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Research article

Differential insecticide resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae) field populations in the Punjab Province of Pakistan

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A R T I C L E I N F O

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A B S T R A C T

The cotton whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has a propensity for developing high-level resistance to insecticides. Management of *B. tabaci* in cotton grown in Pakistan depends on insecticide use, resistance monitoring has become essential to minimize the development of resistance. In this study, resistance was monitored in adult whiteflies collected from cotton fields in the Bahawalpur, Faisalabad, Lodhran, Multan, and Vehari districts of the Punjab Province, Pakistan during 2017, 2018, and 2019. Resistance monitoring was carried out for two insect growth regulators (pyriproxyfen and buprofezin) four neonicotinoids acetamiprid, imidacloprid, thiamethoxam, thiacloprid, and the historically used pyrethroid, bifenthrin and organophosphate, chlorpyrifos. Results based on resistance ratio (RR) showed that moderate to high level of resistance against noenicitinoids insecticides have been observed in all four districts while whiteflies exhibited very low to low resistance to pyriproxyfen and buprofezin. The RRs for acetamiprid, imidacloprid, thiamethoxam, thiacloprid varied from 7.60 to 50.99, 19.32 to 65.72, 17.18 to 54.65 and 6.49–47.49-fold, respectively. Bifenthrin and chlorpyrifos showed very low toxicity against whiteflies in all districts except Faisalabad, with RRs of 12.28–50.56-fold and 7.94–26.24-fold, respectively. The results will facilitate ‘smart’ selection and guide rates of insecticide applications for whitefly management in cotton for effective whitefly management while also delaying the development of resistance.

1. Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) cryptic species (De Moya et al., 2019), is a damaging insect pest and virus vector that infests fiber and food crops, worldwide, including cotton (*Gossypium hirsutum* L.), legumes, vegetables, and ornamentals (Jeschke et al., 2010; Basit et al., 2011; Wang et al., 2017; Horowitz et al., 2020). Whiteflies cause damage by feeding cell sap and excrete the honey dews on leaves leading to development of sooty mold which reduces photosynthesis, collectively decreasing crop yield and quality (Jones, 2003). *B. tabaci* also transmits plant viruses i.e., *Begomovirus* (Family, *Geminiviridae*). Taxonomically, *B. tabaci* is considered a cryptic specie, consisting of at least five major phylogenomic groups, of which two are represented throughout Asia (Brown, 2010; De Moya et al., 2019). Genetic and behavioral differences between cryptic species are known to influence host range, mating compatibility, insecticide resistance, and virus transmission competency (Bedford et al., 1994; Legg, 1996; Brown, 2007, 2010; Pan et al., 2018; Chen et al., 2019).

In Pakistan, insecticides have been used to control *B. tabaci* in commercial cotton plantings since 1970's when whitefly outbreaks began to increase in prevalence (Hussain and Ali, 1975), resulting in damage caused by whitefly feeding and diseases caused by the cotton leaf curl virus complex (Mansoor et al., 2003; Amin et al., 2006). In cotton-vegetable cropping systems throughout much of the world, *B. tabaci* has a propensity to develop resistance to different classes of insecticides, including carbamates, organophosphates, pyrethroids and several chemistries recently introduced for use in Pakistan (Ahmad et al., 2010; Basit et al., 2011; Ahmad and Khan, 2017; Shah et al., 2021) which

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resulted in failure of whitefly control and lead to significant damage to the cotton crop.

During 1990' s pyriproxyfen and neonicotinoid were developed with new modes of action, which became widely used to control *B. tabaci* (Basit et al., 2013). Pyriproxyfen is a juvenile hormone analog or insect growth regulator (IGR), introduced in 1996 for controlling whiteflies and other phloem-feeding insects, which prevents the maturation of immature instars into adults and disrupts egg-laying (Streibert et al., 1988; Kayser and Eilinger, 2001; Ali, 2011; Kumar et al., 2014). Neonicotinoids (acetamiprid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) are synthetic chemistries that can be applied as a foliar, to soil or as a seed treatment (Nauen et al., 2002). The use of neonicotinoids against whiteflies and other phloem feeding insects increased rapidly during 2000s (Horowitz et al., 2004; Nauen and Denholm, 2005; Millar and Denholm, 2007). They disrupt acetylcholine receptors in the central nervous system and are effective against a broad range of arthropods (Jeschke et al., 2010; Wang et al., 2018). However, widespread neonicotinoid uses to control *B. tabaci* resulted in the development of resistance, particularly in China, Cyprus, India, Israel, Pakistan, Spain and USA (Horowitz et al., 2004; Fernández et al., 2009; Vassiliou et al., 2011; Ahmad and Khan, 2017; Zheng et al., 2017). In Pakistan, *B. tabaci* populations were found to harbor moderate to high-level resistance to imidacloprid (Ahmad and Khan, 2017). The objective of this study was to monitor populations of *B. tabaci* for resistance to the most commonly used insecticides in the primary cotton-growing districts in Punjab Province.

2. Material and methods

2.1. Whitefly reference colony

The susceptible reference colony was established from *B. tabaci* adults collected from untreated cotton plants in Faisalabad, Punjab Province in 2010. It was identified as the Asia II-1 mitotype by PCR amplification, sequencing, and comparative analysis of the mitochondrial cytochrome oxidase I gene (authors, data not shown) using previously published methods (Paredes Montero et al., 2019). Whiteflies were reared in an insect-free greenhouse at 27 ± 2 °C, 65 ± 2% relative humidity, and 16:8 (L:D) hr photoperiod. The colony was maintained by serially transferring adults to greenhouse-grown insect-free cotton plants every 4–6 weeks for greater than twenty generations, without exposure to insecticides.

2.2. Field populations of Bemisia tabaci

Adult *B. tabaci* were collected from infested cotton, okra (*Hibiscus esculentus* L.), and tomato (*Lycopersicon esculentum* Mill) plants in five districts of the Punjab Province, Bahawalpur, Multan, Lodhran, Vehari and Faisalabad, during 2017, 2018 and 2019 (Figure 1). Field populations of whitefly were sampled from two-hectare block in 8–10 random locations, approximately 100 km apart. Whiteflies were collected from infested leaves (3/plant) using a hand-held aspirator, transferred to a plastic vial, and pooled in a glass container (11 × 11 × 19 cm^3) (50/jar) prior to transporting them to the laboratory. Whiteflies collected in each district were designated as one homogeneous population. Each field population was maintained on young cotton plants (5-leaf stage) in a separate cage in an insecticide-free greenhouse. Resistance bioassays were conducted by transferring whiteflies from caged cotton plants to a glass container, prior to exposure to insecticide-treated leaf discs. The containers were turned upright to attract whiteflies to the upper portion of the glass container and given access to insecticide-treated or untreated leaf discs (Dennehy et al., 1999; Ahmad et al., 2010; Basit et al., 2013).

2.3. Insecticide resistance bioassays

Commercially-available formulated insecticides used for resistance bioassays were acetamiprid (Mospilan 20SP, Arysta Life Sciences Pakistan (Pvt.) Ltd), buprofezin (Buprofezin 25SC, FMC, Pakistan), bifenthrin (Talstar 10EC, FMC, Pakistan), chlorpyrifos (Lorsban 40EC, Arysta Life Sciences Pakistan (Pvt.) Ltd), imidacloprid (Confidor 200SL, Bayer Crop Science, Germany), thiamethoxam (Actra 25WG, Syngenta Pakistan Limited), thiacloprid (Talent 480SC, Kanzo AG, Pakistan), and pyriproxyfen (Pyriproxyfen 10.8EC, Jaffer Agrochemicals Pakistan Limited).

2.4. Leaf-dip bioassay

The leaf-dip bioassay previously described by Ahmad et al. (2010) was used to expose adult whiteflies with treated leaf discs insecticide. Cotton leaf discs (54 mm wide) were immersed for 10 s in the respective insecticide solution and or immersed in water, as the negative experimental control. Leaf discs were air-dried on a paper towel for 20 min. After air-drying, leaf discs were arranged with the adaxial-side down in a Petri dish containing a layer of agar (7 g L^{-1}). The adults were sexed to separate males from females. Each group was placed into a separate plastic vial and stored for −20 °C for 60 s carefully transferred to the treated leaf disc, at 20–25 whiteflies/per disc. Each Petri dish was covered with a translucent ventilated lid. When whiteflies were recovered, the Petri dish was turned to orient leaf discs adaxial side up, and held at 27 ± 2 °C, 65 ± 2% relative humidity for a 16:8 (L:D) hr photoperiod. Mortality was recorded at 48- and 72-hr post-leaf disc exposure. Three replicates were carried out for different concentrations (doses) of each insecticide and for each water-treated negative control. In each year, experiments were repeated. Mortality was scored as positive when whiteflies exhibited no movement. Whitefly mortality in the negative control-treated discs was generally <10%. Mortality data were normalized using mortality incurred by the water control as the baseline (zero), according to a previously published method, referred to as Abbott 's formula (Abbott, 1925). Experiments at each concentration were repeated three time in each year.

2.5. Statistical analysis

Bioassays results for each experiment (sample/insecticide) combination were pooled for probit analysis (Finney, 1971) using POLO-PC (LeOra, 2003). The LC_{50} of each insecticide were determined based on a fiducial limit (FL) of 95% and slope ±SE limit. For any two values compared, the results were considered significantly different if the respective fiduciary limit of 95% did not overlap (Wolfe and Hanley, 2002). The resistance ratio (RR), a standard method used for estimating insecticide resistance (Ahmad et al., 2010) was determined by dividing the LC_{50} value of the field population by the LC_{50} value of the susceptible whitefly reference colony, which characteristically exhibited lower LC values, as expected. The RR results were considered robust estimates of resistance, based on previously described methods (Saleem et al., 2016) and RR scale for which RR ≤ 1 (none), RR = 2–10-fold (very low level), RR = 11–20-fold (low level), RR = 21–50-fold, or moderate level, RR = 51-100-fold, or high level, and RR > 100-fold, or very high level (Ahmad et al., 2010).

3. Results

3.1. LC_{50} for susceptible reference colony

The LC_{50} values for the susceptible reference colony and each insecticide evaluated in this study are shown in Table 1. The LC_{50} values for acetamiprid, buprofezin, pyriproxyfen and thiacloprid were lower than those for bifenthrin, chlorpyrifos and thiamethoxam. Imidacloprid showed lower efficacy, compared to acetamiprid, thiamethoxam, and thiacloprid (95%, FL no-overlap), with the LC_{50} for doses of 10.97 and 4.19 mg/l, respectively. Based on the LC_{50} values of >100 mg/l determined for the susceptible reference colony, the efficacy of chlorpyrifos and bifenthrin was very low at 49.99 mg/l, compared to 37.7 mg/l for the susceptible whitefly colony (Table 1).

3.2. Pyriproxyfen and buprofezin resistance bioassays

The LC_{50} for the buprofezin and pyriproxyfen bioassays were similar for all of the field-collected *B. tabaci*, regardless of the districts sampled, but were significantly different-at (95% FL no-overlap) compared to the susceptible, reference laboratory population (Table 2). The field-collected whitefly populations from all five locations and over the three-year collection period showed very low levels of resistance to pyriproxyfen, compared to the reference colony, at LC_{50} (1.11–1.21 mg/l. For buprofezin, the populations showed very low level of resistance, to low level resistance, at 8–16.66-fold (95% FL no-overlap) respectively (Table 2).

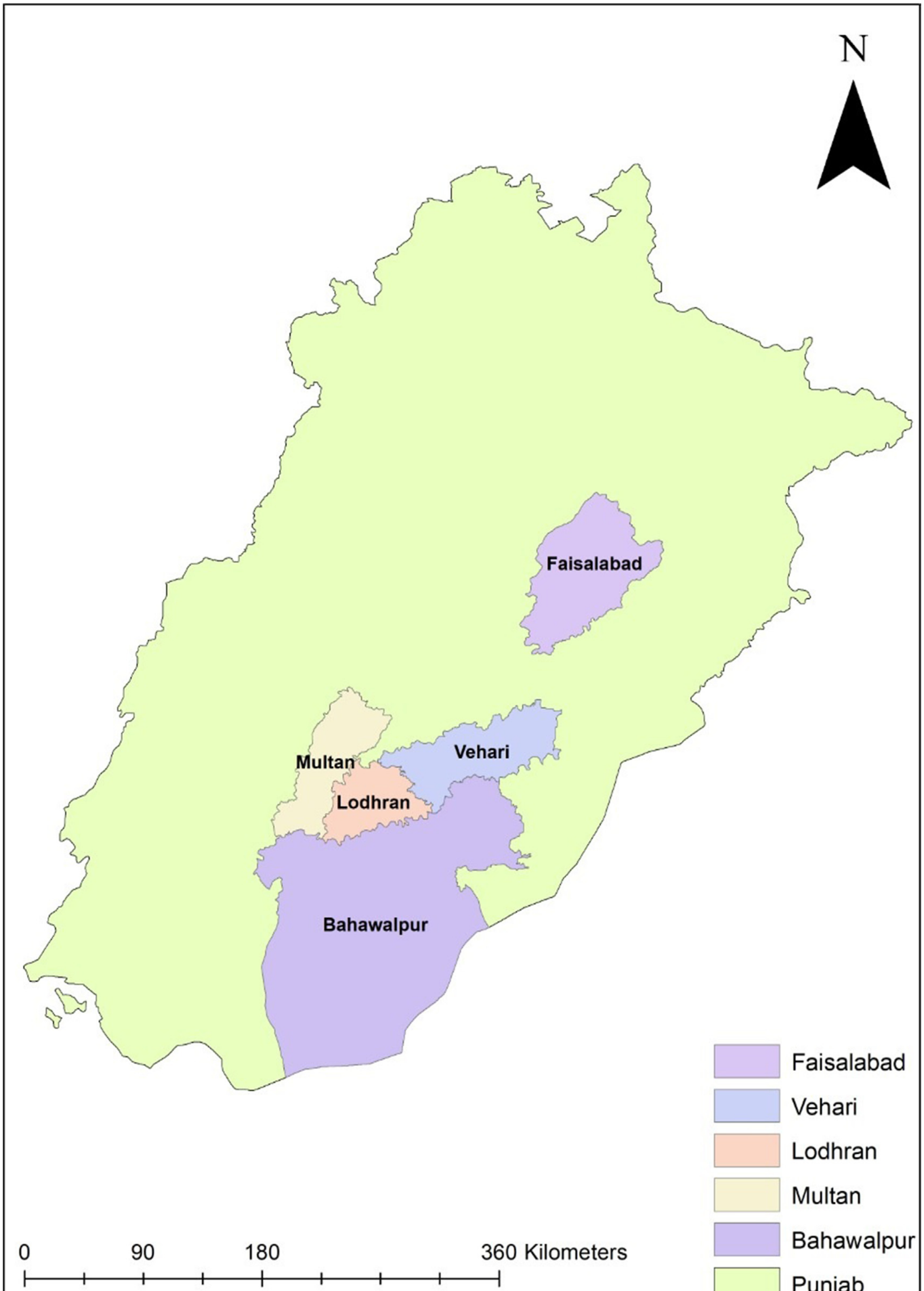

Figure 1. Map of the Punjab Province showing the districts from which whitefly samples were collected during 2017–2019 and used for monitoring insecticide resistance in this study. The study area demonstrating the sample sites.

Table 1. Response of Laboratory susceptible (Lab-PK) strain of *B. tabaci* to insecticides.

Insecticides	n[a]	LC_{50} (mgL^{-1}) 95%FL)[b]	Fit of probit analysis			
			Slope ± SE[c]	χ2[d]	df[e]	P
Pyriproxyfen	240	0.33 (0.19–0.49)	2.14 ± 0.13	1.14	4	0.54
Bupfrofezin	230	0.12 (0.07–0.30)	2.20 ± 0.19	1.99	3	0.38
Imidacloprid	240	10.97 (8.20–16.5)	2.50 ± 0.09	0.15	3	0.91
Acetamaprid	240	5.14 (2.30–7.11)	3.45 ± 0.29	0.32	3	0.67
Thiamethoxam	240	4.19 (3.04–7.4)	2.65 ± 0.31	0.13	3	0.65
Thiacloprid	180	2.99 (2.03–4.10)	2.44 ± 0.22	0.33	3	0.49
Bifenthrin	215	37.7 (22.2–58.5)	2.55 ± 0.21	2.24	3	0.56
Chlorpyrifos	240	49.99 (31.3–79.5)	2.14 ± 0.11	1.11	3	0.72

[a] Numbers of *B. tabaci* adults used in bioassay.
[b] 95% FL refers to fiducial limits of LC_{50} of each insecticide.
[c] Slope and standard error.
[d] Chi-square value.
[e] Degree of freedom as calculated by probit analysis.

3.3. Neonicotinoid efficacy

The efficacy of neonicotinoids on whitefly mortality varied by district, year, and the specific insecticide (Table 3). For imidacloprid, *B. tabaci* populations from all five districts showed moderate to high resistance at 29.66- to 65.72-fold during 2017–2019, compared to the susceptible reference colony, except for the 2018–2019 Faisalabad populations collections for which resistance ranged from 19.32- to 19.78-fold. For acetamiprid, populations showed moderate to high resistance, at 21.59–50.99-fold except the Faisalabad populations collections, which exhibited low resistance during 2018 and 2019 (Table 3). Similarly, for thiamethoxam, field populations from all five districts exhibited moderate to high resistance during 2017–2019 except for the Faisalabad populations collections, which showed low resistance at 17.18-fold in 2019. For thiacloprid, Bahawalpur, Vehari, Lodhran and Multan populations showed moderate levels of resistance of 30.81–43.81-fold during 2017 and 2018, but high resistance, at 47.49-fold in 2019. For the imidacloprid bioassay, the LC_{50} for whiteflies from Bahawalpur were significantly different, at a dose of 721 mg/l, while whiteflies from Multan, Vehari, Lodhran and Faisalabad districts, differed significantly, at 10.97 mg/l compared to the susceptible reference colony. The RR for imidacloprid ranged from 19.32 to 65.72-fold, and the LC_{50} for Bahawalpur, Multan, Vehari, Lodhran and Faisalabad districts for acetamiprid, thiamethoxam, and thiacloprid were statistically different, over doses ranging from 19.4 to 262.1 mg/l. By comparison, RRs for acetamiprid, thiamethoxam, and thiacloprid ranged from 7.60- to 50.99-fold, 17.18–54.65-fold, and 6.49–47.49-fold, respectively (Table 3).

Table 2. Toxicity of two IGRs (pyriproxyfen & buprofezin) insecticides against adult of *B. tabaci* collected from five districts of Punjab, Pakistan from 2017 to 2019.

Year	Insecticides	Location	LC50 (mg/l) (95% FL)[a]	Fit of probit analysis				
				Slope ± SE[b]	χ2[c]	df[d]	P	RR[e]
2017	Pyriproxyfen	Bahawalpur	1.19 (0.97–1.39)	3.12 ± 0.15	7.14	4	0.18	3.61
		Multan	1.16 (1.0–1.41)	2.99 ± 0.19	6.12	4	0.74	3.52
		Lodhran	1.17 (0.99–1.34)	2.81 ± 0.45	5.11	4	0.56	3.54
		Vehari	1.14 (0.91–1.22)	2.11 ± 0.22	4.67	4	0.89	3.45
		Faisalabad	1.11 (1.04–1.20)	3.01 ± 0.18	4.5	3	0.49	3.37
2018		Bahawalpur	1.18 (1.11–1.31)	3.05 ± 0.11	2.14	3	0.64	3.58
		Multan	1.17 (1.10–1.34)	2.87 ± 0.20	3.96	3	0.21	3.55
		Lodhran	1.18 (1.0–1.29)	2.45 ± 0.32	6.31	3	0.56	3.57
		Vehari	1.13 (0.93–1.34)	3.65 ± 0.54	5.32	3	0.89	3.42
		Faisalabad	1.12 (1.08–1.24)	3.10 ± 0.23	4.67	3	0.19	3.40
2019		Bahawalpur	1.21 (1.11–1.41)	3.27 ± 0.13	8.19	3	0.14	3.67
		Multan	1.19 (1.13–1.36)	3.33 ± 0.19	7.15	3	0.91	3.60
		Lodhran	1.20 (1.0–1.44)	2.22 ± 0.66	3.55	4	0.33	3.63
		Vehari	1.16 (0.91–1.39)	3.66	4.51	4	0.67	3.51
		Faisalabad	1.17 (1.12–1.29)	3.11 ± 0.49	6.10	3	0.97	3.55
2017	Buprofezin	Bahawalpur	2.0 (1.5–2.43)	5.12 ± 0.11	5.25	4	0.11	16.66
		Multan	1.10 (1.05–1.31)	4.10 ± 0.91	7.11	4	0.29	9.16
		Lodhran	1.08 (0.99–1.20)	3.22 ± 0.87	6.34	4	0.45	9
		Vehari	1.11 (0.90–1.30)	4.67 ± 0.47	4.89	4	0.66	9.25
		Faisalabad	1.05 (1.0–1.29)	3.31 ± 0.89	4.99	3	0.21	8.75
2018		Bahawalpur	1.87 (1.5–2.10)	3.67 ± 0.99	3.10	3	0.72	15.58
		Multan	1.12 (1.04–1.44)	2.76 ± 0.29	3.11	3	0.11	9.34
		Lodhran	1.35 (1.05–1.78)	4.55 ± 0.63	4.77	3	0.55	11.25
		Vehari	1.20 (1.10–1.40)	3.33 ± 0.67	4.11	3	0.33	10
		Faisalabad	1.0 (0.80–1.14)	3.91 ± 0.12	4.01	3	0.32	8.33
2019		Bahawalpur	1.11 (1.0–1.21)	3.05 ± 0.89	7.11	3	0.62	9.25
		Multan	1.05 (0.54–1.16)	3.81 ± 0.64	4.09	3	0.44	8.75
		Lodhran	1.07 (0.89–1.15)	4.23 ± 0.61	5.10	3	0.55	8.91
		Vehari	1.08 (1.0–1.20)	5.11 ± 0.48	4.10	3	0.91	9
		Faisalabad	0.96 (0.50–1.10)	3.11 ± 0.79	5.23	3	0.54	8

[a] 95% FL refer to fiducial limits.
[b] Slope and standard error.
[c] Chi-square value.
[d] f degree of freedom.
[e] RF = Resistance factor.

Table 3. Toxicity of neonicotinoid insecticides against *B. tabaci* populations collected from five districts of Punjab, Pakistan, from 2017 to 2019.

Year	Insecticides	Location	LC50 (mg/l) (95% FL)[a]	Fit of probit analysis				
				Slope ± SE[b]	χ2[c]	Df[d]	P	RR[e]
2017	Imidacloprid	Bahawalpur	554 (502–689)	3.90 ± 0.11	5.10	3	0.14	50.50
		Multan	388 (320–441)	2.65 ± 0.34	4.23	4	0.47	35.37
		Lodhran	340 (310–380)	3.10 ± 0.23	4.67	4	0.54	30.99
		Vehari	329 (311–349)	2.98 ± 0.39	3.66	4	0.33	29.99
		Faisalabad	277 (1.04–1.20)	3.44 ± 0.99	3.11	3	0.94	25.25
2018		Bahawalpur	699 (613–731)	3.32 ± 0.90	3.20	3	0.46	63.71
		Multan	432 (410–494)	2.07 ± 0.23	4.89	3	0.12	39.38
		Lodhran	460 (415–510)	3.10 ± 0.34	3.54	3	0.44	41.93
		Vehari	420 (380–445)	3.89 ± 0.66	4.69	3	0.56	38.28
		Faisalabad	212 (208–234)	3.11 ± 0.33	5.81	3	0.91	19.32
2019		Bahawalpur	721 (681–749)	3.17 ± 0.19	9.09	3	0.41	65.72
		Multan	519 (493–536)	3.13 ± 0.11	6.12	3	0.19	47.31
		Lodhran	522 (499–545)	4.00 ± 0.19	5.44	4	0.76	47.58
		Vehari	497 (470–510)	3.44 ± 0.42	4.90	4	0.56	45.30
		Faisalabad	217 (202–239)	3.41 ± 0.19	7.22	4	0.79	19.78
2017	Acetamaprid	Bahawalpur	221.0 (195–249)	4.56 ± 0.31	5.25	4	0.11	42.99
		Multan	110 (1.05–1.31)	3.99 ± 0.11	7.11	4	0.29	21.40
		Lodhran	113 (99–130)	4.22 ± 0.45	4.33	3	0.52	21.98
		Vehari	111 (100–131)	3.55 ± 0.89	3.66	3	0.22	21.59
		Faisalabad	44.97 (31.0–59)	3.22 ± 0.19	4.99	3	0.21	8.75
2018		Bahawalpur	236 (210–260)	3.20 ± 0.59	3.10	3	0.72	45.91
		Multan	124 (104–144)	2.11 ± 0.69	3.11	3	0.11	24.12
		Lodhran	130 (100–148)	3.22 ± 0.54	4.30	4	0.78	25.29
		Vehari	120 (99–144)	2.56 ± 0.29	3.67	4	0.45	23.34
		Faisalabad	39.1 (20.3–54.7)	3.71 ± 0.19	4.01	3	0.32	7.60
2019		Bahawalpur	262.1 (219–291)	3.95 ± 0.49	7.11	3	0.62	50.99
		Multan	135 (124–176)	3.11 ± 0.74	8.09	3	0.44	26.26
		Lodhran	145 (105–187)	4.10 ± 0.56	6.44	3	0.65	28.21
		Vehari	129 (110–167)	3.50 ± 0.33	5.10	3	0.71	25.09
		Faisalabad	35.9 (20.0–50.1)	3.21 ± 0.20	5.23	3	0.54	8
2017	Thiamethoxam	Bahawalpur	154 (130–180.9)	2.34 ± 0.59	2.14	3	0.44	36.75
		Multan	130 (106–156.3)	2.33 ± 0.29	1.45	3	0.41	31
		Lodhran	119 (103–145)	3.22 ± 0.47	4.22	4	0.45	23.15
		Vehari	117 (100–133)	3.99 ± 0.77	3.11	4	0.89	22.76
		Faisalabad	78 (43.6–94.2)	3.21 ± 0.49	1.90	3	0.22	18.61
2018		Bahawalpur	204 (178–233.3)	2.78 ± 0.56	2.56	3	0.11	48.68
		Multan	194 (177.3–209)	2.71 ± 0.90	2.11	3	0.67	46.30
		Lodhran	170 (140–210)	3.23 ± 0.34	3.51	3	0.91	40.57
		Vehari	156 (130–189)	2.89 ± 0.51	3.90	3	0.73	37.23
		Faisalabad	76.1 (60.2–89.0)	3.10 ± 0.50	1.99	3	0.10	18.16
2019		Bahawalpur	229 (180–259)	3.16 ± 0.81	1.11	3	0.92	54.65
		Multan	199.2 (179–267)	2.67 ± 0.62	2.99	3	0.78	47.54
		Lodhran	195 (170–240)	2.45 ± 0.34	3.11	3	0.43	46.53
		Vehari	189 (163–230)	3.45 ± 0.67	2.90	3	0.61	45.10
		Faisalabad	72 (54.1–98.3)	3.01 ± 0.50	2.78	3	0.76	17.18
2017	Thiacloprid	Bahawalpur	114 (99–131)	3.15 ± 0.45	3.10	3	0.88	38.12
		Multan	92.1 (78.9–103)	2.98 ± 0.14	1.00	3	0.91	30.81
		Lodhran	97.4 (71.2–114.5)	3.65 ± 0.53	1.45	4	0.33	32.57
		Vehari	91.2 (78.5–119.4)	2.98 ± 0.61	2.55	4	0.12	30.50
		Faisalabad	77.5 (55.2–91.3)	2.21 ± 0.60	4.22	3	0.41	25.92
2018		Bahawalpur	131 (109–149)	2.90 ± 0.39	3.21	3	0.51	43.81
		Multan	94.1 (70.3–127)	3.11 ± 0.99	2.11	3	0.43	31.47
		Lodhran	104 (87–131)	4.11 ± 0.44	3.21	3	0.59	34.78
		Vehari	96.2 (82.1–121.4)	3.65 ± 0.22	2.89	3	0.65	32.17
		Faisalabad	44.2 (30.9–54.1)	2.71 ± 0.19	1.89	3	0.55	14.78
2019		Bahawalpur	142 (119–174.2)	3.95 ± 0.99	2.10	3	0.64	47.49
		Multan	97.4 (72.4–141)	2.21 ± 0.14	1.33	3	0.91	32.57
		Lodhran	103 (88–123)	3.21 ± 0.65	2.67	3	0.56	34.44
		Vehari	99.4 (89.1–114.3)	2.33 ± 0.31	3.89	3	0.44	33.24
		Faisalabad	19.4 (10.3–30.3)	2.27 ± 0.10	1.31	3	0.43	6.49

[a] 95% FL refer to fiducial limits.
[b] Slope and standard error.
[c] Chi-square value.
[d] f degree of freedom.
[e] RF = Resistance factor.

3.4. Conventional insecticide efficacy

The LC_{50} and RR for bifenthrin and chlorpyrifos were high, based on doses of 397.3–1670 mg/l, at 7.94–49.55 for whiteflies collected in the five districts during 2017–2019 sampling (Table 4), suggesting that the efficacy of the traditional insecticides was minimal. The Faisalabad population exhibited low resistance to bifenthrin and chlorpyrifos at 14- and 7.94-fold, respectively, while Bahawalpur, Lodhran, Multan and Vehari populations exhibited moderate to high resistance to the conventional insecticides tested, and had higher LC_{50}'s at 1000 mg/l. Resistance to the conventional insecticides varied widely for whitefly collected in Lodhran and Multan during 2017–2019, while resistance to chlorpyrifos in the Faisalabad populations declined gradually over all three years (Table 4).

4. Discussion

Insecticide resistance monitoring *B. tabaci* has been ongoing in Pakistan for over a decade (Ahmad et al., 2010; Ali, 2011; Basit et al., 2011; Basit, 2019). Based on these and the results reported here, whiteflies have developed resistance to several once-effective insecticides (Mushtaq et al., 2000; Ahmad et al., 2001; Ahmad and Khan, 2017), underscoring the need for continued monitoring to facilitate management of resistance to preserve the chemical efficacies for the long term.

The failure to control *B. tabaci* with traditional pesticides were evidenced in Pakistan throughout the 1990s due to the development of resistance against organophosphates, carbamates and pyrethroids. Consequently, new chemistries such as imidacloprid, acetamiprid, thiamethoxam, and thiacloprid were approved for managing phloem-feeding insect pests, including *B. tabaci* (Basit et al., 2013; Ahmad and Khan, 2017). In Pakistan, IGRs and neonicotinoids are extensively used to control phloem-feeding insect pests infesting cotton and vegetable crops, and ornamental plants (Basit et al., 2011). Whitefly populations collected in the districts of Bahawalpur, Vehari, Lodhran, Multan, and Faisalabad during 2017–2019 showed variation in levels of resistance to the different insecticides evaluated in this study. Among these insecticides, pyriproxyfen was moderately toxic to *B. tabaci* monitored in all five

Table 4. Toxicity of traditional Insecticides to *B. tabaci* collected from five districts of Punjab, Pakistan from 2017 to 2019.

Year	Insecticides	Location	LC50 (mg/l) (95% FL)[a]	Fit of probit analysis				
				Slope ± SE[b]	χ2[c]	df[d]	P	RR[e]
2017	Bifenthrin	Bahawalpur	1670 (1504–1749)	3.12 ± 0.15	7.14	4	0.18	49.55
		Multan	1549 (1456–1641)	2.99 ± 0.19	6.12	4	0.74	45.96
		Lodhran	1521 (1410–1689)	3.21 ± 0.54	5.76	4	0.91	40.34
		Vehari	1599 (1501–1605)	2.56 ± 0.99	4.98	4	0.49	42.41
		Faisalabad	429.5 (304.2–520.4)	3.01 ± 0.18	4.5	3	0.49	12.74
2018		Bahawalpur	911 (885.3–998.1)	3.05 ± 0.11	2.14	3	0.64	27.03
		Multan	857 (789.3–881.5)	2.87 ± 0.20	3.96	3	0.21	25.43
		Lodhran	894 (801–945)	2.11 ± 0.39	2.77	3	0.55	23.71
		Vehari	861 (798–878)	2.65 ± 0.55	2.55	3	0.19	22.83
		Faisalabad	414.1 (399–4531)	3.10 ± 0.23	4.67	3	0.19	12.28
2019		Bahawalpur	1704 (1611–1741)	3.27 ± 0.13	8.19	3	0.14	50.56
		Multan	1312 (1283–1336)	3.33 ± 0.19	7.15	3	0.91	38.93
		Lodhran	1523 (1420–1651)	3.66 ± 0.45	55.77	3	0.59	40.39
		Vehari	1320 (1299–1401)	4.22 ± 0.55	6.22	3	0.81	35.0
		Faisalabad	471.8 (452–529)	3.11 ± 0.49	6.10	3	0.97	14
2017	Chlorpyrifos	Bahawalpur	1230 (1205–1293)	5.12 ± 0.11	5.25	4	0.11	24.60
		Multan	1312 (1305–1331)	4.10 ± 0.91	7.11	4	0.29	26.24
		Lodhran	1303 (1201–1389)	4.22 ± 0.41	6.44	4	0.45	26.06
		Vehari	1221 (1189–1269)	5.34 ± 0.77	7.02	4	0.66	24.42
		Faisalabad	501.6 (456–529)	3.31 ± 0.89	4.99	3	0.21	10.03
2018		Bahawalpur	1412 (1385–1470)	3.67 ± 0.99	3.10	3	0.72	28.24
		Multan	1133 (1104–1174)	2.76 ± 0.29	3.11	3	0.11	22.66
		Lodhran	1234 (1201–1309)	3.22 ± 0.59	2.66	3	0.66	24.68
		Vehari	1150 (1103–1245)	3.89 ± 0.78	3.55	3	0.89	23.00
		Faisalabad	499.1 (480–529)	3.91 ± 0.12	4.01	3	0.32	9.98
2019		Bahawalpur	1157.3 (1133–1291)	3.05 ± 0.89	7.11	3	0.62	23.14
		Multan	766.9 (714–816)	3.81 ± 0.64	8.09	3	0.44	15.34
		Lodhran	821.1 (734–910)	2.66 ± 0.72	7.55	3	0.55	16.42
		Vehari	774.3 (740–867)	3.65 ± 0.51	6.77	3	0.81	15.48
		Faisalabad	397.3 (385–410)	3.11 ± 0.79	5.23	3	0.54	7.94

[a] 95% FL refer to fiducial limits.
[b] Slope and standard error.
[c] Chi-square value.
[d] f degree of freedom.
[e] RF = Resistance factor.

districts. The $LC_{50's}$ of pyriproxyfen to control *B. tabaci* populations in Bahawalpur, Vehari, Lodhran, Multan, and Faisalabad were less than 1.50 mg/L, values that agreed with those reported by Basit et al. (2011, 2013) and Singh (2017), indicating that resistance to pyriproxyfen had not developed in the whitefly populations analyzed here. Further, field populations of *B. tabaci* exhibited greater susceptibility to pyriproxyfen, based on the lower LC_{50} values, when compared to the susceptible laboratory strain. This result may reflect differences in *B. tabaci* populations due to the different collection sites and host plants. For example, rearing insects on diverse host plants had differentially influence insecticide sensitivities and the activities of detoxification enzymes related to differences in plant allelochemicals (Liang et al., 2007; Khorsand et al., 2014; Xie et al., 2014; Wang et al., 2018). Also, higher $LC_{50's}$ were recorded for whitefly *B. tabaci* reared on poinsettia plants for three years compared to whiteflies reared on cabbage, cotton, cucumber, and tomato, and the LC_{50} values among whiteflies reared on different plant hosts differed by as much as 14.80-fold (Xie et al., 2014).

Buprofezin is an insect growth regulator with a long history of efficacy for mortality of whitefly nymphs and egg hatch in whiteflies. Buprofezin was very toxic to *B. tabaci*. Among the five districts monitored here, whitefly populations showed very low resistance to pyriproxyfen and buprofezin. This is consistent with the level of resistance reported previously for whiteflies collected from cotton in the same locations (Basit et al., 2013). Buprofezin, a thiadiazole-chitin synthesis inhibitor, has been used to achieve effective control of *B. tabaci* on cotton and other crops elsewhere (Ishaaya et al., 1988; Horowitz and Ishaaya, 1994; Naranjo et al., 2004; Gogi et al., 2006). Whitefly adults collected from Bahawalpur in 2019 showed a RR of 9.25-fold, while in Multan the RR was 8.75-fold, and in Lodhran, Vehari, and Faisalabad the RR was 8-, 9-, and 8.91-fold, respectively, indicating an increasing sensitivity to buprofezin. Based on the results of this and previous studies (Gogi et al., 2006; Ahmad et al., 2001; Ali, 2011; Basit et al., 2013) both pyriproxyfen & bupfrofezin were effective for controlling whiteflies in the southern Punjab region. Regardless, the pre-determined rotation of insecticides is considered essential for preventing the development of resistance to IGRs and other equally effective chemistries. In recent years, neonicotinoid insecticides have been the fastest growing class of insecticides used in modern crop protection programs, with widespread use against diverse phloem-feeding insects, including aphids, planthoppers, and whiteflies. As powerful agonists they act selectively on nicotinic acetylcholine receptors, their molecular target (Jeschke et al., 2010; Basit et al., 2013; Abd-Ella, 2014).

In this study, the level of insecticide resistance among *B. tabaci* populations was dependent upon the whitefly population, year, and particular insecticide. Imidacloprid was first introduced for use against whiteflies during the early 1990s and it was the first neonicotinoid used in Pakistan for controlling whiteflies and other insect pests. However, gradually reduced efficacy of neonicotinoids for whitefly management has been reported, particularly for imidacloprid. Here, whitefly collected in Faisalabad, Khanewal, Multan, and Vehari (Punjab) exhibited low to moderate degrees of resistance to imidacloprid, acetamiprid, thiamethoxam, and thiacloprid (Basit et al., 2013; Ahmad and Khan, 2017). Results showed that neonicotinoid resistance detected in whitefly from 2017 to 2019 was overall, generally greater for imidacloprid than the other three neonicotinoids, acetamiprid, thiamethoxam, and thiacloprid). The RR for imidacloprid in Bahawalpur, Lodhran, Multan, and Vehari had increased from previous years, while at the same time, resistance to the same compounds in whiteflies collected in Faisalabad cotton during 2018 and 2019 was lower, at the high to moderate level (Table 3). The lower resistance levels observed in this study could possibly be attributed to a reduction in the number of applications of imidacloprid used recently by farmers in Faisalabad. In contrast, acetamiprid resistance of *B. tabaci* populations in Bahawalpur, Lodhran, Multan, and Vehari increased during the three years in which this research was conducted, as the result of widespread acetamiprid use in recent years (Ahmad and Khan, 2017; Basit, 2019) and during this study. Results indicated that *B. tabaci* in the districts of Bahawalpur, Lodhran, Multan, and Vehari developed high resistance to imidacloprid and acetamiprid based on the increasingly greater LC_{50} values observed during this study. By comparison, Ahmad and Khan (2017) found that *B. tabaci* from the three districts, Bahawalpur, Faisalabad, and Multan, exhibited high RR values, ranging from 0.88 to 922 for imidacloprid, 0.84- to 1172- for acetamiprid, 1.2- to 505- for thiamethoxam and 2.6- to 16136-folds for thiacloprid respectively (3.4–3.7). Similar results have been reported from China (Yang et al., 2013; Wang et al., 2020a), Israel (Horowitz et al., 2004), Greece (Horowitz et al., 2020), Spain (Fernández et al., 2009) and the US (Dennehy et al., 1999; Palumbo et al., 2001 and Horowitz et al., 2020). Overall, the monitoring results showed that the continued application of neonicotinoid insecticides is very likely to select for resistant *B. tabaci* populations in many districts of Pakistan. Thiacloprid is the newest insecticide incorporation to the arsenal of neonicotinoids against *B. tabaci* population in Pakistan. Here, LC_{50} values decreased from 2017 to 2019, possibly because of increased farmer awareness of the rapid development of resistance to thiacloprid, which resulted in more judicious use of the compound. Finally, the LC_{50} values of the conventional insecticides, chlorpyrifos and bifenthrin, were higher than 1000 mg/l, indicating they contribute minimally, at such low efficacy to whitefly control, at 3.8 to 3.9. This was consistent with results from a previous study in Pakistan conducted nearly a decade ago (Ahmad et al., 2010). In general, the moderate to high resistance to chlorpyrifos and bifenthrin did not differ to any major extent and remained at steady levels. In addition, no control of *B. tabaci* was evident following applications of the traditional insecticides, bifenthrin and chlorpyrifos, which also has occurred in whitefly populations collected from cotton crop in West Africa (Houndété et al., 2010), China (Islam et al., 2010), Cyprus (Vassiliou et al., 2011), and India (Naveen et al., 2017). In the five districts whitefly populations monitored over a three-year period of time from 2017-2019, buprofezin, pyriproxyfen, and thiacloprid consistently yielded the lowest LC_{50} values, of 0.96, 19.4 and 1.11 mg/l, respectively. Consistent with the results reported here showing that bifenthrin and chlorpyrifos did not provide effective whitefly control, other recent studies in the same locales have documented moderate to high levels of resistance to the traditional and neonicotinoid insecticides in whitefly populations. Finally, buprofezin and pyriproxyfen were found to be highly effective against whitefly in all of the study sites, making them a good fit in IPM/IRM programs for achieving effective control when whiteflies become a serious problem. The routine monitoring of insecticide resistance to aid in the management of *B. tabaci* is highly recommended for farmers. Therefore, monitoring is the most effective way to guide the selection of insecticides for effective control of *B. tabaci* as pest insect and vector of plant viruses. The successes of such monitoring programs in Pakistan and elsewhere underscore the power of implementing such knowledge for the prudent management of whitefly resistance in field populations.

Declarations

Author contribution statement

Muhammad Saleem: Conceived and designed the experiments, Performed the experiments, Analyzed and interpreted the data and wrote the paper.

Dilbar Hussain: Conceived and designed the experiments and Contributed reagents, materials, analysis tools or data.

Muhammad Sagheer: Conceived and designed the experiments, Performed the experiments.

Mansoor ul Hasan: Conceived and designed the experiments.

Muhammad Zubair: Performed the experiments.

J.K. Brown: Analyzed and interpreted the data.

Ghulam Ghouse: Analyzed and interpreted the data and Contributed reagents, materials, analysis tools or data.

Sikander Ali Cheema: Contributed reagents, materials, analysis tools or data.

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Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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