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Determination of insecticide resistance in cotton whitefly in north India

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ABSTRACT

The whitefly, *Bemisia tabaci* (Gennadius) considered as most destructive pest, poses a significant threat to various crop species globally has developed resistance due to the indiscriminate use of synthetic chemicals. A study was carried out in 2018 at ICAR-Indian Agricultural Research Institute, New Delhi to determine insecticide resistance, in five Asia II 1 populations of *B. tabaci* from different cotton (*Gossypium hirsutum* L.) growing regions of north India. The susceptibility of the populations to different insecticide classes, viz. synthetic pyrethroid (cypermethrin); neonicotinoids (imidacloprid and thiamethoxam); thiourea (diafenthiuron) and diamide (cyantraniliprole) were assessed. Results revealed substantial heterogeneity in the responses of these populations to the insecticides. Sriganganagar and Bathinda populations exhibited moderate resistance to cypermethrin, imidacloprid, and thiamethoxam. Low level of resistance was observed in Bathinda and Sriganganagar populations against diafenthiuron. All populations were highly susceptible to cyantraniliprole. No cross-resistance was observed between cyantraniliprole and other insecticides, suggesting its potential as an alternative for managing insecticide resistance. High levels of detoxification enzymes (esterase, cytochrome P450 monooxygenase, and glutathione-S- transferase) in Sriganganagar and Bathinda populations indicated a positive correlation between insecticide resistance and detoxifying enzymes. These findings offer valuable insight for implementing insecticide rotation strategies to combat *B. tabaci* resistance in India.

Keywords: Asia II 1, *Bemisia tabaci,* Detoxifying enzymes, Genetic group, Insecticide resistance, LC_{50}

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the 100 invasive insect species in the world, infesting more than a 1000 species of plants. The populations of whiteflies with varied geographical distribution, virus transmission efficiency, host specificity, and insecticide resistance would have resulted in genetic variability where mere morphological distinctiveness would not be sufficient to distinguish them. Molecular studies categorized *B. tabaci* as a cryptic species complex. Presently, the complex is comprised of as many as 46 morphologically identical and genetically different groups, among which at least 10 are from the Indian subcontinent (Rehman *et al*. 2021). *B. tabaci* sucks the sap from the phloem tissues and acts as a vector in transmitting more than 300 plant pathogenic viruses (Barman *et al*. 2022). Farmers use insecticides more frequently to control whitefly populations in a single crop cycle, which causes the rapid development of insecticide resistance. *Arthropod Pesticide Resistance Database* documented around 720 resistance cases of *B. tabaci* against around 30 insecticide groups with different mode of action worldwide (Mota-Sanchez and Wise 2023). The polyphagous nature, high reproductive potential, short life cycle, abaxial surface habitat, and the dispersal of the adults by wind make the insect unmanageable in field situations. Manifold resistance to various insecticides with a different mode of action has been reported in diverse populations in India (Naveen *et al.* 2017). Using chemicals with a novel mode of action on a rotation basis in the field is suggested to be effective for managing insecticide resistance. In the present study, we estimated the susceptibility status of both nymphal and adult stages of whitefly *B. tabaci* against five insecticides with different mode of actions, to understand the resistance/tolerance level in field conditions.

MATERIALS AND METHODS

Insect: Whiteflies being an important pest of cotton, populations for the study were collected from different cotton growing areas in north India, viz. New Delhi (Pusa-28.64°N 77.17°E), Rajasthan (Sriganganagar-29.52°N 74.78°E), Haryana (Hisar-29.09°N 75.87°E), Punjab (Bathinda-30.20°N 74.95°E) and Madhya Pradesh (Indore-22.8°N 75.73°E) in the month of August and September 2018. The collected whiteflies were reared on a potted cotton plants kept in a separate cages (50 cm × 45 cm × 45 cm) in controlled conditions (temperature of 27±2°C and relative humidity of 60–70% and photoperiod 14:10) (L:D) at Insect Proof Climate Control Chamber (IPCCC),

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ICAR-Indian Agricultural Research Institute, New Delhi. After one generation, iso-female lines were obtained by releasing one pair of whiteflies (male and female) into a fresh cotton plant in a clip cage. The population collected from *Leucaena leucocephala* (Pusa Campus-28.64°N 77.16°E) was maintained as lab culture, which has never been exposed to any insecticides directly.

Genetic group identification: The genetic groups of the collected whitefly populations were confirmed using *B. tabaci* specific mitochondrial cytochrome oxidase (mtCO1) primer (forward primer CI–J–2195 (5'–TTGATTTTTTGGTCATCCAGAAGT–3') and the reverse primer TL2–N–3014 (5'–TCCAATGCACTAATCTGCCATATTA–3'). The DNA extracted was amplified through PCR following specific amplification conditions (Naveen *et al*. 2017). The PCR amplicons were sequenced, and phylogenetic analysis was performed to confirm the genetic group of *B. tabaci* populations using the standard mtCOI reference dataset MEGA 10.0 software.

Bioassay: The selected commercial insecticides, viz. cypermethrin 25 EC (synthetic pyrethroid), imidacloprid 17.8 SL (neonicotinoid), thiamethoxam 25 WG (neonicotinoid), diafenthiuron 50 WP (thiourea compound) and cyantraniliprole 10.26 OD (diamide) were procured from the open market and used for the study. Adult bioassays were carried out with leaf dip method as described by Naveen *et al*. (2017). Mortality was recorded after 72 h of insecticide exposure and the insect with no sign of movement was recorded as dead. Nymphal bioassays were carried out following the leaf dip method described by IRAC (Version 3, Method 016) (http://www.iraconline.org) with minor modifications. 30–40 days old, potted cotton plants were used for the experiment. 10 pairs of adult whiteflies were released into clip cages and each clip cage was fitted into each leaf. After 24 h, adult whiteflies were removed, egg-laying on the undersurface of the leaves was confirmed and kept for 5–7 days to get sessile second instar nymphs. Each leaf with nymphs was independently immersed in insecticide solutions for 30 sec and observed for nymphal development after 6–8 days exposure. The second and fourth nymphal instars were counted and categorized as dead and live, respectively. A single plant was taken for each concentration, and the three leaves were considered as three replicates.

Estimation of detoxifying enzymes: Three detoxifying enzymes, esterase; cytochrome P450 monooxygenases; and glutathione S transferase (GST) were assessed in the adult stages of whiteflies across all populations. The assays were performed in triplicate, and microplate wells without samples served as blanks. The specific activity of these enzymes was calculated per milligram of protein in the samples. The whitefly samples were homogenized in ice-cold sodium phosphate buffer (0.1 M, *p*H 7.5) containing 1 mM EDTA, 1 mM PTU, 1 mM PMSF, and 0.1 mM DTT.

Esterase activity: The substrate used was 1 mM α–naphthyl acetate with fast blue RR salt (1.6 mg/ml), and the absorbance was measured at 450 nm. The enzyme activity was quantified using an α-naphthol standard curve (Guo *et al*. 2014).

Cytochrome P450 monooxygenase activity: Cytochrome P450 monooxygenase activity was assessed using 3,3,5,5'-Tetramethylbenzidine (TMBZ) as the primary substrate and H_2O_2 as the co-substrate. After a 5 min incubation at room temperature, the absorbance was measured at 650 nm. The enzyme activity was calculated using a Cytochrome C standard curve (Penilla *et al*. 2007).

GST activity: GST activity was determined using 1-chloro−2,4−dinitrobenzene (CDNB) and Glutathione reduced (GSH) as substrates. The absorbance was measured at 340 nm, and the GST estimation was performed using the molar extinction coefficient of 2,4−dinitrophenyl−glutathione (ε340 nm = 9.6/mM/cm) (Guo *et al*. 2014).

Statistical analysis: Lethal concentrations (LC_{50}) and fiducial limits were determined for adult and nymphal stages using PoloPlus 2.0 software. Significant differences in LC_{50} values among insecticides were assessed via the overlap of 95% fiducial limits (Robertson *et al*. 2007). The relative resistance ratios were computed to classify RR from 5–10 as low resistance, 10–40 as moderate level, >40 as high level of resistance (Liu *et al*. 2010). Pairwise comparisons were done using Pearson's correlation coefficient ® in SPSS 16.0. Enzyme activity differences were analyzed with one-way ANOVA, and means were compared using Tukey's test (P<0.05) in SPSS software. These methods provided robust statistical insights in a concise manner.

RESULTS AND DISCUSSION

Genetic group identification: The sequences obtained from the Sanger sequencing of the mtCOI genomic region of *Bemisia tabaci* were analyzed using MEGA 10.0 software to delineate them into different genetic groups. The sequences were submitted to the NCBI database, and the accession numbers are MK636819 (lab susceptible), MK636818 (New Delhi), MK636815 (Bathinda), MK636820 (Sriganganagar), MK636817 (Indore), and MK636816 (Hisar). The phylogenetic tree was constructed by keeping Boykin *et al.* (2017) standard, confirming that all the populations collected and used for the study are Asia II 1.

Baseline susceptibility studies: Susceptibility data for the Asia II 1 genetic group indicated varying resistance levels to different insecticides, signifying a heterogeneous response. This is crucial in addressing whitefly resistance as Asia II 1 is prevalent in north India. In adult stages, the LC_{50} values of cypermethrin, imidacloprid, thiamethoxam and diafenthiuron showed a significant difference from the lab susceptible population considering the overlap in 95% fiducial limits. The LC_{50} values among the Asia II 1 populations varied in range; for cypermethrin (151.55–1070.5 mg/litre), imdacloprid (120.17–845.8mg/litre), thiamethoxam (91.42–442.345 mg/litre) and diafenthiuron (65.95–230.82 mg/litre) (Table 1). There was 25- and 19-fold increase in relative resistance ratio for cypermethrin and imidacloprid respectively in Sriganganagar, showing a moderate level of resistance. A decline in the LC_{50} value

of Sriganganagar population against cypermethrin, with an overlapping 95% fiducial limit, when compared with Naveen *et al*. (2017) (LC_{50} = 1362 mg/litre) strengthens the possibility of withdrawal of cypermethrin from cotton fields from 2013 onwards. The other neonicotinoid, thiamethoxam and the thiourea compound diafenthiuron showed the highest LC_{50} in Bathinda (442.35 and 230.822, respectively) with 12- and 7-fold increase in resistance ratio. Barman *et al*. (2022) indicated the expression of diverse level of susceptibility of six populations from West Bengal to neonicotinoids. Sriganganagar and Hisar populations have also shown a low resistance level to diafenthiuron with a relative resistance ratio of six and fivefold. A survey on Brazilian MEAM1 population also indicated a low level of resistance against diafenthiuron whereas more than 100-fold resistance levels were reported for neonicotinoids and pyrethroids (Dangelo *et al*. 2018).

The study revealed that the magnitude of susceptibility is high in cyantraniliprole, the diamide compound to all the populations with lowest LC_{50} value of 3.320 mg/litre in Indore population. There observed no significant difference in LC_{50} values of the tested Asia II 1 populations (Table 1). The baseline susceptibility data can be used for further monitoring of resistance to this insecticide in field conditions. Caballero *et al*. (2013) established baseline data in Florida for the same insecticide, concluding that cyantraniliprole demonstrated efficacy in managing whitefly resistance. The highest LC_{50} value for cyantraniliprole was shown by Pusa

Table 1 Median lethal concentrations (LC_{50}) of insecticides against adult and nymphal stages of different *B. tabaci* population

Insecticide	Population	χ^2	LC_{50}	95% fiducial limits LC_{50}	RR (LC_{50})	χ^2	LC_{50}	95% fiducial limits LC_{50}	RR (LC_{50})
		Adult stage				Nymphal stage			
Cypermethrin	Susceptible	4.34	42.37	33.45–55.59a	1	2.08	29.09	22.79–35.71a	1.00
	Pusa	0.67	151.55	114.42–192.09b	3.57	0.18	72.86	58.59–87.66b	2.5
	Hisar	0.30	477.18	348.75–612.66c	11.26	5.53	208.59	164.50–261.93d	7.17
	Bathinda	1.27	825.50	699.535– 944.64d	19.48	2.22	647.60	525.86–773.95e	22.26
	Indore	0.82	237.04	167.579– 303.59b	5.59	1.80	137.90	117.84–157.75c	4.74
	Sriganganagar	2.34	1070.58	896.28– 1253.06d	25.26	7.49	710.83	648.19–798.41e	24.44
Imidacloprid	Susceptible	1.51	58.19	46.27–70.54a	1	3.45	28.71	23.99–33.79a	1.00
	Pusa	2.11	120.17	72.64–154.79b	2.06	3.09	42.61	34.33–50.05b	1.48
	Hisar	1.47	421.14	351.84–509.73c	7.24	5.98	163.39	112.64–251.81c	5.69
	Bathinda	2.14	765.66	614.42–928. 25d	13.16	2.89	315.14	247.87–378.81cd	10.97
	Indore	3.31	280.12	232.19–328.03c	4.81	10.14	119.58	70.70–196.78c	4.16
	Sriganganagar	3.14	845.85	678.08–1045.49d	14.53	1.49	418.19	354.42–471.90d	14.56
Thiamethoxam	Susceptible	4.31	37.359	29.63–46.66a	1	0.44	10.98	9.31–12.66a	1.00
	Pusa	4.34	91.402	46.022–140.43b	2.4	0.668	25.07	20.63–30.74b	2.51
	Hisar	0.80	323.30	245.85–399.81c	8.6	1.792	62.58	50.041–75.310c	6.26
	Bathinda	0.37	442.35	368.90–520.52cd	11.8	4.278	176.69	122.43–208.37d	17.67
	Indore	2.80	289.20	210.56–362.79c	7.74	3.082	61.82	52.21–72.26c	5.63
	Sriganganagar	0.491	415.33	326.069–500.15cd	11.12	0.916	131.65	106.23–156.78d	11. 89
Diafenthiuron	Susceptible	1.15	34.84	23.23–44.96a	1	4.376	29.91	25.74–34.18a	1.00
	Pusa	4.54	65.95	31.49–106.61a	1.89	0.593	69.94	57.66–83.91b	2.34
	Hisar	3.303	190.92	135.76–249.02c	5.48	5.603	77.19	38.43–118.65b	2.58
	Bathinda	0.75	230.82	190.31–276.96c	6.6	2.253	168.61	126.70–201.26c	5.64
	Indore	4.43	89.78	30.11–140.08ab	2.58	7.519	69.90	37.88–107.29b	2.34
	Sriganganagar	3.74	196.37	154.18–234.79c	5.64	5.283	89.34	57.22–129.14bc	2.99
Cyantraniliprole	Susceptible	0.85	1.803	1.439–2.25a	1	5.926	0.017	0. 012–0.022a	1.00
	Pusa	3.91	4.89	3.622–6.257b	2.7	4.082	0.065	0.055–0.077c	3.89
	Hisar	2.03	3.89	2.603–5.24b	2.16	1.120	0.056	0.048–0.065c	3.29
	Bathinda	3.45	4.31	3.167–5.48b	2.40	0.477	0.070	0.057–0.086c	4.12
	Indore	2.79	3.32	2.349–4.31b	1.84	2.200	0.033	0.028–0.039b	1.94
	Sriganganagar	2.04	4.58	3.038–6.31b	2.54	3.108	0.062	0.050–0.075c	3.5

Different letters indicate significant difference between populations for an insecticide based on overlapping 95% CL of LC_{50} value.

population (LC_{50} = 4.897 mg/litre) and this might be due to the use of other diamide insecticides such as flubendamide, chlorantraniliprole etc. against other insects in the field conditions. Resistance reported in whiteflies against cartap hydrochloride and chlorantraniliprole in Brazil confirmed the frequent use of these two insecticides against other insect pests, but not against *B. tabaci* (Dangelo *et al*. 2018).

The nymphal stages of Asia II 1 *B. tabaci* populations followed a similar susceptibility trend like adult bioassay against the insecticides tested (Table 1). The LC_{50} values of cypermethrin for nymphal stages were highest in Sriganganagar (710.837 mg/litre) followed by Bathinda (647.595 mg/litre) with relative resistance ratios 24.44 and 22.26, respectively. In all the populations studied, among the neonicotinoids the efficacy of thiamethoxam was observed to be higher compared to imidacloprid. Similar results were observed by El Zahi *et al.* (2017), where thiamethoxam was reported to have significantly higher efficacy against the 2nd instar nymphs compared to imidacloprid. The nymphal stage of Bathinda population is comparatively less susceptible to diafenthiuron (LC_{50} = 168.610 mg/litre) with a resistance ratio of 5.64 whereas, for Sriganganagar, LC_{50} value was 86.399 mg/litre. Cyantraniliprole is proved to be highly susceptible to the nymphal stages of all populations with no significant difference between their LC_{50} values considering 95% fiducial-limits. A comparison of LC_{50} values of adult and nymphal stages displayed a significant difference in the median lethal concentration in thiamethoxam (t value = 2.57*) and cyantraniliprole (t value = 8.18*) whereas for other insecticides cypermethrin (0.87), imidacloprid (1.51) and diafenthiuron (1.42) the values were non-significant. Relatively more sensitivity of nymphs of *B. tabaci* was reported by many workers. Relatively more susceptibility of nymphs was reported against neonicotinoids (Gravalos *et al*. 2015) and cyantraniliprole and thiamethoxam (Chen *et al.* 2018).

Pair-wise comparison of the log LC_{50} values revealed substantial cross-resistance between cypermethrin, neonicotinoids as well as diafenthiuron (Table 2). Notably, a significant correlation was observed among the LC_{50} values of neonicotinoids and diafenthiuron in adult and nymphal stages of *B. tabaci*. Although diafenthiuron operates through a distinct mode of action compared to synthetic pyrethroids and neonicotinoids, a weak correlation was found between these insecticides. Many workers have reported cross resistance of insecticides in *B. tabaci* from different countries (Wang *et al*. 2023). No correlation between cyantraniliprole and the other insecticides tested except with diafenthiuron at nymphal stage (r = 0.856). A 138.4-fold cyantraniliprole resistant strain through selection pressure showed no cross-resistance to synthetic pyrethroids, neonicotinoids and other newer chemicals tested (Wang *et al*. 2020a).

From the data, it is also noticeable that Asia II 1 genetic group (Bathinda and Sriganganagr populations) have developed multiple resistance to insecticides. Such reports have already been identified from China (Wang *et al.* 2020b) in MED and MEAM1 genetic groups. Resistance monitoring can document the response of populations to selection pressure both geographically and temporally, thereby providing an early warning of the impending resistance problems in field conditions and also assessing the effectiveness of various resistance management tactics. Recent worldwide investigations suggested established insecticide resistance to different insecticide groups in the genetic groups, MEAM 1 (B), Asia I and Asia II 1 (Horowitz *et al*. 2020). The compilation by Basit (2019) described reports of high to low levels of resistance to insecticides from Asian, European and African countries. The use of field recommended dose of cyantraniliprole against *B. tabaci* can act as a high dose strategy for managing multiple insecticide resistance in the field.

Many reports of increased esterase cytochrome P450 monooxygenase and GST activities have been documented in *B. tabaci* (Horowitz *et al*. 2020). The knowledge about underlying insecticide resistance mechanisms can help elucidate details of the colonization of *B. tabaci* populations and pest-host plant interactions. The specific activity of esterase and cytochrome P450 monooxygenases was found significantly enhanced in all the tested Asia II 1 populations (Table 3). The highest esterase activity was observed in Sriganganagar followed by Bathinda population with 7.75±0.21 and 7.28±0.24 µmol/min/mg of protein,

Table 2 Correlation coefficients of pairwise comparisons between the log LC_{50} values of the insecticides evaluated against adult and nymphal stages of *B. tabaci* populations

Insecticide	Stage of *B. tabaci*	Cypermethrin	Imidacloprid	Thiamethoxam	Diafenthiuron
Imidacloprid	Adult	0.98^{8**}			
	Nymph	0.984^{**}			
Thiamethoxam	Adult	0.95^{8**}	0.970^{**}		
	Nymph	0.981^{**}	0.972^{**}		
Diafenthiuron	Adult	0.97^{8**}	0.970^{**}	0.953^{**}	
	Nymph	0.879^{*}	0.808^{ns}	0.914^{*}	
Cyantraniliprole	Adult	0.762^{ns}	0.671^{ns}	0.681^{ns}	0.737^{ns}
	Nymph	0.747^{ns}	0.648^{ns}	0.725^{ns}	0.856^{*}

**Significance $P<0.01$; * Significance $P<0.05$; ns, Not significant.

Table 3 The specific activity of detoxification enzymes against different *B. tabaci* population

Population	Esterase activity Mean±SE (µmol/min/mg of protein)	Ratio	Cytochrome P450 monoxygenase activity (nmol/min/mg of protein)	Ratio	GST activity Mean±SE (µmol/min/mg of protein)	Ratio
Susceptible	1.75±0.02[a]	1.00	3.14±0.14[a]	1.00	1.29±0.06[a]	1.00
Pusa	3.96±0.12[b]	2.26	4.30±0.12[b]	1.37	1.60±0.01[a]	1.24
Bathinda	7.28±0.24[c]	4.16	8.37±0.2[0]d	2.85	1.84±0.0[2]a	1.42
Hisar	4.58±0.09[b]	2.6	6.06±0.21[c]	1.93	1.74±0.02[a]	1.34
Indore	4.28±0.30[b]	2.44	4.39±0.14[b]	1.39	1.62±0.02[a]	1.25
Sriganganagar	7.75±0.21[c]	4.4	8.66±0.1[8]d	3.07	1.72±0.01[a]	1.33
F ratio	142.15		176.34		0.955	
P value	<0.0001		<0.0001		0.465	

Means with different letters denote the significance ($P \leq 0.05$) difference after Tukey's HSD test.

respectively. Cytochrome P450 specific activity was highest in Sriganganagar (8.66±0.18 µmol/min/mg of protein) and Bathinda (8.37±0.2 nmol/min/mg of protein). The GST activity of all populations was found to be on par with the lab susceptible population with highest value in Bathinda 1.84±0.02 µmol/min/mg of protein, and the lowest activity in Pusa (1.60±0.01 µmol/min/mg of protein).

Basij *et al.* (2017) reported a 205.2-fold imidacloprid resistant population showing 17-fold enhanced cytochrome P450 activity and confirmed the role of the enzyme in neonicotinoid resistance in nine Iranian populations of *B. tabaci.* Six populations of *B. tabaci* from a province in China have exhibited low to moderate degree of resistance against neonicotinoid insecticides and have shown a notable positive correlation between their resistance levels and the activity of cytochrome P450 monoxygenase and GST (Wang *et al*. 2020c). The increased esterase and cytochrome monooxygenase activity observed in Sriganaganagar and Bathinda populations, maybe due to the multiple resistance development of the populations against various insecticides. The present study also depicted a non-significant correlation between cyantraniliprole and all enzyme activities investigated which agreed with Wang *et al*. (2020a).

Managing *B. tabaci* is challenging due to its high polyphagous nature and rapid reproduction. Insecticides, while initially effective, can lead to resistance development passed down through generations. Detecting resistance and understanding its distribution is crucial. Knowing baseline susceptibility and cross-resistance to new insecticides aids in informed resistance management. Rotating insecticides with novel modes of action is an effective strategy to combat *B. tabaci* populations in India.

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