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Susceptibility of *Bemisia tabaci* Gen. (Homoptera: Aleyrodidae) to Selected Insecticides

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Abstract.- Bioassay studies were conducted using the leaf dip method for endosulfan, imidacloprid, acetameprid and diafenthiuron to determine the susceptibility of *Bemisia tabaci* Gen. from three districts (Bahawalpur, Faisalabad, and Multan) of the Punjab, Pakistan. It was observed that all the *B. tabaci* populations were susceptible to these pesticides. Comparative resistance ratios of these insecticides at LC_{50} were1.75-3.60 folds for endosulfan, 1.18-2.09 folds for imidacloprid, 1.01-4.29 folds for acetameprid and 1.06-2.0 folds for diafenthiuron. In the present studies, the variability in the tolerance of *B. tabaci* is measured for the first time against endosulfan, imidacloprid, acetameprid and diafenthiuron against *Bemisia tabaci* Gen. in Pakistan. The wise use of these insecticides for controlling *B. tabaci* Gen. field populations in the presence of conventional insecticides will help for its better management.

Key Words: *Bemisia tabaci,* insecticide resistance, endosulfan, imidacloprid, acetamiprid, diafentthiuron.

INTRODUCTION

Whitefly (*Bemisia tabaci* Gen.) B biotype, is considered a major pest of cotton in Pakistan. Although it damages the crop by sucking the cell sap, inviting sooty mould on its honey dews and injecting toxins in the plants, yet more damage results from its ability to transfer leaf curl virus in cotton crop. In 1993-1994, Pakistan beard a tremendous loss (30-40%) in the cotton yield only due to the spread of this disease in cotton crop (Mansoor *et al*., 1999). Pakistan, being agricultural country relies heavily on cotton both for industrial requirements and foreign exchange. Therefore, the need to save this cash crop from this whitefly is inevitable. *B. tabaci* has been controlled by conventional insecticides including organochlorines, organophosphates, carbamates and pyrethroids since long. The reports regarding development of insecticide resistance against conventional chemistries (Ahmad *et al*., 1999, 2000, 2001) have raised serious concerns about their efficacy in the field. But the introduction of neonicotinoids in the form of imidacloprid and acetamiprid proved an immediate relief for cotton growers. This group had different mode of action as compared to the previous insecticides. Imidacloprid was the first member of this family and was effective against many insects showing resistance to carbamates, organophoshates and pyrethroids (Cox, 2001). Acetamiprid belongs to second generation of the nicotinoids (Yamado *et al.*, 1999). It is systemic insecticide with translaminar activity and both contact and stomach actions. Its foliar sprays provided even more effective control of whitefly compared to imidacloprid (Horowitz *et al*., 1998a). These insecticides proved invaluable additions in the tools for controlling cotton whitefly and are used extensively on cotton. Diafenthiuron is a thiourea derivative and it is very useful entry in the available chemical insecticides against whitefly. It was introduced in Pakistan since the start of 21^{st} century. It has unique mode of action; it disturbs the insect respiratory system by inhibiting the oxidative phosphorylation and mitochondrial ATP synthesis (Ruder and Kayser, 1993). Endosulfan is the only organochlorine which is still being used on cotton whitefly effectively. It belongs to cyclodiene sub class of organochlorines and is considered as antagonist of the GABA receptors-chloride channel complex (Anthony *et al*., 1995).

These insecticides gave good control of whitefly due to their different mode of actions coupled with absence of cross resistance to the conventional chemistries (Prabhaker *et al.,* 1997).

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But their continuous and indiscriminate use particularly on cotton may decrease their efficiency. Moreover, various report regarding development of resistance in whitefly particularly against endosulfan (Denholm *et al.,* 1996) and imidacloprid (Rauch and Nauen, 2003) have further enhanced the need to measure the variability in the tolerance of *B. tabaci* to endosulfan, imidacloprid, acetamiprid and diafenthiuron in different cotton growing regions of Pakistan. Therefore, the present study was undertaken to evaluate the current resistance status of *B. tabaci* to these novel insecticides along with endosulfan to make the whitefly management programme more effective.

MATERIALS AND METHODS

Collection of insects

Adult whitefly populations were collected from various locations of Punjab viz., Faisalabad, Bahawalpur, and Multan with the use of battery operated suckers during 2005-2007. Adult whitefly was then placed in wide mouth jars covered with muslin cloth to avoid mortality. Jars were placed in cool box containing ice and were taken back to laboratory. Before toxicity tests, the jars were inverted on the racks. The healthy ones climbed to the upper portion of the jars. The dead and unfit individuals remained on the bottom and were discarded. Bioassays were conducted within 1-2 hrs of arrival in the laboratory.

Insecticides

Insecticides used for bioassay were endosulfan (Thiodan 35 EC, Syngenta, Basel, Switzerland), diafentheuron (Polo metabolite 240 SC, Syngenta, Basel, Switzerland), acetamiprid {Mospilan 20 SP, Arysta Life Science Corporation, Japan) and imidacloprid (Confidor 200 SL, Bayer Crop Science, Leverkusen, Germany).

Bioassays

Serial dilutions of test insecticides were prepared. Cotton leaf discs of diameter 5 cm were cut and were dipped in test solution for 20 seconds. Leaf discs were then air dried on towel tissue papers. Agar-agar was prepared by dissolving 9 gram in 400 ml water and then cooled. Agar-agar solution was dispensed on inverted Petri-dishes using syringes. Inverted leaf discs were then placed on agar agar layer to avoid desiccation of leaves. Adult whitefly (mixed sex population) was immobilized by the CO_2 gas. 30-40 adults were placed in each Petri- dish and covered with lid containing sieve for ventilation. Insecticide solutions were freshly prepared in distilled water with Stapple as surfactant (Stapple, Dupont Pakistan) at 5µg/ml to enhance the adhesiveness of insecticides. Each trial consisted of eight treatments and four replicates. For control, only distilled water and Stapple were used. Lab temperature was maintained at 25±2°C, 60±5% relative humidity and 14: 10 light dark period.

Data analysis

Data was recorded 24 hours after treatment. Adults with inverted body position were considered dead. Data was corrected for percent mortality using Abbot formula (1925). Data was analyzed using Probit analysis (Finney, 1971). Comparative ratios were determined by dividing LC_{50} of field populations by LC_{50} of population showing minimum value. Pair wise comparison of LC_{50} values of bioassays was made to measure their inherent variability. Two treatments were considered significant if their 95 % CL were not overlapping each other (Litchfield and Wilcoxon, 1949). To determine cross resistance among insecticides, tested against field populations of whitefly, pair wise correlation co-efficient of LC_{50} values were also calculated for each insecticide by CoStat statistical computer program (CoStat-1998-2005).

RESULTS

In the absence of baseline data for the test insecticides, the whitefly populations exhibiting the lowest LC_{50} values among all the test populations were used to calculate the comparative ratios of populations showing higher LC_{50} values.

Endosulfan

The probit analysis of the data recorded from the bioassays of endosulfan against *B. tabaci* is shown in the Table 1.The CR values based on LC_{50}

for all the populations ranged from 1.00 to 3.60 folds. Except Mtn-1, Bwp-1 and Bwp-3 strains, all the strains showed CR values more than 2. The slope of regression line remained more than two for most of strains. Fsd-2 strain showing highest CR *i.e.*, 3.60 among all the test populations also exhibited highest slope (4.02) of regression line.

Imidacloprid

The results of bioassays of imidacloprid against field populations of *B. tabaci* Gen. (Tabe I) revealed that all the test populations collected from Faiaslabad, Multan and Bahawalpur showed CR values less than 2 except Fsd-1 strain collected from cotton in 2005. These values indicated very low level of resistance against this compound in whitefly. The slope of regression line remained less than two for most of strains.

Acetamiprid

Results of monitoring of insecticide resistance in whitefly against acetamiprid are expressed in Table I. CR values of all the whitefly populations showed very low level of resistance. Except Fsd-1(CR = 4.29) and Bwp-2(CR = 2.25), all the populations showed CR less than 1.5. The slope of regression line remained less than two for most of strains.

Diafenthiuron

Very low level of CR of the bioassays of diafenthiuron against whitely (Table 1) resistance indicated that all the test populations were highly susceptible to this thiourea product. Maximum CR value was exhibited by Fsd-2, which was only 2. All the other populations showed CR values < 2. The slope of regression line remained more than two for most of the strains.

Pair wise correlation between LC_{50} values of different insecticides

Correlation between endosulfan and imidacloprid was found to be significant (Table II), however its correlation with acetamiprid and diafenthiuron was non significant. Similarly, imidacloprid was significantly correlated with acetamiprid at P<0.05, but non significant correlation was found between imidacloprid and diafenthiuron. Negative and non significant correlation was observed between the LC_{50} values of acetamiprid and diafenthiuron.

DISCUSSION

Endosulfan is being used against whitefly on cotton and other crops in Pakistan, since last two decades. In the present studies, all the test populations revealed very low level of resistance (up to 3.6-folds) towards endosulfan. These findings are in conformity with those of Kranthi *et al.* (2001), who reported very low level of resistance (up to 5-folds) in field populations of whitefly against endosulfan. Similar results were described by Prabhaker *et al.* (1996). Bouharroud *et al.* (2007) also reported very low level of resistance in all the test populations of whitefly against endosulfan (not more than 2). However, high level of resistance (58-fold) was found in green house populations of *B. tabaci* (Roditakis *et al.,* 2005). Sethi and Dilawari (2008) also reported high degree of resistance for imidacloprid and endosulfan. Denholm *et al.* (1996) documented varied levels of resistance from 20 to 360-folds in different strains of whitefly from different countries against endosulfan. Anthony *et al.* (1995) suggested that whitefly resistance to endosulfan was due to the replacement of amino acid within the gene (Rdl), encoding γ- amino butyric acid (GABA) receptor sub unit.

Comparative resistance ratio for imidacloprid was found very low (<2) for most of the populations in the present studies. Comparable results were presented by Prabhaker *et al.* (1997), who found no evidence of resistance in the field populations of *B. tabaci.* Similarly, in Arizona, a slight decline in the *B. tabaci* susceptibility was found in the laboratory bioassays (Dennehy *et al*., 1999). Conversely, Rauch and Nauen (2003) and Roditakis *et al.* (2005) reported a high level of resistance against imidacloprid populations collected from Germany, Israel, Spain, Greece and Morroco.

The CR values of acetamiprid against whitefly populations were up to 4.29-folds in the present laboratory bioassays, which demonstrated very low level of resistance. These findings are comparable with those of Horowitz *et al.* (1998b),

Table I.- Toxicity of some insecticide against whitefly (*Bemisia tabaci* Gen.) during 2005-07.

Insecticide	Location	Host	Date tested	No. tested	Fit of probit line				LC_{50} ppm(95%CL)	CR at LC_{50}
					Slope(±SE)	X^2	df	I		
Endosulfan (Thiodan35EC)	Fsd-1	Cotton	06.09.05	1969	1.64 ± 0.43	78.25	5	2	1.37(2.08-0.87) bc	2.80
	Fsd -2	Cotton	10.07.06	674	4.02±0.43	33.56	2	5	1.73(2.44-1.22) c	3.60
	Fsd-3	Cotton	01-08-06	975	1.68 ± 0.26	29.63	4	4	1.24(1.74-0.88) bc	2.58
	Mtn-1	Cotton	15-08-06	1075	2.52 ± 0.16	6.88	4	3	0.48(0.54-0.43) a	1.00
	Bwp-1	Cotton	23-08-06	1079	2.11± 0.30	24.36	4	3	0.86(1.13-0.66) b	1.79
	Mtn-2	Cotton	19-09-06	1030	1.82± 0.11	9.32	3	2	1.29(1.47-1.14) c	2.68
	Bwp-2	Cotton	26-09-06	1011	1.72±0.16	9.58	4	2	1.25(1.52-1.03) bc	2.60
	Mtn-3	Cotton	13-10-06	936	2.19±0.28	18.77	4	4	1.42(1.79-1.13) bc	2.95
	Bwp-3	Cotton	19-10-06	1079	2.08 ± 0.30	24.44	4	3	0.84(1.10-0.64) b	1.75
	Fsd-4	Cotton	03.06.07	1079	2.21 ± 0.32	31.16	4	4	1.42(1.88-1.07) bc	2.95
Imidacloprid (Confidor 200 SL)	Fsd-1	Cotton	06-09-05	1979	0.87 ± 0.10	16.63	5	2	7.24(9.94-5.32) b	2.09
	Fsd-2	Cotton	10-07-06	757	2.05 ± 0.21	9.54	4	3	5.11(6.23-4.19) ab	1.47
	Fsd-3	Cotton	01-08-06	1090	1.87 ± 0.20	14.24	4	4	4.11(5.13-3.29) a	1.18
	Mtn-1	Cotton	15-08-06	1169	1.48 ± 0.26	26.57	4	3	3.46(4.88-2.45) a	1.00
	Bwp-1	Cotton	23-08-06	840	1.90 ± 0.12	4.03	4	2	4.38(5.00-3.84) a	1.26
	Mtn-2	Cotton	19-09-06	979	2.21 ± 0.27	17.92	4	4	4.95(6.26-3.91) ab	1.43
	Bwp-2	Cotton	26-09-06	897	1.42 ± 0.22	28.04	4	3	6.45(9.06-4.60) ab	1.86
	Mtn-3	Cotton	13-10-06	970	2.01 ± 0.13	4.19	4	2	5.39(6.08-4.78) ab	1.55
	Bwp-3	Cotton	19-10-06	1007	1.49 ± 0.20	13.68	4	3	5.00(6.50-3.84) ab	1.44
	Fsd-4	Cotton	02-06-07	995	2.10 ± 0.30	26.28	4	4	6.28(8.32-4.73) bc	1.81
Acetamiprid (Mospilan 20 SP)	Fsd-I	Cotton	07-09-05	295	1.34 ±0.17	26.25	4	4	15.60(21.30-11.05) b	4.29
	Fsd-2	Cotton	11-07-06	1152	2.89 ± 0.39	17.99	4	4	5.05(6.72-4.10) a	1.39
	Fsd-3	Cotton	01-08-06	1158	1.81 ± 0.36	49.10	4	4	3.63(5.46-2.42) a	1.00
	Mtn-I	Cotton	15-08-06	1061	1.82 ±0.12	1.88	4	2	3.70(4.19-327) a	1.01
	Bwp-I	Cotton	23-08-06	982	2.69 ±0.32	17.73	4	3	4.87(5.94-4.00) a	1.34
	Mtn-2	Cotton	19-09-06	989	1.95 ±0.12	1.56	4	2	4.66(5.24-4.15) a	1.28
	Bwp-2	Cotton	26-09-06	867	1.37 ±0.31	33.43	4	3	8.17(13.43-4.97) a	2.25
	Mtn-3	Cotton	13-10-06	902	1.63 ±0.09	5.48	4	3	5.14(5.79-4.57) a	1.41
	Bwp-3	Cotton	19-10-06	1065	1.46 ±0.19	14.93	4	3	4.77(6.23-3.65) a	1.31
	Fsd-4	Cotton	02-06-07	1235	1.88 ± 0.26	26.25	4	3	4.46(5.88-3.38) a	1.22
Diafentthiuron (Polo-Metabolite 250 SL)	Fsd-2	Cotton	10-07-06	805	2.09 ± 0.20	11.57	4	3	1.30(1.59-1.06) a	1.64
	Fsd-3	Cotton	02-08-06	1069	2.50 ± 0.23	11.36	4	3	0.96(1.13-0.82) a	1.21
	Mtn-1	Cotton	15-08-06	1313	1.52 ± 0.30	24.86	4	2	0.79(2.31-1.08) a	1.00
	Bwp-1	Cotton	23-08-06	984	2.21 ± 0.22	10.97	4	3	0.84(1.01-0.69) a	1.06
	Mtn-2	Cotton	19-09-06	979	2.16 ± 0.12	5.46	4	2	1.38(1.08-0.58) a	1.74
	Bwp-2	Cotton	26-09-06	1182	0.44 ± 0.23	22.21	4	4	0.99(1.37-0.72) a	1.25
	Mtn-3	Cotton	13-10-06	986	1.57 ± 0.24	3.38	4	3	0.94(2.02-0.99) a	1.18
	Bwp-3	Cotton	19-10-06	932	1.70 ± 0.22	5.75	4	3	1.51(6.10-5.00) b	1.91
	Fsd-4	Cotton	03-06-07	1075	2.04 ± 0.41	51.65	4	4	1.58(2.31-1.08) a	2.00

Table II.- Pairwise correlation matrix between LC_{50} values of selected insecticide against field populations of whitefly (*Bemisia tabaci* Gen.)

	Endosulfan	Imidacloprid	Acetamiprid
Imidacloprid	0.69 *		
Acetamiprid	0.29^{ns}	0.71*	
Diafenthiuron	0.39^{ns}	0.50^{ns}	-0.05^{ns}

* = Significant at P ≤5%; ns = Non significant

who reported no apparent resistance in *B. tabaci* to imidacloprid or acetamiprid. However, 5 to 10-folds tolerance was observed to this compound in green house populations of *B. tabaci* (Horowitz *et al.*, 1998a). Both imidacloprid and acetamiprid, target the nicotine acetylcholine receptors in the insect nervous system. According to Rauch and Nauen (2003), whitefly resistance to imidacloprid, acetamiprid and thiamethoxam is correlated with over-expression of cytochrome P450 dependent monooxygenases activity.

The evaluation of resistance in whitefly against diafenthiuron indicated very low level of resistance; the CR did not exceeded 2 fold. This indicates high susceptibility of test populations to this insecticide. Similar findings were reported by Otiodobiga *et al.* (2003), who studied the toxicity of diafenthiuron against 24 populations of whitefly and found that its LC_{50}s ranged from 3.5-6.7 mgL^{-1}, which were much lower than its field recommended dose. Diafenthiuron can suppress the progeny formation in the adult females and has strong chemical effect on whitefly nymphs (Ishaaya *et al.*, 1993).

Pair wise comparison of the LC_{50} values of selected insecticides showed positive and significant correlation between imidacloprid and endosulfan. However, further studies are required to confirm these findings because, in the past Prabhaker *et al.* (1997) reported absence of cross resistance to endosulfan, in imidacloprid selected strain of *B. argentifolii.* Imidacloprid exhibited positive and significant correlation with acetamiprid. Elbert and Nauen (2000) also confirmed the presence of strong cross resistance between imidacloprid, acetamiprid and thiamethoxam against whitefly.

These results are encouraging for the farmers' point of view in the sense that they can use all these insecticides to control the pest. But despite of these, the sole dependency on neonicotinoids should be strictly avoided because both imidacloprid and acetamiprid have similar mode of action and possess strong cross resistance. Diafenthiuron is a relatively newer entry in the market and present studies indicate absence of its cross resistance with neonicotinoids and endosulfan. Therefore it may successfully be used in the whitefly spray programme in order to reduce the frequency of neonicotinoids application.

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