

## RESEARCH ARTICLE

# Evaluation of insecticide resistance and underlying resistance mechanisms in selected whitefly populations in Sri Lanka

JP Marasinghe<sup>1</sup> and SHPP Karunaratne<sup>2\*</sup>

<sup>1</sup> Horticulture Research and Development Institute, Department of Agriculture, Peradeniya.

<sup>2</sup> Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya.

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**Abstract:** Whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* cause high economic damage to commercial crops in Sri Lanka. Insecticide resistance was tested in *B. tabaci* collected from Kandy, Anuradhapura, Badulla, Matale and Hambantota districts and *T. vaporariorum* collected from Puttlam District against six insecticides using leaf dip bioassays. LC<sub>50</sub> values and percentage mortalities for the recommended dosages were determined by log-probit mortality curves. Metabolic resistance caused by increased activities of esterases, glutathione S-transferases and cytochrome P<sub>450</sub>-monooxygenases, and the insensitive target site resistance caused by acetylcholinesterases were evaluated by biochemical assays. Overexpression of CYP6CM1 monooxygenase protein in *B. tabaci* was tested using a lateral flow assay kit. The highest and the lowest resistance factors (RFs) were obtained for etofenprox (1311) and profenofos (4), respectively. Resistance percentages of whitefly populations to the recommended dosages were 37–83% for thiamethoxam, 50–83% for acetamiprid, 71% for imidacloprid, 2–36% for profenofos, 15–53% for carbosulfan and 68–89% for etofenprox. Anuradhapura, Badulla and Matale populations had elevated esterases, all populations had elevated GST activities and Badulla population had elevated monooxygenase activities. Populations were homozygous resistant or heterozygous for altered acetylcholinesterase mechanism. High esterase activities were parallel to high neonicotinoid resistance. CYP6CM1 overexpression was linked to thiamethoxam resistance than to acetamiprid resistance. Both the enhanced esterases and altered target sites were associated with high carbosulfan resistance. This is the first report on the insecticide resistance mechanisms of whiteflies in Sri Lanka. The outcome of the present study can be effectively utilized for a proper whitefly control programme in Sri Lanka, avoiding indiscriminate insecticide usage.

**Keywords:** CYP6CM1, insecticide resistance, insensitive acetylcholinesterases, metabolic resistance, whiteflies.

## INTRODUCTION

Whiteflies in vegetables cause extensive economic damage. The silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Insecta: Hemiptera: Aleyrodidae) is a injurious pest in agriculture (Bedford *et al.*, 1994) and listed as one of the 100 worst invasive species in the world (Global Invasive Species Database, <http://www.issg.org/database>). The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Insecta: Hemiptera: Aleyrodidae), although not included in the invasive species list, is considered as another injurious whitefly present globally (Brødsgaard & Albajes, 1999; Karatolos, 2011). In Sri Lanka, both *B. tabaci* and *T. vaporariorum* cause high economic damage to commercial crops. The number of host plant species of *B. tabaci* has been estimated to be more than 600 plant species (Basu, 1995) whereas *T. vaporariorum* is also known as a highly polyphagous species having a wide host plant range (Brødsgaard & Albajes, 1999). However, the host range of these pests could be even higher at present with its wider global distribution. Out of all the whiteflies, *B. tabaci* has become prominent because of its ability to transmit a large number of begomoviruses causing complete destruction of crops (Brown & Bird, 1992). *Bemisia tabaci* is now considered as a cryptic species complex, rather than different biotypes, due to

\* Corresponding author (shppk@pdn.ac.lk;  <https://orcid.org/0000-0002-2537-0548>)



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high genetic variations found among populations (De Barro *et al.*, 2011). Both the physiological traits and genetic variations are crucial in determining its species status, and Surendran *et al.* (2019) had identified that the predominant species type of *B. tabaci* in Sri Lanka is Asia-I.

Naturally, the control of whiteflies is difficult due to their high reproductive rate, wide host range and habit of lodging underneath leaves. On top of these natural features, the acquired resistance to insecticides by them is the most challenging at present. Insecticide resistance of whiteflies is a global issue. *Bemisia tabaci* is found to be resistant to more than 40 active ingredients used worldwide (Arthropod Pesticide Resistance Database, [www.pesticideresistance.org](http://www.pesticideresistance.org)). In brief, insecticide resistance is a heritable trait acquired by a population to withstand the toxicity of an insecticide repeatedly at its recommended dosage (Insecticide resistance action committee, <https://irac-online.org/about/resistance>). High resistance of *T. vaporariorum* to many insecticide groups including pyrethroids, organophosphates and neonicotinoids has been documented elsewhere (Cahill *et al.*, 1995; Gorman *et al.*, 2002; Nauen & Denholm, 2005; Karatolos, 2011). The two major mechanisms of insecticide resistance in insects are; increased metabolism or detoxification by enzymes (mainly through esterases, mono-oxygenases and glutathione-S-transferases), and target site insensitivity by mutations (i.e. altered sodium channels for pyrethroids, altered acetylcholinesterase for organophosphates and carbamates, and altered nicotinic acetylcholine receptors for neonicotinoids) (Karunaratne, 1998; WHO, 1998).

Previously, we reported high resistance to insecticides in three whitefly populations based on laboratory bioassays raising concerns over the existing dosages recommended for chemical control (Marasinghe *et al.*, 2017). The objective of the present study was to investigate the present status of whitefly resistance to

insecticides in more detail by analysing the whitefly populations collected from six districts of the country against three neonicotinoids, one organophosphate, one carbamate and one pyrethroid. The activities of metabolic enzymes and altered acetylcholinesterase target site were also evaluated to understand the mechanisms underlying the insecticide resistance of this injurious pest category.

## METHODOLOGY

### Whiteflies

*Bemisia tabaci* populations were collected from several intensive agricultural localities from five districts belonging to four agroecological zones of Sri Lanka, i.e. Gannoruwa in Kandy, Huruluwewa in Anuradhapura, Bandarawela in Badulla, Matale in Matale, Suriyawewa in Hambantota and Kalpitiya in Puttalam. All collections were made during the 2016–2018 period and the details are given in Table 1. *Bemisia tabaci* collected from Puttalam were not enough for experiments. *Trialeurodes vaporariorum* dominated the whitefly collections from Puttalam district, and those samples were used for experiments for comparison.

Whiteflies were transported to the entomology laboratory of the Horticulture Research and Development Institute, Gannoruwa in cool boxes within few hours of collection. They were reared separately on young brinjal, tomato or cucumber plants in fine-mesh aluminium cages of 80 × 40 × 60 cm<sup>3</sup> under room temperature up to 5–6 generations.

Species identification of both species were re-confirmed using adult/nymphal phenotypic features and the egg-laying pattern, i.e. *B. tabaci* adults are yellowish in colour and comparatively smaller with wings held vertical and parallel to body axis at rest. The fourth instar nymph (pupa) is almost circular in shape without

**Table 1:** Whitefly collection localities and associated host plants

Locality / district	Collected species	Agricultural zone	Host plants
Gannoruwa/Kandy	<i>Bemisia tabaci</i>	Mid country wet	Tomato, Brinjal, Cucurbits
Huruluwewa/Anuradhapura	<i>B. tabaci</i>	Low country dry	Brinjal, Cucurbits
Bandarawela/Badulla	<i>B. tabaci</i>	Up country intermediate	Brinjal, Bean, Tomato
Matale/Matale	<i>B. tabaci</i>	Mid country intermediate	Brinjal
Suriyawewa/Hambantota	<i>B. tabaci</i>	Low country dry	Brinjal
Kalpitiya/Puttalam	<i>Trialeurodes vaporariorum</i>	Low country dry	Brinjal, Cucurbits

long threads. Eggs are laid in a scattered manner on the underside of host leaf. *Trialeurodes vaporariorum* adults hold their wings in a flattened and overlapped position at rest. The fourth instar nymph is oval/ elliptical in shape with long erect threads. Eggs are laid in a circular pattern on the underside of the host leaf. Slide-mounted pupae were also examined for characteristic morphological features to differentiate the two species (Hodges & Evans, 2005). A set of specimens from each population was stored at -20 °C for biochemical assays.

### Chemicals and equipment

Chemicals were purchased from Sigma Chemicals, UK unless otherwise stated. Formulated insecticides of thiamethoxam (Actara from Deccan Fine Chemicals-India Pvt Ltd.), acetamiprid (Mospilan from Nippon Soda Company Ltd.), imidacloprid (Admire from Bayer A.G. Crop Science, Germany), profenofos (Calcron from Coromandel International Ltd., India), carbosulfan (Marshal from FMC Corporation, USA), etofenprox (Trebos from Mitsui Chemicals Agro Inc., Japan) were purchased from the agrochemical sales outlets in Kandy, Sri Lanka. UVmaxELx800TM absorbance microplate reader was from Molecular devices, Bio-Tek, USA. Protein assay kit was purchased from BIO-RAD, UK. Whitefly test kit for the detection of CNI (neonicotinoid) resistance was a gift from Bayer CropScience AG, Germany.

### Bioassays

Bioassays were carried out with three neonicotinoids (thiamethoxam 25% WG, acetamiprid 20% SP and imidacloprid 70% WG), an organophosphate (profenofos 500 g/L SC), a carbamate (carbosulfan 200 g/L SC) and a pyrethroid (etofenprox 100 g/L EC) using leaf dip method as previously described (IRAC, 2009; Marasinghe *et al.*, 2017). In brief, squarecut brinjal leaves ( $3 \times 3$  cm<sup>2</sup>) were immersed in aqueous solutions of insecticides for 5 s and then air dried. Thirty whiteflies were exposed to each insecticide-treated squarecut leaf held in ventilated transparent containers. The containers were kept under normal day/night conditions at room temperature for 24 h. Seven to eight concentrations of each insecticide (five replicates with 150 whiteflies per concentration) that gave mortalities between 0 to 100% were used for testing each population, i.e. thiamethoxam 10, 100, 250, 1000, 2500, 5000, 1000 ppm; acetamiprid 10, 100, 200, 500, 1000, 2000, 5000, 10000 ppm; carbosulfan 10, 100, 300, 500, 1000, 2000, 5000, 10000 ppm; imidacloprid 10, 100, 200, 500, 1000, 2000, 5000, 10000 ppm; etofenprox 10, 100, 500, 1000, 2000, 10000, 15000 ppm and profenofos

10, 50, 100, 250, 500, 800, 1000, 2000 ppm. Distilled water without insecticides was used as the control. Final mortality was assessed after the 24 h exposure period.

### Biochemical assays

Adult whiteflies in batches of 20 individuals totalling up to a minimum of 200 individuals from each population were subjected to esterase, glutathione S-transferase (GST), monooxygenase, protein and acetylcholinesterase assays using a kinetic microplate reader (Bio-Tek, USA). Mass homogenates of the batches of 20 whiteflies were prepared separately in 150 µL of distilled water. Crude homogenate was directly used for acetylcholine esterase assay. The supernatant after centrifugation for 2 min at 10,000 g was used for esterase, GST, monooxygenase and protein assays. All the biochemical assays were carried out in microtiter plates. All the tests were replicated and controls were run with distilled water instead of the crude homogenate or supernatant. The assaying methods described in the Field and Laboratory Manual of the World Health Organization (WHO, 1998) were adopted with slight modifications as previously described for agricultural insect pests (Damayanthi & Karunaratne, 2005; Karunaratne & Weerakoon, 2007).

#### Esterase assay

Ten microlitres of homogenate was mixed with 200 µL of 1 mM p-nitrophenyl acetate (pNPA) working solution in 50 mM sodium phosphate buffer (pH 7.4). At 405 nm, the absorbance of the reaction was measured kinetically for 2 min and converted to moles using an extinction coefficient of 6.53 mM<sup>-1</sup> (corrected for a path length of 0.6 cm).

#### Glutathione S-transferase (GST) assay

Ten microlitres of homogenate was mixed with 200 µL of substrate solution prepared using 10.5 mM reduced glutathione (95 parts GSH in 100 mM phosphate buffer) and 63 mM 1-chloro-2,4-dinitrobenzene (CDNB) (5 parts of CDNB in methanol). The reaction rate was measured at 340 nm for 5 min. The absorbance was converted to moles using an extinction coefficient of 5.76 mM<sup>-1</sup> (corrected for a path length of 0.6 cm).

#### Monoxygenase assay

Twenty microlitres of homogenate was mixed with 80 µL potassium phosphate buffer (pH 7.2) + 200 µL 6.3 mM tetramethyl benzidine (TMBZ) working solution (0.01 g TMBZ dissolved in 5 mL methanol and then in 15 mL

of sodium acetate buffer at pH 5.0) + 25  $\mu$ L of 3 % H<sub>2</sub>O<sub>2</sub> solution in a microtitre plate well. The mixture was left for 2 h for incubation under room temperature and the absorbance was read as an end point assay at 630 nm. The amount of monoxygenase was expressed as equivalent units of cytochrome P<sup>450</sup> (Brogdon *et al.*, 1997).

### Protein assay

Protein assays were conducted to obtain protein concentrations of the homogenates to determine specific activities of the enzymes. BIO-RAD protein determination kit, with bovine serum albumin as the standard protein was used. Ten microlitres of the homogenate was mixed with 300  $\mu$ L of BIO-RAD working solution (prepared according to the manufacturer's instructions) and after a 5 min incubation period at room temperature, the reaction was read at 570 nm as an end point assay.

### Acetylcholinesterase (AChE) assay

Two  $\times$  25  $\mu$ L of crude homogenate were placed in two consecutive wells of a microtiter plate, each containing 145  $\mu$ L of 1 % Triton buffer in 100 mM phosphate buffer at pH 7.8 with 10  $\mu$ L of 10 mM dithiobis 2-nitrobenzoic acid (0.0396 g of DTNB in 100 mM phosphate buffer at pH 7.0). An aliquote of 25  $\mu$ L of 10 mM acetylthiocholine iodide (ASCHI) (0.0578 g ASCHI in 20 mL distilled water) was added to one of the wells while 25  $\mu$ L of 10 mM ASCHI with propoxur (10 mL of 10 mM ASCHI + 20  $\mu$ L of 0.1 M propoxur in acetone) was added to the other. Acetylcholinesterase (AChE) activity was measured at 405 nm for 5 min. Results were expressed as the percentage remaining activity in the inhibited fraction compared with the control (uninhibited) activity.

### Detection of the CYP6CM1 protein in *B. tabaci*

A field portable ELISA based lateral flow assay designed to detect monoxygenase CYP6CM1 protein was used following the manufacture's protocol (Bayer CropScience). Five to ten whiteflies from each population were homogenised in conjugated polyclonal antibodies in a homogenization vial. After a 2–3 min incubation, streptavidin-gold solution was added to the vial and gently inverted 3–4 times. The mixture was then poured into the sample cavity of the test strip and observed for 5 min. Respective distinct lines at C and T points indicated the expression levels of the CYP6CM1 enzyme by the control and the sample, respectively.

### Data analysis

Bioassay data were used only when the control mortality was less than 20 %. Mortalities were adjusted with control mortalities using Abbott's formula (Abbott, 1987). Adjusted mortalities were Probit transformed using Sigma Plot (version 10) software and plotted against the log values of the insecticide concentrations. The LC<sub>50</sub> and the percent mortality for the Department of Agriculture recommended dosages (DOA, 2015) were estimated by regression analysis. Chi square values ( $\chi^2$ ) were calculated by evaluating the goodness-of-fit of the linear regression to log-probit transformed data. The software provides the slope and the 95 % confidence limits for each mortality line. Resistance factors (RF) were calculated using LC<sub>50</sub> values reported for susceptible populations of the same species elsewhere and used to evaluate resistance levels (Cahill *et al.*, 1995; Karatolos, 2011; Vassiliou, 2011).

## RESULTS AND DISCUSSION

A total of 48,989 whiteflies from *B. tabaci* and *T. vaporariorum* were subjected to insecticide bioassays. Probit analysis separately done for the bioassay results obtained for the six populations with six insecticides are shown in Table 2 together with RFs. For thiamethoxam, LC<sub>50</sub> values of both *T. vaporariorum* and *B. tabaci* were in a similar range. However, the LC<sub>50</sub> values estimated for profenofos, carbosulfan and etofenprox for *T. vaporariorum* were higher than those obtained for *B. tabaci*. Among *B. tabaci* populations, the highest and the lowest LC<sub>50</sub> values for thiamethoxam were respectively detected from Badulla and Matale populations. In contrast, for acetamiprid, the highest and the lowest were respectively from Matale and Badulla populations. The highest and the lowest values for profenofos were from Badulla and Kandy populations, respectively. Matale and Hambantota populations showed the highest and lowest LC<sub>50</sub> values, respectively, for carbosulfan, whereas Kandy and Matale populations gave the highest and the lowest LC<sub>50</sub> values for etofenprox, respectively.

Chi square value ( $\chi^2$ ) gives the goodness-of-fit of a dose-mortality line and significant  $\chi^2$  estimates indicate deviation from linear response showing a heterogeneity of the population. For etofenprox, all the populations except Matale exhibited a heterogeneous response. For the other insecticides tested, most of the whitefly populations showed a homogeneous response (Table 2). For carbosulfan, RFs could not be calculated

since no LC<sub>50</sub>s had been reported for susceptible whitefly populations. Similarly, no LC<sub>50</sub>s were found for a susceptible *T. vaporariorum* for acetamiprid, profenofos

and etofenprox. RFs were very high for etofenprox (281–1311) and the lowest RFs were estimated for profenofos (4–71) (Table 2).

**Table 2:** Log-dose probit mortality results obtained for bioassays conducted with whitefly populations of *B. tabaci* and *T. vaporariorum* against thiamethoxam, acetamiprid, imidacloprid, profenofos, carbosulfan and etofenprox. *Trialeurodes vaporariorum* was tested only from Puttalam and *B. tabaci* was tested only from all the other districts.

Insecticide	Population	N	LC <sub>50</sub> ppm	95% CI	Slope	χ <sup>2</sup>	RF
Thiamethoxam	Kandy	1710	579	282–1123	0.61	8.9	<sup>1</sup> 127
	Anuradhapura	1365	294	109–447	0.56	5.7	<sup>1</sup> 64
	Badulla	1093	1090	578–2024	0.86	12.7	<sup>1</sup> 239
	Matale	1472	25	<10–70	0.68	5.0	<sup>1</sup> 5
	Hambantota	1751	223	53–605	1.01	30.4****	<sup>1</sup> 49
	Puttalam	1478	398	49–563	0.70	14.5*	<sup>2</sup> 21
Acetamiprid	Kandy	1799	230	60–605	0.87	13.3	<sup>1</sup> 23
	Anuradhapura	1814	1259	622–3703	0.44	3.2	<sup>1</sup> 125
	Badulla	1739	208	121–321	1.00	8.5	<sup>1</sup> 21
	Matale	1633	1753	930–4943	1.0	9.7	<sup>1</sup> 172
	Hambantota	2051	985	278–5231	0.76	43.1****	<sup>1</sup> 98
	Puttalam	NT	NT	NT	NT	NT	NC
Imidacloprid	Puttalam	1540	806	335–2240	1.35	44.9****	<sup>2</sup> 47
Profenofos	Kandy	1893	27	<10–61	1.07	12	<sup>3</sup> 4
	Anuradhapura	1870	121	55–230	1.21	5.6	<sup>3</sup> 20
	Badulla	1696	434	217–1562	1.51	13.5*	<sup>3</sup> 71
	Matale	1733	88	33–183	1.55	35.7****	<sup>3</sup> 14
	Hambantota	1432	58	16–126	1.69	11.7*	<sup>3</sup> 10
	Puttalam	1754	509	355–817	1.89	5.2	NC
Carbosulfan	Kandy	1644	71	45–112	0.7	0.9	NC
	Anuradhapura	1775	321	211–434	0.97	1.4	NC
	Badulla	1513	237	17–531	0.81	5.4	NC
	Matale	1400	659	240–3073	0.80	17.1**	NC
	Hambantota	953	12	<10–25	0.59	0.2	NC
	Puttalam	1761	760	371–1778	0.79	12.5	NC
Etofenprox	Kandy	1988	1704	795–3225	1.02	12.6*	<sup>3</sup> 1311
	Anuradhapura	1890	587	274–1188	0.93	15.4*	<sup>3</sup> 452
	Badulla	1567	1314	398–9306	0.82	31.1****	<sup>3</sup> 1011
	Matale	1558	365	240–2699	1.30	10.5	<sup>3</sup> 281
	Hambantota	1861	371	128–2240	1.14	35.0****	<sup>3</sup> 285
	Puttalam	1256	2512	1223–7830	0.98	18.5**	NC

N - number tested; CI - confidence intervals; χ<sup>2</sup> - chi square value evaluating the goodness-of-fit of the linear regression to log-probit transformed data; RF - resistance factor; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001; <sup>1</sup> susceptible data used from Vassiliou *et al.* (2011), <sup>2</sup> from Karatolos (2011), <sup>3</sup> from Cahill *et al.* (1995); NT- not tested; NC- not calculated due to unavailability of susceptible data.

Mortality of whitefly populations to the insecticide dosages recommended by the Department of Agriculture (DOA) of Sri Lanka (DOA, 2015) as determined by using the mortality curves are presented in Table 3. Percentage mortalities of populations varied from 11–98 %. Whiteflies from Kandy, Matale and Hambantota gave more than 90 % mortalities for the DOA recommended profenofos dosage, and for other three districts 3–9 fold dose increment was required to achieve a 90 % mortality. All the other tested insecticides required 3– >80-fold increase of the recommended dosage to achieve

90 % mortality in these populations. *Trialeurodes vaporariorum* gave a similar mortality as for *B. tabaci* to DOA recommended dosage except for etofenprox, which showed a lower efficacy for *T. vaporariorum* (Table 3). Imidacloprid could be tested only against *T. vaporariorum* and 29 % mortality obtained for the DOA recommended dosage indicates the high imidacloprid resistance in whiteflies. In general, high resistance to all the tested insecticides is evident in whitefly populations except for profenofos in a few populations (Table 3).

**Table 3:** Percentage mortalities estimated for *B. Tabaci* from Kandy, Anuradhapura, Badulla, Matale, Hambantota and *T. vaporariorum* from Puttalam, to the Department of Agriculture (DOA) recommended dosages of six insecticides

Insecticide	Mode of action*	DOA recommended dosage per 16 L tank	Percentage mortality					
			Kandy	Anuradhapura	Badulla	Matale	Hambantota	Puttalam
Thiamethoxam 25 WG	4A	8 g	30 %	43 %	17 %	63 %	32 %	36 %
Acetamiprid 20 SP	4A	16 g	50 %	39 %	49 %	17 %	29 %	NT
Imidacloprid 20 SL	4A	16 ml	NT	NT	NT	NT	NT	29 %
Profenofos 50 EC	1B	32 mL	95 %	73 %	64 %	95 %	98 %	68 %
Carbosulfan 20 EC	1A	48 mL	75 %	60 %	72 %	49 %	85 %	47 %
Etofenprox 10 EC	3A	24 mL	13 %	30 %	20 %	31 %	32 %	11 %

\*as described by the Insecticide Resistance Action Committee: 4A = nicotinic acetylcholinreceptor competitive modulators-neonicotinoids; 1B = acetylcholinesterase inhibitors-organophosphates; 1A = acetylcholinesterase inhibitors-carbamates; 3A = sodium channel modulators-pyrethroids; NT - not tested

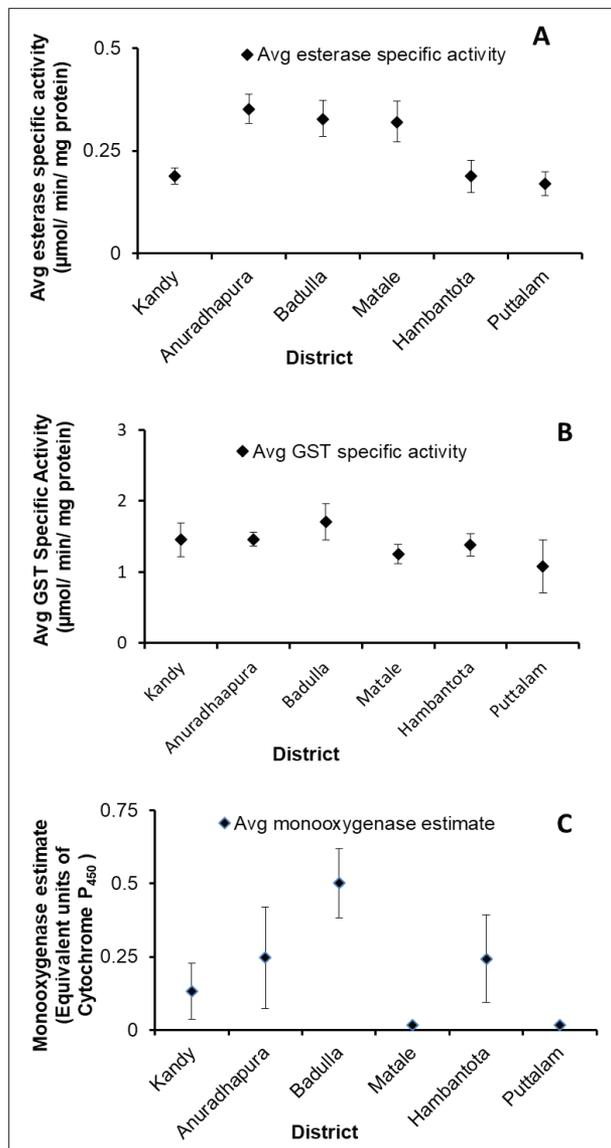
Both the species, *B. tabaci* and *T. vaporariorum*, had similar activity levels in their insecticide metabolizing enzymes (Figure 1). In *B. tabaci*, specific activities of esterases and GSTs were in the ranges of 0.18–0.35 and 1.25–1.71  $\mu\text{mol}/\text{min}/\text{mg}$ , respectively. For *T. vaporariorum*, the respective values were 0.17 and 1.08  $\mu\text{mol}/\text{min}/\text{mg}$ . Monooxygenase amounts in *B. tabaci* were in the range 0.02–0.50 equivalent units of cytochrome P<sub>450</sub> and that of *T. vaporariorum* was 0.02. Vassiliou *et al.* (2011) have reported esterase and GST activities for a susceptible *B. tabaci* population. However, the esterase specific activities cannot be used to compare with the present results since the substrate used for the assay was different to that of the present study. Using the same methodology of the present study, a GST specific activity of 0.2  $\mu\text{mol}/\text{mg}/\text{min}$  has been obtained for their susceptible population, which is about 5–8 times lower than the GST activities detected in our whitefly populations (Vassiliou *et al.*, 2011). No

other enzyme activity data from susceptible strains are available for whiteflies or for any other insect pest of agriculture for comparison. However, for anopheline mosquitoes discriminating values are available in the literature for the same assays to determine the enhanced activities; above 0.25  $\mu\text{mol}/\text{mg}/\text{min}$  for enhanced esterase activity, above 0.40  $\mu\text{mol}/\text{mg}/\text{min}$  for enhanced GST activity, and 0.35 equivalent units of cytochrome P<sub>450</sub> for enhanced monooxygenase amounts (Perera *et al.*, 2008). According to this classification, Anuradhapura, Badulla and Matale populations have elevated esterases, all six whitefly populations have elevated GST activities and Badulla population has elevated monooxygenase activities (Figure 1). In aphids and mosquitoes, elevation of esterases is a powerful mechanism against organophosphates and carbamates, which have molecular structures rich with esterase bonds (Karunaratne *et al.*, 2018). Thiamethoxam resistant *B. tabaci* has been synergized and reversed using the esterase inhibitor

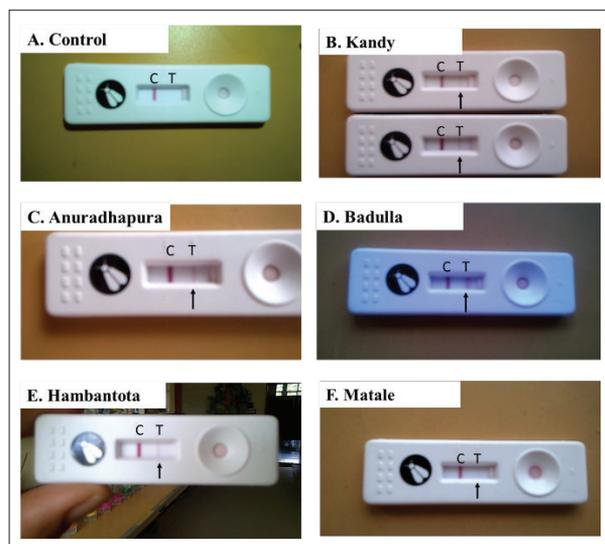
S,S,S-tributyl phosphorotrithioate (DEF) showing its importance in thiamethoxam resistance (Kandil *et al.*, 2008). The present study also reveals high esterase activity levels parallel to high neonicotinoid resistance,

indicating the contribution of esterases to neonicotinoid resistance. Although GSTs are primarily important in dechlorination of organochlorines, elevated levels of GST activity have been reported in organophosphate, organochlorine and pyrethroid resistant mosquitoes (Che-Mendoza *et al.*, 2008). High GST levels observed in the studied whitefly populations may have a wider general role giving resistance to all insecticide groups.

Results of the ELISA based lateral flow assay for CYP6CM1 monooxygenase protein also showed a clear overexpression of CYP6CM1 monooxygenase protein in Badulla *B. tabaci* population (Figure 2). The CYP6CM1 expression levels of lateral flow assay nicely matched with the biochemical assay data indicating that CYP6CM1, a typical microsomal P<sub>450</sub> member of CYP6 family, is a major candidate of enhanced monooxygenases of the tested whitefly populations. Imidacloprid resistance in both the B and Q biotypes of *B. tabaci* has been tightly related to the up-regulation of the cytochrome P450-dependent monooxygenase gene CYP6CM1 (Karunker *et al.*, 2008). Overexpression of CYP6CM1 gene has also been detected in thiamethoxam resistant *B. tabaci* too (Yang *et al.*, 2013). Imidacloprid resistance in *B. tabaci* has not been tested in our study and thus, the association with imidacloprid resistance could not be traced.



**Figure 1:** Insecticide metabolizing enzyme activities of whitefly populations. *Trialeurodes vaporariorum* was tested only from Puttalam and *B. tabaci* was tested only from all the other districts. A - esterase specific activity; B - glutathione-S-transferase (GST) specific activity; C - monooxygenase amounts in equivalent units of cytochrome P<sub>450</sub>. A minimum of 100 insects (five batches, 20 individuals per batch) were tested per assay per site. Error bars indicate the standard errors.

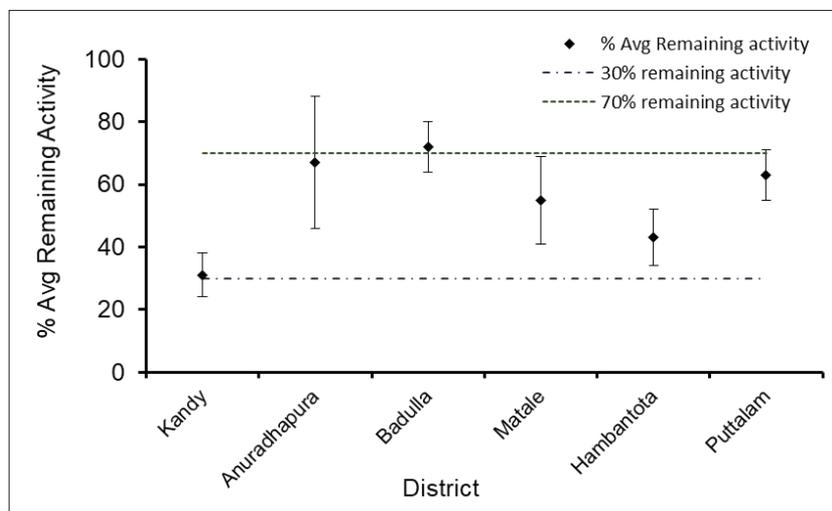


**Figure 2:** Monoxygenase CYP6CM1 expression level in *B. tabaci* populations from Kandy (B), Anuradhapura (C), Badulla (D), Hambantota (E) and Matale (F) as detected by the diagnostic kits. Control (A) does not show a 2<sup>nd</sup> line; C - control line; T - CYP6CM1 monooxygenase expression line

According to our bioassay results from Badulla and Matale populations, it can be concluded that CYP6CM1 is more important in thiamethoxam resistance than in acetamiprid resistance.

Percentage remaining activities of propoxur inhibited target site AChE are shown in Figure 3. The same AChE assay is used by WHO to categorize mosquito populations as susceptible homozygous (SS)

(< 30% remaining activity), heterozygous (RS) (30–70% remaining activity) and resistant homozygous (RR) (> 70% remaining activity) for altered or insensitive AChE target site mechanism (WHO, 2016). According to these guidelines, Badulla *B. tabaci* population was homozygous resistant with > 70% remaining activity while all other populations including *T. vaporariorum* population were heterozygous for altered AChE mechanism with a remaining activity of 30–70% (Figure 3).



**Figure 3:** Percentage remaining activities of propoxur inhibited acetylcholinesterases of *B. tabaci* except for Puttalam where *T. vaporariorum* was tested. A minimum of 100 insects (five batches, 20 individuals per batch) were tested per assay per site. Two dotted lines show the WHO recommended discriminating remaining activities for mosquitoes (< 30% susceptible; 30–70% heterozygous resistance; > 70% homozygous resistance) (WHO, 2016). Error bars indicate the standard errors.

High organophosphate and carbamate resistance is expected from the altered AChE target sites and elevated esterases activities (Karunaratne *et al.*, 2018). Acetylcholinesterase insensitivity has been previously reported in OP and/or carbamate resistant whiteflies (Dittrich & Ernst, 1990; Byrne & Devonshire, 1991). Therefore, the high profenofos and carbosulfan resistance detected in Badulla and Matale districts for *B. tabaci* populations must be due to these two mechanisms.

Whitefly control has become a serious problem in agriculture due to reduced efficacy of recommended insecticides. Severity of the problem has intensified with the economical importance of *B. tabaci* being a begomovirus vector of many crops. A large number of insecticides belonging to different chemical groups have

been recommended for whitefly control in Sri Lanka (MOAAL, 1997; DOA, 2009). Organophosphates and carbamates have a long history of use for more than 4 decades. Pyrethroids were introduced in the 1990s and neonicotinoids in early years of this century for whitefly control (DOA, 2009). The popularity of neonicotinoids among farmers increased not only due to their high efficacy at the introductory phase, but also due to the wide crop range applicability and the long residual activity. The present study evaluated the efficacy of representative samples of these insecticide groups against *B. tabaci* and *T. vaporariorum* using laboratory bioassays with the leaf dip method, which resembles the foliar application method practiced in the field. The percentage mortalities estimated using probit regression lines revealed high resistance of whiteflies to all the tested insecticides,

except for profenofos. Since repeated use of less effective insecticides would further increase resistance generation with environment pollution, taking different approaches to control whiteflies at this stage is necessary. The underlying mechanisms of insecticide resistance in Sri Lankan whitefly populations were evaluated for the first time using biochemical assays. It was evident that both the metabolic resistance and insensitive target site resistance mechanisms have been developed to varying degrees in Sri Lankan whitefly populations. In *T. vaporariorum* also the resistance levels and resistance mechanisms are found to be comparable to those of *B. tabaci*.

Etofenprox is a non-ester pyrethroid having a mode of action similar to other pyrethroids, i.e. by interacting with voltage gated sodium channels (VGSC) of insect neurons. Cross resistance studies have shown no effect of enhanced metabolic enzyme activities on etofenprox toxicity in mosquitoes possibly due to the relatively simple structure of the etofenprox molecule compared to most of the other pyrethroids (Hemingway, 1995). Also, the monooxygenase synergist piperonyl butoxide (PBO) and GST synergist diethyl maleate (DEM) were able to synergize permethrin resistance, but not etofenprox resistance in *Anopheles* mosquitoes (Gross & Bloomquist, 2018). With the high resistance observed in all whitefly populations against etofenprox, and with a least chance for metabolic resistance mechanisms to be operative, there is a high possibility that knock-down type (*kdr*) target site mutations are involved in this resistance. Future studies are required to investigate the presence of *kdr*-type *VGSC* gene mutations in Sri Lankan whiteflies.

## CONCLUSIONS

High percentages of the tested whitefly populations have developed resistance to the DOA recommended dosages of thiamethoxam (37–83%), acetamiprid (50–83%), imidacloprid (71%), carbosulfan (15–53%) and etofenprox (68–89%) insecticides. Least resistance was shown to the organophosphate profenofos (2–36%). Both the increased detoxification by esterases, glutathione S-transferases and monooxygenases, and AChE target site insensitivity contribute in varying degrees to the observed resistance. Involvement of *kdr* type mutations in pyrethroid resistance should be further investigated. This is the first report confirming the resistance status of Sri Lankan whiteflies with underlying resistance mechanisms and the outcome can be utilized effectively for developing a proper whitefly control programme in Sri Lanka avoiding indiscriminate use of insecticides.

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