

## RESEARCH ARTICLE

# Control failure of Sri Lankan whitefly (*Bemisia tabaci* Genn.) is due to high resistance development against recommended insecticides

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**Abstract:** Whitefly, *Bemisia tabaci* Genn. is a major pest of vegetables. Control of the whitefly has become a major problem in agriculture pest control programmes despite the use of several recommended insecticides. No studies have been carried out previously in Sri Lanka to evaluate the insecticide resistance status of *B. tabaci*. The present study focused on identifying the resistance status of *B. tabaci* from three main climatic zones of Sri Lanka. Field collected or laboratory reared (up to 5<sup>th</sup> generation) adults from Gannoruwa (n = 3612), Kahatagasdigiya (n = 3385) and Bandarawela (n = 4146) were exposed to different concentrations of recommended insecticides, *i.e.* two neonicotinoids (thiamethoxam and acetamiprid), an organophosphate (profenofos), a carbamate (carbosulfan) and a pyrethroid (etofenprox), using leaf-dip method. Log-probit mortality curves were obtained for each insecticide, and compared with published data available for susceptible populations and the dosages recommended by the Department of Agriculture (DOA) of Sri Lanka. Resistance factors (RF) ranged from 2.74 – 789.48 and the highest resistance was observed for etofenprox. Moderate to high resistance to neonicotinoids and moderate resistance to carbosulfan was evident. All three populations were largely susceptible for profenofos. The populations exhibited significant heterogeneous responses at least for one insecticide except for carbosulfan. A 6 to 800 fold increase of the DOA recommended dosages are needed to gain 90 % mortality of the populations except for profenofos. An integrated approach to manage insecticide resistance guided with a regular resistance monitoring programmes is urgently required for effective control of the whitefly in horticultural crops in Sri Lanka.

**Keywords:** Agricultural pests, *Bemisia tabaci* G., insecticide resistance, log-probit mortality curves, WFRM.

## INTRODUCTION

Whitefly, *Bemisia tabaci* Genn. (Aleyrodidae: Hemiptera) is a serious pest that is used to be controlled by insecticides in many horticultural crops including beans, brinjal, tomato, cucurbits and ornamental plants in Sri Lanka. Nymphs and adults of *B. tabaci* feed on the lower surface of the leaves and remove plant sap causing loss of vigour of the plants under severe infestations. It secretes honey dew and spreads many viruses causing a significant economic loss to the grower. Due to the wide host range of the pest and prevailing overlapping cropping pattern, a well-planned year round programme is needed to control whiteflies. Neonicotinoids (thiamethoxam, acetamiprid and imidacloprid), organophosphates, carbamates and pyrethroids are used as integral tools for the management of whiteflies all over the world (Caballero *et al.*, 2013). In Sri Lanka, thiamethoxam, acetamiprid, carbosulfan and profenofos have been recommended by the Department of Agriculture (DOA) for the control of whiteflies. Some other insecticides such as etofenprox are also applied by farmers to their crops infested with whiteflies, although these chemicals have not been recommended specifically for whitefly control.

Multiple applications of insecticides create a selection pressure for resistance development in the field populations of whitefly (Palumbo *et al.*, 2001; Caballero *et al.*, 2013) leading to the appearance of different biotypes (Horowitz *et al.*, 2005). *B. tabaci* has been ranked as the

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5<sup>th</sup> top resistant insect species among agricultural insect pests, being resistant to 54 insecticide compounds with 555 recorded cases throughout the world (Sparks & Nauen, 2015). Acetamiprid, thiamethoxam, profenofos, carbosulfan and etofenprox are among the compounds to which reduced susceptibility levels have been detected (Li *et al.*, 2000; Farghaly, 2010; Houndete *et al.*, 2010).

Insecticide resistance in a population is commonly measured by calculating the resistance factor (RF), which is the ratio of  $LC_{50}$  between a test population and a known susceptible population. A RF of 10 or greater has been used as a threshold to declare a resistance development (Mota-Sanchez *et al.*, 2008). Discriminating dosages or resistance diagnostic dosages, which are supposed to kill all the susceptible individuals leaving the resistant ones, have also been used to determine the resistance (Mota-Sanchez *et al.*, 2008). This diagnostic dosage can be used to differentiate the homozygous susceptible (SS) individuals from homozygous resistant (RR) and heterozygous resistant (RS) individuals for a particular insecticide.

Knowledge on the resistance status of a particular insect pest is vital to evaluate the validity of the insecticides in use and their recommended dosages, in order to achieve effective management of the pest. Insecticide resistance management (IRM) is a group of strategies that helps to maintain the susceptibility status of insects to available insecticides, and maintain their effectiveness by preventing or delaying the development of resistant strains (Tejeda *et al.*, 2009). Thus, the data obtained from resistance studies are essential for integrated IRM programmes for an effective pest management with a minimum risk to health and environment.

In this study, we report the resistance status of three whitefly populations collected from the Wet (annual rainfall > 2,500 mm), Dry (annual rainfall < 1,750 mm) and Intermediate (annual rainfall 1,750 – 2,500 mm) Zones of Sri Lanka to thiamethoxam, acetamiprid, etofenprox, carbosulfan and profenofos. Compounds from each of the major classes of insecticides were evaluated in order to find out whether the whitefly populations have developed broader resistance spectrum to different insecticide classes.

## METHODOLOGY

### Whiteflies

Three populations of the whitefly, *B. tabaci* were collected from the agricultural fields of Gannoruwa in

the Wet Zone, Kahatagasdigiliya in the Dry Zone and Bandarawela in the Intermediate Zone of Sri Lanka during mid 2014 to mid 2015. The adult flies were collected to plastic vials using aspirators, from brinjal, tomato and bean crops. They were chilled and transported to the Entomology Laboratory of the Horticulture Research and Development Institute at Gannoruwa and reared in separate fine mesh aluminium cages of 80 × 40 × 60 cm under room temperature. Potted brinjal, tomato and cucumber plants (2 – 8 weeks) placed in the cages served as host plants for the whiteflies.

### Insecticide bioassays

Two neonicotinoid insecticides (thiamethoxam 25 % WG and acetamiprid 20 % SP), an organophosphate (profenofos 500 g/L EC), a carbamate (carbosulfan 200 g/L SC) and a pyrethroid (etofenprox 100 g/L EC) were used for insecticide bioassays. The leaf dip method, as modified by the Insecticide Resistance Action Committee (IRAC, 2009), was adopted as it is closely related to the exposure pattern of the recommended foliar spraying.

Immature brinjal leaves were cut into square shapes of 3 × 3 cm<sup>2</sup> with petioles dipped in different concentrations of commercial formulations of the insecticides for 5 s and left to air dry approximately for 30 mins. Insecticide dilutions were prepared in distilled water and the dosages giving mortality between 0 – 100 % were determined after a preliminary range finding study. The leaves dipped in distilled water alone served as the controls. The insects were exposed to the insecticide treated leaves inside transparent yoghurt cups. Prior to the assay a portion of the centre of the lid (4 cm diameter) and a portion of one side (2 × 4 cm<sup>2</sup>) of the cup were removed to cover with fine net cloth for proper ventilation. The petiole of a leaf was inserted through a small tight hole made at the base of the cup, and the tip of the petiole coming out of the cup was covered with a swab of moistened cotton wool. About 30 male and female adult whiteflies were introduced into each cup through the side opening for each assay. Each treatment was replicated three times and the mortalities were recorded after 24 hrs.

Data were taken only if the control mortalities were less than 20 % and the actual mortalities of test experiments were corrected with control mortalities using the Abbott's formula (Abbott, 1987). Active ingredient concentration present in each dilution was calculated using the information given in the label of each insecticide formulation. Percentage mortalities (probability axis) were plotted against the concentration

of the active ingredient (log axis). Lethal concentration, which kills 50 % of the population ( $LC_{50}$ ) and 90 % of the population ( $LC_{90}$ ), slope of the mortality curve, 95 % confidence interval, chi square at 0.05 probability, and correlation coefficient ( $r^2$ ) were obtained by probit analysis using Sigma Plot (Version 10.0) software.

Table 1 shows the dosages of thiamethoxam, acetamiprid, profenofos and carbosulfan recommended by the Department of Agriculture (DOA), Sri Lanka for whitefly control. Mortalities for these concentrations of the active ingredients were obtained for Gannoruwa, Kahatagasdigiliya and Bandarawela populations using log-probit mortality curves. A DOA recommendation for whitefly control for etofenprox was not available; therefore recommended dosages for the control of sucking insects of the same family were used. Since a susceptible local whitefly population or reported data on a local susceptible whitefly population could not be found,  $LC_{50}$  values available for susceptible populations and discriminating dosages for neonicotinoids and

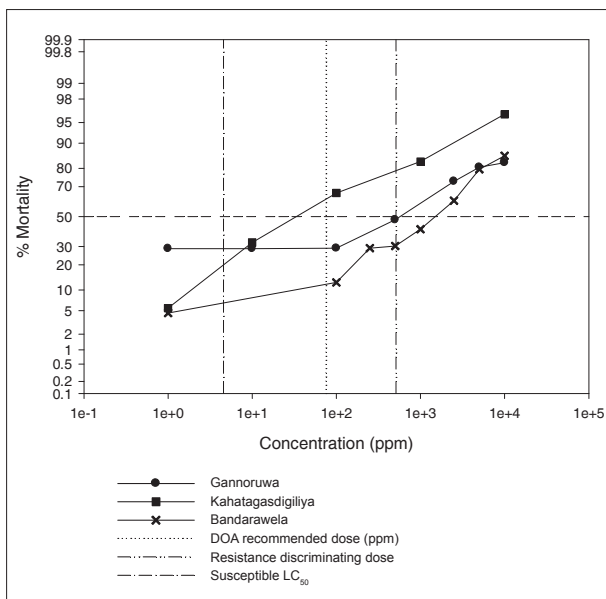
pyrethroid insecticides from elsewhere were used to calculate the resistance factors (RF).

**Table 1:** Insecticides and their dosages recommended by the DOA for the control of whitefly in Sri Lanka

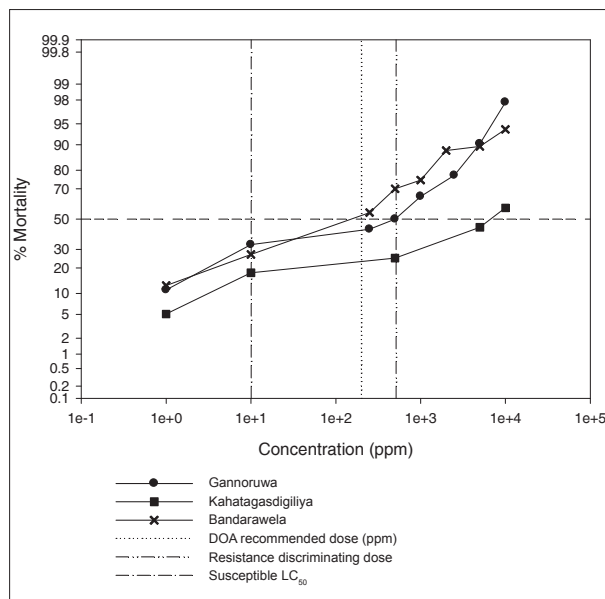
Insecticide	Mode of action <sup>a</sup>	Recommended dosage/ 10 L	A.I.C. <sup>b</sup> (ppm)
Thiamethoxam 25 WG	4A	5 g	125
Acetamiprid 20 SP	4A	10 g	200
Profenofos 50 EC	1B	20 mL	1000
Carbosulfan 20 EC	1A	30 mL	600

<sup>a</sup>As listed by the Insecticide Resistance Action Committee: 4A = nicotinic acetylcholine receptor competitive modulators - neonicotinoids; 1B = acetylcholinesterase inhibitors - organophosphates; 1A = acetylcholinesterase inhibitors - carbamates

<sup>b</sup>A.I.C. = Active ingredient concentration in the DOA recommended dosage



**Figure 1:** Log-probit mortality curves for the whitefly populations from Gannoruwa (n = 647), Kahatagasdigiliya (n = 685) and Bandarawela (n = 701) exposed to thiamethoxam using bioassays by leaf-dip method. Dosage recommended by the DOA for whitefly control in Sri Lanka,  $LC_{50}$  for a susceptible population (Vassiliou, 2011) and a resistance discrimination dosage (Huang *et al.*, 2007) are also shown for comparison.

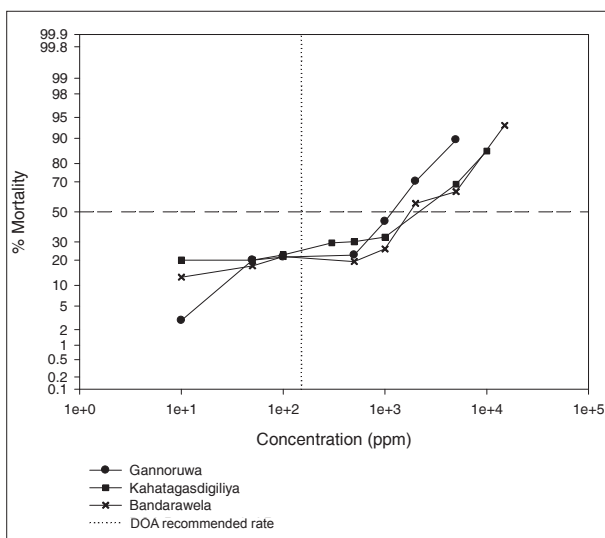


**Figure 2:** Log-probit mortality curves for the whitefly populations from Gannoruwa (n = 1028), Kahatagasdigiliya (n = 364) and Bandarawela (n = 951) exposed to acetamiprid using bioassays by leaf-dip method. Dosage recommended by the DOA for whitefly control in Sri Lanka,  $LC_{50}$  for a susceptible population (Vassiliou, 2011) and a resistance discrimination dosage (Huang *et al.*, 2007) are also shown for comparison.

## RESULTS AND DISCUSSION

Bioassay data with mortalities between 0 – 100 % were successfully obtained for different concentrations of the insecticides for all three populations of whiteflies. Log-concentration probability-mortality curves for all three insect populations for the insecticides tested are shown in Figures 1 – 5. The results of the probit analysis are given in Tables 2 and 3.

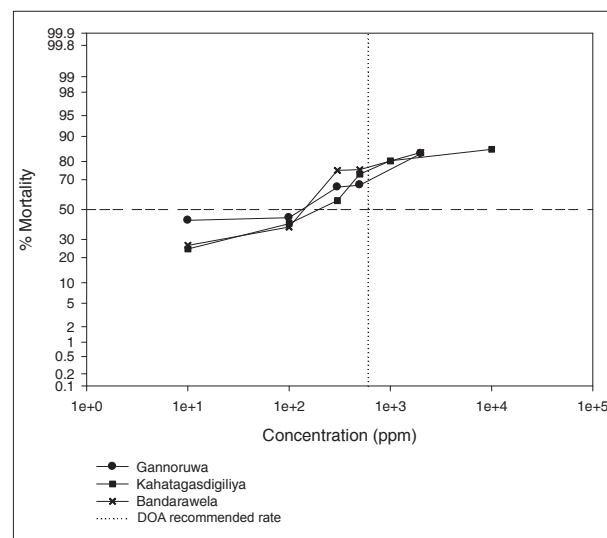
For thiamethoxam  $LC_{50}$  ranged from 53.11 – 1088.43 ppm. If the mortality curve is fitted into a linear curve, the population is considered as a homogeneous population (Karunaratne *et al.*, 2007). Linearity of the curve (response to different concentrations) was rejected for Gannoruwa and Bandarawela populations indicating the heterogeneity of these populations. The highest heterogeneity was observed in Gannoruwa population ( $p < 0.001$ ; Figure 1). All three populations showed a high resistance ( $RF > 10$ ) with the highest shown by the Bandarawela population ( $RF = 238.69$ ). At the dosage recommended by the DOA, thiamethoxam has shown a low efficacy giving a mortality between 26.01 % and 61.59 %. According to the estimations, the recommended dosage has to be increased by  $\times 16$ ,  $\times 566$  and  $\times 800$  folds to obtain 90 % mortality of Kahatagasdigiliya, Bandarawela and Gannoruwa populations, respectively.



**Figure 3:** Log-probit mortality curves for the whitefly populations from Gannoruwa ( $n = 821$ ), Kahatagasdigiliya ( $n = 925$ ) and Bandarawela ( $n = 982$ ) exposed to etofenprox using bioassays by leaf-dip method. Dosage recommended by the DOA for whitefly control in Sri Lanka, is also shown for comparison.

$LC_{50}$  of acetamiprid ranged from 11.55 – 21.80 ppm. Linearity of the mortality response to the concentration was rejected for Gannoruwa population, which exhibited the highest heterogeneity ( $p < 0.01$ ; Figure 2). Gannoruwa and Kahatagasdigiliya populations showed a high resistance ( $RF > 10$ ) with the highest by Kahatagasdigiliya ( $RF = 789.48$ ). Mortality estimated for the DOA recommended dosage of acetamiprid ranged from 26.59 % to 56.59 % showing a low efficacy. To achieve 90 % mortality  $\times 30$ ,  $\times 45$  and  $\times 500$  fold increase of the recommended dosage of the insecticide is needed for Bandarawela, Gannoruwa and Kahatagasdigiliya populations, respectively.

The  $LC_{50}$  range of etofenprox for the three populations was 749.89 – 1270.57 ppm. Linearity of the mortality curve was rejected in all three cases tested and a high heterogeneity was exhibited ( $p < 0.001$ ). Resistance factors could not be established for etofenprox due to the unavailability of  $LC_{50}$  value for a susceptible population. At the DOA recommended dosage, the estimated mortality was very low for all three populations ranging from 24.21 % to 29.69 %. The recommended dosage of etofenprox has to be increased by  $\times 93$ ,  $\times 476$  and  $\times 666$  folds to obtain 90 % mortality of Gannoruwa, Bandarawela and Kahatagasdigiliya populations, respectively.



**Figure 4:** Log-probit mortality curves for the whitefly populations from Gannoruwa ( $n = 469$ ), Kahatagasdigiliya ( $n = 726$ ) and Bandarawela ( $n = 811$ ) exposed to carbosulfan using bioassays by leaf-dip method. Dosage recommended by the DOA for whitefly control in Sri Lanka, is also shown for comparison.

**Table 2:** Analysis of mortality data resulted from the bioassay experiments by leaf-dip method for *B. tabaci* populations exposed to different dosages of neonicotinoids thiamethoxam and acetamiprid, organophosphate profenofos, carbamate carbosulfan and pyrethroid etofenprox

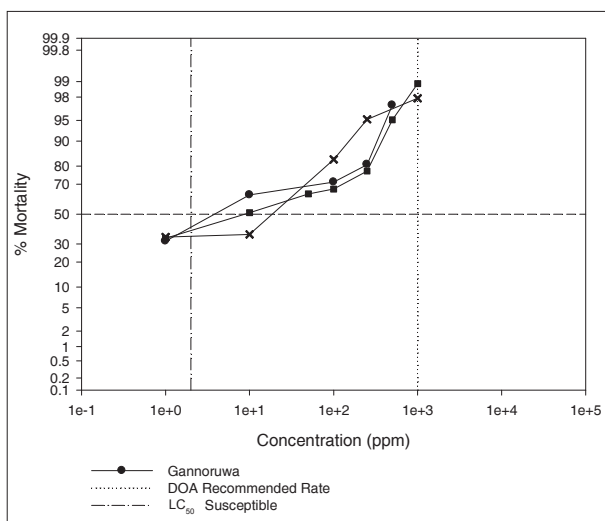
Insecticide	L	N	LC <sub>50</sub> (ppm)	95 % CI	Slope	χ <sup>2</sup> (df)	r <sup>2</sup>
Thiamethoxam	G	647	141.25	14.98 – 1029.91	15.39	** 17.88 (6)	0.81
	K	685	53.11	25.11 – 108.94	23.23	2.32 (4)	0.98
	B	701	1088.43	387.25 – 3650.90	20.97	* 14.32 (7)	0.87
Acetamiprid	G	1028	115.45	21.16 – 375.66	20.62	* 15.69 (7)	0.93
	K	364	7957.93	1189.05 > 100000	11.55	4.6 (4)	0.85
	B	951	72.97	46.00 – 115.45	21.80	2.49 (7)	0.97
Etofenprox	G	821	749.89	323.59 – 2488.86	29.81	** 18.91(6)	0.88
	K	925	1101.54	331.89 – 11272.00	21.70	** 20.67 (7)	0.78
	B	982	1270.57	374.11 – 8260.38	23.84	** 29.19 (7)	0.76
Carbosulfan	G	469	54.82	33.11 – 2293.76	18.64	4.05 (4)	0.89
	K	726	123.88	33.96 – 294.44	22.54	3.06 (5)	0.91
	B	811	82.41	7.85 – 266.68	28.17	5.95 (4)	0.83
Profenofos	G	647	5.4800	< 0.1 – 34.04	21.51	3.14 (4)	0.83
	K	685	8.4500	0.43 – 50.00	21.96	11.12 (6)	0.81
	B	701	6.6500	0.44 – 22.08	25.93	** 15.95 (5)	0.89

L - location; G - Gannoruwa; K - Kahatagasdigiliya; B - Bandarawela; N - number tested; LC<sub>50</sub> - lethal concentration which kills 50 % of the population; CI - confidence interval; χ<sup>2</sup> - Chi square goodness-of-fit for concentration-mortality response; df - degrees of freedom; \* p < 0.01; \*\* p < 0.001; r<sup>2</sup> - correlation coefficient

**Table 3:** Characterisation of mortality data obtained for whitefly populations exposed to the insecticides thiamethoxam, acetamiprid, etofenprox, carbosulfan and profenofos

Insecticide	L	LC <sub>50</sub> (ppm)	LC <sub>50</sub> -S (ppm)	RF	LC <sub>90</sub> (ppm)	% M	RDD (ppm)
Thiamethoxam	G	141.25	4.56 *	30.97	> 100000	49.29	512
	K	53.11	4.56 *	11.64	1939.10	61.59	512
	B	1088.43	4.56 *	238.69	70794.60	26.01	512
Acetamiprid	G	115.459	10.08 *	11.45	8912.51	56.59	512
	K	7957.93	10.08 *	789.48	> 100000	26.59	512
	B	72.97	10.08 *	7.23	5953.88	26.59	512
Etofenprox	G	749.89	N/A	–	13995.99	24.21	N/A
	K	1101.54	N/A	–	> 100000	29.69	N/A
	B	1270.57	N/A	–	71449.60	24.21	N/A
Carbosulfan	G	54.82	N/A	–	18620.90	69.01	N/A
	K	123.88	N/A	–	12078.10	75.17	N/A
	B	82.41	N/A	–	3828.25	76.92	N/A
Profenofos	G	5.48	2.0**	2.74	344.82	94.51	N/A
	K	8.45	2.0**	4.23	344.82	95.46	N/A
	B	6.65	2.0**	3.32	167.64	98.42	N/A

LC<sub>50</sub>-S: LC<sub>50</sub> susceptible population values from \*Vassiliou, 2011 and \*\*Huang *et al.*, 2007; N/A: not available; RF: resistance factor; % M: % mortality at DOA recommended dilution rate; RDD: resistance discriminating dosage values from Huang *et al.*, 2007



**Figure 5:** Log-probit mortality curves for the whitefly populations from Gannoruwa ( $n = 469$ ), Kahatagasdigiliya ( $n = 685$ ) and Bandarawela ( $n = 701$ ) exposed to profenofos using bioassays by leaf-dip method. Dosage recommended by the DOA for whitefly control in Sri Lanka, and  $LC_{50}$  for a susceptible population (Huang *et al.*, 2007) are also shown for comparison.

Linearity of the mortality curves was not rejected with all three populations suggesting the homogeneity of the response to carbosulfan by the three populations ( $p > 0.05$ ). The  $LC_{50}$  range was between 54.82 – 123.88 ppm. Resistance factors could not be established for carbosulfan due to the unavailability of  $LC_{50}$  value for a susceptible population. A moderate level of resistance with a mortality rate of 69.01 % to 76.92 % could be achieved at the DOA recommended dosage for carbosulfan. A  $\times 6$ ,  $\times 20$  and  $\times 3$  fold increase of the recommended dosage is needed to kill 90 % of the Bandarawela, Kahatagasdigiliya and Gannoruwa populations, respectively.

For profenofos, mortality response of the Bandarawela population was not linear ( $p < 0.001$ ) and the  $LC_{50}$  values ranged from 5.48 – 8.45 ppm. All three populations exhibited low resistance factor levels. The RF values were 2.74, 3.32 and 4.23, respectively for Gannoruwa, Bandarawela and Kahatagasdigiliya populations. A high efficacy of the DOA recommended dosage for profenofos giving mortalities above 95 % was observed for all three populations.

Monitoring programmes have revealed that the patterns of insecticide usage (*i.e.* continuous, mosaic or alternate) have a significant impact on the development of

resistance in the whitefly, *B. tabaci* (Palumbo *et al.*, 2001; Servin-Villegas *et al.*, 2006; Castle & Prabhakar, 2013) apart from the influence from host plant interactions (Li *et al.*, 2000; Castle *et al.*, 2008), environmental stresses (Castle & Prabhakar, 2013), and different biotypes (Dennehy *et al.*, 2005; Li *et al.*, 2012; Xie *et al.*, 2014). Thus, continuous monitoring of resistance to different insecticides can detect the change of susceptibility levels in a population allowing the implementation of necessary changes to insecticide usage pattern. Insecticide resistance status of *B. tabaci* has been well documented in other countries (Denholm *et al.*, 1998; Dennehy *et al.*, 2004; Vassiliou *et al.*, 2011; Xie *et al.*, 2014). Although insecticide resistance of selected insect pests and natural enemies of rice (Hemingway *et al.*, 1999; Karunaratne *et al.*, 1999; 2007; Karunaratne & Weerakoon, 2007) and horticultural crops (Damayanthi & Karunaratne, 2005) has been reported previously from Sri Lanka, this is the first report to show the insecticide resistance status of Sri Lankan whitefly populations.

Uniform high resistance levels were observed with etofenprox in all three populations suggesting that the whitefly populations have developed resistance to this particular pyrethroid insecticide. Moderate to very high levels of resistance were observed with neonicotinoids. For carbosulfan, the resistance was moderate while for profenofos, the populations were highly susceptible. Comparison of mortality responses with those of susceptible populations of whiteflies reported for neonicotinoids by Vassiliou *et al.* (2011) and for profenofos by Huang *et al.* (2007) was possible, since the methodologies used were similar. Resistance factors could not be obtained for etofenprox and carbosulfan due to the unavailability of reported mortality data of a susceptible population. High heterogeneity was observed with all three populations for all the insecticides tested except for carbosulfan, suggesting the possible existence of different biotypes.

At present, neonicotinoids, thiamethoxam and acetamiprid are widely used for whitefly control in chili, potato, bean, cucurbits and floriculture in Sri Lanka (DOA, 2009). Outbreaks of whitefly populations were observed in the areas of Bandarawela and Kahatagasdigiliya recently, despite several repeated applications of neonicotinoids. Whitefly resistance to neonicotinoids has been reported from many countries (Vassiliou *et al.*, 2011; Castle & Prabhakar, 2013). The pyrethroid, etofenprox, has not been recommended for whitefly control in Sri Lanka. However the brinjal crop, which is one of the host plants from which the pest was collected for the present study, is generally sprayed with etofenprox during pest control programmes as etofenprox

is recommended for brinjal shoot and pod borer control (DOA, 2009). Thus, it is obvious that the whiteflies are exposed to this insecticide regularly, developing the resistance. However, the Bandarawela *B. tabaci* population, which was collected from bean cultivations where etofenprox is not routinely applied, has also shown a high resistance level against etofenprox. Therefore, it is important to further examine the mechanism of cross resistance development to etofenprox in future studies. Whitefly resistance to etofenprox has also been reported from other countries (Cahill *et al.*, 1995). Both carbosulfan and profenofos are recommended for the control of chili leaf curl virus vectors including whiteflies, and the farmers tend to apply these on other crops also due to the broad spectrum pest control ability of the insecticides. High effectiveness of profenofos against the whitefly as shown by the present study highlights the importance of incorporation of profenofos in whitefly resistance management (WFRM) programmes.

The underlying mechanisms for the observed differences in resistance levels, and the efficacy for carbamate and organophosphate classes need to be studied in detail as both groups share the same target site, acetylcholinesterase. Insecticide resistance of insects can be developed due to qualitative and/or quantitative changes of the insect metabolic enzyme systems and due to mutated target sites (Karunaratne, 1998). The resistance to neonicotinoids in *B. tabaci* has been associated with enhanced oxidative detoxification by cytochrome P450 mono-oxygenases (Rauch & Nauen, 2003; Jones *et al.*, 2011). Metabolic detoxification by esterases and monooxygenases, and target site mutations in acetylcholinesterases have been associated with insecticide resistance in *B. tabaci* (Alon *et al.*, 2008; Reditakis *et al.*, 2009). Further studies to investigate the underlying molecular mechanisms for the high insecticide resistance of whitefly populations will be useful for the future agricultural pest control programmes of Sri Lanka.

According to our results, all the tested insecticides except profenofos require  $\times 6$  to  $\times 800$  fold increase of the recommended dosage to keep the efficacy at 90 % level, which is not economically or environmentally acceptable. Repeated application of insecticides for which pests have already developed resistance, has led to further development of resistance in the whitefly populations establishing resistance genes in the populations giving high reproductive dynamics (Heong *et al.*, 2011). An integrated approach including physical and biological control strategies together with effective insecticides followed by regular resistance monitoring programmes for whitefly control is essential for future WFRM programmes of the country.

## CONCLUSION

Whitefly populations from the Wet, Intermediate and Dry Zones of Sri Lanka show high resistance to thiamethoxam, acetamiprid, carbosulfan and etofenprox demanding  $\times 6$  to  $\times 800$  fold increase of the recommended dosages for a 90 % mortality. Since this is not an economically or environmentally viable option, these insecticides should be removed from whitefly control programmes with immediate effect. Organophosphates can be used as alternatives. An integrated approach with effective insecticides followed by regular resistance monitoring is urgently required for effective control of whitefly populations in Sri Lanka.

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