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# Spectrum of Insecticide Resistance in Whitefly from Upland Cotton in Indian Subcontinent

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Abstract: Cotton, Gossypium hirsutum L. is a chief fiber crop of India and contributes significantly to Indian agricultural and industrial economy. Its economic cultivation is very much affected by insect pest infestations. Whitefly, Bemisia tabaci (Genn.) has attained a status of key pest among these insect pests and the main reliance has been on the use of insecticides for its management. Insecticide resistance develops more readily due to their injudicious and indiscriminate use by growers. Thus, it is imperative to test the level of resistance to design a successful insecticide resistant management program. In this study, the resistance level to triazophos, endosulfan and imidacloprid were determined in whitefly populations from cotton growing areas of India. Whitefly populations from seven different locations; Guntur, Coimbatore, Kolar, Ludhiana, Bathinda, Sri Ganganagar and Sirsa were tested for resistance at two discriminating doses (0.25 and 1%) of each insecticide. Populations from all locations displayed widespread resistance to triazophos, endosulfan and imidacloprid with highest in Bathinda and lowest in Coimbatore. High resistance levels were recorded in populations from North India compared to populations from south India against all three insecticides. Whitefly populations from all locations did not show any resurgence against any insecticide.

**Key words:** *Bemisia tabaci*, triazophos, endosulfan, imidacloprid, discriminating dose, resurgence, survey, cross-resistance

#### INTRODUCTION

Cotton (Gossypium hirsutum L.) is an important cash crop of India and adds around 30% to the gross domestic product of Indian agriculture. Its area (8-9 million ha) accounts for 25% of the world's total cotton area and ranks third after the United States and China. However, as with many cotton growing areas of the world, a major limiting factor in its production is damage due to insect pests (Bennett et al., 2004). Among these insect pests, whitefly Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae) is a very important pest worldwide (Byrne and Bellows, 1991; Perring et al., 1993; Bedford et al., 1994; Brown et al., 1995; Viscaret et al., 2003). Epizootic outbreaks of whitefly populations began in seventies in Sudan and the Middle East (Dittrich et al., 1985), reached the USA in the eighties (Johnson et al., 1982) and later on expanded to most tropical and subtropical countries, including Australia, Canada, Japan and The Netherlands (Gerling and Mayer, 1996).

Whitefly causes severe damage accounting for hundreds of millions of dollars annually (Menn, 1996; Naranjo et al., 1996; Chu et al., 1998). Damage is due to feeding and deposition of honeydew (Schuster et al., 1996), physiological disorders (Yokomi et al., 1990) and transmission

of gemniviruses (Bedford et al., 1994; Markham et al., 1996; Idris and Brown, 2004). No significant economic damage had been caused to crops in Asia until population outbreaks started in early nineties. Among the suggested causes of whitefly outbreaks are climatic factors (Jayaraj et al., 1986; Byrne et al., 1992), cropping practices (Byrne et al., 1992) and use of broad-spectrum insecticides that induce resistance development (Dittrich et al., 1985; Prabhaker et al., 1985, 1989) and/or disrupt control by natural enemies (Eveleens, 1983). The resurgence in whitefly has been reported due to the repeated use of pyrethroids (Jayaraj et al., 1986; Patil et al., 1990; Butter and Kular, 1999). Many insecticides are known to alter the physiology and behavior of whitefly through hormoligosis (Abdullah et al., 2006; Dutcher, 2007).

Now, insecticide resistance is a serious threat to whitefly control and has been reported from USA (Prabhaker *et al.*, 1985), Israel (Perry, 1985; Byrne *et al.* 1994), Guatemala, Sudan, Turkey (Dittrich *et al.*, 1990a), Central America, Ethiopia, Mexico, Peru (Lemon, 1992; Martinez-Carrillo, 2006), Cyprus UK, Yemen (Byrne and Devonshire, 1993), Pakistan (Cahill *et al.*, 1994, 1995; Ahmad *et al.*, 1999, 2000, 2001) and India (Butter and Kular, 1999; Jayaraj *et al.*, 1986; Patil *et al.*, 1990). To manage this pest efficiently, resistance management strategies should be implemented. This highlights need to assess and monitor the responses to insecticides in the target population, to enable the timely use of alternative control measures such as rotation of different insecticides, reduction in the number of applications, or the use of synergists (Stansly *et al.*, 1997; McAuslane *et al.*, 1993). Therefore, in order to assure a sustainable resistant management program, it is essential to survey the insecticide resistance levels in cotton growing areas (Nibouche, 1994). Thus, in the present study we determined how widespread is the problem of resistance in whitefly in cotton growing areas of India against the most commonly used insecticides belonging to three different groups; organophosphates, cyclodienes and neonicotinoids.

#### MATERIALS AND METHODS

The commercial formulations of three insecticides viz. organophosphate Hostathion 40 EC (triazophos), cyclodiene Thiodan 35 EC (endosulfan) and neonicotinoid Confidor 200 SL (imidacloprid) at two discriminating concentrations (0.25 and 1.0%) were used to investigate the level of resistance. Bioassays were conducted at grower's cotton fields from 7 different locations representing 6 states of India; Guntur (Andhra Pradesh), Coimbatore (Tamil Nadu), Kolar (Karnataka), Ludhiana and Bathinda (Punjab), Sri Ganganagar (Rajasthan) and Sirsa (Haryana) (Fig. 1). Treated cotton leaf discs were placed in the Petri dishes with agar (Sethi *et al.*, 2008) and were dipped in an aqueous solution of formulated insecticide for 10 sec and allowed to dry for 1 h. Untreated cotton leaf discs were used as control. Thirty whitefly adults (unsexed) were collected from different sites of the same field at each location and released in the Petri dish. Each insecticide concentration level had 5 replications and mortality was recorded after 24 h. Percentage mortality was analyzed by ANOVA using Proc GLM (SAS Institute, 2003) at each insecticide concentration level. Tukey's Honestly Significant Difference (HSD) test with a significance level of  $\alpha = 0.05$  (SAS Institute, 2003) was used for post hoc means separation.

In the absence of any baseline susceptibility data for triazophos, endosulfan and imidacloprid against *B. tabaci*, mortality of the Coimbatore population at 0.25% was used as a base population for calculating resistance ratio (RR = mortality of selected population/mortality of Coimbatore population) because of exhibiting highest mortality among all the populations. Resurgence index in whitefly population for each insecticide was calculated using the following formula (Henderson and Tilton, 1955; Dutcher, 2007):

Resurgence (%) = 
$$\left(1 - \frac{(T_a \times C_b)}{(T_b \times C_a)}\right) \times 100$$

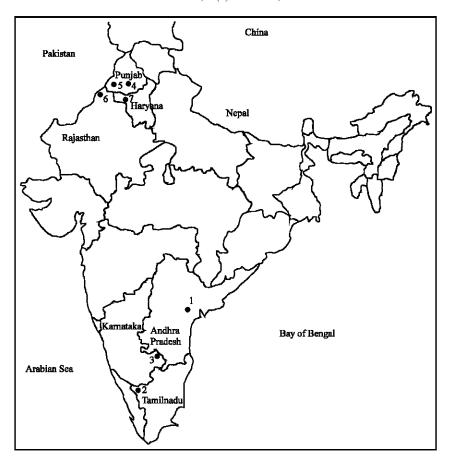


Fig. 1: Regions of India from where bioassays on whitefly populations were done for insecticide resistance: (1) Guntur, (2) Coimbatore, (3) Kolar, (4) Ludhiana, (5) Bathinda, (6) Sri Ganganagar and (7) Sirsa

## Where:

T<sub>a</sub> = Percentage of whitefly survived on treated leaf disc after treatment

C<sub>b</sub> = Percentage of whitefly on control leaf disc before treatment

T<sub>b</sub> = Percentage of whitefly on treated leaf disc before treatment

C<sub>a</sub> = Percentage of whitefly survived on control leaf disc after treatment

If whitefly survival was greater in the treated than control leaf discs, the above formula would result in a negative value indicating that resurgence has occurred after the insecticide application in the field over the years.

#### RESULTS

The percentage mortality in whitefly control (untreated leaf discs) populations was  $11\pm0.83$  (Mean±SEM; Guntur),  $8.4\pm1.8$  (Coimbatore),  $13.4\pm0.81$  (Kolar),  $7\pm0.54$  (Ludhiana),  $6\pm0.83$  (Bathinda),  $9\pm1.4$  (Sri Ganganagar) and  $5\pm1.4$  (Sirsa).

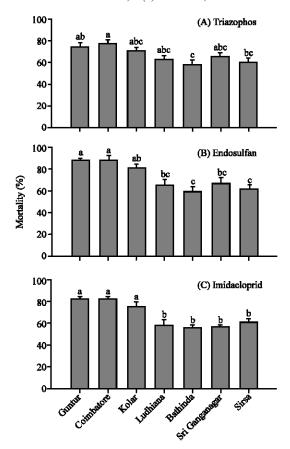


Fig. 2: Whitefly mortality among different populations from India at 0.25% concentration of commonly used insecticides. Error bars indicate SEM. Bars topped with different letters differ significantly at the 0.05 level (Tukey's HSD test)

#### Triazophos

Whitefly mortality at 0.25% concentration was significantly different among all locations (F = 4.08; df = 6, 28; p = 0.0046) (Fig. 2A). Coimbatore population exhibited highest and Bathinda population exhibited lowest mortality. Bathinda population possessed highest resistance ratio (1.32-fold) (Table 1). Values for resurgence index were positive at all locations (Table 2).

Whitefly mortality at 1% concentration was not significantly different among all locations (F = 1.85; df = 6, 28; p = 0.1246) (Fig. 3A).

## Endosulfan

Whitefly mortality at 0.25% concentration was significantly different among all locations (F = 10.02; df = 6, 28; p = <0.0001) (Fig. 2B). Whitefly populations from Coimbatore and Guntur exhibited highest mortality and Bathinda population exhibited lowest mortality. Bathinda population possessed highest resistance ratio (1.47-fold) (Table 1). Values for resurgence index were positive at all locations (Table 2).

Whitefly mortality at 1% concentration was significantly different among all locations (F = 3.59; df = 6, 28; p = 0.0091) (Fig. 3B). Bathinda population exhibited lowest mortality.

<u>Table 1: Resistance ratio of whitefly populations from different regions of India against commonly used insecticides</u>
Resistance ratio

Locations	Triazophos	Endosulfan	Imidacloprid	
Guntur	1.04	1.00	1.01	
Coimbatore	1.00	1.00	1.00	
Kolar	1.10	1.08	1.09	
Ludhiana	1.22	1.34	1.41	
Bathinda	1.32	1.47	1.48	
Sri Ganaganagar	1.18	1.32	1.46	
Sirsa	1.28	1 42	1 36	

Table 2: Whitefly resurgence index (%) against commonly used insecticides in India

	Triazophos		Endosulfan	ı	Imidaclopri	d
Locations	0.25%	1%	0.25%	1%	0.25%	1%
Guntur	71	96	87	98	79	94
Coimbatore	75	95	87	100	80	95
Kolar	65	88	79	98	71	92
Ludhiana	60	94	63	97	55	86
Bathinda	55	89	57	94	52	82
Sri Ganganangar	62	95	64	100	52	88
Sirsa	58	93	60	96	58	84

#### **Imidacloprid**

Whitefly mortality at 0.25% concentration was significantly different among all locations (F = 14.30; df = 6, 28; p = <0.0001) (Fig. 2A). Populations from northern states (Bathinda, Sri Ganganagar, Ludhiana and Sirsa) exhibited less mortality compared to southern states (Coimbatore, Guntur and Kolar). Bathinda population possessed highest resistance ratio (1.48-fold) (Table 1). Values for resurgence index were positive at all locations (Table 2).

Whitefly mortality at 1% concentration was significantly different among all locations (F = 7.04; df = 6, 28; p = 0.0001) (Fig. 3B). Whitefly populations from northern states exhibited lowest mortality compared to southern states.

# DISCUSSION

Analysis of *B. tabaci* populations among different locations in India detected a high degree of resistance in every location. The highest degree of resistance as measured by resistance ratio was found for imidacloprid and endosulfan and followed by triazophos. The pattern of resistance closely followed the frequency of type of insecticides used by growers, as imidacloprid and endosulfan being two of the most popular compounds with growers for whitefly control. Roditakis *et al.* (2005) also found a high level insecticide resistance in whitefly against pirimiphos-methyl (18-fold), endosulfan (58-fold) and imidacloprid (730-fold) in southern Greece. Kranthi *et al.* (2001, 2002) could not detect any resistance in whitefly against triazophos and endosulfan. A high level of resistance against imidacloprid had been reported by Rauch and Nauen (2003) for populations collected from Germany, Israel and Spain,. Whitefly populations from all the locations did not show resurgence against any insecticide.

At present, organophosphates, neonicotinoids and endosulfan (a cyclodiene) are being used extensively because of their effectiveness against important pests of agricultural crops. However, the major concern with intensive use of these insecticides is the potential for cross-resistance due to possible shared target sites and similar degradation pathways (Golenda and Forgash, 1985; Scott, 1989; Bisset *et al.*, 1997). This cross-resistance could cause serious impact on the control of insect pests by reducing effectiveness of many new insecticides. Insecticide resistance results due to two mechanisms, mutations of the target protein decreasing affinity to the respective insecticide and increased detoxification by enzymes, such as esterases, cytochrome P450-dependant monoxygenases or gluthatione S-tranferases. Whitefly resistance to organophosphates is due to the enhanced detoxification by non-specific esterases and cytochrome P450-dependant monoxygenases

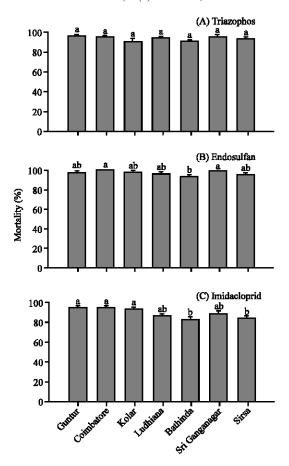


Fig. 3: Whitefly mortality among different populations from India at 1% concentration of commonly used insecticides. Error bars indicate SEM. Bars topped with different letter(s) differ significantly at the 0.05 level (Tukey's HSD test)

(Prabhaker *et al.*, 1988; Dittrich *et al.*, 1990b; Bloch and Wool, 1994) and also due to the insensitivity of the synapse acetylcholinesterase (Anthony *et al.*, 1998; Byrne and Devonshire, 1997). Perez *et al.* (2000) suggested whitefly resistance mechanism against endosulfan is due to oxidative and hydrolytic degradation. Whitefly resistance to endosulfan is associated with a replacement of a single amino acid (Alanine to Serine or Glycine substitution at position 302) within the resistance to dieldrin gene (*Rdl*) encoding the γ-aminobutyric acid (GABA) receptor subunit which is the main target of cyclodiene insecticides (Anthony *et al.*, 1995). Neonicotinoids resistance in whitefly is due to the overexpression of cytochrome P450-dependant monoxygenases (Rauch and Nauen, 2003). Insecticide resistance management strategies are generally considered after resistance has been developed. Resistance management program can be much more effective if the likelihood of resistance development to novel insecticides can be predicted before their use. Therefore, studies on resistance mechanisms with their chances of cross-resistance can facilitate us to design novel strategies to prevent or minimize the spread and development of resistance and also to develop a successful program to overcome already attained resistance.

Whitefly populations from northern areas; Bathinda and Ludhiana (Punjab), Sirsa (Haryana) and Sri Ganganagar (Rajasthan) showed a higher level of resistance against all the three insecticides

compared to southern areas; Guntur (Andhra Pradesh), Coimbatore (Tamil Naidu) and Kolar (Karnataka). This suggests that insecticide pressure is exceptionally higher in the northern states. Nearly 54% of the total pesticides used in India are applied on cotton crop alone and cotton accounts only for 5% of the total cultivated area (Puri, 1995). Punjab and Haryana states are classified under category I states (5,000 MT annually) based on the consumption of pesticides; whereas Andhra Pradesh, Tamil Nadu, Karnataka and Rajasthan category II (1000-5000 MT annually) (FAO, 2002). Punjab consumes 923 g ha<sup>-1</sup> of technical grade of insecticide which is the highest in India (Agnihotri, 2000). Bathinda area in Punjab, largely grows cotton, is known for excessive use of pesticides. Though the Punjab Agricultural University at Ludhiana recommends only 7 sprays on cotton in 6 months, Bathinda growers have been known to spray as many as 32 times (Dhawan, 2002). Hence, this explains that resistance levels are appropriate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that the resistance levels rise with higher insecticide usage. The pesticides create very high pressure for resistant genotypes of pest population by affecting frequencies of resistant and susceptible alleles (Dobzhanky, 1951; Roush and Daly, 1990). This suggests that indiscriminate use and heavy dependence on pesticide will further complicate the already worsened situation and hints at aiming for insecticide resistant management strategies. As there are few alternative materials for growers to use at present, insecticide resistance will likely to become an increasing challenge for growers and can jeopardize their ability to produce high quality export cotton in near future. A recent step made to Insecticide Resistant Management (IRM) program in India highlights the necessity for experimental learning of growers about complex technologies, such as integrated pest management through participation in training programs (Peshin et al., 2007). Results from our study so far imply that whitefly resistance is widespread against commonly used insecticides and may likely to expand and intensify among cotton growing areas in India.

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