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RESEARCH REPORT

Biochemical basis of conventional and novel mode of action insecticides resistance in field population of *Diaphorina citri* collected from Southern Punjab, Pakistan

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Abstract

Diaphorina citri Kuwayama is a phloem-feeding pest of citrus worldwide and vectors the putative causal agent of citrus greening, "*Candidatus* Liberibacter asiaticus" (*C*Las). Baseline toxicity and activities of esterases (EST), and glutathione-S-transferases (GST) sensitivity to bifenthrin, chlorpyrifos, thiamethoxam, acetamiprid, nitenpyram, chlorfenapyr and imidacloprid were established with field populations of *D. citri*. The highest calculated toxicity was observed in thiamethoxam treated *D. citri* with LC_{50} of 4.6 µg/mL and minimum in chlorpyrifos and imidacloprid with LC_{50} values of 96.9 and 91.9 µg/mL, respectively. The EST activity recorded in *D. citri* treated with different insecticides ranged from 11.8 to 52.1 µmol/min/mg of protein, while GST activity ranged from 10.9 to 51.3 µmol/min/mg of protein which subsequently linked with insecticide resistance. The present results provide a specific baseline to study levels of detoxifying enzymes against commonly used insecticides in *D. citri* that's indirectly correlated with level of resistance.

Key Words: Insecticides; HLB; Esterase; GST; resistance; citrus psylla; detoxification

Introduction

Diaphorina citri Kuwayama (Hemiptera: Sternorrhyncha: Liviidae), Asian citrus psyllid (Burckhardt and Ouvrard, 2012), a worldwide pest of citrus (Sapindales: Rutaceae) (Halbert and Manjunath, 2004; Bové, 2006) and primarily important due to its role as fastidious vector of *Candidatus (Ca.)* Liberibacter (L.) *africanus*, *Ca.* L. *asiaticus*., and *Ca.* L. *americanus* associated with "citrus greening" also known as Asian huanglongbing (HLB) (Pelz-Stelinski *et al.*, 2010; Boina and Bloomquist, 2015). Citrus trees infected by this disease produce small, discolored, lopsided and misshapen fruit over a shortened life span (Bové, 2006; Gottwald *et al.*, 2012; Grafton-Cardwell *et al.*, 2013) and infected trees can cause 33% yield loss of citrus fruits (Salcedo *et al.*, 2016). HLB management programs include the removal of infected trees, use of disease-free nursery stock and psyllid vector control by pesticide sprays (Belasque *et al.*, 2010; Tiwari *et al.*, 2010; Grafton-Cardwell *et al.*, 2013).

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The injudicious use of different insecticides puts *D. citri* under pressure and the insects use different mechanism (increased detoxification, reduced penetration, alterations of target sites and behavioral adaptations) for evolving resistance (Brattsten *et al.*, 1986; Chen *et al.*, 2006; Sétamou *et al.*, 2010) that may be found among *D. citri* populations. The enzymatic mechanism involves increased activities of esterases (EST) and glutathione-S-transferases (GST) (Hemingway *et al.*, 2004; Tiwari *et al.*, 2011a,b,c). A positive correlation between level of insecticide resistance and detoxifying enzymes has already been documented in several insect pests (Scott, 1999; Maymo *et al.*, 2002; Srigiriraju *et al.*, 2009). The insecticide resistance management involves reducing the frequency of resistance alleles within a field population so that the efficacy of a specific insecticide can be preserved (Georghiou, 1994).

EST are detoxifying enzymes produced through biotransformation of xenobiotics and directly linked to insecticides resistance (Devorshak and Roe, 1998) EST are known to detoxify organophosphate insecticides by hydrolyzing the ester moiety (Oakeshott *et al.*, 2005) and are studied in several insects like *Culex quinquefasciatus* (Diptera: Culicidae) (Sahgal *et al.*, 1994), *Blattella germanica* (Blattodea: Ectobiidae) (Anspaugh *et al.*, 1994), and

Table 1 List of Insecticides used for bioassays

Insecticide	Trade name and manufacturer	Target site of Action	Group of Insecticide
chlorpyrifos	(Lorsban®, 400 EC; Dow Agro Sciences)	Acetyl cholinesterase inhibitors	Organophosphates
bifenthrin	(Talstar®,10 EC; FMC)	Sodium channel modulators	Pyrethrins
imidacloprid	(Confidor®, 20 SL; Bayer Crop Sciences)	Agonists of nicotinic acetylcholine receptor	Neonicotinoids
acetamiprid	(Mospilan®, 20 SP; Arysta Life Sciences)	Agonists of nicotinic acetylcholine receptor	Neonicotinoids
thiamethoxam	(Actara®, 25WG; Syngenta)	Agonists of nicotinic acetylcholine receptor	Neonicotinoids
nitenpyram	(Paranol®, 10 EC; Kanzo, Agrochemicals)	Agonists of nicotinic acetylcholine receptor	Neonicotinoids
chlorfenapyr	(Pirate®, 360 SC; BASF Chemicals and Polymers)	Uncouplers of oxidative phosphorylation via disruption of the proton gradient	Pyrroles

Trichoplusia ni (Lepidoptera: Noctuidae) (Ishaaya and Casida, 1980). Similarly, increased level of GST is correlated with insecticide resistance (Li *et al.*, 2007). The enhanced oxidative and hydrolytic enzyme activities of GST expression have been associated with organophosphate and synthetic pyrethroid resistance in *Musca domestica* (Diptera: Muscidae) (Clark *et al.*, 1984; Tang *et al.*, 1990); *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Srinivas *et al.*, 2004), and *Spodoptera litura* (Lepidoptera: Noctuidae) (Armes *et al.*, 1997; Kranthi *et al.*, 2001).

The understanding of baseline activity of enzymes is necessary to suggest better management tactics and to minimize insecticide resistance in *D. citri* (Boina *et al.*, 2011; Tiwari *et al.*, 2011a). The present study intended to examine the activities of EST and GST after application of conventional and noval mode of action insecticides in field populations of *D. citri* so that better management program involving insecticides can be devised.

Materials and methods

Insects

The adult *D. citri* were collected from citrus orchard of Khanewal, Punjab, Pakistan through an aspirator and released on small citrus plants in Plexiglas cages 40×40×40 cm under laboratory conditions. Insects were reared in Laboratory of Insect Microbiology, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan until specific numbers were obtained to be used in bioassays. The rearing conditions were maintained at 27 ± 2 ºC and 60-65% relative humidity with a (12: 12h) light: dark photoperiod.

Insecticides

Seven insecticides belonging to different toxicological groups were chosen on the basis of their current use in field (Table 1).

Insecticidal Bioassays

The leaf dip method was used to determine the toxicity of different insecticides against *D. citri*. Serial dilutions of insecticides with selected concentrations (through hit and trial method) were formed for bioassay with distilled water (Naeem *et al.*, 2016). Fresh shoots/flushes of citrus were exposed for about 10 sec with different insecticide dilutions and were allowed to dry for 1 h at room temperature, while distilled water was used to treat control insects instead of insecticide dilution. In each treatment fifteen (5-7 days old) adult psyllids were released on treated citrus flushes within plastic jars (9×6×6 cm) covered with net from two sides for good aeration. Each treatment was replicated three times under similar conditions as described earlier. The mortality was recorded after 48, 72 and 96 h for conventional, neonicotinoid and pyrrole insecticides, respectively. The treatment samples for further biochemical assay were collected after 24, 48 and 72 h during bioassay.

Biochemical assays

Subsequent to the insecticidal bioassay, collected alive adult *D. citri* of different treatment levels and control were processed for further biochemical assay. Firstly, three to five psyllids were taken from each treatment and crushed by using the 0.15 M NaCl in the Eppendorf tube. For centrifugation the final volume was made 400 µl. The samples were spun at 3000 rpm for 10 mins at 5 ± 1 °C using the centrifuge machine (Z216MK, Hermle Labortechnik, Germany) to obtain the supernatants. The supernatants (*D. citri* homogenate) were further used to determine EST and GST activities.

Determining the activities of EST and GST
Determination of Protein Contents

The protein contents were determined in *D. citri* homogenates through Bradford (1976) procedure, using bovine serum albumin as standard at 25 °C with a spectrophotometer (UV3000, O.R.I. Germany).

Table 2 Toxicity of conventional and novel mode of action insecticides against *D. citri*

Insecticide	LC_{50} (µgmL^{-1}) (FD)	Slope	χ^2	*df*	*P*[a]	n[b]
chlorpyrifos	91.88 (62.67-144.85)	1.35 ± 0.25	2.722	4	0.61	270
bifenthrin	40.14 (23.05-80.21)	0.95 ± 0.23	0.231	4	0.99	270
imidacloprid	96.91 (49.17-487.05)	0.70 ± 0.22	1.218	4	0.88	270
acetamiprid	67.42 (43.13-135.74)	1.18 ± 0.26	0.024	4	1.00	270
thiamethoxam	4.64 (3.15- 6.61)	1.25 ± 0.22	1.195	4	0.88	270
nitenpyram	6.10 (4.25- 9.67)	1.25 ± 0.23	1.599	4	0.81	270
chlorfenapyr	23.99 (16.58-37.89)	1.21 ± 0.22	0.363	4	0.99	270

[a]*P*-values are based on Chi-square goodness of fit test. *P* values > 0.05 suggest goodness of fit of the model
[b]Number of *D. citri* used in the bioassay, including the control

EST activity

EST activity was measured by using 1 mM 4-Nitrophenyl acetate as a substrate (Damayanthi and Karunaratne, 2010). In each replicate, 90 µl of phosphate buffer (PBS, pH 6.5) and 100 µl of 0.6 M aNa (or bNa) was added to 10 µl of *D. citri* homogenate. After 30 min incubation, 100 µl of Fast Garnett BC solution was added to stop the reaction. The concentration of the final product was determined at 405 nm as an endpoint calculated from standard curves of a- and b-Naphtol, respectively.

GST activity

The GST activity was determined according to Caballero *et al.* (2008). For this purpose, 1- CDNB (chloro-2, 4-dinitrobenzene) (1 mM) was used as a substrate in ultraviolet (UV) semi-micro cuvettes (4 ml) with 5 mM reduced glutathione, 0.1 M Tris buffer pH 8 and 30 µl of *D. citri* homogenates. Enzyme activity was determined by monitoring continuous changes in absorbance at 340 nm for 4 min at 25 °C with a spectrophotometer.

Statistical analysis

The mortality data was evaluated by probit analysis (Finney, 1971) using the software package EPA probit analysis version 1.5. The enzymatic activities were analyzed through split plot design. Significance was accepted at α = 0.05. The enzymatic activities in *D. citri* homogenates were expressed by plotting graphs using Graph PrismPad 6.02.

Results

Effect of insecticides on D. citri

The toxicity of two groups of insecticides (conventional and novel mode of action) was evaluated against *D. citri* and their LC_{50} are given in Table 2. The highest percentage mortalities by chlorpyrifos, bifenthrin, imidacloprid, acetamiprid, thiamethoxam, nitenpyram and chlorfenapyr were 75.0, 66.0, 62.0, 59.0, 82.0, 73.0 and 71.0, respectively at their highest concentrations µgmL^{-1} (Table 3). However, thiamethoxam with lowest LC_{50} value of 4.6 (3.2-6.6) µgmL^{-1} proved to be most potential insecticide against *D. citri* as compared to imidacloprid with LC_{50} value of 96.9 (49.2-487.1) µgmL^{-1}, chlorpyrifos 91.9 (62.7-144.8) µgmL^{-1}, acetamiprid 67.4 (43.1-135.7) µgmL^{-1}, bifenthrin 40.1 (23.0-80.2) µgmL^{-1}, chlorfenapyr 23.9 (16.6-37.9) µgmL^{-1} and nitenpyram 6.10 (4.2-9.7) µgmL^{-1}, respectively.

EST activity

Activity of EST in D. citri after the exposure to conventional insecticides

In case of conventional insecticides bifenthrin showed maximum inhibition of EST i.e., (3.3 ± 0.7) µmol/min/mg of protein (F= 278.0, df= 5, P= 0.0000) observed after 48 h of treatment, while chlorpyrifos (34.6 ± 2.3) µmol/min/mg of protein (F= 2118.7, df= 5, P= 0.0000) expressed highest EST activity at 200 µgmL^{-1} after 24 h (Fig. 1).

Activity of EST in D. citri after the exposure to novel mode of action insecticides

The maximum inhibition of EST was expressed in chlorfenapyr treated *D. citri* (2.8 ± 0.4) µmol/min/mg of protein (F= 391.6, df= 5, P= 0.0000), while maximum EST activity was recorded in nitenpyram (52.1 ± 5.8) µmol/min/mg of protein (F= 45.2, df= 5, P= 0.0000) followed by imidacloprid, chlorfenapyr, thiamethoxam and acetamiprid.

In case of imidacloprid maximum activity (24.4 ± 1.9) µmol/min/mg of protein (F= 703.2, df= 5, P= 0.0000) was expressed in 24 h treatment as compared to 48 h and 72 h. In case of acetamiprid highest activity (11.8 ± 1.5) µmol/min/mg of protein (F= 368.5, df= 5, P= 0.0000) was observed after 48 h and lowest in 72 h post treatment. On the other hand, in case of nitenpyram, chlorfenapyr and thiamethoxam highest activity (12.9 ± 1.5) µmol/min/mg of protein (F= 110.7, df= 5, P= 0.0000) was expressed in 24 h than 48 h and 72 h samples (Fig. 1).

Table 3 Percent mortalities of *D. citri* against conventional and novel mode of action insecticides

Insecticides	Concentration (µgmL^{-1})	%Mortality	F-value	*P*-value	LSD
chlorpyrifos	200	75.5 ± 8.4 A	24	0.000	23.9
	100.0	44.4 ± 1.9 B			
	50.0	40.0 ± 3.3 BC			
	25.0	26.6± 3.3 BCD			
	12.5	17.7 ± 1.9 CD			
	control	4.4 ± 1.9 D			
bifenthrin	100	66.6 ± 3.3 A	41.1	0.000	16.7
	50	57.7 ± 3.8 AB			
	25	46.6 ± 3.3 B			
	12.5	28.8 ± 1.9 C			
	6.2	24.4 ± 1.9 C			
	Control	6.6 ± 1.9 D			
imidacloprid	150	62.2 ± 1.9 A	26.8	0.000	18.3
	75	44.4 ± 3.8 AB			
	37.5	37.7 ± 3.8 B			
	18.7	28.8 ± 1.9 B			
	9.4	28.8 ± 5.1 B			
	Control	2.2 ± 1.9 C			
acetamiprid	100	59.9 ± 3.3 A	28.5	0.000	18.3
	50	46.6 ± 5.7 AB			
	25	33.3 ± 3.3 BC			
	12.5	22.2 ± 1.9 CD			
	6.2	15.5 ± 1.9 CD			
	Control	4.4 ± 1.9 D			
thiamethoxam	20	82.2 ± 3.8 A	45.4	0.000	20.2
	10	66.6 ± 5.7 AB			
	5	46.6 ± 3.3 BC			
	2.5	31.1 ± 3.8 C			
	1.2	28.8 ± 1.9 C			
	Control	2.2 ± 1.9 D			
nitenpyram	14	73.3 ± 8.4 A	31.9	0.000	21.1
	7	46.6 ± 1.9 B			
	3.5	40.0 ± 3.3 BC			
	1.7	22.2 ± 3.3 CD			
	0.8	17.7 ± 1.9 DE			
	Control	2.2 ± 1.9 D			
chlorfenapyr	60	71.1 ± 5.1 A	50.3	0.000	16.7
	30	53.3 ± 3.3 B			
	15	39.9 ± 3.3 BC			
	7.5	26.6 ± 0.0 CD			
	3.7	17.7 ± 1.9 DE			
	Control	2.2 ± 1.9 E			

GST activity

Activity of GST in D. citri after the exposure of conventional insecticides

In case of conventional insecticides, maximum inhibition of GST i.e., (5.3 ± 0.9 µmol/min/mg) of protein was due to chlorpyrifos at 200 µgmL^{-1} after 72 h of treatment (F= 2196.4, df= 5, P= 0.0000), while bifenthrin expressed highest GST activity (47.6 ± 3.6 µmol/min/mg of protein) (F= 122.1, df= 5, P= 0.0000) at 100 µgmL^{-1}, 24 h post treatment (Fig. 2).

Activity of GST in D. citri after exposure of novel mode of action insecticides

Chlorfenapyr expressed maximum GST inhibition (2.7 ± 1.6 µmol/min/mg of protein) (F= 220.1, df= 5, P= 0.0000) followed by thiamethoxam and acetamiprid. In contrast, nitenpyram expressed maximum GST activity. The GST activities of novel mode of action insecticides were as follows: imidacloprid (10.9 ± 2.5 µmol/min/mg of protein), acetamiprid (24.2 ± 1.9 µmol/min/mg of protein) (F= 38.6, df= 5, P= 0.0000), nitenpyram

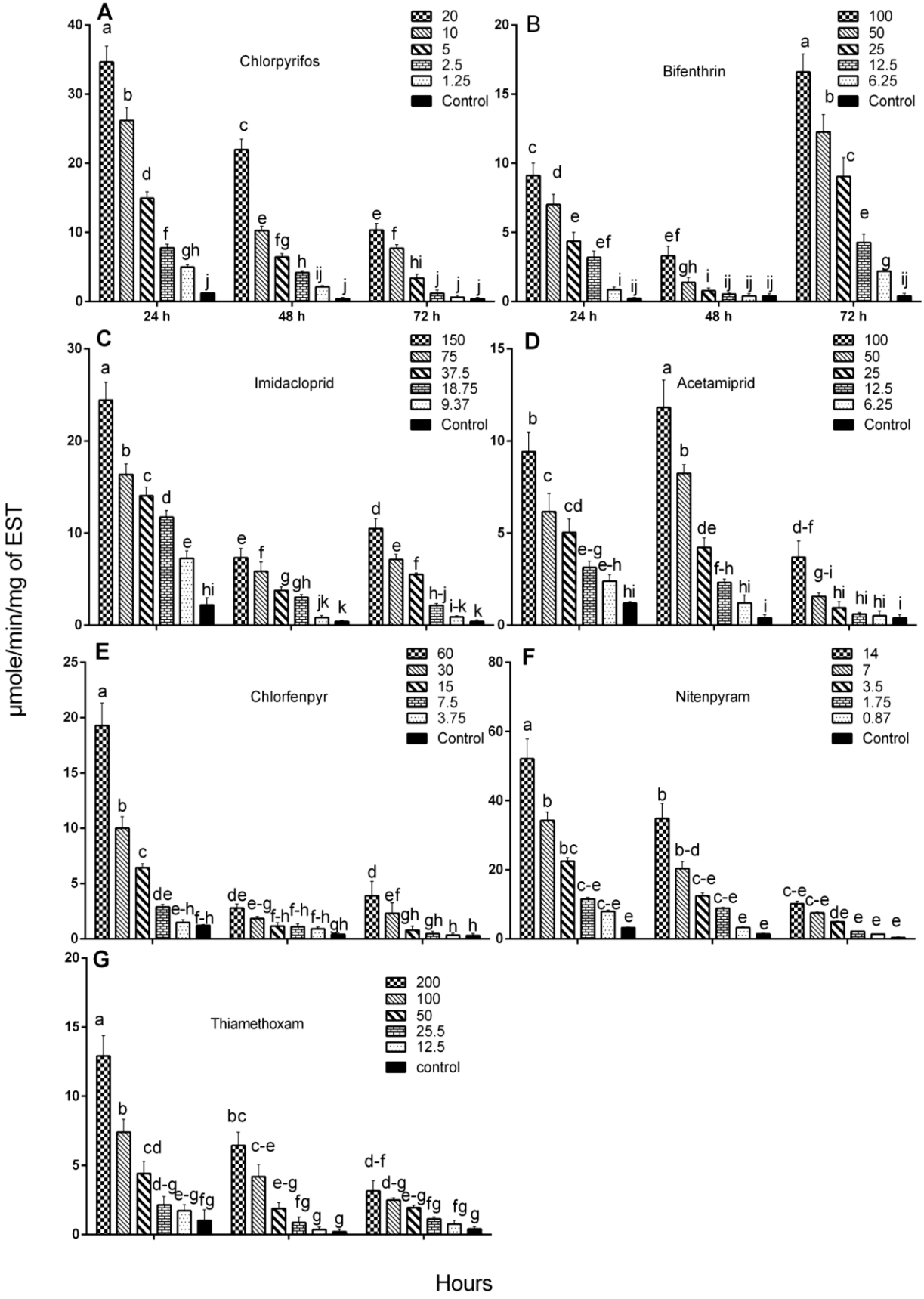

Fig. 1 EST specific activity expressed as mean in bars followed by the same letter within columns indicate no significant difference ($P \leq 0.05$) in a Tukey's test after the exposure of different insecticides in *D. citri*. The line in the bars represents the mean standard error

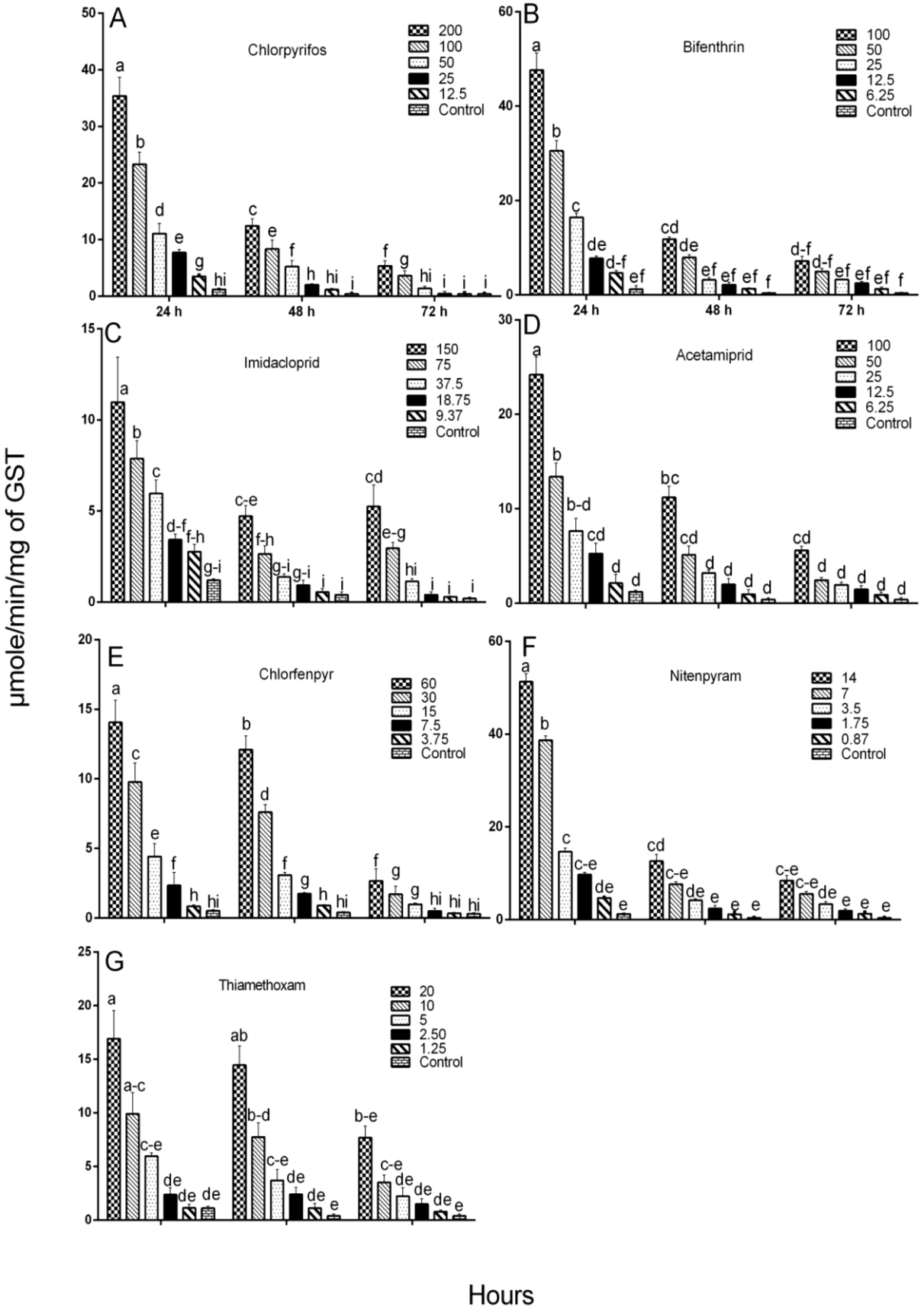

Fig. 2 GST specific activity expressed as mean in bars followed by the same letter within columns indicate no significant difference ($P \leq 0.05$) in a Tukey's test after the exposure of different insecticides in *D. citri*. The line in the bars represents the mean standard error

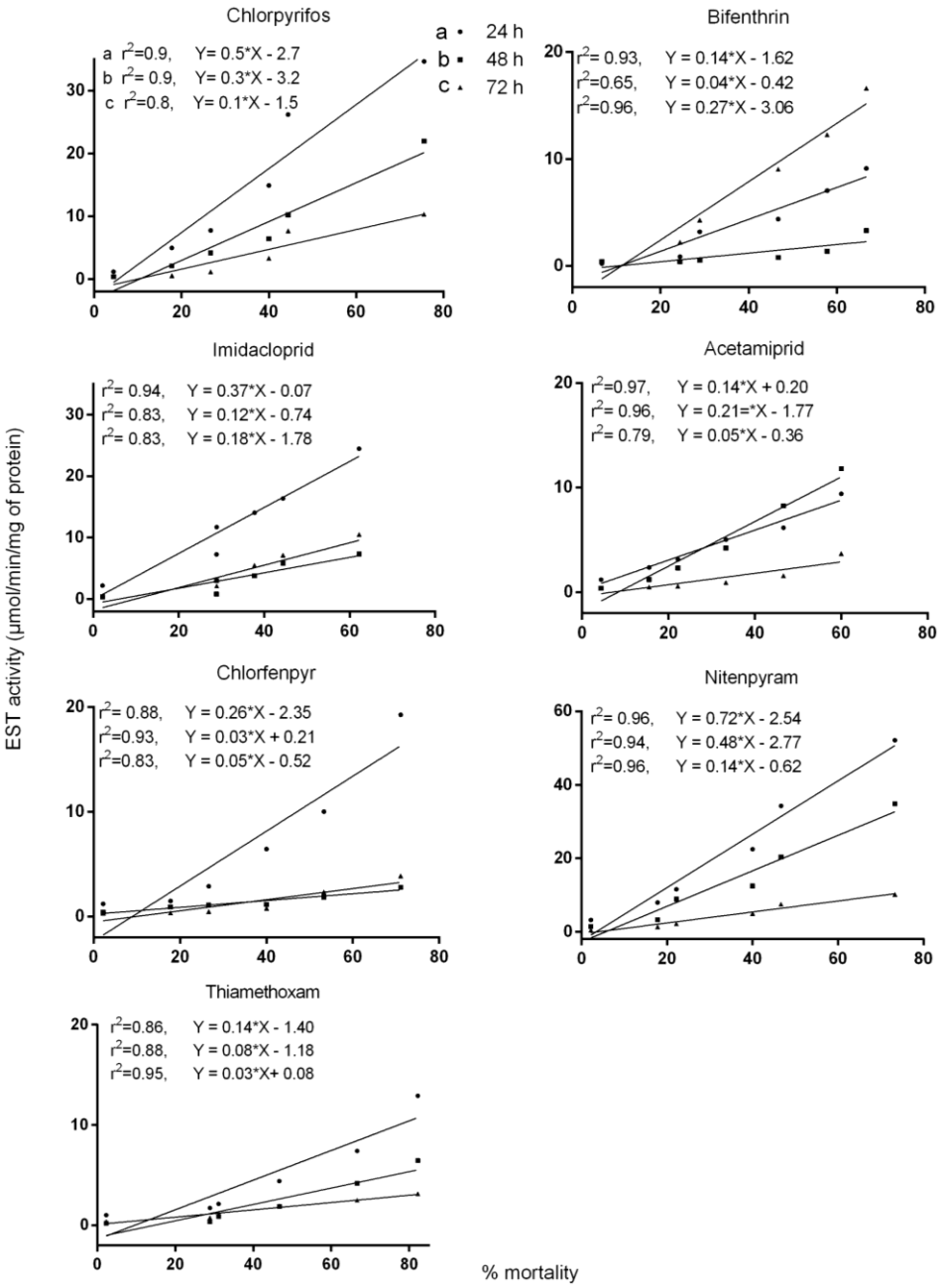

Fig. 3 Linear regression analysis showing the relation between percent mortality and EST enzymatic expression with respect to different hours ($P \leq 0.05$)

(51.3 ± 1.7 µmol/min/mg of protein) (F= 95.7, df= 5, P= 0.0000), thiamethoxam (16.9 ± 2.6 µmol/min/mg of protein) and chlorfenapyr (14.0 ± 1.6 µmol/min/mg of protein) after 24 h of treatment (Fig. 2).

Relation between percent mortality of D. citri and enzymatic expression

The relation between percent mortality and EST (Fig. 3) and GST (Fig. 4) was expressed by drawing regression lines activity in *D. citri.* The difference between the slopes showed significant values.

Discussion

Asian HLB has grown into a great risk to the world citrus industry (Bové, 2006; Hall *et al.*, 2013) as the vector *D. citri* has developed resistance to a wide range of insecticides (Naeem *et al.,* 2016; Chen and Stelinski, 2017). Detoxification enzymes like EST and GST are described as the defense mechanism against foreign toxic compounds (Li and Liu, 2007) and play important roles in maintaining the normal physiological activities in the insect's body (Chen *et al.*, 2017). For example, due to neurotoxic action of insecticides, insects release the

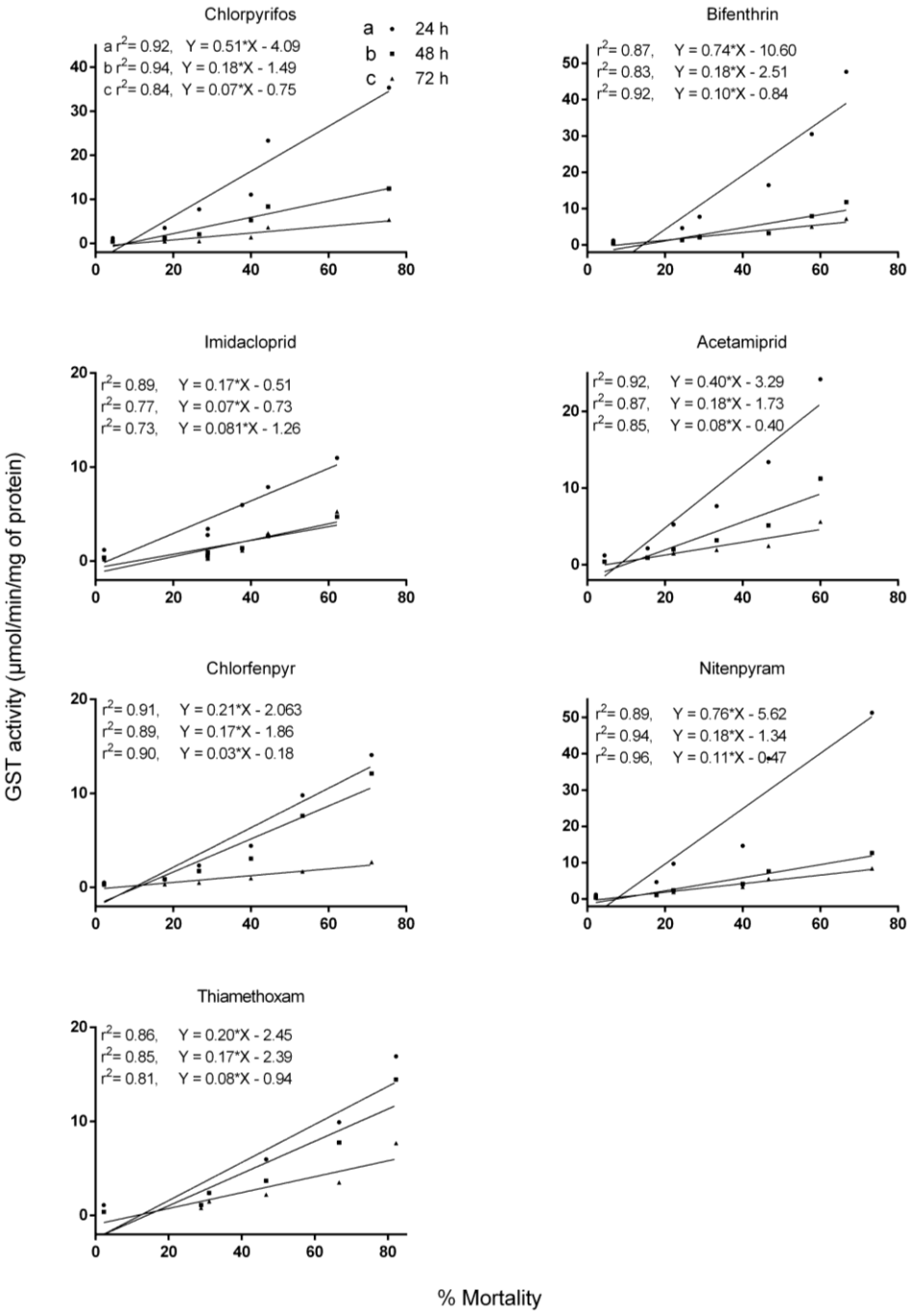

Fig. 4 Linear regression analysis showing the relation between percent mortality and GST enzymatic expression with respect to different hours ($P \leq 0.05$)

detoxifying enzymes which correlate with insecticide resistance (Bilal *et al.*, 2018). The biochemical mechanisms of insecticide resistance in *D. citri* have received significant recent attention (Liu *et al.*, 2016; Chen *et al.*, 2018).

Tiwari *et al.* (2011a) reported high levels of detoxifying enzymes activity at higher toxicity level of insecticide (thiamethoxam, imidaclorpid, bifenthrin, chlorpyrifos and fenpropathrin) in *D. citri* populations. Similarly, our result showed higher enzymatic function at higher toxicity level of insecticides as compared to control *D. citri* population.

Our results suggested that *D. citri* showed maximum EST (52.1 ± 5.8 µmol/min/mg of protein) after 24 h of exposure to nitenpyram. In relation with percent mortality 73.3% regression showed highly significant correlation with EST activity after 24 h of exposure (F= 124.3, P= 0.0004) However, our results indicated that acetamiprid (11.8 ± 1.5)

µmol/min/mg of protein induced reduced activity of EST whereas, imidacloprid (10.9 ± 2.5) µmol/min/mg of protein induced a reduction in activity of GST.

Results declared some inner connection between enzyme activity, time, percent mortality and concentrations of insecticide. For example, in case of EST activity for acetamiprid, chlorfenpyr and thiamethoxam slope was positively related with percent mortalities at specific time i.e., 24 h, 48 h and 72 h, respectively as compared to other insecticides. Its means that increase in EST enzyme release was directly linked with percent mortality, which might be a reason for resistance development at specific time of exposure of insecticide. Whereas, GST had significantly negative slope between percent mortality and enzyme activity for all insecticides with respect to time. Suppression of detoxification enzymes indicates that these enzymes play no role in the detoxification of tested compounds and may increase the susceptibility of insect pests to these insecticides (Abd-Elaziz and El-Siad, 2009).

Previous studies revealed that GST enzymes contribute to pyrethroid resistance (Grant and Matsumura, 1989; Tiwari *et al.*, 2011a, c). Our results revealed that bifenthrin expressed (47.6 ± 3.6) µmol/min/mg of protein GST activity which coincides with previous study. In contrast, bifenthrin revealed very low EST activity i.e., 3.3 ± 0.7 µmol/min/mg of protein. Current research showed that in vivo, metabolism of imidacloprid changed to the main metabolite 4/5-hydroxyimidacloprid, a product of oxidative degradation, in honeybee *Apis mellifera* (Suchail *et al.*, 2004). Similarly, the main metabolite of imidacloprid i.e., 5-hydroxyimidacloprid was detected in imidacloprid-resistant strains of *Bemisia tabaci* (Rauch and Nauen, 2003). Parallel to previous results of our studies, imidacloprid showed EST and GST activities i.e., 24.4 ± 1.9 and 10.9 ± 2.5 µmol/min/mg of protein, which subsequently linked with imidacloprid resistance. In future, there is crucial need to reduce P450 activity which increase the susceptibility to imidacloprid through silencing of *CYP4* genes in adult *D. citri* (Killiny *et al.*, 2014).

Insecticide resistance management becomes a serious threat due to insecticide failures in the field. By monitoring detoxifying enzymes of currently used insecticides we practiced a strategy to obtain optimal resistance management. Use of enzyme inhibitors, such as piperonyl butoxide, diethyl maleate and triphenyl phosphate, for cytochrome P450, GST and carboxylesterase, respectively may be useful in cases where increased enzymatic detoxification is contributing to resistance (Terriere, 1984; Ishaaya, 1993). Biochemical assays explain that the insensitivity of different insecticides is likely the biochemical basis of conventional and novel mode of action insecticides resistance in *D. citri.* A proactive approach has been taken to characterize the molecular and biochemical response of *D. citri* to insecticides for optimization of rotation schedules (Tiwari *et al.*, 2011a; Coy *et al.*, 2016).

The data presented here on sensitivity of EST and GST for inhibition and on specific activity of various detoxifying enzymes. The enzymatic levels for the tested insecticides were highly variables. The confirmation of resistance associated enzymes (EST and GST) through analyzing enzymatic levels provide an additional baseline to previously reported toxicological data (Naeem *et al.,* 2016) and helpful in citrus management programs that include rotation of insecticide classes, use of biological and cultural control practices.

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References

Abd El-Aziz MF, El-Said YA. Toxicity and biochemical efficacy of six essential oils against *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae). Egypt. Acad. Biolog. Sci. 2: 1-11, 2009.

Anspaugh DD, Rose RL, Koehler PG, Hodgson E, Roe RM. Multiple Mechanisms of pyrethroid resistance in the German Cockroach *Blattella germanica* (L.). Pestic. Biochem. Physiol. 50: 138-140, 1994.

Armes NJ, Wightman JA, Jadhav DR, Ranga Rao GV. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh. Indian Pestic. Sci. 50: 240-248, 1997.

Belasque J, Bassanezi RB, Yamamoto PT, Ayres AJ, Tachibana A, Violante AR, *et al*. Lessons from huanglongbing management in Sao Paulo State, Brazil. J. Plant Pathol. 92: 285-302, 2010.

Bilal M, Freed S, Ashraf MZ, Rehan A. Resistance and detoxification enzyme activities to bifenthrin in *Oxycarenus hyalinipennis* (Hemiptera: Lygaeidae). Crop Prot. 111: 17-22, 2018.

Boina D, Youn Y, Foliminova S, Stelinski LL. Effects of pymetrozine, an antifeedant of Hemiptera, on Asian citrus psyllid, *Diaphorina citri*, feeding behavior, survival and transmission of *Candidatus* Liberibacter *asiaticus*. Pest Manage. Sci. 67: 143-155, 2011.

Boina DR, Bloomquist JR. Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Manage. Sci. 71: 808-823, 2015.

Bové JM. Huanglongbing: a destructive, newly-emerging century-old disease of citrus. J. Plant Pathol. 88: 7-37, 2006.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254, 1976.

Brattsten LB, Holyoke CW, Leeper JR, Raffa KF. Insecticide resistance: Challenge to pest management and basic research. Science. 231: 1255-1260, 1986.

Burckhardt D, Ouvrard D. A revised classification of the jumping plant-lice (Hemiptera: Psylloidea). Zootaxa. 3509: 1-34, 2012.

Caballero C, López-Olguín J, Ruiz M, Ortego F, Castañera P. Antifeedant activity and effects of terpenoids on detoxication enzymes of the beet armyworm, *Spodoptera exigua* (Hübner). Span. J. Agric. Res. 6: 177-184, 2008.

Chen XD, Sanada-Morimura S, Yanagi SI, Nakasuji F. Rapid recovery of susceptibility under harsh environmental conditions in fenvalerate-

resistant strains of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Appl. Entomol. Zool. 41: 641-650, 2006.

Chen XD, Seo M, Stelinski, LL. Behavioral and hermetic effects of the butenolide insecticide, flupyradifurone, on Asian citrus psyllid, *Diaphorina citri*. Crop Prot. 98: 102-109, 2017.

Chen XD, Stelinski LL. Rapid detection of insecticide resistance in *Diaphorina citri* (Hemiptera: Liviidae) populations using a bottle bioassay. Fla. Entomol. 100: 124-133, 2017.

Chen XD, Gill TA, Nguyen CD, Killiny N, Pelz-Stelinski KS, Stelinski LL. Insecticide toxicity associated with detoxification enzymes and genes related to transcription of cuticular melanization among color morphs of Asian citrus psyllid. Insect Science. *In press,* DOI: 10.1111/1744-7917.12582, 2018.

Clark AG, Shamaan NA, Dautreman WC, Hayaoka T. Characterization of multiple glutathione s-transferases from the house flies, *Musca domestica* (L). Pestic. Biochem. Physiol. 22: 51-59, 1984.

Coy MR, Liu B, Stelinski LL. Reversal of insecticide resistance in Florida populations of *Diaphorina citri* (Hemiptera: Liviidae). Fla. Entomol. 99: 26-32, 2016.

Damayanthi BT, Karunaratne SHPP. Biochemical characterization of insecticide resistance in insect pests of vegetables and predatory ladybird beetles. J. Natl. Sci. Found. Sri Lanka. 33: 115-122, 2010.

Devorshak C, Roe RM. The role of esterases in insecticide resistance. Rev. Toxicol. 2: 501-537, 1998.

Finney DG. Probit Analysis, 3rd edition. Cambridge University Press, UK, 1971.

Georghiou GP. Principles of insecticide resistance management. Phytoprot. 75: 51-59, 1994.

Gottwald TR, Graham JH, Irey M, McCollum G, Wood B. Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer and disease progress. Crop Prot. 36: 72-82, 2012.

Grafton-Cardwell EE, Stelinski LL, Stansly PA. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annu. Rev. Entomol. 58: 413-432, 2013.

Halbert SE, Manjunath KL. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87: 330-353, 2004.

Hall DG, Richardson ML, Ammar ED, Halbert SE. Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomol. Exp. Appl. 146: 207-223, 2013.

Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochem. Mol. Biol. 34: 653-665, 2004.

Ishaaya I, Casida JE. Properties and toxicological significance of esterases hydrolyzing permethrin and cypermethrin in *Trichoplusia ni* larval gut and integument. Pestic. Biochem. Physiol.14: 178-184, 1980.

Karunaratne SHPP, Hemingway J, Jayawardena KGI, Dassanayaka V, Vaughan A. Kinetic and molecular differences in the amplified and non-amplified esterases from insecticide resistant and susceptible *Culex quinquefasciatus* mosquitoes. J. Biol. Chem. 270: 31124-31128, 1995.

Killiny N, Subhas H, Tiwari S, Gowda S, Stelinski LL. Double-stranded RNA uptake through topical application, mediates silencing of five *CYP4* genes and suppresses insecticide resistance in *Diaphorina citri*. Plos One. 9: 110536, 2014.

Kranthi KR, Jadhav DR, Wanjari LR, Alis SS, Russel D. Carbamate and organophosphate resistance in cotton pests in India 1995-1996. Bull. Entomol. Res. 91: 37-46, 2001.

Li X, Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52: 231-253, 2007.

Li XZ, Liu YH. Diet influences the detoxification enzyme activity of *Bactrocera tau* (Walker) (Diptera: Tephritidae). Acta Entomol. Sin. 50, 989-995, 2007.

Liu B, Coy MR, Wang JJ, Stelinski LL. Characterization of the voltage-gated sodium channel of the Asian citrus psyllid, *Diaphorina citri*. Insect Sci. 24: 47-59, 2016.

Maymo AC, Cervera A, Sarabia R, Martinez-Pardo R, Garcera MD. Evaluation of metabolic detoxifying enzyme activities and insecticide resistance in *Frankliniella occidentalis*. Pest Manage. Sci. 58: 928-934, 2002.

Naeem A, Freed S, Jin FL, Akmal M, Mehmood M. Monitoring of insecticide resistance in *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) from citrus groves of Punjab, Pakistan. Crop Prot. 86: 62-68, 2016.

Oakeshott JG, Claudianos C, Campbell PM, Newcomb RD, Russell RJ. Biochemical genetics and genomics of insect esterases. In Comprehensive Molecular Insect Science - Pharmacology. Elsevier, Oxford. 5, 309-381, 2005.

Pelz-Stelinski KS, Brlansky RH, Ebert TA, Rogers ME. Transmission parameters for *Candidatus* Liberibacter *asiaticus* by Asian citrus psyllid (Hemiptera: Psyllidae). J. Econ. Entomol. 103, 1531-1541, 2010.

Rauch N, Nauen R. Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). Arch. Insect Biochem. Physiol. 54: 165-176, 2003.

Sahgal A, Kumar S, Pillai MKK. Microplate Assay of elevated esterase-activity in individual pyrethroid-resistant mosquitos. J. Biosci. 19: 193-199. 1994.

Salcedo D, Mora G, Covarrubias CC, Hinojosa R, De Paolis F, Mora S. Evaluación del impacto económico de la enfermedad de los cítricos huanglongbing (HLB) en la cadena *citrícola mexicana*. http://repiica.iica.int/docs/b2146e/b2146e.pdf.2016

Scott JG. Cytochromes P450 and insecticide resistance. Insect Biochem. Mol. Biol. 29: 757-777, 1999.

Sétamou M, Rodriguez D, Saldana R, Schwarzlose G, Palrang D, Nelson SD. Efficacy and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leaf miner, two foliar feeding citrus pests. J. Econ. Entomol. 103: 1711-1719, 2010.

Srigiriraju L, Semnter PJ, Anderson TD, Bloomquist JR. Esterase based resistance in the tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in the eastern United States. Arch. Insect Biochem Physiol. 72: 105-123, 2009.

Srinivas R, Udikeri SS, Jayalakshimi SK, Sreeramula K. Identification of factors responsible for insecticide resistance in *Helicoverpa armigera*. Comp. Biochem. Physiol. C. 137: 169-261, 2004.

Suchail S, De Sousa G, Rahmani R, Belzunces LP. In vivo distribution and metabolisation of 14C-imidacloprid in different compartments of *Apis mellifera.* Pest Manage. Sci. 60: 1056-1062, 2004.

Tang ZH, Wood RJ, Cammak SL. Acetyl cholinesterase activity in organophosphorous and carbamate resistance and susceptible strains of the *Culex pipens* complex. Pestic. Biochem. Physiol. 37: 192-199, 1990.

Terriere LC. Induction of detoxification enzyme in insect. Annu. Rev. Entomol. 29: 71-88, 1984.

Tiwari S, Lewis-Rosenblum H, Pelz-Stelinski K, Stelinski LL. Incidence of *Candidatus* Liberibacter *asiaticus* infection in abandoned citrus occurring in proximity to commercially managed orchards. J. Econ. Entomol. 103: 1972-1978, 2010.

Tiwari S, Mann RS, Rogers ME, Stelinski LL. Insecticide resistance in field populations of Asian citrus psyllid in Florida. Pest Manage. Sci. 67: 1258-1268, 2011a.

Tiwari S, Pelz-Stelinski K, Stelinski LL. Effect of *Candidatus* Liberibacter *asiaticus* infection on susceptibility of Asian citrus psyllid, *Diaphorina citri,* to selected insecticides. Pest Manage. Sci. 67: 94-99, 2011b.

Tiwari S, Pelz-Stelinski K, Mann RS, Stelinski LL. Glutathione Transferase and Cytochrome P450 (General Oxidase) activity levels in *Candidatus* Liberibacter *Asiaticus*-Infected and uninfected Asian Citrus Psyllid (Hemiptera: Psyllidae). Annu. Entomol. Soc. Am. 104: 297-305, 2011c.