Sains Malaysiana 43(1)(2014): 31–36

Insecticide Susceptibility of *Bemisia tabaci* to Karate® and Cydim Super® and its Associated Carboxylesterase Activity

(Kerentanan *Bemisia tabaci* terhadap Racun Serangga Karate® dan Cydim Super®
serta Kaitannya terhadap Aktiviti Karboksilesterase)

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ABSTRACT

Bemisia tabaci *is a pest of several economic crops worldwide and is mostly managed in Ghana by farmers through the use of insecticides. However, vegetable farmers have recently expressed concerns about the susceptibility of* B. tabaci *to insecticides. Hence, the susceptibility status of field populations of* B. tabaci *on cassava, okra and tomato in vegetable growing sites in Accra to two commonly used insecticides, Karate® 2.5* EC *(λ-cyhalothrin) and Cydim Super® (36 g Cypermethrin + 400 g Dimethoate per litre), using a modified dipping method and their carboxylesterase activity levels were assessed. The* LC_{50} *values of the* B. tabaci *populations to the insecticides were low and ranged from 0.12-0.55 mL/L to 0.07-0.36 mL/L for Karate® and Cydim Super®, respectively. There was a wide variation in carboxylesterase activity levels of the insect populations with high levels recorded in some of the populations. The elevated activity levels could negatively impact on future whitefly management methods. This study provides baseline information on the insecticide resistance status and carboxylesterase levels of whitefly populations in these areas and for monitoring future insecticide resistance development.*

Keywords: Bemisia tabaci; *carboxylesterase; insecticides; susceptibility; vegetables*

ABSTRAK

Bemisia tabaci *ialah salah satu serangga perosak kepada beberapa tanaman ekonomi di seluruh dunia dan kebanyakannya berjaya dikawal di Ghana oleh para petani melalui penggunaan racun serangga. Walau bagaimanapun, para petani menyatakan kebimbangan tentang kerentanan* B. tabaci *terhadap racun serangga pada masa hadapan. Oleh itu, status kerentanan populasi bidang* B. tabaci *pada ubi kayu, bendi dan tomato di tapak semaian di Accra bagi dua racun serangga biasa digunakan, Karate® 2.5* EC *(λ-cyhalothrin) dan Cydim Super® (36 g Cypermethrin + 400 g Dimethoate setiap liter), menggunakan kaedah pencelupan diubah suai dan tahap aktiviti karboksilesterase mereka telah diuji. Nilai* LC_{50} *populasi* B. tabaci *bagi racun serangga didapati rendah, masing masing antara 0.12-0.55 mL/L dan 0.07-0.36 mL/L untuk Karate® dan Cydim Super®. Terdapat satu variasi luas bagi tahap aktiviti karboksilesterase bilangan serangga dengan tahap paling tinggi direkodkan dalam beberapa populasi. Ketinggian tahap aktiviti ini menggambarkan impak negatif pada masa hadapan tentang cara pengurusan bena putih. Kajian ini memberikan maklumat asas tentang kerentanan racun serangga dan karboksilesterase oleh populasi bena putih di kawasan ini bertujuan untuk memantau keboleh-rentanan racun serangga pada masa akan datang.*

Kata kunci: Bemisia tabaci; *karboksilesterase; kerentanan; racun serangga; sayur-sayuran*

INTRODUCTION

The sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important pest of crops such as cotton, cassava, vegetables and ornamentals (Oliveira et al. 2001). It is widely distributed (Boykin et al. 2007; Dinsdale et al. 2010) and affects over 900 host plants (GISD 2012). Initially, it was thought to be made up of several biotypes (Perring 2001) but has recently been named as a complex of 11 groups with a subset of about 24 cryptic species (De Barro et al. 2011). The two biotypes (cassava and okra) previously described in Ghana (Gadelseed 2000; Omondi 2003) belong to the high level sub-Saharan African group (De Barro et al. 2011) and share cowpea, tomato and garden egg as common hosts (Omondi et al. 2005).

Bemisia tabaci is a major pest of vegetables in Ghana and damages plants by its sap-sucking activity, vectoring plant diseases and secreting honeydew that promotes the growth of sooty mould (Obeng-Ofori 2007). An estimated 96.7% of the total land mass of Ghana is susceptible to whitefly problems (Ortiz 2006) and the dominant strategy used by farmers to control *B. tabaci* and other insect pests is the use of synthetic insecticides. The dependency on these chemicals has led to the development of insecticide resistance and negative effects on natural enemies (Gonzalez-Zamora et al. 2004). Insecticide resistance is due to mutations of target site proteins and/or the insects' ability to metabolise insecticides using esterases, microsomal oxidases and glutathione-S-transferases (GSTs) (ffrench-Constant et al. 2004; Li et al. 2007). Esterases have been associated with pyrethroid resistance of several insects (Li et al. 2007), whiles increased carboxylesterase metabolism and target site protein mutation were associated with organophosphate (OP) resistance in *B. tabaci* biotype B (Alon et al. 2008).

In this study, toxicological studies of *B. tabaci* from vegetable growing sites to two commonly used insecticides Karate® 2.5 EC (λ-cyhalothrin) and Cydim Super®, a binary insecticide (Dimethoate and Cypermethrin) and their carboxylesterase activity levels were conducted to ascertain and monitor resistance development patterns due to the reported decline in efficacy of insecticides by farmers.

MATERIALS AND METHODS

INSECTS *BEMISIA TABACI*

Adults were collected from plants at Airport, Ashaiman, Dzorwulu, Haatso and Ridge (all suburbs of Accra, Ghana) into vials using aspirators in the morning. The insects were collected from cassava (*Manihot esculenta*), okra (*Abelmoschus esculentus*) and tomato (*Lycopersicon esculentum*) and transported in a cool-box to the laboratory for various bioassays. A susceptible population on the ornamental *Duranta* sp in a semi-enclosed environment at ARPPIS which had then never been exposed to insecticides served as the reference population. The susceptible whiteflies were reared on their host plant for 2-3 generations and the adults were used for the assays. Before treatment, vials containing the insects were inverted and healthy individuals that climbed to the top due to positive phototaxis were used for the toxicity test.

INSECTICIDES

Commercial formulations of test insecticides Cydim Super® (36 g Cypermethrin + 400 g Dimethoate per litre) and Karate® 2.5 EC (λ-cyhalothrin) were used. At least six serial dilutions of the insecticides were prepared in distilled water and each treatment replicated thrice. Distilled water was used as a control. The recommended dose of these insecticides for use by farmers is 2.3 mL/L for Cydim Super® and 10 mL/L for Karate®.

BIOASSAY

The assay was done according to the modified dipping method of Owusu et al. (1995). One end of a glass tube (30 × 20 mm length/internal diameter) was sealed with a piece of nylon cloth. Unsexed *B. tabaci* adults (15) were introduced into the tube and the other end sealed with parafilm to prevent escape of the insects. The tube was inverted to allow the insects to move up to the nylon covered end. This end was then dipped in concentrations of the test solutions for 5 s. Insects that stayed onto the sides of the test tube were made to fall onto the cloth by gently tapping the sides of the tube. Excess liquid was removed by blotting on a piece of tissue paper. The whiteflies were then transferred into petri dishes containing excised insecticide-free leaves of the respective host plants from which they were sampled. The setup was left under room temperature (27.0 ± 2.0°C) and a photoperiod of 12:12 h light: dark. Mortality was recorded after 24 h. An insect was presumed dead if it showed no movement when probed.

CARBOXYLESTERASE (CAE) ACTIVITY

ENZYME PREPARATIONS

Individual whiteflies were homogenized in 0.3 mL of phosphate buffer pH7.0 in a glass well and the resultant homogenate was used as enzyme source for carboxylesterase assay (Owusu et al. 1995) in the DABCS insectary at 27.0 ± 2.0°C.

PROTEIN ASSAY

Reagent B (2 mL) (50 mL of 2% Na_2CO_3 in 0.1 M NaOH added to 0.5 mL each of $CuSO_4$ and $NaC_4O_62H_2O$ to obtain a total ratio of 50:1) was added to 0.1 mL of insect's homogenate in a test tube and allowed to stand for 20 min. About 3 min to the end of this period, 0.25 mL of a phenol: water mixture prepared in a 1:1 ratio was added to the contents of the test tube. The set up was allowed to stand for 20 min to ensure colour development. Absorbance readings were taken at 750 nm against the control on a CAMSPEC M106 spectrophotometer.

CARBOXYLESTERASE ACTIVITY ASSAY

Enzyme homogenate (100 μL) and 100 μL of 30 mM α-Naphthyl acetate (α – Na) substrate were pipetted into test tubes containing 2.8 mL of phosphate buffer pH7.0 (Owusu et al. 1995). This was incubated at 40°C in a water bath for 10 min. After incubation, 0.5 mL of a solution mixture of sodium dodecyl sulphate-fast blue salt (SDS-FBS) was added to stop the reaction and effect colour development. Absorbance was measured at 600 nm on a CAMSPEC M106 spectrophotometer against the control. Carboxylesterase activity was expressed as nmol α-naphthol produced/min/mg protein.

DATA ANALYSIS

Abbott's (1925) formula was used to correct the control mortality. Concentration-mortality responses of various populations of *B. tabaci* were analyzed using probit analysis package (EPA 2006). The probit parameters estimated include LC_{50} values and their corresponding 95% fiducial limits (FL) and slopes of regression. Mortality responses between populations were considered significant if there was no overlap between their corresponding 95% FL. The resistance ratios (RR) were calculated by dividing the LC_{50} of the various field populations by the LC_{50} of the reference population.

RESULTS AND DISCUSSION

The use of insecticides is one of the common methods of controlling the whitefly *B. tabaci* in the field. Due to the widespread application of insecticides against *B. tabaci*, it has developed resistance to several insecticide classes (Bacci et al. 2007; Horowitz et al. 2007; Houndete et al. 2010; Palumbo et al. 2001; Roditakis et al. 2009). In this study, the use of insecticides by vegetable farmers was predominant in all the study sites. The insecticide application records (Table 1) of farmers from the study sites were inconsistent and these were frequently disrupted since farmers applied insecticides whenever they detected the presence of insects. Against Karate®, there were no significant differences between the various field populations of the whiteflies and the reference population although the insects on tomato at Airport showed about 4.6 fold tolerance whilst the most susceptible was the population on cassava at Ridge with RR of about 1.6-fold (Table 2).

The pattern of susceptibility of the populations against Cydim Super® was similar to Karate®. The airport population on okra showed over 5-fold resistance (Table 3) whilst the population on cassava at Ridge was the most susceptible (1.1-fold RR). The whitefly populations sampled on crops at the study locations were susceptible to both Karate® and Cydim Super®. Cydim Super®, which is a binary insecticide, had a higher toxic effect on *B. tabaci* populations compared to Karate®. Though insecticide resistance to λ-cyhalothrin and dimethoate has been reported in *B. tabaci* populations in neighbouring West African countries (Houndete et al. 2010), λ-cyhalothrin continues to be effective in reducing populations of some insect species (Salem et al. 2009) including *B. tabaci* (Li et al. 2009) as observed in this study. In variance with this study, cypermethrin was ineffective in controlling *B. tabaci* populations (Bacci et al. 2007) while resistance to α-cypermethrin in Q- biotype (Roditakis et al. 2009; Wang et al. 2010) and B biotype (Wang et al. 2010) have also developed.

Whitefly populations from commercial vegetable growing areas such as Airport, Ashaiman and Haatso showed higher tolerance to the insecticides compared to Ridge and this may be due to the greater insecticide use and hence higher insecticide pressure on the insects at the former sites than the latter site. The populations appear to have responded homogeneously to Karate® than Cydim Super® as evidenced by the slopes for the probit plots

TABLE 1. Insecticide application history of Karate® and Cydim Super® on host plants

Site	Host	Spray frequency	
		Karate®	Cydim Super®
ARPPIS	Ornamental	None	None
Ridge	Cassava	None	None
Ridge	Okra	None	None
Ashaiman	Tomato	Weekly	Weekly
Ashaiman	Okra	Weekly	Weekly
Haatso	Okra	Weekly	Weekly
Haatso	Tomato	None	None
Dzorwulu	Okra	Weekly	Weekly
Dzorwulu	Tomato	Weekly	Weekly
Airport	Tomato	Weekly*	Weekly*
Airport	Okra	Weekly*	Weekly*

*Above recommended dosage

TABLE 2. Toxicity of Karate® (λ-cyhalothrin) against *Bemisia tabaci* adults

Sites	Hosts	N[1]	LC_{50} (mL/L) (95% FL)	Slope (±SE)	χ^2 (df[2])	RR
ARPPIS	Ornamental	315	0.12 (0.00-0.22)	3.06 (±1.52)	0.82(6)	___
Airport	Okra	315	0.44 (0.03-0.65)	3.86 (±1.62)	1.32(6)	3.67
Airport	Tomato	315	0.55 (0.01-0.77)	4.73 (±2.19)	0.85(6)	4.58
Ashaiman	Okra	315	0.44 (0.00-0.69)	3.67 (±1.81)	0.88(6)	3.67
Ashaiman	Tomato	315	0.32 (0.02-0.52)	3.42 (±1.36)	1.24(6)	2.67
Dzorwulu	Okra	315	0.31 (0.04-0.50)	3.07 (±1.12)	2.34(6)	2.58
Dzorwulu	Tomato	315	0.36 (0.03-0.55)	3.95 (±1.60)	0.98(6)	3.00
Haatso	Okra	315	0.46 (0.03-0.65)	4.38 (±1.86)	0.85(6)	3.83
Haatso	Tomato	315	0.35 (0.01-0.59)	2.99 (±1.27)	1.03(6)	2.92
Ridge	Cassava	315	0.19 (0.01-0.32)	3.65 (±1.49)	1.81(6)	1.58
Ridge	Okra	315	0.23 (0.02-0.40)	3.52 (±1.34)	0.96(6)	1.92

[1]N = number of insects tested; [2]df = Degree of freedom

which were relatively high and low, respectively (Tables 2 & 3). It is not surprising that higher LC_{50} values were recorded for Karate® than Cydim Super® since Karate® has been in use by farmers longer than Cydim Super® and thus the insects could be more tolerant to it as compared to Cydim Super®. The higher efficacy of Cydim Super® could also be due to its binary insecticide composition and thus has an additive toxic effect on the whiteflies. Such joint synergistic effect by binary mixtures of pyrethroids and organophosphates on *B. tabaci* has been reported (Ahmad 2007; Ellsworth & Martinez-Carrillo 2001; Prabhaker et al. 1998). The enhanced toxicity could also be attributed to the inhibition of the resistance mechanisms of *B. tabaci* to these insecticide classes (Dittrich et al. 1990).

There was a wide variation in the mean CaE activity levels of whitefly populations (Table 4). The whitefly populations at Airport had the highest CaE activity levels with the populations on tomato having a 13-fold activity ratio compared with the reference population. It was observed that at Airport, insecticide cocktail (Karate®, Cydim Super® and Attack® [Emamectin Benzoate]) were widely used with doses exceeding the recommended limits and hence incurring unnecessarily high control costs due to insecticide purchases. The application of higher insecticide doses at frequent intervals could exert a higher insecticide selection pressure on local whitefly populations leading to the development and spread of insecticide resistance. High insecticidal use frequency in addition to biological features of *B. tabaci* such as short developmental time and high fecundity and its behavioral tendency of a high dispersal capacity play major roles in insecticide resistance development (Dittrich et al. 1990; Ellsworth & Martinez-Carrillo 2001; Prabhaker et al. 1998). There is therefore the need for farmers to strictly adhere to the recommended dosage levels since these insecticides continue to provide adequate control over *B. tabaci* field populations.

In nature, there are interactions between herbivorous insects and host plants and these interactive effects could induce changes in detoxification enzymes in the insects that may reflect in their levels of tolerance to insecticides (Xie et al. 2011). Susceptibility of *B. tabaci* tomato populations to acetamiprid, carbosulfan, chlorpyrifos and bifenthrin were higher compared with populations on poinsettia, cabbage, cucumber and cotton whilst carboxylesterase activities of populations on cabbage and cucumber were higher than those on poinsettia, cotton and tomato (Xie et al. 2011). In another study, greenhouse *B. tabaci* cucumber populations had lower LC_{50} to abamectin, deltamethrin and omethoate compared to cotton populations (Liang et al. 2007) whilst field populations on cotton and cantaloupes had lower LC_{50} to bifenthrin and endosulfan than populations on some brassica (Castle et al. 2009).

Though, okra and cassava at Ridge were unsprayed, these were in close proximity to *Duranta* sp. in the same vicinity which had a monthly insecticide spray regime. This predisposed the whiteflies on these crops to insecticide selection pressure through drift. There is also the likelihood of migration of whiteflies from the

TABLE 3. Toxicity of Cydim Super® (Cypermethrin + Dimethoate) against *Bemisia tabaci* adults

Site	Host	N[1]	LC_{50}(mL/L)(95% FL)	Slope (±SE)	χ^2 (df[2])	RR
ARPPIS	Ornamental	315	0.07 (0.00-0.16)	2.26 (±1.12)	0.94(6)	___
Airport	Okra	315	0.36 (0.04-0.55)	3.97 (±1.61)	1.69(6)	5.14
Ashaiman	Tomato	315	0.19 (0.00-0.37)	2.52 (±1.02)	2.83(6)	2.71
Dzorwulu	Okra	315	0.17 (0.01-0.33)	2.25 (±0.79)	1.59(6)	2.42
Haatso	Okra	315	0.28 (0.00-0.42)	4.99 (±2.51)	0.46(6)	4.00
Ridge	Cassava	315	0.08 (0.00-0.23)	1.55 (±0.70)	1.50(6)	1.14

[1] N = number of insects tested; [2] df = Degree of freedom

TABLE 4. Mean carboxylesterase activity of *Bemisia tabaci* adults on host plants

Population	Host plant	No. tested	[a]Specific CaE activity ± S.E.	[b]RR
ARPPIS	Ornamental	30	0.45 ± 0.07	-
Airport	Okra	30	5.17 ± 0.23	11.5
Airport	Tomato	30	6.23 ± 0.14	13.8
Ashaiman	Okra	30	1.44 ± 0.08	3.2
Ashaiman	Tomato	30	1.32 ± 0.30	2.9
Dzorwulu	Okra	30	1.26 ± 0.10	2.8
Dzorwulu	Tomato	30	1.32 ± 0.09	2.9
Haatso	Okra	30	1.77 ± 0.13	3.9
Haatso	Tomato	30	1.53 ± 0.19	3.4
Ridge	Cassava	30	0.85 ± 0.15	1.9
Ridge	Okra	30	1.33 ± 0.17	2.9

[a] Mean specific CaE activity (nmol α-naphthol produced/min/μg protein)

[b] RR= Specifc CaE activity of population/ Specific CaE activity of ARPPIS population

ornamental onto these crops and vice versa. The cassava biotype does not colonise okra and the okra biotype does not also colonise cassava (Omondi 2003), hence, there is likely to be differential pressures on the two biotypes (if the *B. tabaci* are strictly of these respective biotypes) invariably leading to variant biotype-specific resistance levels. Omondi (2003) stated that the okra biotype was more tolerant to insecticides than the cassava biotype and in this study the whiteflies on okra had higher CaE levels and insecticide tolerance than those on cassava. The ineffectiveness of the insecticides as claimed by the vegetable farmers could be due to factors like ecophysiology, method and time of insecticide application and the management practices on the farm (Owusu et al. 1995). It could also be due to an expectation of rapidity of result (death or paralysis of insects) by farmers' moments after insecticide application since some farmers perceived paralysis as evidence of insecticide efficacy. However, laboratory bioassays may not be wholly reflective of what happens in the field (Owusu et al. 1995) since test populations are less heterogenous compared with field populations. The resistance status of *B. tabaci* field populations to insecticides could be dynamic and fluctuate within years (Ahmad et al. 2010). Hence, there is the need for constant monitoring of resistance in *B. tabaci* populations in these areas and other vegetable growing sites to these insecticides and other insecticides used to manage them.

Even though, CaE activity of *B. tabaci* on okra at Haatso was about 3-fold lower than that on okra at Airport, it was more tolerant to Karate®. It is likely that some esterase isozymes present in Haatso and Ashaiman okra populations did not effectively hydrolyse the 1-naphthylacetate substrate since some colorimetric substrates such as p-nitrophenyl acetate (PNPA) and 1-naphthylacetate were not effectively hydrolysed by carboxylesterase isozymes (Wheelock et al. 2005).

CONCLUSION

The resistance status of Ghanaian populations of *Bemisia tabaci* from vegetable farms in the capital city, Accra to Karate® and Cydim Super® and their carboxylesterase levels were studied. The *B. tabaci* populations were susceptible to both insecticides, though the degree of susceptibility to the binary insecticide, Cydim Super® was higher. This shows that these insecticides continue to be effective in managing whitefly populations from these fields. However, since the susceptibility status of *B. tabaci* to insecticides could alter from year to year (Ahmad et al. 2010), monitoring of their resistance status to these and other insecticides should be sustained whilst farmers are encouraged on the judicious use of insecticides. Though the insecticides were lethal to the whitefly populations, some populations especially the Airport populations had elevated CaE levels. Widespread elevated CaE levels in whitefly populations from the metropolis could result in resistance management problems in the near future. This emphasizes the need for thorough investigations of other biochemical resistance mechanisms such as Glutathione-S-transferases (GSTs) and Mixed Function Oxidases (MFO) in *B. tabaci* populations in the Accra metropolis so as to detect or delay the onset of resistance.

ACKNOWLEDGEMENTS

The cooperation of the authorities and farmers of the various study sites and a TWAS-USM fellowship to SWA which were helpful in completing the manuscript are gratefully acknowledged.

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Received: 6 January 2013
Accepted: 23 April 2013