradesh for the control of H. armigera.

Insecticides used

The following technical grade insecticides were used for bioassays on H. armigera: cypermethrin (90% w/w; Zeneca Agrochemicals, UK), endosulfan (94% w/w; Excel Industries, India), monocrotophos (73% w/w; Khatau Junker Ltd, India), quinalphos (72% w/w; Zeneca Agrochemicals, UK), methomyl (73% w/w; DuPont, India), fenvalerate (90% w/w; DuPont, India), chlorpyriphos (98% w/w; DeNocil, India).

Bioassays

Newly moulted second-, third- and fifth instar larvae from the F_1 laboratory generations were exposed to different insecticides using the leaf dip technique as recommended by the Insecticide Resistance Action Committee (IRAC) of GIFAP [7]. Formulations of test compounds were prepared in distilled water as parts per millions of active ingredients. i.e., 100 ppm. Leaf discs of cotton (5 cm diameter) were punched out from 2-week-old plants and immersed into the serial dilutions (0.1, 1, 2, 4, 8, 16, 32, 64, 100 and 1000 ppm) for fifteen seconds. Control leaves were dipped in diluent only. They were allowed to surface-dry on a paper towel and then placed into petri dishes containing moistened filter papers to avoid desiccation of leaves. Larvae were transferred to the leaf disks by tapping lightly to dispense 5 larvae per petri dish per replicate. Each treatment was replicated 5 times along with an untreated control under complete randomized design. All rearing and bioassay operations were carried out at 25 ± 2°C under a 12:12h light: dark regime and mortality was assessed 48 and 72 hours after treatment.

Data analysis

Data from the replicates were pooled and dose-mortality regressions, LD_{50}s and their fiducial limits were computed by probit analysis using POLO-PC [6]. Corrections for control mortality were made using Abbott's formula [1]. Resistance factors (RFs) were calculated as LD_{50} of the field strain /LD_{50} of the susceptible strain.

RESULTS

Cypermethrin

The Amaravati population recorded a maximum LD_{50} value to cypermethrin (285.3 µg/larva) followed by the population from Coimbatore (220.4µg/larva), Nalgonda (165.6), Raichur (148.2) and Nagpur (136.3). The lowest LD_{50} value was observed in the population from Nanded (15.01µg/larva) followed by Madurai (15.21), Dharwad (18.23) and Wardha (22.01). The resistance was found to be highest for the population of Amaravati (919-fold) followed by Coimbatore (712-fold), Nalgonda (532-fold), Raichur (479-fold) and Nagpur (439-fold). The Amaravati strain which showed the highest resistance to cypermethrin (919-fold) was also highly resistant to fenvalerate (213-fold) and quinalphos (170-fold). The least resistance was observed in the population of Nanded (48-fold) followed by Madurai (49-fold), Dharwad (58-fold) and Wardha (71-fold) and slopes of regression lines ranged from 2.0-2.4 for seven strains and <2 for the remaining strains. The Raichur and Amaravati strains showed higher slope values of 2.41 and 2.32 respectively (table 2).

Fenvalerate

The Coimbatore population recorded a maximum LD_{50} value to fenvalerate (113.21µg/larva) followed by the population from Nagpur (109.1µg/larva), Amaravati (98.21) and Raichur (80.07). The lowest LD_{50} value was observed in the population from Madurai (4.91µg/larva) followed by Akola (6.28), Dharwad (8.21), Nanded (9.27) and Yavatmal (12.22) and. resistance to fenvalerate was very variable, ranging from 11-fold in the Madurai strain to 245-fold in the Coimbatore strain. Like cypermethrin

Table 3. Response of field strains of *Helicoverpa armigera* for fenvalerate bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Akola susceptible	57	0.46	0.30-0.46	1.89 ± 0.21	
Madurai	68	4.91	2.54-5.33	1.99 ± 0.19	11
Akola	74	6.28	4.21-7.09	1.17 ± 0.11	14
Nagpur	85	109.1	87.1-133.2	1.09 ± 0.23	237
Wardha	90	29.01	21.7-35.7	2.36 ± 0.14	63
Amaravati	58	98.21	74.2-112.3	1.05 ± 0.01	213
Nanded	65	9.27	7.56-10.99	2.25 ± 0.11	20
Yavatmal	87	12.22	10.6-13.29	1.15 ± 0.07	27
Raichur	80	80.07	65.9-96.8	1.8 ± 0.15	174
Dharwad	80	8.21	6.44-9.91	1.01 ± 0.04	18
Guntur	85	61.02	45.3-66.7	1.68 ± 0.02	132
Medak	65	17.41	13.14-20.1	1.74 ± 0.03	38
Khammam	35	34.21	29.0-40.1	2.04 ± 0.02	74
Nalgonda	68	79.2	63.4-94.3	1.78 ± 0.14	172
Coimbatore	65	113.21	82.3-223.5	1.99 ± 0.23	245

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error, RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

Table 4. Response of field strains of *Helicoverpa armigera* for quinalphos bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Madurai susceptible	70	0.22	0.18-0.26	1.18 ± 0.21	
Akola	57	2.92	1.91-5.50	2.25 ± 0.11	13
Madurai	58	2.37	1.21-5.22	1.31± 0.041	11
Nagpur	50	20.41	15.9-23.8	2.19 ± 0.02	91
Wardha	85	16.01	12.5-19.4	1.63 ± 0.22	73
Amaravati	95	37.32	31.4-44.5	2.22 ± 0.12	170
Nanded	90	2.74	2.13-6.14	2.34 ± 0.24	12
Yavatmal	48	4.21	3.02-7.98	2.02 ± 0.14	19
Raichur	50	30.22	23.9-36.5	1.86 ± 0.17	136
Dharwad	65	3.32	2.21-6.21	2.01 ± 0.31	15
Guntur	78	18.02	14.1-22.5	1.57± 0.20	82
Medak	75	6.32	5.23-8.31	2.31 ± 0.12	29
Khammam	70	17.11	13.5-19.7	2.11 ± 0.17	77
Nalgonda	70	31.73	24.1-37.5	1.81 ± 0.01	144
Coimbatore	50	40.01	32.3-49.1	2.43 ± 0.14	182

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

and endosulfan resistance to fenvalerate increased sharply after 2003. The Wardha, Nanded and Khammam strains showed higher slope values of 2.36, 2.25 and 2.04 respectively (table 3).

Quinalphos

The Coimbatore population recorded a maximum LD_{50} value to quinalphos (40.01µg/larva) and the lowest LD_{50} value were observed in the population from Madurai (2.37µg/larva). Resistance monitoring during 2001-2005 indicated moderate to high (11- to 182-fold) levels of resistance to quinalphos in field strains of H. armigera collected from main cotton growing districts of Maharashtra and south India. There did not appear to be a clear relationship of RFs between years and different crops. However, RFs in the Coimbatore (182-fold) and Amaravati (170-fold) strains, collected after the 2004 outbreak of H. armigera when insecticides were used frequently for its control, were particularly high. The resistance was found to be highest for the population of Coimbatore (182-fold) followed by Amaravati (170-fold), Nalgonda (144 fold), Raichur (136-fold) and Nagpur (91-fold). The least resistance was observed in the population of Madurai (11-fold) followed by Nanded (12-fold), Akola (13-fold), Dharwad (15-fold), and Yavatmal (19-fold). Slopes of regression lines ranged from 2.0-2.4 for nine strains and <2 for the remaining strains. The Coimbatore and Nanded strains showed higher slope values of 2.43 and 2.34 respectively (table 4).

Endosulfan

The Coimbatore population recorded a maximum LD_{50} value to endosulfan (30.01µg/larva) followed by the population from Raichur (18.01µg/larva), Nagpur (17.31) and Amaravati (16.74). The lowest LD_{50} value was observed in the population from Akola (4.71µg/larva) followed by Khammam (4.81), Madurai (4.97), Dharwad (5.72) and Medak (6.55). The resistance was found to be highest for the population of Coimbatore (79-fold) followed by Raichur (47-fold), Nagpur (45-fold), Amaravati (44-fold) and Nalgonda (37-fold). Out of 14 strains tested, five strains showed resistance factors of <20. Like other insecticides, resistance to endosulfan increased after the 2003 cotton season. In 9 of 14 strains, slopes of regression lines were approximately equal to or below 1.5. The Coimbatore, Raichur and Amaravati strains showed higher slope values of 2.54, 2.21 and 2.15 respectively (table 5).

Monocrotophos

The Coimbatore population recorded a maximum LD_{50} value to monocrotophos (35.31µg/larva) followed by the population from Amaravati (32.8µg/larva), Raichur (25.21), Nalgonda (22.01) and the lowest LD_{50} value was observed in the population from Akola (1.12µg/larva) followed by Nanded (1.33), Yavatmal (3.11), Khammam (3.17) and Dharwad (5.91). Resistance to monocrotophos was very variable, ranging from 2-fold in the Akola strain to 50-fold in the Coimbatore strain. There did not appear to be a clear relationship of RFs between years and different crops. Slopes of regression lines ranged from 2.0-2.6 for seven strains and <2 for the remaining strains. The Coimbatore and Guntur strains showed higher slope values of 2.6 and 2.4 respectively (table 6).

Methomyl

The Coimbatore population recorded a maximum LD_{50} value to methomyl (18.51µg/larva) followed by population from Amaravati (17.52µg/larva), Nalgonda (15.01), Raichur (13.61), Nagpur (12.48) and Wardha (12.21). The lowest LD_{50} value was observed in the population from Akola (0.31µg/larva) followed by Madurai (0.81µg/larva), Yavatmal (1.28) and Medak (1.62). Table 1 shows that RFs for methomyl ranged from 1 to 49-fold. The resistance was found to be highest for the population of Coimbatore (49-fold) followed by Amaravati (46-fold), Nalgonda (39-fold), Raichur (36-fold), and the least resistance was observed in the population of Akola(1-fold) followed by Madurai (2-fold), Yavatmal (3-fold), Medak(4-fold), Dharwad (5-fold) and Nanded (8-fold). In 8 of 14 strains, slopes of regression lines were below 2. The Wardha, Nanded and Raichur strains showed higher slope values of 2.91, 2.41 and 2.25 respectively (table 7).

Chlorpyriphos

The Coimbatore population recorded a maximum LD_{50} value to chlorpyriphos (35.24µg/larva) followed by the population from Raichur (31.02µg/larva), Wardha (30.02), Guntur (19.14) and the lowest LD_{50} value was observed in the population from Madurai (1.01µg/larva) followed by Dharwad (1.11), Yavatmal (1.23), Akola (1.31) and Khammam (1.52). The resistance was found to be highest for the population of Coimbatore (38-fold) followed by Raichur (33-fold), Wardha (32-fold), Guntur (20-fold), Nagpur (19-fold) and Nalgonda (15-fold). Out of 14 strains tested, ten strains showed resistance factors of <20. Slopes of regression lines ranged from 2.0-2.3 for five strains and <2 for the remaining strains. The Guntur and Amaravati strains showed higher slope values of 2.3 and 2.2 respectively (table 8).

The results of bioassay studies conducted on field population of H. armigera collected from infested cotton plants during 2001-2005 as well as on laboratory maintained strain of H. armigera revealed a higher LD_{50} values for majority of the strains from central and south zone of India than that of laboratory reared strain

Table 5. Response of field strains of *Helicoverpa armigera* for endosulfan bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Akola susceptible	65	0.38	0.30-0.46	1.69 ± 0.21	
Madurai	60	4.97	3.79-7.21	1.50 ± 0.13	13
Akola	68	4.71	3.32-7.01	1.3 ± 0.22	12
Nagpur	65	17.31	15.3-24.9	1.03 ± 0.04	45
Wardha	61	11.91	10.7-17.2	1.38± 0.11	31
Amaravati	54	16.74	14.1-23.7	2.15 ± 0.14	44
Nanded	50	12.91	11.5-19.3	1.34 ± 0.23	34
Yavatmal	39	10.12	9.44-15.9	1.02 ± 0.01	27
Raichur	45	18.01	16.2-25.5	2.21 ± 0.11	47
Dharwad	63	5.72	4.97-8.99	1.99 ± 0.19	15
Guntur	58	12.52	12.5-18.2	1.47 ± 0.12	33
Medak	58	6.55	5.90-11.02	1.12 ± 0.14	17
Khammam	80	4.81	3.47-7.91	2.01 ± 0.23	13
Nalgonda	64	14.21	13.4-22.2	1.09 ± 0.23	37
Coimbatore	68	30.01	23.7-36.8	2.54 ± 0.15	79

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

Table 6. Response of field strains of *Helicoverpa armigera* for monocrotophos bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Akola susceptible	70	0.71	0.28-0.48	1.16 ± 0.13	
Madurai	98				
Akola	47	1.12	.78-1.24	1.02 ± 0.21	2
Nagpur	58	18.13	14.8-21.2	1.50 ± 0.12	25
Wardha	68	20.35	16.7-24.3	2.40 ± 0.21	29
Amaravati	95	32.81	25.7-37.4	1.98± 0.27	46
Nanded	90	1.33	.92-1.99	2.01± 0.17	2
Yavatmal	78	3.11	2.0-5.89	1.25 ± 0.14	4
Raichur	65	25.21	20.1-29.9	1.81 ± 0.01	36
Dharwad	68	5.91	4.73-8.01	2.31 ± 0.12	8
Guntur	78	20.1	16.5-24.3	2.41 ± 0.021	28
Medak	70	6.87	5.81-9.31	2.19 ± 0.21	10
Khammam	54	3.17	2.21-5.51	1.49 ± 0.21	4
Nalgonda	50	22.01	18.5-27.1	2.36 ± 0.25	31
Coimbatore	60	35.31	30.6-44.6	2.61 ± 0.26	50

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

and the general LD_{50} values recorded were far higher indicating the existence of resistance to almost all classes of insecticides tested. Among the different insecticides tested, the resistance level was high for cypermethrin (RF=48-919) followed by fenvalerate (RF=11-245), quinalphos (RF=11-182), endosulfan (RF=12-79), monocrotophos (RF=2-50), methomyl (RF= 1-49) and chlorpyriphos (RF= 1-38). The results are consistent with the existence of moderate to high levels of resistance (RF=29-73) in H. armigera of Wardha ecosystem to all of the insecticides tested during the survey (2001-2005). However, the resistance drastically differs from location to location within the South Indian cotton ecosystem. Our study indicates that resistance levels to all of the insecticides rose sharply after the 2003 cotton season. There was a severe outbreak of H. armigera on cotton during September- October in 2004. Therefore, farmers applied frequent sprays of insecticides (18 to 30 applications per season) against H. armigera.

DISCUSSION

This study with cyclodiene (endosulfan) and some organophosphates (monocrotophos, quinalphos, chlorpyriphos) and carbamate (methomyl), as well as with two pyrethroids (fenvalerate and cypermethrin), clearly demonstrated that the H armigera population has lost susceptibility/developed resistance to commonly used insecticides and their further usage on cotton needs to be properly monitored. The development of insecticide resistance is influenced by genetic, behavioural, and agroecological factors which regulate the proportion of the total population selected with insecticides and the selection pressure exerted on sprayed populations [17].

Resistance to endosulfan has been reported from Australia [19], India and Nepal [31, 11, 28], Pakistan [4] and Indonesia [32]. Armes et al. [11] reported the highest resistance levels of 28-fold to endosulfan in H. armigera strains from Andhra Pradesh. In the current study, resistance to endosulfan in Andhra Pradesh (South India) was found to range from 17 –37-fold and comparatively high resistance recorded in Central Indian strains. The excessive use of insecticides led to problems of insecticide resistance in Central India. Endosulfan is the single largest selling insecticide in Central India, with an estimated 85% of it used on cotton [28]. Gunning et al. [22] and McCaffery et al. [31] stated that endosulfan is inherently not very effective against H. armigera larvae.

Resistance to methomyl has been reported earlier in field strains from Australia [22], India, Nepal and Pakistan [11, 27] indicating the risk of introduction of these genotypes in other parts of the world and their further selection.

Resistance to chlorpyrifos has been reported in field strains from India [28] and Pakistan [4] but with low RFs in most cases. Armes et al. [11] reported the absence of resistance to monocrotophos, but observed resistance levels of up to 59-fold to quinalphos in H. armigera field strains in India. Significant resistance to monocrotophos has been widely reported from China [12, 45, 46] and Pakistan [4] and recently Kranthi et al. [27] reported resistance levels of up to 65-fold to monocrotophos, in H. armigera strains collected from Bhatinda in North India during November 1998. Toxicity of the phosphate group of organophosphate insecticides such as monocrotophos is unaffected by oxidase inhibitors [19] and resistance to such compounds has been mostly attributed to insensitive acetylcholine-esterase based mechanisms [34]. The toxicology data from Dittrich et al. [16] suggest that one major resistance gene is common for AChE insensitivity and the AChE variant contributes to the substantial resistance to monocrotophos. Previous studies have shown a very strong correlation between AChE insensitivity and increased metabolism of insecticides [33].

Resistance to cypermethrin has been reported earlier in field strains from Andhra Pradesh in South India [31, 11, 28], Tamilnadu in South India [11] and Maharashtra in Central India [11, 26]. The cotton bollworm, H. armigera had developed high resistance as the season advanced and reached highest between January-February. A similar seasonal pattern of cypermethrin resistance frequencies was reported in the discriminating dose monitoring studies conducted by ICRISAT Asia Form [10]. Prior to this study, in Varanasi area in Uttar Pradesh, pyrethroid resistance was recorded in H. armigera larvae collected from early pigeonpea in November 1991 and from chickpea in March 1992 [9]. These details reveal that the pyrethroid resistance has already moved from South India to other parts of India. In India, the migratory movements of resistant individuals together with the absence of refugia have been proposed to explain the high pyrethroid resistance that is currently prevalent in that subcontinent [11].

Enhanced monooxygenase activity as a mechanism of resistance to pyrethroids in H. armigera had been reported from India [38, 25]. Monooxygenase was involved in H. armigera resistant to cypermethrin and fenvalerate [25, 29]. It is notable that resistance was substantially lower to all classes of insecticides than to cypermethrin. This indicates that isomeric content may have a marked effect on the development of resistance to pyrethroids as also reported by Forrester et al. [19] & Ahmad et al. [5]. However, Forrester et al. [19] reported that changes in the acid moiety had little effect on the extent of resistance development in Helicoverpa armigera in Australia.

Table 7. Response of field strains of *Helicoverpa armigera* for methomyl bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Madurai susceptible	95	0.38	0.30-0.46	1.89 ± 0.21	
Akola	90	0.31	.20-.52	1.99 ± 0.19	1
Madurai	78	0.81	.57-.99	1.32 ± 0.11	2
Nagpur	65	12.48	8.76-12.35	1.09 ± 0.23	33
Wardha	68	12.21	8.96-12.9	2.91± 0.51	32
Amaravati	78	17.52	13.7-20.1	2.01 ± 0.01	46
Nanded	40	3.21	2.56-3.99	2.41 ± 0.02	8
Yavatmal	42	1.28	1.21-2.18	1.52 ± 0.21	3
Raichur	74	13.61	9.94-14.3	2.25 ± 0.14	36
Dharwad	74	2.01	1.84-2.63	1.78 ± 0.11	5
Guntur	48	11.82	7.48-11.1	1.99 ± 0.23	31
Medak	84	1.62	.87-1.91	2.07 ± 0.12	4
Khammam	80	3.81	3.11-4.91	2.05 ± 0.10	10
Nalgonda	65	15.01	12.2.-19.0	1.8 ± 0.15	39
Coimbatore	63	18.51	14.0-23.2	1.67 ± 0.12	49

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

Table 8. Response of field strains of *Helicoverpa armigera* for chlorpyriphos bioassay.

Location/strain	Sample size*	LD_{50}	95% FL	Slope ± S.E.	RF
Madurai susceptible	54	0.93	0.59-1.97	1.10 ± 0.11	
Akola	65	1.31	1.54-2.33	1.99 ± 0.19	1
Madurai	50	1.01	1.07-2.12	1.01 ± 0.11	1
Nagpur	50	18.02	13.5-22.3	2.11 ± 0.21	19
Wardha	37	30.02	23.1-36.4	1.38 ± 0.11	32
Amaravati	84	12.41	11.9-17.1	2.26 ± 0.25	13
Nanded	49	2.81	2.11-3.58	1.78 ± 0.05	3
Yavatmal	58	1.23	.96-1.29	1.27 ± 0.01	1
Raichur	65	31.02	24.0-36.7	1.79 ± 0.23	33
Dharwad	47	1.11	.91-1.23	1.96 ± 0.11	1
Guntur	48	19.14	14.5-23.1	2.31 ± 0.12	20
Medak	70	4.12	4.11-5.88	1.98 ± 0.04	4
Khammam	74	1.52	1.01-1.92	2.01 ± 0.12	2
Nalgonda	78	14.4	12.1-19.3	1.67 ± 0.12	15
Coimbatore	57	35.24	27.3-41.5	2.09 ± 0.23	38

*Number of larvae per location
Abbreviations: LD_{50}= median lethal dose, FL= Fiducial limits, SE= standard error,
RF= resistance factor estimated as RF =LD_{50} field strain/LD_{50} susceptible strain (see text).

In some strains (e.g. Amaravati, Coimbatore and Nalgonda) resistance levels were high and such high levels of resistance to these compounds may be mediated through different mechanisms. Several mechanisms of resistance have been identified in H. armigera populations in various parts of the world. Mechanisms of pyrethroid resistance in H. armigera include reduced penetration [21, 9, 25, 26] decreased nerve sensitivity [2, 21, 44] and enhanced metabolism [3]. Insect behaviour may modulate insecticide resistance dynamics. The major behavioural factor affecting the evolution of insecticide resistance is the result of the gene flow concomitant with immigration processes regulating the gene pool of local populations [13]. A facultative migrant gene flow in H. armigera can result in resistant alleles reaching untreated populations [13] or vice versa. Although H. armigera is more sedentary and closely associated with crops than other species belonging to the Helicoverpa/Heliothis complex [17]. Daly & Gregg [14] demonstrated significant gene flow between populations of H. armigera in Australia due to its high vagility.

A survey of insecticide resistance in H. armigera during 2001-2005 revealed that resistance levels were highest in the intensive cotton growing regions of Maharashtra and South Indian cotton ecosystem where excessive application of insecticides is common. Armes et al. [11] also reported that the most highly resistant populations of H. armigera were generally found in the central and southern regions of India. The resistance levels in these regions (heavy insecticide usage areas) are due to heavy dependence on insecticides. Due to the indiscriminate use of insecticides to control it, several reports of development of resistance in this pest in India [41, 37, 38, 31, 10] and other parts of the world [20, 29] have been documented.

The development of insecticide resistance is primarily a result of the selection pressure exerted on sprayed populations increasing the frequency of resistant individuals. The study conducted by Forrester [18] clearly revealed that resistance levels rose when pyrethroids were used but fell significantly when they were withheld. Thus, the pesticides were creating very high selection pressure for resistant genotypes. However, it has been observed that several regions of the country where insecticides are used in a very low quantity, resistance in this pest can be expected over space and time [43]. A crucial agroecological component determining the extent to which insecticide resistance may evolve is the proportion of the total population sprayed [17].

One of the basic aspects of resistance management is to devise approaches to minimize reliance on insecticides so that the selection pressure can be alleviated. In order to rationalize the pesticide use on the farms, it is imperative to stress the importance of economic threshold levels in the application of pesticides and to follow the integrated pest management practices to bring down the expenditure and to increase the effectiveness of plant protection measures in cotton. This study does provide a warning that indiscriminate use of insecticides is leading towards reduced efficacy and higher control costs for growers. Resistance to methomyl and chlorpyriphos was low to moderate in most of the strains tested from central and south zone of India and hence these compounds may still be effectively used here. The findings of present investigations clearly pointed out the possibility of resistance phenomenon operating in H. armigera population of these localities. Further, the outcome of the survey clearly indicates the need for genetic investigations of the geographic populations of bollworm and the formulation of population specific integrated pest management (IPM) modules.

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