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*Research Paper*

# A survey of insecticide resistance in *Helicoverpa armigera* in central and South Indian cotton ecosystems

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## Abstract

**Monitoring for insecticide resistance was carried out on *Helicoverpa armigera* (Hubner) collected from main cotton-growing districts of Central and South India for the period 2007-2009. Four chemicals, including cyclodiene (endosulfan), organophosphate (monocrotophos) and pyrethroids (fenvalerate and cypermethrin) were tested against second-, third- and fifth-instar H. armigera larvae. Data from the replicates were pooled and dose-mortality regressions 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=41-700) to cypermethrin. The *H. armigera* strain from Coimbatore, South India exhibited highest level of resistance (700-fold) to cypermethrin and the lowest resistance (3- fold) was recorded in field strain from Akola, Central India. A substantial inter-strain variation in insecticide resistance was evident.**

**Keywords** Endosulfan, fenvalerate, *Helicoverpa armigera*, insecticide resistance, monocrotophos.

## Introduction

Cotton, the major commercial crop, is grown extensively in Karnataka, Tamil Nadu, Andhra Pradesh, Punjab, Haryana, Gujarat, Maharashtra and Rajasthan. Cotton occupies only 5% of the total cultivable area in India but consumes more than 55% of the total insecticides used in the country [1]. Andhra Pradesh alone consumes more than 33% of all insecticides used in the country, with over 54% of this on cotton [2]. The pest spectrum of cotton is very complex since as many as 1326 species of insect pests have been listed on this crop in the world [3]. The cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), is one of the most damaging cotton pests in India. Losses up to Rs.10, 000 million have been reported solely due to this pest in crops like cotton, pigeonpea, chickpea, groundnut, sorghum, pearl millet, tomato, and other crops of economic importance [4].

The indiscriminate use of insecticides, particularly during the 1980s and 1990s, contributed to the emergence of cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) as a primary pest of cotton in recent years. This pest has developed resistance to all the major insecticide classes and it has become increasingly difficult to control its population not only in India but also in Australia and Thailand [5, 6, 7, 8]. In India, the first case of control failure after spraying synthetic pyrethroids was from Guntur in Andhra Pradesh [9]. To understand the level of susceptibility /resistance of *H. armigera* to recommended and commonly used insecticides, the present study was undertaken in cotton- ecosystems of Central and South India where considerable amount of insecticides are used.

## Materials and Methods

A survey to assess the levels of resistance was carried out in major cotton growing regions of India, during a three years period (2007 to 2009). The larvae of *Helicoverpa armigera* collected from four major cotton-growing states (Maharashtra, Andhra Pradesh, Tamilnadu and Karnataka) in India (Table 1). In the regions surveyed, an average of 11 to 20 applications of insecticides is common on cotton, with preference for cypermethrin and monocrotophos.

### Insecticides used

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

### Bioassays

Larvae of *H. armigera* were reared on a semi-synthetic diet described by Armes *et a*l [10]. Larvae from $F_1$ generation of the field strains were used for bioassays using a topical application procedure as recommended by the Entomological Society of America [11]. All rearing and bioassay operations were carried out at 25 ± 2°C under a 12:12h light: dark regime and mortality was assessed 72h after treatment.

### Data analysis

Data from the replicates were pooled and dose-mortality regressions were computed by probit analysis [12]. Results were expressed as percentage mortalities, corrected for untreated (check) mortalities using Abbott's formula [13]. Resistance factors (RFs) were calculated as $LD_{50}$ of the field strain /$LD_{50}$ of the susceptible strain.

## Results and Discussion

### Endosulfan

The Coimbatore population recorded a maximum $LD_{50}$ value to endosulfan (28.01µg/larva) followed by the population from Raichur (17.01µg/larva), Nagpur (16.31) and Amaravati (15.74). 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 (75-fold) followed by Raichur (49-fold), Amaravati (45-fold), and Nalgonda (37-fold). Out of 14 strains tested, seven strains showed resistance factors of <20. Like other insecticides, resistance to endosulfan increased after the 2008 cotton season. In 7 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 2.59, 2.41 and 2.35 respectively (Table 2).

### Monocrotophos

The Coimbatore population recorded a maximum $LD_{50}$ value to monocrotophos (30.31µg/larva) followed by the population from Amaravati (28.8µg/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 Khammam (3.57), Yavatmal (3.71), Nanded (4.73), and Dharwad (6.91). Resistance to monocrotophos was very variable, ranging from 3-fold in the Akola strain to 55-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 2.07-2.71 for five strains and <2 for the remaining strains. The Coimbatore and Guntur strains showed higher slope values of 2.7 and 2.52 respectively (Table 3).

### Fenvalerate

The Coimbatore population recorded a maximum $LD_{50}$ value to fenvalerate (120.21µg/larva) followed by the population from Amaravati (91.21) and Raichur (85.07). The lowest $LD_{50}$ value was observed in the population from Madurai (4.91µg/larva) followed by Nanded (6.27), Dharwad (8.21), Akola (9.28), and Yavatmal (12.22) and. resistance to fenvalerate was very variable, ranging from 10-fold in the Madurai strain to 255-fold in the Coimbatore strain. Like cypermethrin and endosulfan resistance to fenvalerate increased sharply after 2008. The Guntur, Amaravati and Coimbatore strains showed higher slope values of 2.68, 2.55 and 2.49 respectively (Table 4).

**Cypermethrin**

The Amaravati population recorded a maximum $LD_{50}$ value to cypermethrin (275.3 µg/larva) followed by the population from Coimbatore (200.4µg/larva), Nalgonda (160.6), Raichur (150.2) and Nagpur (121.3). The lowest $LD_{50}$ value was observed in the population from Dharwad (11.23), Nanded (14.01µg/larva) followed by Madurai (15.21)) and Wardha (22.01). The resistance was found to be highest for the population of Coimbatore (700-fold) followed by Amaravati (600-fold), Nalgonda (482-fold), Raichur (460-fold) and Nagpur (435-fold). The least resistance was observed in the population of Nanded (41-fold) followed by Madurai (46-fold), Dharwad (58-fold) and Wardha (69-fold) and slopes of regression lines ranged from 2.0-2.53 for eight strains and <2 for the remaining strains. The Raichur and Amaravati strains showed higher slope values of 2.53 and 2.39 respectively (Table 5).

**Table 1: Sampling sites of *Helicoverpa armigera* in Central and South India (2007-2009)**

| Location/strain | Origin* | Collection date |
|---|---|---|
| Madurai | Cotton | Mar. 2006, Dec. 2007, Sep. 2008 |
| Akola | Cotton | Mar. 2006, Dec. 2007, Dec. 2008, |
| Nagpur | Pigeonpea | Sep. 2006, Jan. 2007, Jan. 2008 |
| Wardha | Pigeonpea | Dec. 2006, Sep. 2007, Mar. 2007, Jan. 2008 |
| Amaravati | Cotton | Feb. 2007, Dec. 2007, Feb. 2008 |
| Nanded | Cotton | Mar. 2006, Dec. 2007, Feb. 2007, Mar. 2008 |
| Yavatmal | Cotton | Mar. 2006, Dec. 2007, Dec. 2008, |
| Raichur | Tomato | Feb. 2007, Dec. 2007, Feb. 2008 |
| Dharwad | Chickpea | Sep. 2006, Jan. 2007, Jan. 2008 |
| Guntur | Cotton | Mar. 2006, Dec. 2007, Feb. 2007, Mar. 2008 |
| Medak | Chickpea | Mar. 2006, Dec. 2007, Dec. 2008, |
| Khammam | Sunflower | Dec 2006, Dec. 2007, Feb. 2007, Mar. 2008 |
| Nalgonda | Potato | Mar. 2006, Dec. 2007, Dec. 2008, |
| Coimbatore | Cotton | Mar. 2006, Dec. 2007, Dec. 2008, |

* Range of host plants

**Table 2: Response of field strains of *Helicoverpa armigera* for endosulfan bioassay**

| Location/strain | Sample size* | $LD_{50}$ | 95% FL | Slope ± S.E. | RF |
|---|---|---|---|---|---|
| Akola susceptible | 66 | 0.38 | 0.31-0.42 | 1.89 ± 0.21 | |
| Madurai | 50 | 4.97 | 3.69-7.01 | 1.50 ± 0.13 | 13 |
| Akola | 63 | 4.71 | 3.32-7.01 | 1.32 ± 0.22 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| Nagpur | 65 | 16.31 | 17.3-24.3 | 1.03 ± 0.04 | 35 |
| Wardha | 57 | 11.91 | 10.7-17.2 | 1.38± 0.11 | 31 |
| Amaravati | 54 | 15.74 | 14.1-23.7 | 2.35 ± 0.14 | 45 |
| Nanded | 56 | 12.91 | 11.5-19.3 | 1.64 ± 0.23 | 34 |
| Yavatmal | 39 | 10.12 | 8.44-17.9 | 1.02 ± 0.01 | 27 |
| Raichur | 46 | 17.01 | 16.2-25.5 | 2.41 ± 0.11 | 49 |
| Dharwad | 60 | 5.72 | 4.57-7.99 | 1.99 ± 0.19 | 15 |
| Guntur | 55 | 12.52 | 12.5-18.2 | 1.87 ± 0.12 | 33 |
| Medak | 48 | 6.55 | 4.90-10.02 | 1.12 ± 0.14 | 17 |
| Khammam | 70 | 4.81 | 3.77-6.91 | 2.01 ± 0.23 | 13 |
| Nalgonda | 69 | 14.21 | 11.4-20.2 | 1.09 ± 0.23 | 37 |
| Coimbatore | 71 | 28.01 | 22.7-35.8 | 2.59 ± 0.15 | 75 |

*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 3: Response of field strains of *Helicoverpa armigera* for monocrotophos bioassay**

| Location/strain | Sample size* | $LD_{50}$ | 95% FL | Slope ± S.E. | RF |
|---|---|---|---|---|---|
| Akola susceptible | 74 | 0.71 | 0.27-0.38 | 1.16 ± 0.13 | |
| Madurai | 80 | | | | |
| Akola | 47 | 1.12 | .74-1.20 | 1.01 ± 0.21 | 3 |
| Nagpur | 52 | 18.13 | 14.8-21.2 | 1.50 ± 0.12 | 25 |
| Wardha | 68 | 20.35 | 16.7-24.3 | 2.07 ± 0.21 | 29 |
| Amaravati | 87 | 28.81 | 25.7-37.4 | 1.78± 0.27 | 46 |
| Nanded | 89 | 4.73 | 22-1.99 | 1.91± 0.17 | 7 |
| Yavatmal | 78 | 3.71 | 20-5.89 | 1.25 ± 0.14 | 4 |
| Raichur | 65 | 25.21 | 23.1-29.9 | 1.51 ± 0.01 | 36 |
| Dharwad | 68 | 6.91 | 4.73-8.01 | 2.34 ± 0.12 | 8 |
| Guntur | 78 | 20.1 | 16.5-24.3 | 2.52 ± 0.021 | 28 |
| Medak | 76 | 6.87 | 5.81-9.31 | 1.99 ± 0.21 | 10 |
| Khammam | 56 | 3.57 | 2.21-5.51 | 1.47 ± 0.21 | 4 |
| Nalgonda | 47 | 22.01 | 18.5-27.1 | 2.46 ± 0.25 | 31 |
| Coimbatore | 71 | 30.31 | 37.6-49.6 | 2.71 ± 0.26 | 55 |

*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 fenvalerate bioassay**

| Location/strain | Sample size* | $LD_{50}$ | 95% FL | Slope ± S.E. | RF |
|---|---|---|---|---|---|
| Akola susceptible | 45 | 0.46 | 0.30-0.46 | 1.89 ± 0.21 | |
| Madurai | 60 | 4.91 | 2.04-5.33 | 1.99 ± 0.19 | 10 |
| Akola | 74 | 9.28 | 7.21-7.09 | 2.17 ± 0.11 | 20 |
| Nagpur | 85 | 52.1 | 87.1-123.2 | 1.09 ± 0.23 | 227 |
| Wardha | 92 | 29.01 | 21.7-35.7 | 1.36 ± 0.14 | 63 |
| Amaravati | 58 | 91.21 | 71.2-112.3 | 2.55 ± 0.01 | 203 |
| Nanded | 65 | 6.27 | 4.56-10.99 | 1.25 ± 0.11 | 14 |
| Yavatmal | 67 | 12.22 | 20.6-13.29 | 1.15 ± 0.07 | 27 |
| Raichur | 80 | 85.07 | 65.9-96.8 | 1.8 ± 0.15 | 174 |
| Dharwad | 70 | 8.21 | 5.44-9.91 | 1.01 ± 0.04 | 18 |
| Guntur | 75 | 61.02 | 39.3-60.7 | 2.68 ± 0.02 | 152 |
| Medak | 65 | 17.41 | 13.14-20.1 | 1.74 ± 0.03 | 30 |
| Khammam | 38 | 34.21 | 29.0-40.1 | 2.24 ± 0.02 | 64 |
| Nalgonda | 60 | 79.2 | 63.4-94.3 | 1.78 ± 0.14 | 152 |
| Coimbatore | 55 | 120.21 | 79.3-200.5 | 2.49 ± 0.23 | 255 |

*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 5: Response of field strains of *Helicoverpa armigera* for cypermethrin bioassay.**

| Location/strain | Sample size* | $LD_{50}$ | 95% FL | Slope ± S.E. | RF |
|---|---|---|---|---|---|
| Madurai susceptible | 50 | 0.31 | 0.18-0.26 | 1.18 ± 0.21 | |
| Akola | | | | | |
| Madurai | 55 | 15.21 | 10.3-14.3 | 1.32 ± 0.21 | 46 |
| Nagpur | 37 | 121.3 | 99-140 | 2.11 ± 0.11 | 435 |
| Wardha | 50 | 22.01 | 14.0-19.3 | 2.07 ± 0.20 | 69 |
| Amaravati | 53 | 275.3 | 101-309 | 2.39 ± 0.12 | 600 |
| Nanded | 75 | 14.01 | 10.2-19.5 | 2.01 ± 0.31 | 41 |
| Yavatmal | 61 | 77.07 | 50.9-80.1 | 1.72 ± 0.12 | 233 |
| Raichur | 65 | 150.2 | 99-150 | 2.53 ± 0.11 | 460 |
| Dharwad | 75 | 11.23 | 14.1-23.5 | 1.09 ± 0.23 | 58 |
| Guntur | 65 | 102.2 | 72.2-131 | 1.21 ± 0.13 | 345 |
| Medak | 70 | 39.11 | 31.3-47.1 | 2.03 ± 0.11 | 116 |
| Khammam | 82 | 41.21 | 30.4-41.1 | 2.21 ± 0.13 | 127 |
| Nalgonda | 63 | 160.6 | 102-149 | 2.15 ± 0.14 | 482 |
| Coimbatore | 68 | 200.4 | 30.7-200 | 1.78± 0.14 | 700 |

*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).

This study with cyclodiene (endosulfan) and organophosphate (monocrotophos,) 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 [14].

In some strains 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 decreased nerve sensitivity and enhanced metabolism [15, 16, 17]. 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 [18]. A facultative migrant gene flow in *H. armigera* can result in resistant alleles reaching untreated populations [18] or vice versa. Although *H. armigera* is more sedentary and closely associated with crops than other species belonging to the *Helicoverpa*/*Heliothis* complex.

## Conclusion

The findings of present investigations clearly pointed out the possibility of resistance phenomenon operating in *H. armiger*a 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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