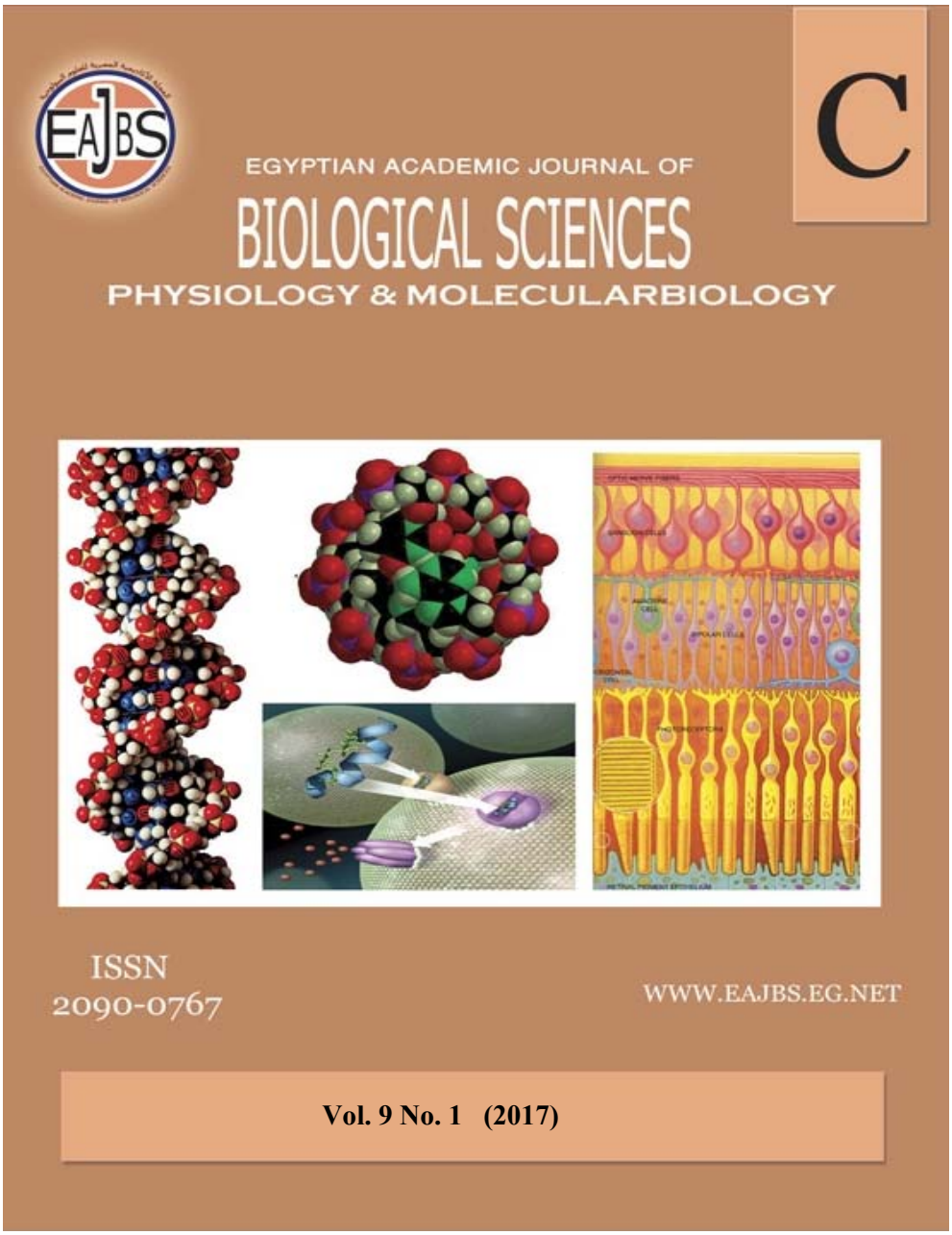

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Efficacy of Certain Pesticides Against Larvae of Tomato Leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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ABSTRACT

Recently the tomato leafminer, *Tuta absoluta* become one of the most devastating pests of tomato in Egypt. Chemical control has been the main method of controlling it. To select the best insecticide for control this insect, susceptibility tests were made under laboratory conditions. Nine insecticides were tested against field *T. absoluta* 2nd instar larvae. Imidacloprid and Thiocyclam-H.O. were the most powerful insecticides and the insect had no resistance to them. Lufenuron, Dinotefuran, Fenoxycarb, Diflubenzuron and Phenthoate gave moderate toxic effect and resistance level except Phenthoate and Fenoxycarb. The two bio-insecticides had the lowest effect on larvae with low level of resistance, but *Bacillus thuringiensis kurstaki* is more toxic than Nuclear Polyhedrosis Virus (NPV). Treatment of *T absoluta* 2nd instar larvae with LC_{50} of tested insecticides decrease the total protein content in the whole body homogenate of treated larvae compared with that of control insects. A significant depletion of protein concentration presented in Phenthoate (-46.8%), Imidacloprid (-43.0%) and Dinotefuran (-39.1%) treatments. LC_{50} treatment of tested insecticides elevated the activity of Cytochrom P450 (PCMAN-demethylase monooxygenase) and Superoxide dismutase (SOD) enzymes in tissues of treated larvae than control. The highest significant increase in PCMAN-demethylase monooxygenase activity (157.1%) was detected in tissues of Phenthoate larvae. The more pronounce increasing in (SOD) activity was observed in Imidacloprid larvae. Fractionation of the total protein contents in whole body homogenate of *T. absoluta* larvae on SDS-PAGE revealed that the depletion of protein concentration correlated to disappearance of several proteins and reduction of bands intensity in treated larvae compared with control. The highest difference in protein pattern (37%) presence between control and treated larvae with Phenthoate , Imidacloprid and Dinotefuran. The aforementioned results revealed that the all tested insecticides had toxic effect on the tomato leafminer and caused biochemical disturbance in their bodies. So that, we can be use small amount from the effective chemical insecticides in rotation with IGRs and bio-insecticides in integrated pest management (IPM) program of this pest.

INTRODUCTION

Tomato plants are attacked by great variety of insects. The tomato leaf miner, *Tuta absoluta* (Meyrick), (Lepidoptera: Gelechiidae) is the most destructive pest to tomato in several countries and in Egypt (Mohammed, 2010). Both yield and fruit quality were reduced by the direct feeding of *T. absoluta* and secondary pathogens that may enter through the wounds made by the insect (Medeiros *et al.*, 2005and Cristina *et al.*, 2008).

The insect deposits eggs usually on the underside of leaves, stems and to a lesser extent on fruits. After hatching, young larvae penetrate into tomato fruits and leaves on which they feed and develop creating mines and galleries. Tomato plants may be attack at any developmental stage, from seedlings to mature stage (OEPP, 2005).

Chemical control has been the main method of control used against *T. absoluta* and the growers normally choose the insecticide in a diversity of options officially registered and recommended (Braham and Hajji, 2011: Hanafy and El-Sayed, 2013). The farmers apply insecticide from 8 to 25 times in a season (Temerak, 2011).The indiscriminate use of synthetic chemical pesticides to control this pest resulted in the rapid development of resistance (Dittrich *et al.,* 1990) and several risks as harmful pesticide residues in fruits (Malhat *et al.*,2012), undesirable effects on humans and natural environments, eliminated natural enemies from crop ecosystems and estimates proved that only a small portion of pesticides applied to the crop reaches the target pest while the major portion reaches to the non-target organisms (Erayya *et al*., 2013).

Applying new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful alternatives in the integrated management approach (Parsaeyan *et al.*, 2013).

Microbial biopesticides are environmentally safe, self perpetuating in nature, specific to target pests etc. Among the microbial biopesticides, bacterial, fungal and virus products occupy a special space in managing several pests (Bidyarani- Devi *et al.*, 2016). Bacterial insecticides were the earliest developed and the most widely used microbial pesticides in the world. One of the most common bacterial insecticides contains *Bacillus thuringiensis* (Bt), which specific to larvae of lepidopteran pests (Peng, 1992). The application of *B. thuringiensis* in Egypt started at 1960 against young larvae of the cotton leaf worm, *Spodoptera littoralis* (El-Husseini, 1981). Each of the three subspecies of *B. thuringiensis* attacks larvae of a specific order, i.e., *B. thuringiensis kurstaki* for Lepidoptera, *B. thuringiensis israelensis* for Diptera and *B. thuringiensis tenebionis* for Coleoptera (El-Husseini, 2006). Viruses are sub microscopic, intracellular and obligate pathogenic entities with nucleic acid and protein. These viruses are often genus or species specific and highly virulent to their hosts. Several groups of viruses having a potential in controlling many pests. Among these groups of viruses, Nuclear Polyhedrosis Virus (NPV) which belongs to the family baculoviridae was exploited widely as a microbial control (Herniou *et al*., 2012). Entomopathogenic viruses are currently used as alternatives to traditional insecticides. Its use should not be generalized because each pest has its own case. In specific cases, viruses proved very effective in managing populations of certain pests as Lepidoptera and Hymenoptera (El-Husseini, 2006). The virus enters the nucleus of infected cells, and reproduces until the cell is assimilated by the virus and produces crystals in the fluids of the host. These crystals will transfer the virus from one host to another (Chiu *et al*., 2012). NPV forming polyhedra like occlusion bodies kills most important crop pests such as *Helicoverpa armigera* and *S. litura* (Bidyarani- Devi *et al.*, 2016).

The exposure to insecticide can lead to physiological and behavioral changes in the organism (Hyne and Maher, 2003). These changes in pest leading to insecticide avoidance, altered penetration, sequestration, and target site

alteration or bio-degradation. Metabolic changes occur through increased bio-degradation of the insecticide, usually through overproduction of detoxification enzymes (Hemingway and Ranson, 2000). Cytochrom P450s are the primary enzyme family associated with resistance to most insecticides. Elevated levels of P450 activity are frequently observed in resistant insects (Hemingway and Ranson, 2000; Brooke *et al.*, 2001). Also, pesticides and allelochemicals cause oxidative stress, characterized by exposure to in vivo excessive reactive oxygen species (ROS) is involved in organisms (Amin and Hashem, 2012). Reactive oxygen species cause oxidative stress leading to the damage of biomolecules such as proteins, lipids, and nucleic acids, resulting in disturbance of homeostasis and cellular death if not eliminated (Hermes-Lima and Zenteno-Savin, 2002). Insect's posses an antioxidant defense system that consists of both enzymatic and non-enzymatic components. Superoxide dismutase enzyme (SOD) is one from the enzymatic components of antioxidant defense system (Krishnan and Kodrík, 2006; Krishnan *et al.*, 2009).

This work aims to evaluate toxic and biochemical effects of the chemical and bio-insecticides on second instar larvae of the tomato leaf miner, *T.absoluta*.

MATERIALS AND METHODS

Insect:

Tuta absoluta larvae were collected from infested tomato plants of Qalubyia Governorate in April 2015 and kept under laboratory conditions (25 ± 2° C, 65 ± 5% R.H. and a photoperiod of 16 L:8 D) on untreated tomato plants for one generation in Central Agriculture Pesticides Laboratory.

Insecticides:

Nine insecticides were used:

1-Two biopesticides:

-*Bacillus thuringiensis* subsp. *kurstaki (*Bt, Dipel DF WG 32×10^6 International Units\ gm- Valent BioSciences -Canada).

-Nucleopolyhedrovirus(NPV, Littovir SC 2×10^{12}International Units\L Bienvenue Chez Andermatt Biocontrol,Switzerland).

2-Two chitin synthesis inhibitor insecticides:

-Lufenuron (Match 5%EC-Syngenta, Switzerland).

-Diflubenzuron (Dimilin 48%SC-Chemetura Europe Limited, United Kingdom).

3-One Juvenile Hormone Mimic insecticide:

-Fenoxycarb (Ethylcarbamate, Insegar 25% WP- Sumitomo Chemical Corporation Japan).

4-Two neonicotinoid insecticides:

- Imidacloprid (Admire 20% SC-Bayer Crop Science Ltd, India)

- Dinotefuran (Oshin 20% SG- Mitsui chemicals Agro, Japan).

5-One organophosphorus insecticide:

-Phenthoate (Elsan 50%EC- Nissan Chemical Industries Ltd, Japan)

6-One Thiocyclam insecticide:

-Thiocyclam-Hydrogen-Oxalate (Evisect S 50%SP- Nippon Kayaku Co., Japan.)

Bioassay:

Leaf-dip bioassay method used to detect the response of second instar larvae of *T. absoluta* F1 progeny to tested insecticides. Ten serial aqueous concentrations of each pesticide were prepared. Fresh tomato leaflets were individually dipped in each prepared concentration of the tested insecticides for 10 seconds with genital agitation, control leaflets were dipped in water only (Five replicates for each insecticide concentration and control), then the leaflets were left to dry. The dried leaflets were placed on a slightly moistened filter paper covering the bottom of clean glass cages (15D× 15H) to keep the leaf material turgi1d throughout the bioassay period. Ten 2nd instar larvae were carefully placed using

a fine soft brush in each cage and kept under lab conditions (IRAC, 2010). Mortality was recorded after 72 hr. of treatment and corrected by Abbott's formula (Abbott, 1925).

Sub lethal concentrations, LC_{50} and LC_{95} of treated larvae were calculated by using SAS probit program (SAS, 1997). To assess the resistance of a given population, the resistance coefficient (Wegorek *et al.,* 2011) was calculated as follows:

Resistance Coefficient (RC) = LC_{95} of field insects/ recommended field concentration.

Biochemical assay:

After toxicological experiments, the survivor larvae of *T.absoluta* from untreated (control) and LC_{50} treatments were removed and frozen for subsequent biochemical analysis. The total protein content, fractionated proteins and activity of Cytochrom P450 (PCMA N-demethylase monooxygenase) and Superoxide dismutase (SOD) enzymes were determined in the whole body tissues of control and treated larvae.

Protein and enzymes extract:

Samples of 500 mg from control and treated larvae were homogenized in 1 ml Sodium Phosphate buffer (0.1M pH7) using Teflon glass homogenizer and centrifuged at 10.000rpm for 15 min at 4°C (five replicates of each sample).The supernatant was used as a source of protein and SOD enzyme tests.

For Cytochrom P450(PCMAN-demethylase monooxygenase) activity ,100 mg of control and treated insects were homogenized in 0.2 ml Sodium Phosphate buffer (0.1M pH7.8) containing 10% glycerol ,1mM DTT(1,4-dithiothreitol) 1mM EDTA (ethylene diamine tetra acetic acid),1mM PMSF (phenyl methane sulfonyl floride) and 1mM PTU (N-phenyl thiourea) (five replicates of each sample). The samples were centrifuged at 10.000rpm for 10 min at 4°C. The supernatants were centrifuged at 18.000 rpm for 30 min at 4°C. The produced supernatants were collected and used as enzyme source ((Chen *et al*., 2011; with some modifications).

Total content of proteins:

Total protein content was determined based on Biuret test (Henry, 1964), using Kit purchase from dp international laboratory. A mixture of 1.0 ml of the total protein reagent (0.2N Sodium hydroxide, 18mM/L Sodium Potassium tartrate, 12mM/L Potassium iodide and 6mM/L Cupric sulfate), 20µl of each sample and 20µl of deionized water then incubated for 5 minutes at 25^{o}C. Read and recorded the absorbance at wave length of 546 nm versus the reagent blank as reference and Standard. The total protein concentration in the whole body homogenate of control and treated larvae was represented as µg/mg body weight of insects.

Activity of Cytochrom P450 enzyme (PCMAN-demethylase monooxygenase):

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), 0.4unite 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-chloroaniline was quantified by comparing absorbance at 445 nm to simultaneously determined standard

curve (0-50nmol). The activity of enzyme was represented as n moles of p-chloroaniline /mg protein^{-1}/min^{-1}.

Activity of Superoxide dismutase (SOD) enzyme:

The activity of Superoxide dismutase enzyme (SOD) was measured according to method of Beauchamp and Fridovich (1971) with some modification by Krishnan *et al*., (2002) tests the ability of SOD to inhibit the reduction of Nitro blue tetrazolium by the superoxide anion generated photo chemically. One milliliter of assay mixture consisted of 50 mM Sod. Phosphate buffer, pH 7.8, 13 mM Methionine, 75 µM Nitro blue tetrazolium, 2 µM riboflavin, 0.1 mM EDTA, and enzyme extract. Riboflavin was added last, the samples were placed inside a light box contains three comptalux bulbs (100 W, Philips Ltd,) and the reaction was allowed to run for 15 min. The reaction was stopped by switching off the light. A non irradiated reaction mixture, which was run in parallel, did not develop color and served as a control. The absorbance was read at 560 nm. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

Total protein content and enzyme activities of all samples are reported as mean ± standard error and statistically analyzed using Excel Microsoft Office and Student's t-test Program. Differences were considered significant at $p< 0.05$ level.

Fractionation of proteins:

The basic principle of proteins fractionation by electrophoresis is the movement of the charged molecules towards an electrode with the opposite charge through a supporting medium. Proteins of tissues were separated on 11% Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). In a solution of SDS and 2-mercaptoethanol, proteins dissociated into subunits with rod like shape, the rod diameter is thought to be constant, while the long axis varies in proportion to the molecular weight. The later value can be determined by comparing the electrophoretic mobility of unknown proteins with the mobility of known standard protein markers. After fractionation process the gel was photographed, scanned and analyzed with Gene Tools program. Similarity index, commonality band ratio and polymorphism in samples protein were calculated according to Nei and Li (1979).

RESULTS

Bioassay:

Toxic effect of the nine tested insecticides against 2nd larval instar of field *T. absoluta* was shown in Table 1. Imidacloprid was the most powerful insecticide on tomato leaf miner larvae ($LC_{50=}$6.32 ppm) followed by Thiocyclam-H.O. ($LC_{50=}$9.98 ppm), Lufenuron ($LC_{50=}$14.65 ppm), Dinotefuran ($LC_{50=}$15.11 ppm), Fenoxycarb ($LC_{50=}$16.88 ppm), Diflubenzuron ($LC_{50=}$19.65 ppm) and Phenthoate ($LC_{50=}$20.76 ppm).The two bio-insecticides had the lowest effect on these larvae, but Bt is more toxic ($LC_{50=}19.7x10^3$ IU/Ml) than NPV virus($LC_{50=}3.1x10^5$ IU/Ml).

Comparison the LC_{95s} of tested insecticides with the recommended concentration of them revealed that the tomato leaf miner larvae had no resistance (Resistance Coefficient, RC=0.5fold) to Imidacloprid and Thiocyclam and low resistance (1. 9 folds) to NPV virus. On the other hand these larvae responded to Dinotefuran, *B. thuringiensis*, Lufenuron and Diflubenzuron by medium resistance (2.7-4.9 folds), to Phenthoate by high resistance (5.1 folds) and gave very high resistance (10.6 folds) with Fenoxycarb treatment.

Table 1: Toxic effect of chemical and bio- insecticides on2nd instar larvae of the field tomato leafminer, *Tuta absoluta*.

Insecticide	Slope ± S.E.	LC_{50} (ppm) (95%CL)	LC_{95} (ppm) (95%CL)	T.I. (%)	Rec. Con. (ppm)	RC (fold)
Imidacloprid	1.56 ± 0.36	6.32 (3.41 - 8.77)	129.56 (69.85 -251.10)	100	250	0.5
Thiocyclam H.O.	1.28 ± 0.52	9.98 (6.05 - 17.34)	594.24 (354.21 -829.17)	63.3	1250	0.5
Lufenuron	1.51 ±0.24	14.65 (5.28 - 26.60)	182.66 (70.08-669.49)	43.1	40	4.6
Dinotefuran	1.12 ± 0.43	15.11 (10.49 - 18.19)	685.35 (316.85-2341.47)	41.8	250	2.7
Fenoxycarb	1.15 ± 0.26	16.88 (12.79 - 24.47)	1322.78 (832.34 - 2254.71)	37.4	125	10.6
Diflubenzuron	1.65 ± 0.41	19.65 (14.32 - 28.84)	1470.09 (567.92 - 4514.48)	32.2	300	4.9
Phenthoate	1.68 ± 0.31	20.76 (15.63- 35.28)	1178.54 (824.33- 3368.29)	30.4	230	5.1
Bio-insecticide	Slope ± S.E.	LC_{50} (IU/Ml) (95%CL)	LC_{95}(IU/Ml) (95%CL)	T.I. (%)	Rec.Con. (IU/Ml)	RC value
B.thuringiensis	1.54 ±0.28	$19.7x10^3$ ($12.4 x10^3$ – $26.2x10^3$)	$172.3x10^3$ ($88.3 x10^3$-$304.5x10^3$)	100	($64x10^3$)	2.7
NPV	1.75 ±0.24	$3.1 x10^5$ ($2.3 x10^5$ – $4.6 x10^5$)	$9.6 x10^5$ ($6.7 x10^5$- $14.7 x10^5$)	6.35	($5x10^5$)	1.9

CL = Confidence Limits, T.I. =Toxicity Index,
Rec.Con. =Recommended Concentration, RC = Resistance Coefficient

Biochemical assay:

Total content of proteins:

Treatment of *T absoluta* 2nd instar larvae with LC_{50} of tested insecticides caused a decreasing in the total protein content of larval tissues compared with that of control insects (434.7±17.42 µg/mg of body weight). The values of protein concentration reached to 352.4±25.53, 335.6±8.55, 311.5±18.48, 304.2±9.33, 298.1±12.37, 285.9±22.86, 264.8±38.64, 247.8±15.82 and 231.3±29.02 µg/mg of body weight in the whole body homogenate of larvae treated with Diflubenzuron, Thiocyclam-H.O., Fenoxycarb, NPV, Lufenuron, Bt, Dinotefuran, Imidacloprid and Phenthoate, respectively. These data revealed a presence of high significant depletion of protein concentration in Phenthoate (-46.8%), Imidacloprid (-43.0%) and Dinotefuran (-39.1%) treatments. A significant decreasing in insect proteins (-34.2, -31.4, -30.0 and -28.3%) was detected in *B. thuringiensis*, Lufenuron, NPV and Fenoxycarb treatments, resp. Thiocyclam-H.O. and Diflubenzuron produced a medium decrease (-22.8 and -18.9%) in proteins of treated insect (Table 2).

Activity of Cytochrom P450 (PCMAN-demethylase monooxygenase) enzyme:

The activity of Cytochrom P450 (PCMAN-demethylase monooxygenase) was highly increased in the whole body homogenate of *T. absoluta* larvae post treatment with LC_{50} of the tested insecticides than control ones (Table2). This activity reached to 2.8±1.24 n moles of p-chloroaniline/mg^{-1} protein/ min^{-1}in tissues of control insects. The highest significant increase in enzyme activity (157.1 %) was recorded in tissues of treated larvae with Phenthoate. Also, a very high elevation of enzyme activity (125.0, 67.9, 64.3 and 46.4%) was detected in Imidacloprid, NPV, Fenoxycarb and *B. thuringiensis* treated larvae, resp. Lufenuron and

Diflubenzuron produced medium elevation of enzyme (28.6 and 21.4%) in treated larvae. Thiocyclam-H.O. and Dinotefuran caused the lowest induction (14.3 %) to enzyme in treated larvae.

Activity of Superoxide dismutase (SOD) enzyme:

The activity of Superoxide dismutase (SOD) enzyme increased in *T. absoluta* 2nd instar larvae after exposure to LC_{50} of the tested insecticides (Table 2). The highest increase in enzyme activity was detected in Imidacloprid (93.3%) treated larvae. Avery high significant increase (83.2, 70.1, 66.3, and 49.7%) presented in Fenoxycarb, Thiocyclam-H.O., Phenthoate and *B. thuringiensis* treated larvae, resp. Dinotefuran treatment produced significant elevation (39.1%) of enzyme activity but Lufenuron, Diflubenzuron and NPV caused a slight activation (13.9, 9.2 and 4.3%, resp.) of larval enzyme.

Table 2: Concentration of total proteins and activity of Cytochrom P450 (PCMAN- demethylase monooxygenase) & Superoxide dismutase (SOD) enzymes in the whole body homogenate of *Tuta absoluta* 2nd instar larvae treated with LC_{50} of tested insecticides

Insecticide	Total protein concentration (µg/ mg B.W.)		Activity of Cytochrom P450(PCMAN-demethylase monooxygenase) (nmole of p- chloroaniline /mg^{-1} protein /min^{-1})		Activity of Superoxide dismutase SOD (U/mg^{-1} Protein)	
	Activity ± S.E	Change %	Activity ± S.E	Change %	Activity ± S.E	Change %
Imidacloprid	247.8^{d} ±15.82	(-) 43.0	6.3^{b} ±0.86	(+) 125.0	94.5^{a} ±1.42	(+) 93.3
Thiocyclam H.O.	335.6^{b} ±8.55	(-) 22.8	3.2^{e} ±0.79	(+) 14.3	83.2^{b} ±3.15	(+) 70.1
Lufenuron	298.1^{c} ±12.37	(-) 31.4	3.6^{e} ±0.54	(+) 28.6	55.7^{d}±10.24	(+) 13.9
Dinotefuran	264.8^{d} ±38.64	(-) 39.1	3.2^{e} ±1.15	(+) 14.3	68.0^{c} ±6.87	(+) 39.1
Fenoxycarb	311.5^{c} ±18.48	(-) 28.3	4.6^{c} ±0.81	(+) 64.3	89.6^{a} ±1.35	(+) 83.2
Diflubenzuron	352.4^{b} ±25.53	(-) 18.9	3.4^{e} ±1.07	(+) 21.4	53.4^{d} ±9.86	(+) 9.2
Phenthoate	231.3^{d} ±29.02	(-) 46.8	7.2^{a} ±0.46	(+) 157.1	81.3^{b} ±4.32	(+) 66.3
B.thuringiensis	285.9^{c} ±22.86	(-) 34.2	4.1^{d} ±0.82	(+) 46.4	73.2^{c} ±5.76	(+) 49.7
NPV	304.2^{c} ±9.33	(-) 30.0	4.7^{c} ±0.63	(+) 67.9	51.0^{d}±11.18	(+) 4. 3
Control	434.7^{a} ±17.42	0.0	2.8^{e}±1.24	0.0	48.9^{d} ±6.11	0.0

S.E =standard error, (+) increase, (-) decrease

Mean of total protein concentration and enzyme activity values in the same column followed by different letters are significantly different ($P < 0.05$).

Change%= mean of treated larvae-mean of control larvae / mean of control larvae x 100

Fractionation of proteins:

The total protein contents in control and LC_{50} treated 2nd instar larvae of *T. absoluta* were fractionated on SDS polyacrylamide gel electrophoresis (Fig. 1 and Table 3). Nineteen bands appeared in control and treated larval tissues according to their molecular weights and relative mobility on the gel. The total numbers of protein bands were 19, 14, 13, 15, 13, 12, 12, 14, 14 and 12 appeared in the whole body homogenate of control (C), *B. thuringiensis* (Bt), NPV(V), Diflubenzuron (D), Lufenuron (L), Phenthoate (P), Imidacloprid (I), Thiocyclam-H.O.(T), Fenoxycarb (F) and Dinotefuran (Df) treated larvae. There were eleven common bands; 1, 2, 4, 6, 7, 10, 11, 15, 16, 17and 18 with Molecular Weights of 201.42, 194.56, 170.89, 130.78, 115.23, 65.36, 57.55, 26.65, 21.37, 17.52 and 14.13 (KDa) and Relative Mobility values of 0.053, 0.097, 0.171, 0.248, 0.283, 0.494, o.536, 0.772, 0.808,0.834 and 0.863 (Rm),respectively, appeared in control and treated insects. Band no.9 (83.57 KDa and 0.412 Rm) was common in all larval samples except Df. Band no.5 (159.22 KDa and 0.202 Rm) shared in five samples C, D, T, Fand

Df. Also, band no.13 (38.68 KDa and 0.667 Rm) appeared in five samples C, Bt, V, D and L. Band no.8 (94.49 KDa and 0.376 Rm) shared in four samples C, D, T and F. Band no.14 (32.16 KDa and 0.710 Rm) presented in C & Bt and disappeared in other samples. Bands no. 3, 12, 19 (M.W. 185.38, 45.95&8.56 KDa and 0.125, 0.623&0.930 Rm) are specific proteins to control larvae and disappeared in tissues of treated ones. The denistometeric scanning of protein pattern revealed that the concentration of bands in treated larvae was very low compared with those of control. The highest commonality band ratio and similarity index (1) with no difference in SDS protein pattern presented between V&L, P&I and T&F treated larvae. A highest similarity and commonality band ratio (0.96 and 0.90) and the lowest polymorphism (10%) in protein bands were presented between Bt & V larvae. Also, a high similarity and commonality band ratio (0.92 and 0.84) with low polymorphism (16%) in protein bands were presented between Df & P & I. A moderate value of similarity, commonality and polymorphism in protein (0.88 and 0.79 and 21% , rep.) was presented between Control & D and (0.85, 0.74 and 26%, rep.) in Control & Bt, T and F larvae, resp. Low value of similarity and commonality (0.81 and 0.68) and high polymorphism (32%) were detected between Control &V & L larvae,. The highest difference (37%) and very low similarity and commonality protein band ratio (0.77 and 0.63) were presented between Control & P, I and Df larvae.

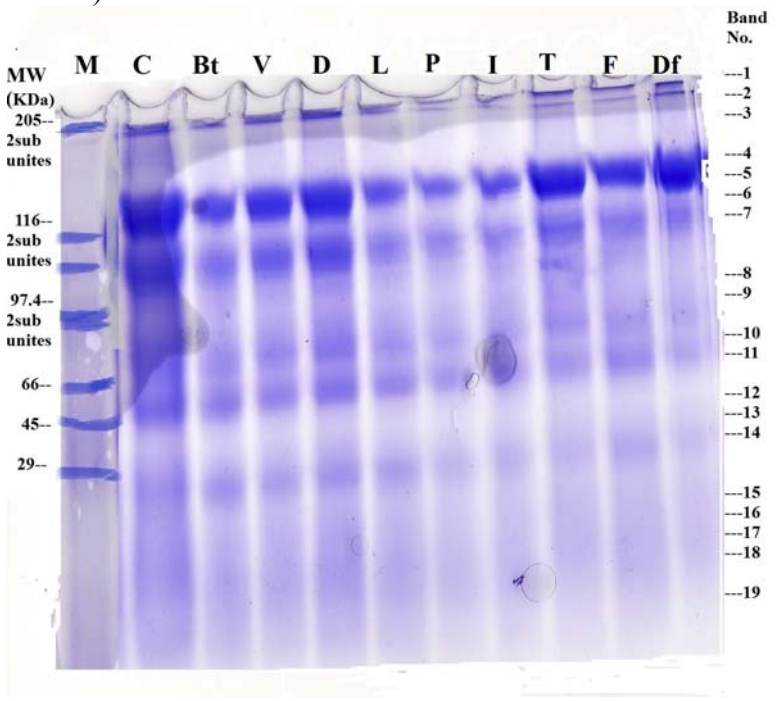

Fig.1: SDS Polyacrylamide gel of denatured protein patterns in the whole body homogenates of *Tuta absoluta* 2nd instar larvae treated with LC_{50} of tested insecticides . Lane: M for the protein molecular weights marker. Lane :C for control larvae, Lanes; Bt, V, D, L, P, I, T, F and Df for treated larvae with LC_{50} of *B.thuringiensis*, NPV, Diflubenzuron, Lufenuron, Phenthoate, Imidacloprid, Thiocyclam H.O., Fenoxycarb and Dinotefuran respectively. Protein band numbers are indicated on the right side of the gel. Molecular weights of marker bands are indicated on the left side of the gel.

Table 3: Relative mobility values and molecular weights of SDS protein bands detected in the whole body homogenates of *Tuta absoluta* 2nd instar larvae treated with LC_{50} of tested insecticides

Band No.	Relative mobility value (Rm)	Molecular weight (KDa)	Presence of bands									
			C	Bt	V	D	L	P	I	T	F	Df
1	0.053	201.42	+	+	+	+	+	+	+	+	+	+
2	0.097	194.56	+	+	+	+	+	+	+	+	+	+
3	0.125	185.38	+	-	-	-	-	-	-	-	-	-
4	0.171	170.89	+	+	+	+	+	+	+	+	+	+
5	0.202	159.22	+	-	-	+	-	-	-	+	+	+
6	0.248	130.78	+	+	+	+	+	+	+	+	+	+
7	0.283	115.23	+	+	+	+	+	+	+	+	+	+
8	0.376	94.49	+	-	-	+	-	-	-	+	+	-
9	0.412	83.57	+	+	+	+	+	+	+	+	+	-
10	0.494	65.36	+	+	+	+	+	+	+	+	+	+
11	0.536	57.55	+	+	+	+	+	+	+	+	+	+
12	0.623	45.95	+	-	-	-	-	-	-	-	-	-
13	0.667	38.68	+	+	+	+	+	-	-	-	-	-
14	0.710	32.16	+	+	-	-	-	-	-	-	-	-
15	0.772	26.65	+	+	+	+	+	+	+	+	+	+
16	0.808	21.37	+	+	+	+	+	+	+	+	+	+
17	0.834	17.52	+	+	+	+	+	+	+	+	+	+
18	0. 863	14.13	+	+	+	+	+	+	+	+	+	+
19	0.930	8.56	+	-	-	-	-	-	-	-	-	-
Total band no.			19	14	13	15	13	12	12	14	14	12

(+) Present (-) Absent

DISCUSSIONS

Data revealed that the chemical pesticides proved high toxic effect on field *T. absoluta* 2nd instar larvae than IGRs and bio-insecticides. Results showed that Imidacloprid and Thiocyclam-H.O. are the highly effective insecticides and the insect had no resistance to them. Lufenuron, Dinotefuran, Fenoxycarb, Diflubenzuron and Phenthoate gave moderate toxic effect and resistance level except Phenthoate and Fenoxycarb. The two bio-insecticides had the lowest effect on larvae with low level of resistance. These results are in conformity with the findings of several researchers such as *Santos et al*., (2011) who mentioned that the chemical pesticides continue to be an important component of insect pest management even with the development of other control methods. Susceptibility of field populations of *T.absoluta* to insecticides was positively correlated with the number of chemical sprays in the field. The reduction of susceptibility observed, is more probably due to cross-resistance with other insecticides more widely applied in the past (i.e. organophosphates and pyrethroids). The resistant populations have been collected in locations where the presence of the pest has lead to the use of a high number of insecticide sprays during the crop season (Reyes *et al*., 2012*).* The reduced-risk insecticides, Imidacloprid providing rapid knockdown and mortality followed by residual antifeedant activity on rose chafer beetle adults (Isaacs *et al*., 2002). Indoxacarb, Spinosad, Imidacloprid,

Deltamethrin and *B. thuringiensis* var. *kurstaki* have successfully been used to control of *T. absoluta* larval infestations in Spain (Russell, 2009). Moussa *et al.*, (2013) reported that the tomato leafminer causes severe damage to the foliage and fruit of tomato in Egypt at 2010 and 2011 seasons. Chemical pesticides such as Chlorant-raniliprole, Chlorfenapyr, Indoxcarb, Chlofenapyr mixed with Indioxcarb, Spinosad, Spinosad mixed with Abamectin, Emamectin benzoate and Imidacloprid provide excellent control against insect while a biopesticide *B. thuringiensis* provides moderate control. Nazarpour *et al*., (2016) indicated that Bt, Azadirachtin and mixture of them significantly suppressed the larval density and caused 100% reduction in fruit and foliage damage compared to the untreated plants. Microbial biopesticides acts as a solution as they are environmentally safe, self perpetuating in nature, specific to target pests etc. Among the microbial biopesticides, viruses after bacterial and fungal products occupy a special space in managing several pests (Bidyarani-Devi *et al.*, 2016).

Insect Growth Regulators (IGRs) have a much slower mode of action than synthetic chemical insecticides but they cause malformations of treated insects. IGRs include juvenile hormone mimics (Fenoxycarb) and chitin synthesis inhibitors (Lufenuron) inhibit the production of chitin, a major component of the insect exoskeleton. The treated insects became unable to synthesize a new cuticle, and therefore unable to successfully moult into the next stage (El-Aswad**,** 2007). Treatment of *S. littoralis* last instar larvae with Novaluron (IGR) resulted in some features of impaired morphogenesis and remarkably accelerated the ovarian maturation because Novaluron prohibited this vital process (Hamadah *et al*., 2015).

Proteins are important for individual level fitness associated traits such as body size, growth rate, and fecundity, and at higher levels of organization they have been linked to population dynamics, life histories and even biological diversification (Fagan *et al*., 2002). Treatment of *T. absoluta* larvae with the LC_{50} of tested insecticides produced high significant reduction in larval tissue proteins, especially Phenthoate and Imidacloprid treatments. The same observations were detected by Abdel-Hafez *et al*., (1988) who mentioned that the IGR; Diflubenzuron and Triflumuron treatments reduced the level of proteins and free amino acids in laboratory and resistant strains of *S. littoralis.* Etebari *et al*., (2005) showed that many insecticides treatments decrease feeding efficiency and protein amount of insects. The decreasing of the total soluble protein contents in supernatant of the homogenated *Musca domestica* larvae post- LC30 treatment with *B. thuringiensis* β exotoxin as compared to control (Abuldahab *et al.*, 2011). Piri *et al.*, (2014) revealed that treatment of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) larvae with sublethal concentrations (LC_{30} and LC_{40}) of spinosad reduced the protein content in larvae compared to control.

Biochemical mechanisms including the insensivity of target sites to insecticides and enhanced detoxification rate by several detoxifying mechanisms. MFO family (Cytochrom P450s) acts as effectively reducing the efficacy of insecticides on pests (Wang *et al*., 2009). High increase of Cytochrom P450 (PCMAN-demethylase monooxygenase) activity was detected in *T. absoluta* larvae treated with LC_{50} of the tested insecticides. Phenthoate and Imidacloprid treatments had the highly significant increasing effect on insect enzyme. The evaluating mechanisms would be involved in insecticide resistance of *T. absoluta* insect, presenting an increased MFO activity in populations (Reyes *et al.*, 2012). The enhanced oxidative

metabolism mediated by cytochrom P450 monooxygenase was a major mechanism for insecticide resistance in the western flower thrips (Chen *et al.*, 2011). By pinpointing the key enzymes associated with insecticide resistance we can begin to develop new tools to aid the implementation of control interventions and reduce their environmental impact on Earth. Recent technological advances are helping us to build a functional profile of the P450 determinants of insecticide metabolic resistance in mosquito insects (David *et al*., 2013).

Cells have interdependent antioxidant defense mechanisms that protect against damage from oxidative stress. Insect's posses an antioxidant defense system that consists of both enzymatic and non-enzymatic components. The enzymatic components are superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase ((Krishnan and Kodrík, 2006; Krishnan *et al.*, 2009). The non-enzymatic and enzymatic antioxidant defense systems play a major role in detoxification of pro-oxidant endobiotics and xenobiotics (Kolawole *et al*., 2014.). The reactive oxygen species, such as superoxide radical (O_2^-), hydrogen peroxide H_2O_2, and hydroxyl radical (OH^-) are generated in aerobic organisms under normal metabolism and when exposed to various abiotic and biotic factors during life spans (Halliwell and Gutteridge, 2001). LC_{50} treatment of the tested insecticides caused elevation of SOD activity in tissues of treated larvae and Imidacloprid had the superior effect. Al-Barty (2014) studied the effects of Methylamine avermactine on the oxidative stress indicator, and antioxidant enzyme superoxide dismutase activity in *Sitophilus oryzae* tissues. There were significant increases in SOD activities in the LC_{50} treated insect compared to the control. The insecticide treatment causes an increase in oxidative stress which induces antioxidant defense mechanisms. SOD was stimulated by scavenging superoxide radical to protect the insect from insecticide stress.

Fractionation of the total protein contents in the whole body homogenate of *T. absoluta* larvae on SDS-PAGE revealed that the depletion of protein concentration correlated to disappearance of several proteins and reduction of bands intensity in treated larvae compared with control ones. The highest difference in protein pattern (37%) presence between control and Phenthoate, Imidacloprid and Dinotefuran treated larvae. These findings are in accordance with results obtained by Zidan *et al.*, (2002) who mentioned that treatment of the spiny bollworm, *Earias Insulana* larvae with LC_{50} of Abamectin and Esfenvalerate caused disappearance and appearance of numerous protein bands with different intensity. Treatment of the med fly, *Ceratitis capitata* with LC_{50} from Vertimec, sumi-gold and Tracer caused disappearance, appearance and degrees of difference (22, 29 and 38% resp.) in fractionated proteins of insect tissues compared with control (Radwan and El-Malla, 2008).

CONCLUSION

The tested insecticides are effective against the tomato leaf miner, *T. absoluta* 2nd instar larvae. Chemical insecticides proved high toxic effect on insect than IGRs and bio-insecticides. Imidacloprid and Thiocyclam-H.O were more potent toxicant and the insect had no resistance to them. Lufenuron, Dinotefuran, Fenoxycarb, Diflubenzuron and Phenthoate gave moderate toxic effect and resistance level except Phenthoate and Fenoxycarb. The two bio-insecticides had the lowest effect on larvae with low level of resistance. Treatment of tomato leaf miner larvae with the LC_{50} of tested insecticides produced a high significant reduction in

larval tissue proteins. This depletion in protein concentration was correlated to disappearance of several proteins and reducing of bands intensity in treated larvae. LC_{50} treatment of tested insecticides cause elevation of detoxification enzyme, Cytochrom P450 (PCMAN-demethylase monooxygenase) and antioxidant enzyme, superoxide dismutase. Finally, all tested insecticides had toxic effect on the tomato leafminer and caused biochemical disturbance in their bodies. So that, we can be use small amount from the effective chemical insecticides in rotation with IGRs and bio-insecticides in integrated pest management (IPM) program of this pest.

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RABIC SUMMERY

فاعلية بعض المبيدات على يرقات حشرة صانعة أنفاق الطماطم

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المعمل المركزى للمبيدات- مركزالبحوث الزراعية- دقى- جيزة- مصر

أصبحت حشرة صانعة أنفاق الطماطم فى الآونة الأخيرة واحدة من أكثرالآفات المدمرة لنباتات الطماطم فى مصر. ومن أهم الطرق المتبعة فى مكافحة هذه الآفة هو استخدام المبيدات الكيماوية. ولاختيار أفضل مبيد يستخدم فى مكافحة هذه الآفة الخطيرة لابد من إختبار حساسية تلك الآفة للعديد من المبيدات تحت الظروف المعملية .ولذا فقد اختبرت فاعلية تسعة مبيدات ضد العمر اليرقى الثانى لسلالة حقلية من حشرة صانعة أنفاق الطماطم. وتشير النتائج الى أن مبيدى أميداكلوبريد وثايوسكليم هما الأكثر سمية ليرقات العمر اليرقى الثانى لحشرة صانعة أنفاق الطماطم ولم تبدى الحشرة لهما أى مقاومة . وكان لمبيدات ليفينيرون و داى نوتيفران و فينوكسى كارب وداى فليبنزيرون و فينثويت تأثير إبادى ومستوى مقاومة متوسطين عدا مبيدى فينثويت و فينوكسى كارب . وكان المبيدين الحيويين هما الأقل كفاءة إبادية مع ظهور مستوى قليل من مقاومة الحشرة ولكن كان المبيد البكتيرى أكثر كفاءة من المبيد الفيرسى.

وقد تسببت معاملة يرقات العمر اليرقى الثانى لحشرة صانعة أنفاق الطماطم بالتركيز القاتل لنصف العشيرة فى نقص المحتوى الكلى للبروتين فى أنسجة الحشرات المعاملة بالمقارنة بالحشرات غير المعاملة. كما تشير النتائج الي ان أكبر نقص معنوى فى بروتينات اليرقات وجد فى معاملة كل من فينثويت(- ٤٦,٨ %) و أميداكلوبريد(-٤٣,٠%) و داى نوتيفران (-٣٩,١%). وامتد تأثيرنفس المعاملة الى زيادة هائلة فى نشاط إنزيمين من أهم إنزيمات الحماية فى أنسجة الحشرة أحدهما مضاد للتسمم وهو سيتوكروم ب ٤٥٠ (مونوأوكسيحينيز) والآخر مضاد للأكسدة وهو سوبر أوكسيد دسميوتيزفى أنسجة أجسام اليرقات المعاملة مقارنة بالحشرات غير المعاملة. حيث أحدثت المعاملة بمبيد فينثويت إرتفاع معنوى كبير جدا لنشاط إنزيم سيتوكروم ب ٤٥٠ (مونوأوكسيحينيز) (١٥٧,١ %) وكان لمبيد أميداكلوبريد نفس التأثير (٩٣,٣ %) على إنزيم سوبر أوكسيد دسميوتيز. كما أوضحت نتائج تفريد المحتوى الكلى لبروتين أنسجة اليرقات المعاملة وغير المعاملة على إس دى إس بولى أكريل إيميد جيل الكتروفريزيس أن النقص فى المحتوى الكلى للبروتين مرتبط بإختفاء العديد من البروتينات ونقص عدد حزم البروتين فى اليرقات المعاملة عن اليرقات غير المعاملة، فقد ظهر أكبر إختلاف (٣٧%) فى البروتينات بين اليرقات غير المعاملة و المعاملة بمبيدات فينثويت و أميداكلوبريد و داى نوتيفران.

وتوضح النتائج السابق ذكرها أن كل المبيدات المختبرة لها تأثير إبادى على صانعة أنفاق الطماطم وتسبب خلل كيميائى حيوى فى أجسامها . ولذلك يمكن ان نستخدم كميات قليلة من المبيدات الكيميائية الفعالة بالتناوب مع منظمات النمو الحشرية والمبيدات الحيوية فى الإدارة المتكاملة لمكافحة هذه الآفة.