CHAPTER III

Monitoring the role of insecticide resistance and management of *Helicoverpa armigera* (Hubner)
I. INTRODUCTION

Among the key pests of cotton, the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) has remained as the major destructive pest of cotton since mid-80s. Insecticide resistant cotton bollworm, *H. armigera*, populations were first reported in southern India in September 1987, when farmers in the coastal districts of Andhra Pradesh were unable to control the very high populations of *H. armigera* on their cotton crops with conventional insecticides. Many farmers applied over 30 insecticide sprays but were unable to contain the pest. Later in the season, pigeonpea and chickpea crops were also badly attacked and insecticides were mostly ineffective against *H. armigera*, in the coastal cotton belt (Sawicki, 1989). High levels of resistance to synthetic pyrethroids were subsequently confirmed by as a major cause of control failure (Dhingra et al., 1988; McCaffery et al., 1989). In economic terms, *H. armigera* caused an estimated loss of 15%, which is equivalent to US $150 million in Andhra Pradesh (Kishor, 1992). In human terms, more than 230 farmers committed suicide in the major cotton districts of Andhra Pradesh (Guntur, Krishna, and Prakasam) during 1987-88 because of financial difficulties arising from loss of income and inability to repay agricultural loans. Prior to 1989, it was believed that insecticide resistance was restricted to populations in the south-east India coastal cotton growing area (Dhingra et al., 1988). However, bioassays conducted between 1989 and 1991 showed that cypermethrin-resistant populations were common throughout southern India and tolerance to endosulfan, some organophosphates and methomyl had increased significantly (Armes et al., 1992). By 1991, resistance to cypermethrin had been reported from cotton crops in Punjab (Mehrotra and Phokela, 1992).

Thus, insecticide resistance continued to be a major factor for *H. armigera*, and in conjunction with *Spodoptera litura* still largely remain uncontrolled since 1997 (Kranthi et al., 1998; 2001a,b). During the 1997/98 cropping season, severe outbreaks of the cotton bollworm and *S. litura* occurred not only on cotton but also on other crops such as pigeonpea, chickpea, sunflower, and chillies in Warangal, Karimnagar, and Khammam districts of Andhra Pradesh. The situation was similar to what had happened in
1987 in the coastal belt of Andhra Pradesh. During 1997, over 350 cotton farmers committed suicide in Andhra Pradesh due to the debts incurred on inputs and could not sustain the control failures in combating *H. armigera* and *S. litura* populations. In view of continued predominance of *H. armigera* as a major cotton pest, the farmers usually had 25-35 applications of insecticides and tank mixtures. Insect pest management in cotton has become the toughest challenge over the years. The problem has exacerbated with the development of insecticide resistance. Cotton and legume crop failures at several locations in Telangana region was attributed due to indiscriminate use of insecticides. Present investigation aims at exploring the understanding of the nature and spread of insecticide resistance in *H. armigera* in Ranga Reddy district of Andhra Pradesh.

Insecticide use against the four important Heliothine species [*H. armigera, Heliothis virescens* (F.), *Heliothis punctigera* (Wallengren) and *Heliothis zea* (Boddie)] has been higher than that on any other insect pests (King, 1994). Insecticide resistance in *H. armigera* was first noticed to synthetic pyrethroids in Australia (Kay, 1977; Gunning et al., 1984; Turkey, 1984), Thailand and Columbia (1984-85), and USA in cotton (1985-86) (Jackson 1989). It commonly destroys more than half the yield with estimates of annual losses in India amounting to US$ 300-500 million to cotton and pulses (King, 1994).

In 1980, the tobacco caterpillar [*Spodoptera litura* (F.)] became a very serious pest on cotton in southern India, and in a 5-year span developed resistance to endosulfan and other organophosphates (Ramakrishnan et al., 1984; Armes et al., 1997). In view of resistance problem, the synthetic pyrethroids were adopted by the Indian farming community, and rapidly became dominant in cotton insect pest control. During 1986, the first case of control failure from suspected insecticide resistance in *H. armigera* was reported from Guntur and Krishna districts of Andhra Pradesh, where poor control was achieved on pigeonpea (*Cajanus cajan* Mill.). In the following crop season, high density larval populations of *H. armigera* appeared in major cotton growing districts of Andhra Pradesh (Guntur, Krishna, and Ongole), and synthetic pyrethroids alone constituted 50-70% of the control applications (Jayaswal, 1989).
Present work was conducted in 2004/05 season in Ranga Reddy district of Andhra Pradesh by collecting samples of eggs and larvae of *H. armigera* on the available hosts including cotton during June-May cropping season, assess the levels insecticide resistance.

**II. MATERIALS AND METHODS**

*Egg/larval sample collection*

Eggs of *H. armigera* were collected from different host crops by removing the plant parts depending on the host parts such as leaves, buds, squares, flowers, buds or panicles. At each sampling around 1000-1500 eggs were collected from the farmers’ fields (approx. 40) at weekly intervals. However, in the peak period of oviposition, an excess of 4000 eggs were collected at weekly intervals from some locations. Bags were placed in insulated cool boxes with ice-bricks to keep them cool until transferred onto artificial diet.

Eggs were removed from the plant parts with a fine sable hair paint brush (size 0) moistened with water, and placed on the side wall of a 7.5-ml cell in a 12-well tissue culture plate (Linbro, ICN Flow Ltd.). Generally one egg was placed in each cell, but at some locations, where high egg mortality was anticipated from field applied insecticides or parasitism, two eggs were placed and after hatching the larvae were transferred individually to one per cell. Placement of eggs on the side-wall resulted in greater hatching compared to directly putting on the moist diet surface. The trays were sealed by stretching a sheet of semi-permeable plastic film (‘Rapfast’ Kent Paper Co., Australia), before fitting the lid to retain moisture content in the diet, and prevent the escape of neonate larvae. The host plants sampled varied based on the season are listed in Table 3.1, Figs. 3.1, 3.2, 3.3 and 3.4.
Table 3.1: Plant hosts sampled for monitoring insecticide resistance in Ranga Reddy district of Andhra Pradesh.

Weeds - *Lagascea mollis*, and *Datura metal*
Crops - Sorghum, cotton, pigeonpea, chickpea, safflower, tomato, and crossandra

Table 3.2. Semi-artificial diet used for rearing *H. armigera* larvae to 30-40 mg weight in 12-well tissue culture plates for discriminating dose bioassays.

1. Diet ingredients (A):

   550 ml  Hot water (approx. 60 °C from tap)
   160 gm  Chickpea flour (Kabuli chana)
   60 gm  Wheatgerm
   3.3 gm  Methyl-4-hydroxybenzoate
   1.7 gm  Sorbic acid
   13.5 ml  10% (v/v) formaldehyde
   2.5 gm  Aureomycin
   5.3 gm  Ascorbic acid

   Mix the above ingredients in Waring Blender.

2. Diet ingredients (B):

   600 ml  Boiling water
   53 gm  Dried bakers yeast
   16 gm  Agar (powdered)

   In boiling water add yeast followed by agar while stirring. Boil for approximately one minute then add to blender and mix thoroughly.

   Pour hot diet into plastic wash-bottles and dispense into diet trays. Prevent diet from setting too quickly by keeping blender jug in sink of hot water.
Fig. 3.1 Heavily infested field of *Lagascea mollis* Cav. during the rainy season. A major host for *H. armigera* in the beginning of kharif season.

Fig. 3.2. *H. armigera* feeding on *Lagascea mollis* Cav.
Fig. 3.3. *Datrua metel* L. growing in the beginning of monsoon season and a major host of *H. armigera*.

Fig. 3.4 Larva of *H. armigera* feeding inside the earhead of sorghum.
Rearing of *H. armigera*

The cotton bollworm rearing and insecticide assays were conducted at constant temperature (25±2°C) maintained with local air-conditioners. The composition of the chickpea based semi-artificial diet used to rear larvae is furnished in Table 3.2. The liquid diet was poured into the cells of the tissue culture plates (5 mm deep) from squeezable plastic wash bottles with an outlet tube cut to 3-cm and the internal tube removed. Approximately 70-75 trays could be made from the above diet mix. Once the diet was cooled, the plates were kept in an incubator at 16°C.

*H. armigera* bioassay

When neonate larvae reached 30-40 mg weight, they were used for dosing which period approximately took 6-7 days after egg collection. As larvae tended to grow rapidly during the instar-1 to -4, their weights were monitored twice daily (morning and late afternoon). Because of large numbers of larvae being processed, it was not feasible to weigh every larva. Under-weight larvae (25-30 mg) were kept in an incubator at 18°C to ensure optimum weight on the following day.

Insecticides

The following technical grade insecticides were used for bioassays: *cis:trans* (50:50 ratio) cypermethrin (77% w/w; ICI Agrochemicals, UK); fenvalerate (97.6% w/w; Sumitomo Corp., Japan); endosulfan (96% w/w; Hoechst, India); quinalphos (70% w/w; Sandoz AG, Switzerland); and profenofos (94% w/w; Ciba Geigy, Switzerland). The synergist, piperonyl butoxide (Pbo) (90% w/w) was obtained from Gooddeed Chemical Co. Ltd., U.K.
**Discriminating doses**

The following standardized discriminating doses were used for routine monitoring of resistance to pyrethroids, cyclodiene, organophosphate, and carbamate insecticides (Table 3.3).

**Table 3.3 Discriminating dosages of insecticides used for monitoring insecticide resistance to *H. armigera*.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Dosage$^{larva}$ (µg/µl)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypermethrin</td>
<td>0.1</td>
<td>LD$_{99}$ for pyrethroid susceptible <em>H. armigera</em> strain originally collected from the Sudan.</td>
</tr>
<tr>
<td>Cypermethrin + pbo</td>
<td>0.1 + 50</td>
<td>Pyrethroid plus synergist combination to determine the extent of esterase mediated pyrethroid resistance.</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>1.0</td>
<td>Used for precise kill of heterozygotes unowned at this stage, but introduced as a twin ‘twin’cypermethrin discriminating dose because of the very high survival at the normal susceptible discriminating dose.</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>10.0</td>
<td>Approximate LD$_{99}$ for susceptibles calibrated on Australian <em>H. armigera</em>.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>0.2</td>
<td>LD$_{99}$ for susceptibles calibrated in Australia and used to compare the resistance in India with Australia.</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>0.75</td>
<td>LD$_{99}$ for NRI susceptible strain of <em>H. armigera</em>.</td>
</tr>
<tr>
<td>Methomyl</td>
<td>1.2</td>
<td>LD$_{99}$ for NRI susceptible strain of <em>H. armigera</em>.</td>
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III. RESULTS

Resistance monitoring in *Helicoverpa armigera*

The larvae of *H. armigera* collected, from Ranga Reddy District, on unsprayed weeds exhibited resistance frequencies of 77-89% to cypermethrin (0.1 µg/µl) in June-August, which gradually increased to 95% by September-November, and reached 100% in December-April (Fig. 3.5). This trend has been consistent throughout the previous seasons. Resistance level of 100% to pyrethroids had been previously recorded from Guntur district of Andhra Pradesh (Armes et al., 1994). Resistance frequencies to the synergistic combination of cypermethrin (0.1 µg/µl) + Pbo (50 µg/µl) were 49-60% in June-August, which remained stable by September-November (Fig. 3.6). This increased trend in the past two seasons may be due to excessive use of insecticides. Also, Pbo alone caused a suppression of 54-81% during December-April, but had no effect on larval populations. Data collected over two seasons in Andhra Pradesh also showed some interesting trends. Resistance frequencies to cypermethrin (1.0 µg/µl) were in general was higher throughout the season in 2004/05 (Fig. 3.7). The resistance frequencies were 61% and 43% in the early season; 52% and 43% in September-November; 51% and 36% in December-February; and 53% and 29% in March-April. Resistance to fenvalerate (0.2 µg/µl) was extremely high as is the case of cypermethrin in 2004/05 season indicating no difference between them (Fig. 3.8).

Resistance to endosulfan (10.0 µg/µl) was initially low in the crop season during June-August with 20% and 15%, but subsequently increased to 40% by the end of February to early March (Fig. 3.9). However, its use was more on vegetables against *H. armigera* in February-March, and thus the resistance frequencies have increased in the last two seasons from 47% to 63%. In general, resistance to quinalphos (0.75 µg/µl) was high in 2004/05 season with 37% and 24% at the beginning of the season in July and August, 33% and 37% in September to November, rose steadily to 50% and 45% in December and February, and reached 55% and 21% in March and April, respectively (Fig. 3.10). Although the resistance has declined considerably, it was losing its susceptibility rapidly. Resistance to methomyl (1.2 µg/µl) was 40% in the beginning of September then dropped to 20% and in the mid-season increased to 60%-70% until mid-March (Fig. 3.11).
Fig. 3.5 – 3.11. Larval mortality of *Helicoverpa armigera* in relation to differential dosages of insecticides.
IV. DISCUSSION

Present investigation is to examine insecticide resistance in *H. armigera* over a broad range of insecticides used in Ranga Reddy district. The results indicated that survival to twin dose of cypermethrin is >65% and fenvalerate ranged between 75-85%. Synergistic action of Cypermethin 0.1 + Piperonyl butoxide 0.50 µg did not suppress the populations of *H. armigera*, and the survival remained between 54 and 81%. This corroborates with high levels of resistance recorded for pyrethroids reported earlier in Andhra Pradesh (McCaffery et al., 1989; Armes et al., 1992), and also indicated that despite the non-preference by the farmers for the use of pyrethroids between late 90’s and early-20s to control cotton pests there is no decline in the resistance levels. Two decades of monitoring in Andhra Pradesh has indicated that resistance to cypermethrin was on the increase from 7- to 2100-fold during 1989 (Armes et al., 1992), and 20- to 6500-fold during 1991-1994 (Kranthi et al., 2001a). These trends suggest that there is an alarming increase the levels of resistance to synthetic pyrethroid in Andhra Pradesh. Further, Kranthi et al. (2002) reported that increased usage of pyrethroids in Andhra Pradesh has resulted in the severe outbreak of *H. armigera* in 1997-98, with over 60% of insecticides applications on cotton alone. Thus, the severity of pyrethroid resistance in India is comparable to the already documented resistance levels of Australia (Forrester 1990; Forrester et al., 1993), and Pakistan (Ahmad et al., 1998). There is an increase in resistance by 17-fold due to cypermethrin in Turkey (Ernst and Dittrich, 1992), 56,911-fold to deltamethrin, and 1361-fold to fenvalerate in China (Shen et al., 1993).

Despite extensive use of endosulfan on cotton during September-December, the resistance levels remained low-moderate, but on vegetables in February-March has increased the resistance levels in Ranga Reddy District of Andhra Pradesh. Earlier reports indicated 28-fold resistance from the strains of *H. armigera* collected in Andhra Pradesh (McCaffery et al., 1989; Armes et al., 1995, 1996; Singh et al., 1998). Endosulfan has contributed to increased levels of resistance in cotton due to 85% usage in Central India (Kranthi et al., 2005), and 25% in Guntur district of Andhra Pradesh (Fakhrudin et al.,
2003), but was slightly higher than reported earlier (Armes et al., 1994a,b; Armes et al., 1996; Rusell et al., 1998). In addition, Kranthi et al. (2005) reported resistancer levels of 2- to 38-fold to methomyl from strains collected from various locations of India, with highest being 162-fold resistant from Guntur district in Andhra Pradesh. Comparative data over the past ten years showed low-moderate levels of resistance to endosulfan in most recorded places in Andhra Pradesh.

*H. armigera* strains assayed against quinalphos showed moderate levels of resistance in Ranga Reddy district. However, Kranthi et al. (2005) reported low and stable levels with 10-15 fold increase in resistance among the strains collected from Guntur and Prakasam districts of Andhra Pradesh. Fakhrudin et al. (2003) reported only 6% usage in Guntur district. Kranthi et al. (2005) reported only eight strains tested were found to be resistant to methomyl, the highest being 22-fold resistance in a strain collected from Prakasam district in Andhra Pradesh. Whereas, Armes et al. (1996) reported resistance levels of 2- to 38-fold to methomyl from different regions of India. Very high levels of >30 fold resistance was reported to methomyl from China (Cheng and Liu, 1996).

Integrated pest management (IPM) and Insecticide resistance management (IRM) strategies against *H. armigera* are being dealt with natural enemies, neem and other plant products, including nuclear polyhedrosis virus (NPV). However, unfortunately, we are far from having good production agencies for mass production of biological agents. Crop health is a major concern, even in the absence of biologicals and now largely depends on the agrochemical industry and the scientific community to conserve and maintain the potential efficacy of their products against target pests and where already the insect pests have become resistant to develop strategies for reduced resistance selection. However, conservation of newer products and using newer chemistries by their mode of action on pests will be useful in future (Casida and Quistad, 1998). Such efforts are essential in enhancing the credibility of resistance management against *H. armigera*. 

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