


Resistance monitoring for conventional and new chemistry insecticides on *Bemisia tabaci* genetic group Asia-I in major vegetable crops from India

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Abstract Whitefly (*Bemisia tabaci* Gennadius) is a devastating pest of vegetables, cotton, and many other agricultural and horticultural crops worldwide. Since control of *B. tabaci* on vegetable crops solely depends on the use of chemical insecticides in India; monitoring the insecticide resistance of *B. tabaci* populations would be very much essential for achieving successful control and for managing the resistance development. Hence, the aim of the present study was to examine the resistance in different field strains of *B. tabaci* to traditional organophosphates and new chemical insecticides in India. The resistance ratios were recorded in the range of 30.67–131.48 fold for acephate, 29.17–83.67 fold for triazophos, 0.38–2.51 fold for

indoxacarb, 4.55–34.52 fold for dinotefuran, 6.26–27.56 fold for tolfenpyrad, 7.87–31.89 fold for spiromesifen, 1.61–30.08 fold for pyriproxyfen, and 3.09–45.92 fold for flonicamid in comparison to that of the susceptible strain in the laboratory. Resistance levels of *B. tabaci* populations against the tested insecticides were significantly variable among localities. The present data will be helpful for the selection of proper insecticides on vegetable crops in the field for successful management of *B. tabaci* in near future.

Keywords Tobacco whitefly · Insecticide resistance · Dinotefuran · Tolfenpyrad · Flonicamid · Vegetables

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Introduction

Cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is categorized as a serious crop pest worldwide (Kontsedalov et al. 2012). Several reasons including indiscriminate use of insecticides (Denholm et al. 1998), manipulation in agronomic practices (Dittrich et al. 1986), and the introduction of unidentified whitefly biotypes (Banks et al. 2001) led to be this insect pest as one of the most intractable and cosmopolitan species in various agricultural and horticultural crops having economic importance (Erdogan et al. 2008; Wang et al. 2017). This phloem feeder is solely responsible to transmit several viral diseases in over 60 economically important crop plants (Navas-Castillo et al. 2011) and thus causes horrific damages to national agricultural production (Reitz 2007). It causes

tremendous loss to agricultural production and to the national economy by feeding on phloem sap and by transmitting several kinds of viral diseases. This particular pest is very hard to control owing to its broader host range, cryptic behavior, viral diseases transmission capabilities, and remarkable potential to develop resistance against insecticides (De Barro et al. 2011; Naveen et al. 2017).

Insecticides from different classes act as the mainstay for successful control of *B. tabaci* in major vegetables and cotton-growing zones of India (Gutierrez et al. 2015), and it (*B. tabaci*) develops resistance to those molecules that have been applied extremely and frequently. Improper selection of chemical insecticides and their unscientific usage by the farmers resulted in the failure of the insecticide-based control mechanism against *B. tabaci* in India (Peshin and Zhang 2014). However, the resistance development against organophosphates (OPs), pyrethroids, and neonicotinoids in *B. tabaci* generally depends on the recurrent use of some insecticides belong to the aforesaid classes of chemistry, having similar active compounds or with identical mode of action and application of excessive doses of those molecules within a specified crop growing season in an area (Kranthi et al. 2002). As a result of resistance development by whitefly against various old generation conventional molecules in India (Nauen et al. 2015), many traditional insecticides have posed obsessive pressure on new molecules introduced commercially in Indian market in the early 2000s. Multiple reports have displayed the resistance development in *B. tabaci* even against the insecticides of ‘bio-rational molecules’ in divers Asian countries, including India (Kranthi et al. 2001, 2002), China (Luo et al. 2010; Wang et al. 2010), and Pakistan (Ahmad et al. 2010). In the areas with acute chemical pesticide use, like vegetables and cotton producing belts of India, it is the utmost importance to monitor the resistance development status among insect pests against regularly used insecticides for choosing their appropriate dosages, and for retaining their field-effectiveness for a long period (Srinivas et al. 2004). A prolonged history of insecticide resistance in whitefly against OPs, carbamates, and synthetic pyrethroids on cotton is already there in India (Sethi and Dilawari 2008; El-Latif and Subrahmanyam 2010); however, being the second largest vegetable producer globally, scanty of report is been documented from this country on the resistance status of *B. tabaci* to chemical insecticides extensively use in vegetable crops.

Examining the resistance of pests against insecticides is essential for designing an effective IRM (Integrated Resistance Management) approach. This not only assists in documenting the geographical and chronological divergence in the response of the pest population towards insecticides, additionally it offers a clear outlook on the status of a particular pest, resistant to commonly used insecticides in the field. The present investigation attempts to evaluate resistance in *B. tabaci* against the selected ‘new chemistry’ insecticides and against some concurrently used conventional molecules, applied in major vegetable growing regions of West Bengal, India; and to gather preliminary information for advanced monitoring processes that will assist to develop a sound strategy for the insecticide resistance management in *B. tabaci*.

Materials and methods

Collection of insects

Field strains of *B. tabaci* were collected during 2016–2017 from different host plants (tomato, brinjal, chilli, and okra) of six districts in West Bengal, India (Fig. 1), which are 100 to 600 km apart from each other [Nadia (23.4710° N, 88.5565° E), Murshidabad (24.2290° N, 88.2461° E), South 24-Parganas (22.1352° N, 88.4016° E), Bankura (23.1645° N, 87.0624° E), Malda (25.1786° N, 88.2461° E) and Coochbehar (26.3234° N, 89.3227° E)]. Geographically, these districts situated under six different agro-climatic zones of West Bengal (India). Major belts of vegetable production, severe whitefly infestation throughout the year, heavy insecticides used by the vegetable growers for complete control of various insect and mite pests including *B. tabaci* (Vanitha et al. 2013), and easy accessibility were the prime reasons for selecting these regions. While collecting the whitefly populations from the field, standard methodology was followed (Naveen et al. 2017) by moving in ‘Z’ pattern at least 2 ha area of the crop fields. During early morning hours, adult whiteflies were collected with the help of an aspirator, and the leaves containing dense nymphal population were brought to the laboratory in ventilated jars containing fresh tender leaves inserted into wet absorbent cotton. Taxonomic identification of collected *B. tabaci* was carried out through binocular stereo zoom microscopy (40x magnification) using the key of Martin (1987). Field

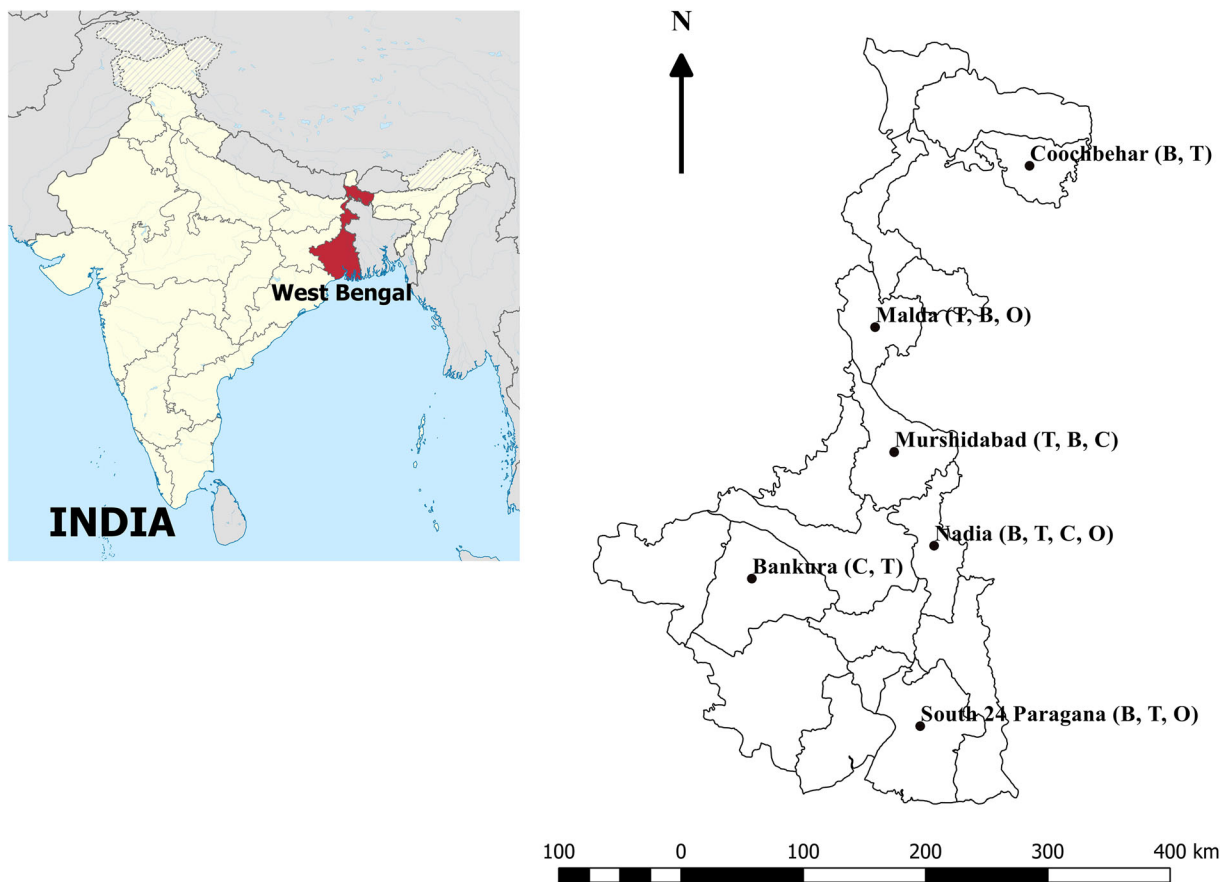


Fig. 1 Collection sites of *B. tabaci* field populations from different vegetable crops in West Bengal, India (T, Tomato; B, Brinjal; C, Chilli; O, Okra)

populations collected from the individual districts were maintained separately and reared for a single generation in the insect and mite culture laboratory at Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal, India, in glass jars (15 cm diameter and 20 cm height) tightly covered with insect rearing nylon mesh on top for adequate ventilation, to achieve a homogenous population and to remove the maternal effects. All the populations were raised on insecticide free tomato (*Solanum lycopersicum* L.) leaves in growth chambers at 26 ± 2 °C temperature and 60–70% relative humidity with a 14 h photoperiod. The insect population was collected from university farm of BCKV, West Bengal, India in 2014 and was fostered for 13 generations in the aforesaid insect and mite culture laboratory devoid of any insecticide exposure to reinstate the genes susceptible against insecticides. From this laboratory strain, insecticide susceptible

reference population was generated in 2016 and designated as Lab-WB by carrying out ‘single pair cross’ technique according to Basit et al. (2011) that could serve as a fundamental line for evaluating insecticide resistance in coming years. The genetic groups of each of the randomly selected ten individual adults per field population were identified using the PCR of specific mitochondrial cytochrome oxidase I (mtCOI) gene and sequencing technique (Dinsdale et al. 2010).

Insecticides selected for bioassays

The selection of insecticides was purely based on the survey data collected from the farmers’ end regarding the usage intensity and duration of different commercially formulated conventional and new generation molecules on vegetable crops in selected vegetable growing zones of West Bengal, India. The selected insecticide

formulations were: acephate (Asataf, 75 SP; Rallis India Ltd.), triazophos (Ghatak, 40 EC; Dhanuka Agritech Ltd.), indoxacarb (Steward, 14.5 SC; DuPont India Pvt. Ltd.), dinotefuran (Token, 20 SG; Indofil Industries Ltd.), tolfenpyrad (Keefun, 15 EC; PI Industries), spiromesifen (Oberon, 240 SC; Bayer Crop Science), pyriproxyfen (Admiral, 10 EC; Sumitomo Chemical Co. Ltd.), and flonicamid (Ulala, 50 WG; United Phosphorus Ltd.).

Bioassays

A modified leaf dip bioassay method was followed (Naveen et al. 2012; Xie et al. 2014) for assessing the resistance of field populations of *B. tabaci* to eight different insecticides, through computing the baseline susceptibility of the reference Lab-WB strain. “Nymphal leaf-dip bioassay” technique was conducted for pyriproxyfen as it is a potent ovicide and effective against immature stages of insects; whereas, “adult leaf-dip bioassay” was followed for rest of the insecticides. Stock solutions of each of the commercial formulations were prepared in acetone, through gradual dilutions in double-distilled water dissolving 0.1 g L⁻¹ of Triton X-100 (a non-ionic wetting agent). The terminal second or the third fully expanded compound leaves (7.5 cm length contains five leaflets), collected from sixteen to twenty-days-old seedlings of a whitefly-susceptible tomato cultivar (cv. NS-521) were dipped in the serially diluted solutions of insecticides for 20 s; then the leaves were subjected to air-dry and placed with their adaxial surface downwards on 2% (w/v) agar slants in Petri plates (9 cm diameter). Compound leaves that were dipped in diluents only, served as controls. Fifteen to twenty-five *B. tabaci* adults were shortly anesthetized using CO₂ and introduced in each Petri plate except pyriproxyfen. On the other hand, thirty second-instar nymphs were transferred onto each of the treated leaves with pyriproxyfen in the similar way (Farnandez et al. 2009). Then all the Petri plates were sealed with adequately perforated lids. Thus, by taking at least five different concentrations of each insecticide, all the treatments were replicated six to seven times. The entire procedures were carried out in a fume hood under laboratory condition. The Petri plates were then placed in an incubator with the prefixed temperature, relative humidity, and photoperiod as mentioned earlier. Mortality was assessed after 48 h exposure to acephate, triazophos, indoxacarb, dinotefuran, tolfenpyrad, and spiromesifen and 72 h exposure to flonicamid (Gorman et al. 2010).

Nymphal mortality to pyriproxyfen was recorded after 20 days when eggs were laid by the survivors after emerging as adults. The nymphs and adult insects were assumed to be dead if an inferior response or no any movement was observed after a soft palp with a 0.1 mm camel hair brush under the light microscope (Naveen et al. 2017).

Data interpretation

Correction of mean mortality values for adults and nymphs of *B. tabaci* was accomplished using Abbott’s formula (Abbott 1925) and analyzed by probit analysis (Finney 1971) through PoloPlus version 2.0 software (LeOra Software Company, USA) for determination of the LC₅₀ values along with their 95% confidence intervals (CI). Then, the LC₅₀ value of the field population was divided by the LC₅₀ value of Lab-WB in order to enumerate the resistance ratio (RR). Calculation of the 95% CI of RR was performed by following Robertson and Preisler (1992). Computed RR values of each individual insecticide were ranged to indicate resistance categories according to Ahmad and Arif (2009): RR = 1–1.99, no resistance; RR = 2–10.99, tolerance; RR = 11–20.99, low resistance; RR = 21–50.99, moderate resistance; RR = 51–100.99, high resistance and RR ≥ 101, extremely high resistance.

Preparation of thematic map

To draw the thematic maps of *B. tabaci* collection sites at different districts of West Bengal, QGIS software (version: 2.18.17) was used. The user-defined classification scale and pre-defined algorithm were used to generate the thematic maps. Joining and enhancing the thematic maps in a single image were done through the Adobe Photoshop (version 7.0) software.

Results

Resistance of *B. tabaci* to insect growth regulator and pyridine carboxamide insecticides

The mitochondrial cytochrome oxidase I sequence analysis revealed that all the *B. tabaci* populations collected from six different locations of West Bengal, India belonged to the Asia-I genetic group.

LC₅₀ values for the *B. tabaci* reference population (Lab-WB)

Pyridine carboxamide insecticide, flonicamid registered the lowest LC₅₀ value against the laboratory reference strain followed by tolfenpyrad, pyriproxyfen, spiromesifen, and dinotefuran. Acephate and triazophos were ascertained to be lower toxic molecules with the LC₅₀ values of 3.22 mg L⁻¹ and 7.08 mg L⁻¹, respectively. Based on LC₅₀ values (>30 mg L⁻¹), acquired with the Lab-WB population, substantially the low toxicity of indoxacarb was observed to adult *B. tabaci* (Table 1).

Resistance of *B. tabaci* to two conventional OP and oxadiazine insecticides

For acephate and triazophos, the LC₅₀ and RR values were significantly high in case of all the field strains (Table 1), implying very poor toxicity of these frequently used conventional molecules against *B. tabaci*. The LC₅₀ values for acephate were in the range of 98.76 to 423.35 mg L⁻¹ and for triazophos were 206.52 to 592.36 mg L⁻¹ among the test populations. The resistance in Bankura population for acephate and South 24-Parganas population for triazophos was the lowest and the resistance in Nadia and Murshidabad populations for the respective insecticides was the highest, with LC₅₀ values >400 mg L⁻¹. Acephate (36.60 fold) and triazophos (45.22 fold) showed a medium level of resistance against the population picked up from Malda. But, Coochbehar population exhibited high resistance level to both the insecticides.

Indoxacarb manifested no resistance against all the collected field strains of *B. tabaci* compared with the Lab-WB except Nadia that showed tolerance (2.51 fold) to this insecticide (Table 1).

Resistance of *B. tabaci* to neonicotinoid, METI, and tetric acid insecticides

The field resistance status of *B. tabaci* to the three different groups of chemistry differed among insecticide molecules and locations (Table 2). Of the six strains of *B. tabaci* examined, populations gathered from South 24-Parganas, Bankura, and Coochbehar showed tolerance (4.55 to 9.52 fold) to dinotefuran (Table 2). Nadia

population exhibited a moderate resistance level (34.52 fold), whereas comparatively low resistance levels were encountered in the remaining *B. tabaci* populations collected from Murshidabad and Malda.

The dose-dependent bioassay results revealed that tolerance to moderate levels of resistance has been shown by tested *B. tabaci* strains likened to the Lab-WB population against tolfenpyrad (Table 2). Population collected from Coochbehar registered a moderate level of resistance (27.56 fold). Low resistance levels (12.23–15.13 fold) were noticed in the strains collected from Murshidabad, Nadia, and Bankura, but, the South 24-Pargana and Malda populations exhibited tolerance to this chemical.

For spiromesifen, the populations of *B. tabaci* collected from Murshidabad and Nadia exhibited moderate resistance levels (21.07 to 31.89 fold). Low level of resistance was encountered in the populations of South 24 Pargana (17.46 fold), Malda (15.08 fold), and Coochbehar (19.45 fold), but Bakura population exhibited tolerance to this insecticide (Table 2).

No resistance to moderate levels of resistance for pyriproxyfen was observed in tested *B. tabaci* populations compared with the reference strain (Table 3). The populations collected from Coochbehar and Malda showed a moderate level of resistance (21.24 to 30.08 fold). South 24-Parganas population found to become susceptible against this insecticide and remaining *B. tabaci* strains registered the low level of resistance (11.61 to 16.96 fold).

The tested *B. tabaci* field populations exhibited the tolerance to moderate levels of resistance to flonicamid (Table 3). Populations collected from South 24-Parganas, Murshidabad, and Nadia showed low levels of resistance (12.80 to 20.85 fold). A moderate resistance level was observed in Malda population (45.92 fold), whereas Bankura and Coochbehar populations showed tolerance to this molecule (3.09 to 3.75 fold).

Discussion

The present study gives an overview of the actual status of insecticide resistance elicited by different field populations of *B. tabaci* (Asia-I genetic group) in vegetable ecosystems across six agro-climatic zones of West

Table 1 Resistance status of OP and oxadiazine insecticides against *Bemisia tabaci* field populations from eastern India

Test insecticides	Selected regions	Collection year	N [†]	χ^2 (df)	Slope \pm SE	P	LC ₅₀ (mg L ⁻¹) [95% CI]	RR [95% CI] [‡]
Acephate ^a	Lab-WB	2014	200	0.03 (4)	0.76 \pm 0.35	0.98	3.22 (2.04–4.69)	1.00
	Murshidabad	2016	200	0.57 (4)	1.23 \pm 0.30	0.91	279.72 (142.24–497.79)	86.87 (44.18–154.59)
	Nadia	2016	200	0.11 (4)	2.05 \pm 0.29	0.96	423.35 (246.25–611.40)	131.48 (76.48–189.88)
	South 24-Parganas	2016	150	0.07 (4)	1.36 \pm 0.42	0.99	204.10 (114.39–342.62)	63.38 (35.52–106.40)
	Bankura	2017	150	0.69 (4)	0.82 \pm 0.33	0.92	98.76 (62.56–181.29)	30.67 (19.43–56.30)
	Malda	2017	200	0.44 (4)	1.45 \pm 0.18	0.89	117.85 (73.16–170.27)	36.60 (22.72–52.88)
	Coochbehar	2017	150	1.01 (4)	0.89 \pm 0.30	0.93	170.83 (98.41–259.56)	53.05 (30.56–80.61)
Triazophos ^a	Lab-WB	2014	200	0.52 (4)	1.56 \pm 0.35	0.95	7.08 (5.95–8.89)	1.00
	Murshidabad	2016	200	2.63 (4)	1.14 \pm 0.35	0.75	592.36 (427.54–739.19)	83.67 (60.39–104.40)
	Nadia	2016	200	0.31 (4)	1.55 \pm 0.31	0.81	436.25 (324.52–586.94)	61.62 (45.84–82.90)
	South 24-Parganas	2016	150	0.94 (4)	1.02 \pm 0.19	0.93	206.52 (133.60–312.94)	29.17 (18.87–44.20)
	Bankura	2017	150	0.55 (4)	1.24 \pm 0.28	0.99	465.47 (320.21–622.74)	65.74 (45.23–87.96)
	Malda	2017	200	1.07 (4)	0.88 \pm 0.31	0.91	320.14 (194.24–497.56)	45.22 (27.43–70.28)
	Coochbehar	2017	150	0.19 (4)	0.97 \pm 0.34	0.89	376.48 (260.32–511.11)	53.18 (36.77–72.19)
Indoxacarb ^b	Lab-WB	2014	200	0.94 (4)	1.45 \pm 0.34	0.99	32.45 (18.32–54.68)	1.00
	Murshidabad	2016	200	0.08 (4)	1.39 \pm 0.39	0.65	64.54 (30.15–106.49)	1.99 (0.93–3.28)
	Nadia	2016	200	0.85 (4)	1.31 \pm 0.40	0.94	81.32 (38.42–146.47)	2.51 (1.18–4.51)
	South 24-Parganas	2016	150	0.22 (4)	1.05 \pm 0.42	0.97	45.20 (24.12–84.75)	1.39 (0.74–2.61)
	Bankura	2017	150	1.02 (4)	1.39 \pm 0.32	0.87	12.45 (5.39–47.45)	0.38 (0.17–1.46)
	Malda	2017	200	0.35 (4)	0.94 \pm 0.30	0.91	27.14 (11.25–46.56)	0.84 (0.35–1.44)
	Coochbehar	2017	150	0.31 (4)	0.99 \pm 0.18	0.82	19.28 (10.47–39.60)	0.59 (0.32–1.22)

[†] Total number of insects used in bioassays, including control

[‡] RR = Resistance ratio with 95% confidence level (CI)

^a OP

^b Oxadiazine

Bengal; the leading vegetable producing state of India. Comprehensive studies along with critical reviews on insecticide resistance of *B. tabaci* has been performed in Asian countries (Mallah 2007; Basit et al. 2013; Wang et al. 2017), and resistance status of this phloem feeder (particularly the Indian population) to different classes of insecticides in cotton has frequently been documented (Jadhav et al. 1999; Singh and Jaglan 2005; Naveen et al. 2017). However, the incidence of new chemical insecticides resistance along with conventional synthetic molecules in *B. tabaci* and especially the Asia-I genetic group from major vegetable belts of India is reported for the first time in the present study. In this study, the *B. tabaci* populations collected in six different locations from 2016 to 2017 showed a long range of resistance against the selected commercial insecticides. However, according to Khan et al. (2013), at least ten-fold of resistance is required for considering an insect

population as ‘resistant’. Results of the present investigation revealed less than ten-fold resistance ratios in *B. tabaci* collected from every location to Indoxacarb, from three locations to dinotefuran, two locations to tolfenpyrad, one location to each of spiromesifen and pyriproxyfen, and two locations to flonicamid due to absolute tolerance rather than any resistance. Although, modern chemistry insecticides have been engrossed worldwide for the sustainable management of different sucking insects, mixing of these chemicals with OP, carbamate, and synthetic pyrethroids could also be one of the prime factors for multiple and cross-resistance. Blending conventional insecticides with new chemistry molecules is a very common practice in India (Kumar et al. 2013) to control various vegetable pests belong to different orders simultaneously, which could create the problem of multiple or cross resistances between the active ingredients of those compounds.

Table 2 Resistance status of neonicotinoid, METI and tetronic acid derivative insecticide against *Bemisia tabaci* field populations from eastern India

Test insecticides	Selected regions	Collection year	N [†]	χ^2 (df)	Slope \pm SE	P	LC ₅₀ (mg L ⁻¹) [95% CI]	RR [95% CI] [‡]
Dinotefuran ^a	Lab-WB	2014	200	0.01 (4)	0.75 \pm 0.28	0.99	1.72 (0.85–3.22)	1.00
	Murshidabad	2016	200	1.42 (4)	0.88 \pm 0.35	0.88	33.40 (15.31–58.45)	19.42 (8.90–33.98)
	Nadia	2016	200	0.75 (4)	1.23 \pm 0.33	0.76	59.37 (40.36–102.14)	34.52 (23.47–59.38)
	South 24-Parganas	2016	150	0.04 (4)	1.34 \pm 0.17	0.92	10.24 (7.74–25.28)	5.95 (4.50–14.70)
	Bankura	2017	150	0.52 (4)	1.04 \pm 0.29	0.72	7.82 (5.12–20.48)	4.55 (2.98–11.91)
	Malda	2017	200	2.23 (4)	1.62 \pm 0.45	0.74	22.18 (13.61–43.45)	12.89 (7.91–25.26)
	Coochbehar	2017	150	0.46 (4)	1.25 \pm 0.30	0.83	16.37 (9.81–30.16)	9.52 (5.70–17.53)
Tolfenpyrad ^b	Lab-WB	2014	200	0.46 (4)	0.82 \pm 0.38	0.99	1.10 (0.21–2.33)	1.00
	Murshidabad	2016	200	0.87 (4)	0.93 \pm 0.42	0.95	16.64 (11.18–28.47)	15.13 (10.16–25.88)
	Nadia	2016	200	1.12 (4)	1.25 \pm 0.32	0.92	14.78 (5.63–25.46)	13.44 (5.12–23.15)
	South 24-Parganas	2016	150	0.29 (4)	1.37 \pm 0.16	0.71	6.89 (4.22–12.18)	6.26 (3.84–11.07)
	Bankura	2017	150	0.54 (4)	0.79 \pm 0.39	0.98	13.45 (7.71–26.98)	12.23 (7.01–24.53)
	Malda	2017	200	0.09 (4)	1.26 \pm 0.30	0.85	9.74 (7.07–19.41)	8.85 (6.43–17.65)
	Coochbehar	2017	150	0.12 (4)	1.38 \pm 0.26	0.69	30.32 (16.34–47.25)	27.56 (14.85–42.95)
Spiromesifen ^c	Lab-WB	2014	200	0.02 (4)	0.77 \pm 0.41	0.92	1.48 (0.52–3.03)	1.00
	Murshidabad	2016	200	1.23 (4)	1.25 \pm 0.14	0.91	31.18 (14.58–56.24)	21.07 (9.85–38.00)
	Nadia	2016	200	2.45 (4)	2.02 \pm 0.34	0.83	47.20 (22.14–117.63)	31.89 (14.96–79.48)
	South 24-Parganas	2016	150	0.75 (4)	1.43 \pm 0.38	0.99	25.84 (12.45–55.82)	17.46 (8.41–37.72)
	Bankura	2017	150	0.08 (4)	1.29 \pm 0.30	0.94	11.65 (9.08–20.76)	7.87 (6.14–14.03)
	Malda	2017	200	0.22 (4)	1.52 \pm 0.37	0.71	22.32 (13.38–38.39)	15.08 (9.04–25.94)
	Coochbehar	2017	150	1.73 (4)	0.90 \pm 0.28	0.82	28.79 (17.22–60.81)	19.45 (11.64–41.09)

[†] Total number of insects used in bioassays, including control

[‡] RR = Resistance ratio with 95% confidence level (CI)

^a Neonicotinoid

^b METI (Mitochondrial Electron Transport Inhibitor)

^c Tetronic acid derivative

In the present study, six different populations of *B. tabaci* exhibited moderate to very high and moderate to high levels of resistance to acephate and triazophos respectively. Erstwhile documentations had clearly transpired the resistance levels of these traditional OP insecticides in the coeval *B. tabaci* populations (Kranthi et al. 2002; Bacci et al. 2007). Impolitic use of insecticides (space, frequency, duration and dose) also induces genetically modification in insects, leads to resistances over a long period (Tabashnik 1989). High to very high scales of resistance to acephate and triazophos marked in the present study in Nadia and Murshidabad strains of *B. tabaci*, with an enormity of resistance encountered being superfluous than previous records, could be ascribed to the widespread usage of these OP compounds by the vegetable growers of India (Ahmad et al. 2015). In West Bengal, acephate and triazophos are among the

most used OP insecticides for the management of major hemipteran pests like whitefly, aphid, jassid, mealybug along with numerous lepidopteran caterpillars, beetles and weevils infesting different vegetable crops (Banerjee et al. 2014). Moreover, wide ranges of resistance of *B. tabaci* (Asia-I genetic group) to triazophos have previously been documented from cotton growing zones of India (Sethi and Dilawari 2008; Naveen et al. 2017) and Pakistan (Ahmad et al. 2010).

In current results, all the tested field populations of *B. tabaci* asserted no resistance levels against indoxacarb. However, resistance to this molecule had previously been observed in different insect pests globally like *Plutella xylostella* (Sayyed et al. 2008), *Spodoptera litura* (Ahmad et al. 2008), *Helicoverpa armigera* (Hussain et al. 2014), *Musca domestica* (Shono et al. 2004) and *Blattella germanica* (Zhu et al.

Table 3 Resistance status of insect growth regulator and pyridine carboxamide insecticide against *Bemisia tabaci* field populations from eastern India

Test insecticides	Selected regions	Collection year	N [†]	χ^2 (df)	Slope \pm SE	P	LC ₅₀ (mg L ⁻¹) [95% CI]	RR [95% CI] [‡]
Pyriproxyfen ^a	Lab-WB	2014	200	0.14 (4)	1.19 \pm 0.35	0.97	1.19 (0.34–2.15)	1.00
	Murshidabad	2016	200	2.24 (4)	0.92 \pm 0.32	0.48	18.73 (14.24–41.82)	15.74 (11.97–35.14)
	Nadia	2016	200	0.07 (4)	0.75 \pm 0.19	0.82	13.82 (10.54–49.70)	11.61 (8.86–41.76)
	South 24-Parganas	2016	150	0.11 (4)	1.11 \pm 0.22	0.93	1.92 (0.47–4.29)	1.61 (0.40–3.61)
	Bankura	2017	150	0.45 (4)	1.61 \pm 0.17	0.99	20.18 (12.33–32.53)	16.96 (10.36–27.34)
	Malda	2017	200	1.06 (4)	2.04 \pm 0.30	0.94	35.79 (15.73–93.10)	30.08 (13.22–78.24)
	Coochbehar	2017	150	1.55 (4)	1.28 \pm 0.34	0.51	25.28 (12.54–45.55)	21.24 (10.54–38.28)
Flonicamid ^b	Lab-WB	2014	200	0.02 (4)	1.63 \pm 0.31	0.99	1.06 (0.10–2.62)	1.00
	Murshidabad	2016	200	1.37 (4)	0.89 \pm 0.37	0.88	18.52 (11.23–28.81)	17.47 (10.59–27.18)
	Nadia	2016	200	2.51 (4)	1.44 \pm 0.31	0.87	22.10 (13.39–40.41)	20.85 (12.63–38.12)
	South 24-Parganas	2016	150	0.45 (4)	1.31 \pm 0.16	0.94	13.57 (4.64–22.38)	12.80 (4.38–21.11)
	Bankura	2017	150	0.58 (4)	0.73 \pm 0.34	0.76	3.28 (1.09–10.58)	3.09 (1.03–9.98)
	Malda	2017	200	0.98 (4)	1.17 \pm 0.30	0.62	48.67 (19.32–77.84)	45.92 (18.23–73.43)
	Coochbehar	2017	150	1.62 (4)	1.46 \pm 0.31	0.81	3.98 (0.92–9.83)	3.75 (0.87–9.27)

[†] Total number of insects used in bioassays, including control

[‡] RR = Resistance ratio with 95% confidence level (CI)

^a Insect growth regulator

^b Pyridine carboxamide

2016). Despite the extensive application of indoxacarb on major vegetable crops for the control of different lepidopteran borers and foliage feeders in India (Saimandir and Gopal 2012; Hasan et al. 2016), this chemical is still efficacious for the sustainable management of *B. tabaci* (Bajya et al. 2015; Jha and Kumar 2017). Therefore, a unique mechanism for the development of indoxacarb resistance or lower usage of this chemical against sucking pests including *B. tabaci* might be the reason for no resistance.

The effectiveness of the third generation neonicotinoid, dinotefuran, was mainly found against various hemipteran sap suckers such as whitefly, aphid, jassid, leaf and plant hoppers (Pappas et al. 2013; Aly 2014) on different vegetable crops in India (Kodandaram et al. 2013). No previous report of dinotefuran resistance has earlier been documented in whitefly populations from the Indian subcontinent. However, the present data has exhibited a significant rise in the resistance level to dinotefuran in the coeval populations of *B. tabaci* in India in comparison to the previous reports of other neonicotinoid insecticides like imidacloprid and Thiamethoxam (Mahalakshmi et al. 2015). Especially, Nadia population showed a moderate level of resistance to dinotefuran (Table 2). A relevant

information should be kept in mind that this particular district has been identified as an endemic zone of tomato leaf curl virus (TLCV) (Saha et al. 2014), yellow vein mosaic (YVMV) disease (Kumar et al. 2017) and enation leaf curl virus (ELCV) of okra (Yadav et al. 2018) transmitted by *B. tabaci*. It has been hypothesized that frequent prevalence of these diseases and consequently large-scale application of neonicotinoid insecticides, including dinotefuran for managing the insect-vector, could have resulted intense selection pressure for the development of resistance in this *B. tabaci* population.

In the case of tolfenpyrad, all the populations of *B. tabaci* showed low levels of resistance except Coochbehar population. The low RR values to tolfenpyrad highlighted in the present findings may be attributable to newly commercialization and minimal application of this insecticide in India (Kodandaram et al. 2016). Additionally, it has also been hypothesized that the high susceptibility levels of several field populations of *B. tabaci* against tolfenpyrad may be a positive consequence of the use of different ready-mix insecticide formulations.

Considerable resistance to spiromesifen noticed in the present investigation could be attributed to the extended utilization of this molecule in the vegetable

ecosystems of West Bengal. Since the introduction of commercial formulation in the Indian market, spiromesifen has extensively been used by progressive farming communities as an ‘insecto-acaricide’ for control of sap-sucking pests including whitefly and phytophagous mites on vegetables (Raj et al. 2012; Mate et al. 2015). Consequently, the standard dose of spiromesifen 240 SC which had earlier conferred protection against whitefly only at 134.2 g a.i. ha⁻¹ in 2010 (Ameta et al. 2010), was later reported to provide protection at 150 g a.i. ha⁻¹ in 2013 (Sujayanand et al. 2013) and at 168.7 g a.i. ha⁻¹ in 2016 (Sathyan et al. 2016). Resistance to spiromesifen was widely documented in different populations of *B. tabaci* in several countries of Asia, Europe and America (Guthrie et al. 2003; Prabhaker et al. 2008; Yukselbaba and Gocmen 2016).

The current findings implied that tolerance to medium resistance levels to pyriproxyfen and flonicamid were encountered in tested field strains of *B. tabaci*. Several global studies documented the resistance status in different populations of *B. tabaci* to these two groups of chemicals (Ma et al. 2010; Roditakis et al. 2014). However, a narrow range of information is there on the resistance status of *B. tabaci* populations of India. This study fairly provided the insecticide resistance and susceptibility levels of Indian populations of whitefly against some traditional OP and new generation novel compounds particularly from vegetable ecosystems. Due to the lack of specific resistance management schemes for *B. tabaci* in India, irrational usage of insecticides is a common practice among the farmers, which could be the most possible reason for the insecticide resistance development (Banerjee et al. 2014). Since India is the second largest producer of vegetables in the world next only to China (Neeraj et al. 2017), vegetable producers make very strong endeavour in order to enhance the production. Insect pest is one of the major constrains in increasing the marketable yield of vegetables in India (Singh et al. 2009) where, *B. tabaci* has caused more than 54% economic damage to tomato, okra, chilli, brinjal, and potato for last two decades (Rai et al. 2014). The vegetable growers’ response was to apply an enormous amount of chemical pesticides to mitigate the insect pests as most of the plant protection recommendations in vegetable crops so far indicated the calendar based application of insecticides (Rai et al. 2014). Moreover, previous studies (Jeyanthi and Kombairaju 2005) indicated that the availability of

the insecticides, educational knowledge and socio-economic conditions of the farming community purely determine their decision making options regarding the selection of chemicals, dosages and the time of application to control several insect pests. Erroneous recommended dose by the pesticide dealers along with the injudicious use of non-selective insecticides and use of unscientific tank mixtures could also be the factual reasons for the exacerbation of the field resistance problems to different groups of chemical insecticide (Sardana et al. 2017).

Third world countries, like India, have issues with the insect pest resistance problem in different fields of agricultural, livestock, medical and household pest management due to injudicious use of chemical compounds (Kranthi et al. 2002; Raghavendra et al. 2017). Rotational scheme of selective insecticides from different chemical classes and use of ready-mix molecules having distinct modes of action could be conducive for the management of resistance problem in *B. tabaci* (Memmi 2010). Since the results of the present study showed a varying degree of resistance to OP, neonicotinoid, METI, tetronic acid derivative, insect growth regulator, and carboxamide insecticides in the *B. tabaci* populations of West Bengal, we strengthen the need for constant investigation on insecticide resistance scenario of different populations across other vegetable growing belts of India. Therefore, a comprehensive IPM programme and proper insecticide resistance management tactics, including resistance-breeding strategies, field sanitation, mechanical control, biological augmentation, and incorporation of novel chemicals will be the ideal option for sustainable management of *B. tabaci* on different vegetable crops in India.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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