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Toxic and biochemical effects of different insecticides on the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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ABSTRACT

Toxic effect of dinotefuran, imidacloprid, fenoxycarb, phenthoate and thiocyclam H.O insecticides on greenhouses population of the tomato leafminer, *Tuta absoluta* (Meyrick) was evaluated in laboratory. Data revealed that the five tested insecticides had high contact toxic effect on moths and 3rd instar larvae of the insect. Moths were more susceptible to the effect of tested insecticides than larvae. Imidacloprid was the superior toxicant against moths and larvae, so it had a very low resistance coefficient (RC= 0.01 and 0.13). Phenthoate and thiocy- clam-H.O. had high toxic effect on two stages. Dinotefuran seemed to have low effect on moths and the same trend was observed with fenoxycarb on larvae. The activity of acetycholinesterase (AChE), glutathione-S-transferase (GST) and monooxygenase (PCMAN-demethylase) was higher in whole body homogenate of untreated moths (1.5, 1.2 and 1.58 times, respectively), than that of 3rd instar larvae. The exposure of moths and 3rd instar larvae to LC_{30}, LC_{50} and LC_{80} of tested insecticides caused significant reduction (51.11and 25.00%) or increasing (41.78 and 28.77%) in activity of AChE, respectively. Phenthoate treatments reduced AChE activity, but imidacloprid and dinotefuran induced this activity in LC_{80} treated insects .Low insecticide treatments produced slight induction (1.41-11.90%) of GST, other two treatments produced reduction or increasing enzyme activity of treated insects. LC_{80} of fenoxycarb had moderate induction (24.79- 27.90%) of moths and larvae GST. A positive correlation between the insecticide concentration and the activity of monooxygenase PCMAN-demethylase was observed in treated insects with five insecticides. Elevation of enzyme activity ranged from 1.59 to 52.12% in moths and 5.83- 59.17% in larvae. Phenthoate and imidacloprid produced the higher induction effect of treated insect enzyme than the other three insecticides.

Keywords: *Tuta absoluta*, Insecticides, Acetycholinesterase, Glutathione-S-transferase, PCMAN-demethylase Monooxygenase.

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill is a vegetable crop of large importance throughout the world. It is the first horticultural crop in Egypt. Tomatoes are grown both under plastic covered greenhouses and in open field. The tomato leafminer, *Tuta absoluta* Meyrick, (Lepidoptera: Gelechiidae) is a serious pest of both outdoor and greenhouse tomatoes. It was originated from South America (Giordano and Silva, 1999) and was recently introduced in Europe and subsequently spread throughout the Mediterranean Basin and Europe (EPPO, 2011). The serious outbreak was reported in many countries, Belgrade (Toševski1 *et al*., 2011), Greece (Roditakis *et al*., 2010), Brazil (Siqueira, *et al*., 2000 a, b), and Egypt (Mohammed, 2010). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas and it is currently considered a key agricultural threat to European and North African tomato production (Desneux *et al*., 2010, Garcia and Vercher, 2010).

Chemical control is the main method for controlling the tomato leafminer, *T.absoluta* (Siqueira *et al*., 2001; Galdino *et al*., 2011). In Argentina organophosphates were initially used for *T. absoluta* control then were gradually replaced by pyrethroids during the 1970s. During the early 1980s, cartap which alternates with pyrethroids and thiocyclam were sprayed showing the good effectiveness of the former. During the 1990s, insecticides with novel mode of actions were introduced such as abamectin, acylurea, insect growth regulators, tenbufenozide and chlorfenapyr (Lietti *et al.,* 2005). Spinosad and Indoxacarb are effective against larvae of *T.absoluta* in Spain (SEWG 2008). Chemical pesticides continue to be an important component of insect pest management even with the development of other control methods (mass-trapping, plant resistance...). The use of insecticides based on different chemistries and with varying modes of action is an important component of an integrated pest management strategy. Hence, insecticides will continue to be an integral component of pest management programs due mainly to their effectiveness and simple use (Braham and Hajji, 2011).

Sublethal insecticide exposure can lead to physiological and behavioral changes in the organism (Hyne and Maher 2003). These responses can be measured using specific biomarkers that provide a measure of sublethal effects, e.g., "fitness" of the survivors (McCarthy and Shugart 1990). Three such biomarkers are acetycholinesterase (AChE), glutathione-S-transferase (GST) and mixed function oxidase (MFO). Acetycholinesterase is the target enzyme for organophosphate and carbamate pesticides, which act by inhibiting its activity. GST is involved in the detoxifcation of a wide range of xenobiotic chemicals. In insects, GST plays an important role in biotransformation of various insecticides (Motoyama 1980, Lamoureux and Rusness 1987); including the degradation of some organophosphorus compounds (Yang 1976). MFO was effectively reducing the efficacy of insecticides on pests (Wang *et al*., 2009).

This work aims to evaluate the efficiency of some insecticides on adult moths and third instar larvae of tomato leaf miner, *T. absoluta*. Effect of the insecticide treatments on activity of acetycholinesterase, glutathione-S-transferases and PCMA N-demethylase monooxygenase in treated insects was also, determined.

MATERIALS AND METHODS

1- Insect

Samples of differant instars *T. absoluta* larvae were collected in March 2011 from commercial tomato greenhouses and kept under laboratory conditions (25 ± 2° C, 65 ± 5% R.H. and a photoperiod of 16 L:8 D) on untreated tomato plants until emergence of the moths from F1generation in Central Agriculture Pesticides Laboratory.

2- Toxicological tests

The insecticides used in this research were the neonicotinoid, Imidacloprid (admire 20%SC-Bayer Crop Science), the neonicotinoid, Dinotefuran (oshin 20%SG-Mitsui chemical), the organophsophate, Phenthoate (elsan 50%EC- Nissan Chemical Industries), the thiocyclam, Thiocyclam-Hydrogen-Oxalate (evisect 50%SP- Arysta Life science) and the juvenile hormone mimic, Fenoxycarb(insegar 25%WP-Sumitomo Chemical Corporation). The stock solution of each formulated insecticide was prepared by dissolving 0.1 ml or 0.1gm from compound in 9ml of distilled water (reach to10ml with acetone) to give the stock material. Ten concentrations (4o, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ppm) were prepared from this stock by diluting with acetone.

2.1- Adulticidal test

Vial method (Plapp *et al*., 1987; Snodgrass1996) was used to evaluate the toxicity of five formulated insecticides against adult moths (one-day old) of *T.absoluta*. Five mL of each concentration of tested insecticides was pipette into 100mL (9Dx15H) glass scintillation vial (five replicates for each treatment). The vials were placed on a hot dog roller (heating elements removed) which was operated until all acetone was evaporated leaving behind insecticidal residues inside the vials. Ten adults were added to each vial containing insecticide concentration and control (treated with acetone only). Vials were then closed with clean muslin squares secured with rubber bands. Vials were kept under lab conditions. The effect of insecticides was determined after 12h of application and expressed as percent mortality of moths at each concentration.

2.2-Larvicidal test

Filter papers impregnated with insecticide molecules method (Salazar and Araya, 1997& 2001; Siqueira *et al*., 2000a, b) was used to evaluate the contact action of the tested insecticides against larvae of *T.absoluta*. Filter paper (Whitman no. 1 cellulose filter paper 9cm) was putted in glass Petri dish with the same diameter then 1 ml of each insecticide concentration was pipette on it, control treatments were applied with acetone only. Five replicates for each treatment and control were used. Ten 3rd instar larvae were added to each replicate after the filter paper was dried and kept in lab conditions for 12h, then the mortality counted.

Sub lethal concentrations LC_{30}, LC_{50}, LC_{80} and LC_{95} of treated moths and larvae were calculated by using SAS probit (1997) program. To assess the resistance of a given population,the resistance cofficient(RC) (Wegorek *et al.,*2011) was calculated as follows: Resistance Cofficient (RC)= LC_{95}/recommended field concentration RC ≤ 1 lack of resistance RC=1.1-2 low resistance RC=2.1-5 medium resistance RC = 5.1-10 high resistance RC ≥ 10 very high resistance.

3-Enzyme activity

After experiments of toxicology, untreated control and survivor treated (LC_{30}, LC_{50} and LC_{80}) moths and 3rd instar larvae of *T.absoluta* were removed and frozen for subsequent enzyme analysis. Acetycholinesterase (AChE), glutathione-S-transferase (GST) and monooxygenase (PCMAN-demethylase) activities were measured in all frozen samples.

3.1-Enzyme extract

For AChE and GST activities ,500 mg of control and each of the treated insects were homogenized in 1 ml sodium phosphate buffer(0.1M,pH7) using Teflon glass homogenizer and centrifuged at 10.000g for 15 min at 4°C (five replicates of each sample).The supernatant was used as a source of enzyme.

For PCMAN-demethylase activity, 100 mg of control and treated insects were homogenized in 0.2 ml sod.phosphate buffer (0.1M,pH7.8) containing 10% glycerol,1mM DTT (1,4-dithiothreitol)1mM EDTA (ethylenediamine tetraacetic acid), 1mM PMSF (phenylmethanesulfonyl floride) and 1mM PTU (N-phenyl thiourea) (five replicates of each sample). The samples were centrifuged at 10.000g for 10 min at 4°C. The supernatants were centrifuged at 18.000g for 30 min at 4°C. The produced supernatants were collected and used as enzyme resource (Chen *et al*., 2011, with some modification).

The total protein content of all samples was determined according to Bradford (1976).

3.2-Acetycholinesterase activity

Activity of this enzyme was measured spectrophotomatricaly as Ellman *et al*., (1961). The reaction mixture consists of 50µl of sample

enzyme, 10µl of 100mM ATCh I, (acetylthiocholine iodide), 10µl 9.2mM DTNB (5,5-dithio-bis (2-nitrobenzoic acid) and potassium phosphate buffer (0.1M ,pH 7.2) up to 1ml (five replicates for each sample). The increment in absorbance at 405 nm &25°C was recorded during 5min. The activity was expressed as nanomoles of acetylthiocholine hydrolyzed/ mg protein^{-1}/min^{-1}.

3.3-Glutathione-S-transferases activity

GST activity was measured based on the method of Habig *et al.,* (1974). The assay was conducted to incubating 50mM of CDNB(1-chloro-2,4-dinitrobenzen) as a substrate with 50mM GSH (reduced glutathione) and 50µl of sample enzyme in 0.1M phosphate buffer (pH7) for 5min. at 27°C (five replicates for each sample). The activity monitored at 340 nm and expressed as nmoles of CDNB conjugated /mg protein^{-1} /min^{-1}.

3.4-PCMAN-demethylase activity

Demethylation of the model substrate P-chloro-N-methylaniline was quantified following the method of kupfer and Bruggerman (1966). The reaction mixture contained 10µM p-chloro-N-methylaniline, 2.5mM glucose-6-phosphate (G6P), 0.4 unite of glucose-6-phosphate dehydrogenase (G6P-dh), 0.5mM nicotinamide adenine dinucleotide phosphate (NADP+) and 7.5mM magnesium chloride (MgCl2). Five replicates for each sample, each replicate contained 50µl of sample enzyme and 400µl of reaction mixture. The reaction proceeded at 37º C for 10 min in a water bath and stopped with the addition of 750µl of p-dimethylaminobenzaldehyde (PDAB) solution, then centrifuged. The product p-choroaniline was quantified by comparing absorbance at 445 nm to simultaneously determined standard curve (0-50nmol). The activity of enzyme was represented as nmoles of p-chloroaniline/mg protein^{-1}/min^{-1}.

3.5-Data analysis:

Enzyme activities were expressed as mean ± standard error (S.E.) and statistically analyzed by using SPSS program V.13. Differences were considered significant at $p< 0.05$ level.

RESULTS AND DISCUSSIONS

Toxicological tests

The efficacy of five insecticides against moths and 3rd larval instar of *T. absoluta* were evaluated in this study. Tables (1&2) demonstrated the insecticidal potency of imidacloprid on both stages than the other insecticides. Imidacloprid's good action on moths and larvae was visible after calculating LC_{30} (95% Cl) = 0.14 (0.7-0.19) and 1.14 (1.05-0-1.85) ppm, LC_{50} (95% Cl) = 0.22(0.15-0.29) and 3.11 (1,98-4.74) ppm, LC_{80} (95% Cl)= 0.93(0.55-0-1.38) and 12.36(10.68-18.57) ppm and LC_{95} (95% Cl)=1.61 (1.00-3.78) and 33.28 (22.36-69.45) ppm, respectively.

Table 1: Susceptibility levels of tomato leafminer, *Tuta absoluta* adult moths to tested insecticides

Insecticide	Slope ± S.E.	LC_{30} (ppm) (95%CL)	LC_{50} (ppm) (95%CL)	LC_{80} (ppm) (95%CL)	LC_{95} (ppm) (95%CL)	RC value
Imidacloprid	1.90 ± 0.31	0.14 (0.07 -0.19)	0.22 (0.15 - 0.29)	0.93 (0.55 - 1.38)	1.61 (1.00 - 3.78)	0.01
Phenthoate	1.73 ± 0.29	0.20 (0.11 -0.35)	0.34 (0.21- 0.54)	1.24 (0.82 -1.73)	5.08 (2.93 - 14.13)	0.01
Fenoxycarb	1.74 ± 0.38	0.38 (0.26 -0.54)	0.57 (0.41 - 0.77)	1.72 (1.32 -3.18)	2.59 (1.48 - 7.29)	0.08
Thiocyclam H.O.	1.79 ± 0.39	0.68 (0.42 -1.11)	1.36 (0.99 - 1.82)	4.64 (2.38 -7.44)	11.29 (6.52 -24.83)	0.02
Dinotifuran	1.99 ± 0.26	1.57 (1.13 -2.64)	2.82 (2.12 - 3.62)	7.66 (4.60- 11.12)	18.93 (14.25 -36.97)	0.30

CL = confidence limits. RC = resistance coefficient

Table 2: Susceptibility levels of tomato leafminer, *Tuta absoluta* 3*rd* instar larvae to tested insecticides

Insecticide	Slope ± S.E.	LC_{30} (ppm) (95%CL)	LC_{50} (ppm) (95%CL)	LC_{80} (ppm) (95%CL)	LC_{95} (ppm) (95%CL)	RC value
Imidacloprid	0.84 ±0.26	1.14 (1.05 -1.85)	3.11 (1.98 - 4.74)	12.36 (10.68-18.57)	33.28 (22.36 - 69.45)	0.13
Dinotifuran	0.65 ±0.18	1.78 (0.66 -3.16)	3.32 (1.46 - 6.24)	62.71 (32.85 -193.20)	297.72 (106.94 - 1391.13)	1.19
Phenthoate	0.99 ±0.31	2.14 (1.73 -3.34)	5.60 (1.68 - 11.70)	35.62 (21.44 -59.87)	110.45 (73.56 - 145.67)	0.48
Thiocyclam H.O.	0.91 ±0.22	2.39 (1.46 -4.92)	5.88 (1.77 -25.85)	51.20 (36.24 - 621.63)	152.6 (167.07 -55528.15)	0.24
Fenoxycarb	0.57 ±0.16	3.14 (1.11-8.43)	6.88 (2.79 - 16.34)	218.37 (75.18 -1137.92)	528.15 (132.34-13135.01)	5.24

CL = confidence limits. RC = resistance coefficient.

The recommended concentration of this insecticide in Egypt is 250 ppm, so the resistance coefficient (0.01 and 0.13) of it was very low in moths and larvae of *T. absoluta* tested population.

Moths were also susceptible to the toxic effect of phenethoate (LC_{50} = 0.34ppm, LC_{95}=2.59) and RC = 0.01), fenoxycarb (LC_{50}=0.57 ppm, LC_{95} = 5.08ppm and RC= 0.08), thiocyclam-H.O. (LC_{50}= 1.36ppm, LC_{95}=11.29 ppm and RC=0.02) and dinotifuran (LC_{50}=2.82 ppm, LC_{95}=18.9 and RC=0.30). Dennehy *et al*., (2005) mentioned that whiteflies collected from different crop fields throughout Arizona (2000-2004) continued to be highly susceptible to imidacloprid (Admire). However, susceptibility to the related neonicotinoid insecticides acetamiprid and thiamethoxam varied widely and was lowest in collections from different crops and greenhouses plants. The reduced-risk insecticides, imidacloprid providing rapid knockdown and mortality followed by residual antifeedant activity on rose chafer beetle adults (Isaacs *et al*., 2004).

The third instar larvae were lowest susceptible (LC_{50} = 3.30 – 6.88 ppm) to effect of tested insecticides than moths. The adult and neonate of insects were more susceptible to effect of the insecticides than old larva and pupa (Campanhola and Plapp, 1989; Abdel-Rahman *et al*., 2002). Comparison the LC_{95s} with the recommended concentration of insecticides revaled that the larvae had low resistance coefficient values (0.24-1.79) to thiocyclam-H.O., phenethoate and Dinotefuran .This value was increased to 5.24 with fenoxycarb treatments, which pointed to high resistance of *T. absoluta* larvae to this insecticide by contact application. Susceptibility of field populations of *T.absoluta* to insecticides was positively correlated with the number of chemical sprays in the field (Reyes *et al*., 2012*).*

Enzyme activities

Biochemical evaluations revealed that the enzymatic systems involved in the susceptibility of *T absoluta* to insecticides. The activities of Acetycholinesterase (AChE), Glutathione-S-transferase (GST) and monooxygenase (PCMAN-demethylase) were higher in whole body homogenate of untreated moths (1.5, 1.2 and 1.58 fold, resp.), than that of 3rd instar larvae. These enzymes were decreased or increased in body homogenate of treated moths and larvae than the untreated ones (Tables 3& 5).

Treatment of *T. absoluta* moths and 3rd instar larvae with LC_{30}, LC_{50} and LC_{80} of tested insecticides produced a significant decrease in AChE activity of insect body homogenates except imidacloprid and dinotefuran (Table 3). There is a negative correlation between the insecticide concentration and the enzyme activity in moth tissues. Phenthoate caused high significant reduction (33.31, 46.40 and 51.11 %) in activity of moth enzyme with LC_{30}, LC_{50} and LC_{80} treatments, resp. than that of control moths (493.48±22.66 nmoles of acetylthiocholine hydrolyzed/mg protein^{-1}/min^{-1}). Fenoxycarb treatments produced 30.95-42.46% decreasing in AChE activity.

Table 3: Activity of Acetycholinesterase enzyme in whole body homogenates of treated *Tuta absloluta* with different concentrations of tested insecticides (nmoles of acetylthiocholine hydrolyzed/ mg protein^{-1}/min^{-1})

Insecticide	Moths						3rd instar larvae					
	LC_{30}		LC_{50}		LC_{80}		LC_{30}		LC_{50}		LC_{80}	
	Activity ± S.E.	Change %	Activity ± S.E.	Change %	Activity± S.E.	Change %	Activity ± S.E.	Change %	Activity ± S.E.	Change %	Activity ± S.E.	Change %
Imidacloprid	423.22^{b} ±14.83	(-) 14.34	288.31^{c} ±21.49	(-) 41.57	699.6^{a} ±35.17	(+) 41.78	305.63^{b} ±13.47	(-) 7.05	376.18^{a} ±11.11	(+) 14.40	404.76^{a} ±23.24	(+) 23.09
Phenthoate	329.12^{c} ±23.45	(-) 33.31	264.50^{c} ±15.24	(-) 46.40	241.2^{c} ±16.87	(-) 51.11	307.85^{b} ±30.18	(-) 6.38	282.17^{b} ±16.25	(-) 14.19	269.76^{b} ±19.25	(-) 17.30
Fenoxycarb	340.75^{c} ±20.89	(+) 30.95	329.26^{b} ±25.99	(-) 33.28	283.9^{c} ±15.69	(-) 42.46	282.47^{b} ±27.88	(-) 14.10	299.15^{b} ±18.36	(-) 9.02	271.55^{b} ±17.25	(-) 17.30
Thiocyclam H.O.	587.63^{a} ±31.98	(+) 19.08	310.51^{b} ±5.24	(-) 36.88	317.6^{c} ±18.81	(-) 35.64	376.24^{a} ±29.76	(+) 14.42	314.54^{b} ±17.22	(-) 4.34	288.33^{b} ±20.19	(-) 12.31
Dinotifuran	352.53^{c} ±19.34	(-) 28.56	398.57^{a} ±17.15	(-) 19.23	517.2^{b} ±14.27	(+) 4.82	246.75^{c} ±14.33	(-) 25.00	352.52^{a} ±16.90	(+) 7.21	423.42^{a} ±21.16	(+) 28.77
Untreated activity ± S.E.	493.48±22.66						328.82±31.46					

S.E =standard error.
Change%= mean activity of treated-mean activity of control/ mean activity of control x100
Mean activity values in the same column followed by different letters are significantly different (P< 0.05).

Table 4: Activity of Glutathione-S-transferase enzyme in whole body homogenates of treated *Tuta absoluta* with different concentrations of tested insecticides (nmoles of CDNB conjugated /mg protein^{-1} /min^{-1}).

Insecticide	Moths						3rd instar larvae					
	LC_{30}		LC_{50}		LC_{80}		LC_{30}		LC_{50}		LC_{80}	
	Activy ± S.E.	Change %	Activty ± S.E.	Change %	Activity ± S.E.	Change %	Activity ± S.E.	Change %	Activity ± S.E.	Chang e %	Activity ± S.E.	Change %
Imidacloprid	334.57^{a} ±17.36	(+) 5.34	313.35^{b} ±14.84	(-) 1.34	290.98^{c} ±11.30	(-) 8.38	276.62^{a} ±16.54	(+) 6.47	244.17^{b} ±6.23	(-) 6.02	228.12^{c} ±12.14	(-) 12.19
Phenthoate	346.72^{a} ±12.83	(+) 9.17	315.31^{b} ±11.68	(-) 0.72	302.87^{b} ±18.43	(-) 4.64	282.29^{a} ±11.06	(+) 8.66	291.16^{a} ±18.36	(+) 12.07	220.56^{c} ±20.65	(-) 15.10
Fenoxycarb	355.39^{a} ±16.72	(+) 11.90	387.87^{a} ±12.15	(+) 22.12	396.35^{a} ±14.35	(+) 24.79	286.04^{a} ±17.16	(+) 10.10	311.14^{a} ±16.83	(+) 19.75	332.28^{a} ±18.63	(+) 27.90
Thiocyclam H-O.	328.12^{a} ±18.55	(+) 3.31	324.23^{b} ±19.55	(+) 2.08	311.44$^{b\,a}$ ±27.33	(-) 1.94	274.32^{a} ±15.45	(+) 5.59	292.84^{a} ±11.22	(+) 12.72	237.53^{c} ±19.79	(-) 8.57
Dinotefuran	342.50^{a} ±15.21	(+) 7.83	293.28^{c} ±12.50	(-) 7.66	284.35^{c} ±18.37	(-) 10.47	263.47^{a} ±8.15	(+) 1.41	278.26^{b} ±11.94	(+) 7.11	267.96^{b} ±11.15	(+) 3.15
Untreated activity ± S.E	317.61±13.86						259.80±21.44					

S.E =standard error.
Change%= mean activity of treated-mean activity of control/ mean activity of control x 100 Mean activity values in the same column followed by different letters are significantly different (P < 0.05).

Table 5: Activity of PCMAN-demethylase enzyme in whole body homogenates of treated *Tuta absoluta* with different concentrations of tested insecticides (nmoles p-chloroaniline /mg protein^{-1}/min^{-1}).

Insecticide	Moths						3rd instar larvae					
	LC_{30}		LC_{50}		LC_{80}		LC_{30}		LC_{50}		LC_{80}	
	Activity ± S.E	Change %	Activity ± S.E	Change %	Activity ± S.E.	Change %	Activity ± S.E	Change %	Activity ± S.E.	Change %	Activity ± S.E.	Change %
Imidacloprid	4.28^{a} ±1.82	(+) 13.23	4.87^{a} ±1.41	(+) 28.84	5.64^{a} ±2.81	(+) 49.21	3.02^{a} ±0.97	(+) 25.83	3.15^{a} ±1.44	(+) 31.25	3.46^{a} ±2.22	(+) 44.17
Phenthoate	4.36^{a} ±2.16	(+) 15.34	4.60^{a} ±1.79	(+) 21.69	5.75^{a} ±4.12	(+) 52.12	3.14^{a} ±1.15	(+) 30.83	3.37^{a} ±2.36	(+) 40.42	3.82^{a} ±1.56	(+) 59.17
Fenoxycarb	4.21^{a} ±1.75	(+) 11.38	4.49^{a} ±2.14	(+) 18.78	4.83^{b} ±1.41	(+) 27.78	2.75^{b} ±1.36	(+) 14.58	2.96^{b} ±1.91	(+) 23.33	3.32^{b} ±1.26	(+) 38.33
Thiocyclam H.O.	3.96^{b} ±2.34	(+) 4.76	4.57^{a} ±2.44	(+) 20.90	4.74^{b} ±3.16	(+) 25.40	2.80^{b} ±0.54	(+) 16.67	3.13^{a} ±1.21	(+) 30.42	3.29^{b} ±1.73	(+) 36.67
Dinotefuran	3.84^{b} ±1.67	(+) 1.59	4.13^{b} ±2.63	(+) 9.26	4.47^{b} ±2.74	(+) 18.25	2.54^{b} ±1.05	(+) 5.83	2.78^{b} ±1.92	(+) 15.83	3.06^{c} ±1.18	(+) 27.50
Untreated activity ± S.E.	3.78±1.33						2.40±0.82					

S.E =standard error.
Change%= mean activity of treated-mean activity of control/ mean activity of control x 100 Mean activity values in the same column followed by different letters are significantly different (P < 0.05).

Thiocyclam- H.O. produced a medium increase (19.08%) in moth enzyme with low concentration and caused significant reduction (36.88 and 35.88%) in this enzyme with other two concentrations. Dinotefuran and imidacloprid caused inhibition (28.56-19.23 and 14.34-41.57%) in enzyme activity with LC_{30}, LC_{50} treatments, while the high concentration induced (4.82 and 41.78%) this activity. The reduction of AChE activity in treated larvae ranged from 6.38 to 17.96% with phenthoate treatments than control larvae (328.82±31.46 nmoles of acetylthiocholine hydrolyzed/mg protein^{-1}/min^{-1}). A medium increase (28.77%) in this activity was detected in LC_{80} treatment of dinotifuran (Table 3). If AChE activity is reduced by > 50%, it is associated with mortality and knockdown (Edwards and Fisher, 1991). Mortality of aphid which exposed to dimethoate in laboratory was negatively correlated with cholinesterase activity (Booth *et al*., 2007).

LC_{30} treatment of five tested insecticides produced increasing (1.41-10.10%) in GST activity of moths (3.31-11.90%) and 3rd instar larval tissues than that of control insects (317.61±13.86 and 259.80±21.44 nmoles of CDNB conjugated/mg protein^{-1} /min^{-1} for moths and larvae, resp.) (Table4). LC_{50} and LC_{80} tests caused inhibition (0.72-10.47and 3.15-15.10%) in moths and larvae enzyme, while these two concentrations of fenoxycarb induced enzyme activity (24.79 and 27.90%) in moths and larvae. A wide variety of synthetic insecticides are known to suppress the activity of key reduction enzymes including GST (Papadopoulos *et al*.,2004; Cossio –Bayugar *et al*., 2002; Wu *et al*.,2009).GST activity in *Spodoptera littoralis* (Boisd.) exposed to lindane for 8 h showed a 1.5 fold elevation in enzyme activity over control (Lagadic *et al*., 1993).

The PCMAN-demethylase activity in moths and 3rd instar larvae (3.78±1.33 and 2.40±0.82 nmoles of p-chloroaniline/mg protein^{-1}/min^{-1}, resp.), was increased with treatments; there is a positive correlation between the concentration of insecticide and the activity of enzyme in moths and larval homogenates. The high significant increase in enzyme activity (52.18 and 59.17%) was recorded with LC_{80} phenthoate in moth and larval homogenates. Dinotefuran caused the lowest induction (18.25 and 27.50%) to moths and larvae enzyme. The evaluating mechanisms would be involved in insecticide resistance of populations of *T. absoluta*, presenting an increased MFO activity in populations (Reyes *et al*., 2012). It appears that enhanced oxidative metabolism mediated by cytochrom P450 monooxygenase was a major mechanism for insecticide resistance in the western flower thrips (Chen *et al*., 2011).

In conclusion

The five tested insecticides had high contact toxic effect on moths and 3rd instar larvae of *T. absoluta*, so that we can use them in control of this insect in greenhouses and open fields. Imidacloprid was the superior toxicant against to moths and larvae of this insect. Phenthoate and thiocyclam H.O. had high toxic effect on two stages. Dinotefuran seemed to have moderate effect on moths and the same trend was observed with fenoxycarb on larvae. The activity of acetycholinesterase (AChE), glutathione-S-transferase (GST) and monooxy- genase (PCMAN-demethylase) was higher in whole body homogenate of untreated moths (1.5, 1.2 and 1.58 times, resp.), than that of 3rd instar larvae. The exposure of moths and 3rd instar larvae to LC_{30}, LC_{50} and LC_{80} of tested insecticides caused significant reduction or slight increasing in activity of AChE and GST. Elevation of

PCMAN-demethylase activity was recorded with all insecticide treatments.

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ARABIC SUMMARY

التأثيرات الإبادية و البيوكيمائية لمبيدات مختلفة على صانعة أنفاق الطماطم

ايمان محمد مصطفى رضوان و حنان صلاح الدين طه
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تم أختبار التأثير الإبادى لمبيدات :داى نوتيفران - أميداكلوبراييد - فينوكسى كارب – ثايوسكليم – فينثويت على حشرة صانعة أنفاق الطماطم المجمعة من الصوب الزراعية تحت الظروف المعملية.

توضح النتائج أن المبيدات الخمسة المختبرة لها تأثير إبادى قوى على فراشات ويرقات العمر الثالث للحشرة بالملامسة ، وكانت الفراشات أكثر حساسية من اليرقات لتاثير المبيدات. وقد أثبت مبيد أميداكلوبراييد كفاءة فائقة ضد كل من الفراشات و اليرقات ولذلك كان معامل مقاومةالحشرة له قليل جدا"(10،- 13،) ،كما كان لمبيدى فينثويت و ثايوسكليم تأثير سام عالى على كلا الطورين وكان مبيد داى نوتيفران هو الأقل كفاءة على الفراشات وكان لمبيد فينوكسى كارب نفس الإتجاه على اليرقات.

وعند تقدير أنشطة إنزيمات : أسيتيل كولين إستريز- جلوتاثيون إس ترانسفيريز- مونوأوكسيجينيز فى طحين أجسام الحشرات غير المعاملة وجد أنها فى الفراشات أعلى (1،5-1،2-1،58 مرة على التوالى) من اليرقات.

وقد تسبب تعرض الفراشات واليرقات لتركيزات تحت مميتة (30، 50، 80 % موت) من المبيدات المختبرة لنقص معنوى (25.00-51،11%) أو زيادة(28،77-41،78 %) فى نشاط إنزيم أسيتيل كولين إستريزفى طحين أجسام الحشرات الحية المعاملة بالمقارنة بالحشرات الغير معاملة. كما أحدثت المعاملة بتركيز 80% لمبيد فينثويت نقص معنوى فى نشاط الإنزيم فى حين تسبب مبيدى أميداكلوبراييد و داى نوتيفران فى زيادة معنوية للنشاط .

كما أحدثت المعاملة بتركيز30% موت من المبيدات المستخدمة زيادة طفيفة (1،48-11،90%) لنشاط إنزيم جلوتاثيون إس ترانسفيريزفى أجسام الحشرات المعاملة وتسبب التركيزين الآخرين فى نقص أو زيادة النشاط. ووضح هذا التأثير فى معاملة 80% لمبيد فينوكسى كارب حيث إرتفع النشاط بقيم متوسطة (24،79-27،90%) فى أجسام الفراشات واليرقات المعاملة.

وتشير النتائج أيضا لوجود تناسب طردى بين الزيادة فى نشاط إنزيم مونوأوكسيجينيز فى أجسام الحشرات المعاملة وتركيز المبيد حيث كانت قيم هذه الزيادة أكثر معنوية فى معاملات التركيز الأعلى. وتراوحت قيم زيادة الإنزيم بين 1،59 الى 52،12% فى الفراشات و5،83 الى59،17% فى اليرقات. وكان لمبيدى فينثويت و أميداكلوبراييد التأثيرالأكبر على زيادة نشاط الإنزيم عن المبيدات الآخرى المستخدمة.