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Field resistance to insecticides in whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) population on okra and cotton

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Abstract
Whitefly, *Bemisia tabaci* is an important sucking insect pest on many crops including vegetables. It has become a pronounced pest by vectoring plant viruses especially begomoviruses. In recent years, there is severe incidence of these viruses in vegetables *viz.*, okra, tomato, chillies etc., This may be due to changes in the whitefly population itself particularly their response to insecticides. The current level of resistance in *B. tabaci* to selected insecticides, *viz.*, thiamethoxam 25% WG, imidacloprid 17.8% SL, thiacloprid 21.7% SC, triazophos 40% EC and chlorpyrifos 20% EC were assayed in Madurai and Salem districts of Tamil Nadu where there was severe incidence of okra enation leaf curl virus (OELCV) and bhendi yellow vein mosaic virus (BYVMV) was noticed. Susceptibility of these populations had varied and however, had shown significant resistance to these insecticides. The highest level of resistance to thiamethoxam, imidacloprid, thiacloprid, triazophos and chlorpyrifos in terms of resistance ratio (fold increase in LC_{50} over the susceptible laboratory population) was registered in the *B. tabaci* population collected from Tirumangalam (34.54), Attur (69.41), Attur (25.15), Melur (30.92) and Usilampatti (38.67) locations, respectively. This level of resistance may lead to increased damage of host crops by whiteflies and associated spread of begomoviruses. Hence, insecticide resistance management strategies are needed to be adopted to smother the whitefly population besides continuously exploring the host plant resistance strategies.

Keywords: *Bemisia tabaci*, insecticides, resistance, management

Introduction
The sweet potato whitefly, *Bemisia tabaci* Gennadius is a notorious pest and is found among the world's top 100 invasive organisms (De Barro *et al*., 2011) [6]. It has attained a major insect pest status by not only damaging the plant by phloem sap sucking but also equally gained importance being a vector of viral diseases and is causing severe economic damage in over 60 crop plants as a (Castillo *et al*., 2011) [19]. Of late, the management of *B. tabaci* has become difficult owing to the wider host adaptability, cryptic species status, and efficient virus transmission capabilities (De Barro *et al*., 2011) [6]. Insecticides are the key agent in *B. tabaci* management, however control failures of insecticides against *B. tabaci* in India were reported (Peshin & Zhang, 2014) [22] and insecticide resistance in *B. tabaci* has been reported for more than 40 active ingredients of insecticides (Whalon *et al*., 2013) [30].
Insecticides have been phased out decade after decade by the invention of new molecules and the inherent environmental and other issues associated with the extant molecules. Thus, neonicotinoids and other compounds of novel chemistry are ruling worldwide from 1990s (Jeschke *et al*., 2010) [12] and are over used now (Nauen *et al*., 2015) [18]. In India, resistance to OPs, pyrethroids, and carbamates in whitefly is already evident from earlier reports (Kranthi *et al*., 2002, and Armes *et al*., 1996) [13, 3] and to neonicotinoids in recent reports (Naveen *et al*., 2017) [21]. In okra, the severe incidence of Bhendi yellow vein mosaic virus (BYVMV) and Okra enation leaf curl virus (OELCV) diseases that are being transmitted only by *B. tabaci* and no other ways, such as either mechanical through plant sap or seed route of transmission are reported, are limiting the economic prospects of small growers of okra and thus discouraging them from cultivating the crop. The OELCV incidence has reached serious proportions in recent years both in Northern India (Sanwal *et al*., 2014) [24] and Southern India as well (Sayed *et al*., 2014) [25]. This may be due to either the changes in the vector itself in the form of resistance to insecticides or the pathogen themselves being a virulent one or the susceptible nature of the okra accessions.

The present investigation attempts to take a snapshot view of resistance development in field populations of *B. tabaci* against selected insecticides currently used for controlling *B. tabaci* in the areas where there was severe incidence of the begomoviruses on okra.

Materials and Methods

Insecticide usage history and cropping details

The surveys were conducted in farmers' fields to collect primary data on the cropping and insecticide usage pattern of the farmers (Table 1).

Whitefly collection, rearing, and maintenance

Adults of cotton whitefly, *Bemisia tabaci* were collected on okra (*Abelmoschus esculentus* L.) and cotton *(Gossypium* spp.) from Madurai and Salem districts of Tamil Nadu, India using an aspirator during early morning. Leaves infested with the nymphs and pupae of *B. tabaci* were also collected and the petioles of leaves were immersed inside the containers filled with water and plugged at mouth with cotton ball. The insects and the infested leaves were brought to the greenhouse of Insectary, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. Infested leaves, properly hydrated were kept in cages for the emergence of fresh adults. The taxonomic identity of collected *B. tabaci* was confirmed by examining the insects under a light microscope using the keys (Martin, 1987) [16]. The collected and emerging *B. tabaci* adults were allowed inside secured insect cages that contained polybag potted healthy 30-45d old insecticide-free cotton plants (cultivar ARBH 1401).

The cotton plants were grown on cocopith and soil medium with proper fertilizers and water. The plants were maintained in cages 150cmx150cmx150cm and covered with 100 micron mesh cloth. Thirty to forty day old pest free fresh plants were introduced inside the culture cages every fortnight. These populations were maintained as large colonies without insecticide selection prior to the bioassays. For collection of naïve whitefly adults for use in experiments individual plants were caged for 3-4 days separately and the adults emerged and trapped inside the 100 micron mesh cloth cage were collected using aspirator.

Insecticides used in the study

Five commercial formulations of insecticides used in this study were thiamethoxam 25% WG, imidacloprid 17.8% SL, thiacloprid 21.7% SC, triazophos 40% EC and chlorpyrifos 20% EC and their details are provided in Table 2.

Bioassay

For assessing the insecticide toxicity to *B. tabaci*, a modified leaf dip bioassay method of Insecticide Resistance Action Committee was followed (Naveen *et al*., 2012) [20]. The five commercial formulations of insecticides were diluted to five to six concentrations with distilled water. Cotton leaves (cultivar ARBH 1401) with petiole, collected from the fifteen to twenty-five days old seedlings were immersed in the serially diluted insecticide solutions for 20 sec; then allowed to air dry on paper towel. Leaves dipped in only diluents served as the untreated control. After drying the petiolated leaves, they were placed in the glass vial filled with water (the petiole should reach the base of the vial) and the vials were sealed with cotton ball. The naïve adults collected from reared units were transferred in batches of 10-15 onto the treated leaves. All such assays were replicated three times for a minimum of five concentrations for each insecticide. Observations on the insect mortality were recorded up to 72h. The adult insect was considered to be dead if not moving or responding to the external stimulus using a gentle probe with a single haired paintbrush. Mortality was estimated by counting the total number of dead and live insects.

Statistical analysis

The mortality data were corrected according to Abbott's formula (Abbott, 1925). The LC_{50} and LC_{90} values, 95% confidence limits, standard errors, the slopes of the regression lines and $\chi 2$ significance tests, were estimated by Finney's probit analysis (Regupathy and Dhamu, 2001) [23]. Resistant ratio (RR) was computed with LC_{50} of resistant population / LC_{50} of susceptible population.

Results and Discussion

Resistance levels of six field populations of whitefly, *B. tabaci* collected from six locations within two different districts of Tamil Nadu to selected insecticides, *viz*., thiamethoxam 25% WG, imidacloprid 17.8% SL, thiacloprid 21.7% SC, triazophos 40% EC and chlorpyrifos 20% EC using different doses were assessed and reported in tables (3 to 7). The whitefly populations showed different degrees of resistance to insecticides.

Thiamethoxam: The Tirumangalam population of *B. tabaci* showed the highest resistance to thiamethoxam with LC_{50} of 0.829 ppm and RR of 34.54 fold over the susceptible population. The *B. tabaci* populations from Attur, Usilampatti, Omalur, Melur and Edappadi registered LC_{50} (ppm) of 0.737, 0.614, 0.612, 0.564 and 0.444 with a RR of 30.70, 25.58, 25.5, 23.5 and 18.5-folds, respectively (Table 3). Resistance to thiamethoxam in whitefly population were reported by Turkey *et al*. (2017) [29] wherein the reported RR ranged from 8.6 to 31.8 for LC_{50} and 9.9 to 40.8 for LC_{90} based on a susceptible laboratory population and they also reported that all the field collected whitefly populations had LC_{90} values that were higher than the recommended field dose of thiamethoxam.

Imidacloprid: The LC_{50} values of imidacloprid varied from 0.549 to 0.833 ppm for the whitefly from six different locations. The LC_{50} was the lowest in Edappadi (0.549 ppm) and the highest (0.833 ppm) in Attur. The Attur population had the highest RR of 69.41-fold over the susceptible population. The other populations had significantly higher LC_{50} values than the susceptible population (0.012 ppm) with a RR of 45.75, 53.58, 65.75, 59.75 and 48.41-fold, respectively (Table 4). In a similar study, Smith *et al.* (2016) [11] reported LC_{50} for the whitefly (laboratory colony) being 0.131 for imidacloprid, wheras for field populations it ranged from 0.901–24.95.

Thiacloprid: The Attur population showed the highest resistance (0.478 ppm) followed by Melur (0.391 ppm) and Usilampatti (0.384 ppm) population. The maximum RR was 25.15-fold for Attur population and minimum of 17.42-fold for Edappadi population (Table 5). Gul Satar *et al.* (2018) [10] examined five *B. tabaci* populations collected in the Mediterranean region of Turkey for resistance to four neonicotinoid insecticides and reported that each population was distinct but in most cases there was a similar pattern of resistance to these four compounds, wherein the most resistant population to thiacloprid recorded 272 fold resistance.

Triazophos: The Edappadi population was highly susceptible to triazophos as indicated from the lowest LC_{50} (0.293 ppm; RR-20.92). A high level of resistance was noticed in Melur population with LC_{50} of 0.433 ppm (RR-30.92) followed by Attur (0.362 ppm; RR-25.85) and Usilampatti (0.342 ppm; 24.42) (Table 6). The prevalence of triazhophos resistance in *B. tabaci* was reported earlier from different locations of India (Sethi and Dilawari, 2008; Naveen *et al.,* 2017) [26, 21].

Chlorpyrifos: The Usilampatti population had the highest RR (38.67-fold) followed by Tirumangalam (31.54-fold) and Omalur (30.12-fold). The order of increase in LC_{50} (ppm) was: Melur (0.360), Attur (0.513), Edappadi (0.821), Omalur (0.934), Tirumangalam (0.978) and Usilampatti (1.199), whereas the susceptible population recorded 0.031 ppm (Table 7). The presence of chlorpyrifos resistance was earlier reported in *B. tabaci* populations (Sethi and Dilawari, 2008; Naveen *et al.,* 2017; Kumar and Grewal, 2018) [26, 21, 15].

Due to the extensive damage caused by whitefly, the usage of insecticides to control the pest is inevitable. The repeated application of insecticides especially newer molecules provides ample chances for further development of resistance and field control failures. The less expensive old chemistry insecticides are common in use owing to cheaper price and at the same time new chemistries are also encouraged in field use. In India, the commercial seeds of cotton and okra are seed dressed with imidacloprid to manage the sucking insects at the seedling phase and thereby insects are experiencing the chemistry. In the present study, resistance to a greater extent to imidacloprid, thiamethoxam, triazophos and chlorpyrifos was noticed among the *B. tabaci* from the selected pockets of two districts of Tamil Nadu. Triazophos and chlorpyrifos resistance is common among the whitefly in India in recent years (Sethi and Dilawari, 2008; Naveen *et al.,* 2017; Kumar and Grewal, 2018) [26, 21, 15] owing to their use over a longer period. The intensity of the insecticides use and long term exposures enforced genetically based resistances in insects over time (Tabashnik, 1989) [27]. Significant resistance to neonicotinoids especially to imidacloprid recorded in this study might be attributed to the long term exposure of this compound in the cotton and okra crops in these ecosystem of the districts of Tamil Nadu wherein farmers use imidacloprid dressed commercial seeds and also farmers might have resorted to repeated application of the neonicotinoids as it gave an excellent control at the introduction of the chemistry based on past experience and not realizing the latest ineffectiveness of the same chemical. Insecticide resistance is a result of either mutation in target proteins and thus, decreasing the affinity to the targeting insecticide and or the increased expression of detoxifying enzymes. The metabolic resistance mechanisms involving carboxylesterases, cytochrome-P450-dependent monooxygenases, and glutathione S-transferases were implicated in *B. tabaci* resistance (Dittrich *et al*., 1990; Ahmad *et al*., 2010; Erdogan *et al*., 2008; Cahill *et al*., 1995; Denholm *et al*., 1998) [8, 2, 9, 4, 7]. Development of multiple resistance mechanisms in response to field application of these insecticides among *B. tabaci* in the past is inevitable and Naveen *et al*., (2017) [21] suggested the need for a detailed analysis of cross resistance in Indian *B. tabaci* populations for devising suitable insecticide resistance management strategies. Begomovirus outbreak and *B. tabaci* insecticide resistance events are concomitant (Kranthi, 2015) [14]. For effective management of whitefly, further research on management strategies may be identified involving more importance to alternate methods in pest management. Host plant resistance is a major, often preventative measure for managing *B. tabaci* (Chu *et al.*, 2001; Thomas *et al*., 2014; Khan *et al*., 2015) [5, 28, 17] Therefore, a comprehensive, integrated pest management and insecticide resistance management strategies, rotation of conventional insecticides with novel molecules including insect growth regulator (IGR) compounds, use of sticky traps and exploitation of native biological control agents shall sustain the management of *B. tabaci*.

Table 1: Insecticide usage pattern of farmers from survey locations of Salem and Madurai districts of Tamil Nadu where from whitefly, *B. tabaci* were collected to assess the insecticide resistance pattern

Location	Agro-Climatic Zone- Tamil Nadu	Latitude and Longitude	Month &Year	Common insecticides used for control of whitefly in the farms	Host plant and stage of collection	Adjacent crops
Attur	Northwestern zone	11.5941° N, 78.6015° E	April 2018	Spiromesifen Dimethoate Thiamethoxam Triazophos Beta cyhalothrin Chlorpyriphos Imidacloprid Thiacloprid Oxydemeton –Methyl Acetamiprid	Cotton (boll formation stage) and Okra (flowering stage)	Cotton and Vegetables
Edappadi	Northwestern zone	11.5848° N, 77.8388° E	April 2018	Azadiractin Thiamethoxam Triazophos Chlorantraniliprole Chlorpyriphos Imidacloprid Thiacloprid	Okra (flowering stage)	Cotton and Vegetables
Omalur	Northwestern zone	11.7428° N, 78.0473° E	May 2018	Acetamiprid Thiamethoxam Triazophos Chlorpyriphos Imidacloprid Thiacloprid	Okra (flowering stage)	Cotton and Vegetables
Usilampatti	South Zone	9.9651° N, 77.7885° E	May 2018	Azadirachtin Dimethoate Thiamethoxam	Cotton (boll formation stage)	Cotton and Vegetables

				Triazophos Chlorpyriphos Imidacloprid Thiacloprid Acetamiprid		
Melur	South Zone	10.0333° N, 78.3359° E	March 2018	Thiamethoxam Triazophos Chlorpyriphos Imidacloprid Thiacloprid Acetamiprid	Okra (flowering stage)	Chillies, brinjal and maize
Tirumangalam	South Zone	9.8236° N, 77.9864° E	March 2018	Dimethoate Thiamethoxam Triazophos Chlorpyriphos Imidacloprid Thiacloprid Lambda cyhalothrin	Okra and Tomato (flowering stage)	Cotton, Sorghum, Pulses Sunflower

Table 2: Detailed information on insecticides tested against sweet potato whitefly *B. tabaci* to assess insecticide resistance in selected locations of Madurai and Salem districts of Tamil Nadu

Chemical Name	Formulation	Trade Name	Manufacturer
Thiamethoxam	25 WG	Dxtar	Nagarjuna Agrichem Ltd., Hyderabad
Imidacloprid	17.8 SL	Confidor	Bayer Crop Science Ltd., Maharashtra
Thiacloprid	21.7 SC	Alanto	Bayer Crop Science Ltd., Maharashtra
Triazophos	40 EC	Hattrick	Indogulf Crop Sciences Ltd., New Delhi
Chlorpyrifos	20 EC	Rusban	Indogulf Crop Sciences Ltd., New Delhi

Table 3: Acute toxicity of thiamethoxam 25% WG to whitefly, *B. tabaci* populations from different locations in Salem and Madurai districts of Tamil Nadu

Population	Regression equation	X^2	LC_{50} (PPM)	Fiducial limits		LC_{95} (PPM)	Fiducial limits		RR
				Lower limit	Upper limit		Lower limit	Upper limit	
Attur	Y = 0.6973x + 5.0949	0.610	0.737	0.234	2.315	229.059	72.889	719.827	30.70
Edappadi	Y = 0.75x + 5.315	0.255	0.444	0.149	1.328	169.319	56.681	505.796	18.5
Omalur	Y = 0.6822x + 5.1726	0.304	0.612	0.189	1.980	325.198	100.520	1052.070	25.5
Usilampatti	Y = 0.755x + 5.1674	0.396	0.614	0.210	1.797	155.346	53.082	454.624	25.58
Melur	Y = 0.7109x + 5.2182	0.270	0.564	0.179	1.772	277.645	88.342	872.590	23.5
Tirumangalam	Y = 0.7456x + 5.1209	0.277	0.829	0.274	2.508	279.853	92.535	846.358	34.54
Susceptible Population	Y = 0.3708x + 5.6248	0.996	0.024	0.003	0.206	1108.108	126.716	9690.185	

LC: Lethal Concentration, RR: Resistance Ratio

Table 4: Acute toxicity of imidacloprid 17.8% SL to whitefly, *B. tabaci* populations from different locations in Salem and Madurai districts of Tamil Nadu

Population	Regression equation	X^2	LC_{50} (PPM)	Fiducial limits		LC_{95} (PPM)	Fiducial limits		RR
				Lower limit	Upper limit		Lower limit	Upper limit	
Attur	Y = 1.0961x + 5.1348	0.834	0.833	0.392	1.767	30.624	14.434	64.973	69.41
Edappadi	Y = 1.081x + 5.3252	0.759	0.549	0.257	1.174	22.123	10.349	47.294	45.75
Omalur	Y = 1.2x + 5.2814	0.926	0.631	0.315	1.265	16.594	8.282	33.249	53.58
Usilampatti	Y = 1.0095x + 5.1542	0.972	0.789	0.352	1.768	38.466	17.159	86.233	65.75
Melur	Y = 1.2118x + 5.2076	0.709	0.717	0.360	1.431	18.415	9.230	36.740	59.75
Tirumangalam	Y = 1.2356x + 5.3282	0.629	0.581	0.294	1.147	14.739	7.459	29.123	48.41
Susceptible Population	Y = 0.5337x + 6.0117	0.999	0.012	0.002	0.064	21.704	4.266	110.427	

LC: Lethal Concentration, RR: Resistance Ratio

Table 5: Acute toxicity of thiacloprid 21.7% SC to whitefly, *B. tabaci* populations from different locations in Salem and Madurai districts of Tamil Nadu

Population	Regression equation	X^2	LC_{50} (PPM)	Fiducial limits		LC_{95} (PPM)	Fiducial limits		RR
				Lower limit	Upper limit		Lower limit	Upper limit	
Attur	Y = 0.8829x + 5.335	0.802	0.478	0.182	1.258	47.534	18.054	125.152	25.15
Edappadi	Y = 1.0124x + 5.5436	0.981	0.331	0.140	0.785	16.445	6.938	38.980	17.42
Omalur	Y = 0.8587x + 5.3883	0.928	0.350	0.135	0.907	30.066	11.589	78.000	18.42
Usilampatti	Y = 0.8863x + 5.428	0.883	0.384	0.147	1.003	36.090	13.821	94.241	20.21
Melur	Y = 1.0367x + 5.4935	0.963	0.391	0.167	0.918	18.622	7.932	43.721	20.57
Tirumangalam	Y = 0.8973x + 5.457	0.928	0.353	0.136	0.916	30.502	11.760	79.113	18.57
Susceptible Population	Y = 0.3854x + 5.6634	0.996	0.019	0.003	0.143	428.409	57.403	3197.273	

LC: Lethal Concentration, RR: Resistance Ratio

Table 6: Acute toxicity of triazophos 40% EC to whitefly, *B. tabaci* populations from different locations in Salem and Madurai districts of Tamil Nadu

Population	Regression equation	X^2	LC_{50} (PPM)	Fiducial limits		LC_{95} (PPM)	Fiducial limits		RR
				Lower limit	Upper limit		Lower limit	Upper limit	
Attur	Y = 0.6599x + 5.3967	0.705	0.362	0.097	1.351	220.619	59.102	823.533	25.85
Edappadi	Y = 0.7036x + 5.4741	0.558	0.293	0.085	1.019	129.626	37.330	450.118	20.92
Omalur	Y = 0.6735x + 5.4589	0.648	0.306	0.083	1.124	164.137	44.669	603.131	21.85
Usilampatti	Y = 0.7506x + 5.4781	0.722	0.342	0.104	1.124	96.246	29.268	316.502	24.42
Melur	Y = 0.7541x + 5.3799	0.470	0.433	0.133	1.404	132.808	40.914	431.099	30.92
Tirumangalam	Y = 0.6585x + 5.4227	0.676	0.333	0.089	1.249	200.954	53.551	754.094	23.78
Susceptible Population	Y = 0.3654x + 5.685	0.990	0.014	0.002	0.120	564.931	64.506	4947.594	

LC: Lethal Concentration, RR: Resistance Ratio

Table 7: Acute toxicity of chlorpyrifos 20% EC to whitefly, *B. tabaci* populations from different locations in Salem and Madurai districts of Tamil Nadu

Population	Regression equation	X^2	LC_{50} (PPM)	Fiducial limits		LC_{95} (PPM)	Fiducial limits		RR
				Lower limit	Upper limit		Lower limit	Upper limit	
Attur	Y = 0.7053x + 5.2868	0.440	0.513	0.158	1.664	218.620	67.395	709.177	16.54
Edappadi	Y = 0.6339x + 5.0921	0.448	0.821	0.229	2.940	550.299	153.603	1971.511	26.48
Omalur	Y = 0.6346x + 5.043	0.291	0.934	0.262	3.328	704.583	197.670	2511.441	30.12
Usilampatti	Y = 0.6981x + 4.9764	0.521	1.199	0.371	3.875	402.647	124.593	1301.236	38.67
Melur	Y = 0.6602x + 5.3398	0.368	0.360	0.104	1.241	228.325	66.179	787.742	11.61
Tirumangalam	Y = 0.7166x + 5.0374	0.625	0.978	0.313	3.058	282.467	90.371	882.892	31.54
Susceptible Population	Y = 0.4542x + 5.6894	0.858	0.031	0.005	0.183	184.654	31.599	1079.071	

LC: Lethal Concentration, RR: Resistance Ratio

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