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Toxicity of Insecticides against Tomato Leaf Miner, *Tuta absoluta,* and Its Predators and Determination of Their Residue Dissipation in Tomato Fruits

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ABSTRACT

Tomatoes are an important vegetable crop in different parts of the world, where they are grown year-round. Currently, the most important problem facing tomato growers in the world is the devastating damage caused by the invasive tomato leaf miner, *Tuta absoluta* Meyrick (Gelechiidae: Lepidoptera). In this study, the efficacy of three bioinsecticides (*Bacillus thuringiensis* formulations, spinosad and emamectin benzoate, and two chemical insecticides (indoxacarb and chlorpyrifos) against *T. absoluta* and their adverse effects on predators were conducted in two different governorates in Egypt, based on recommended doses of the tested insecticides. In addition, the residue dissipation of the tested insecticides was determined in tomato fruits. Results indicated that emamectin benzoate was the most effective insecticide, exhibiting the highest reduction in *T. absoluta* density of 78.05 and 87.11% in Giza and Qualybia governorates, respectively, followed by indoxacarb (77.01%) in Giza and spinosad (80.44%) in Qualybia. In addition, our finding proved that the tested biopesticide formulations, especially Bt formulations, are environmentally friendly to two of the most important predators in tomato cultivation: *Nesidiocoris tenuis* and *Macrolophus pygmaeus* Reuter. Moreover, the analysis of insecticide residues on tomato fruits revealed that bioinsecticide residues dissipated faster than conventional insecticide (chlorpyrifos). The results of this research suggested that bioinsecticides could be used for the management of *T. absoluta* under field conditions.

Keywords: Bioinsecticides, *Macrolophus pygmaeus*, *Nesidiocoris tenuis*, Residue, *Tuta absoluta*.

INTRODUCTION

Tuta absoluta Meyrick has been an invasive pest in the Mediterranean region since 2009 (Mohammed, 2010) and in Africa (Erasmus *et al*., 2021). It has infested 60% of tomato crops in different parts of the world (Santana, 2019), which has led to a drastic reduction in both crop yield and fruit quality (Daniel and Bajarang, 2017; Prasannakumar *et al*., 2021). As its food habitat, in which larvae mine within plant tissue, imposes great challenges for control of *T. absoluta* (Sevcan, 2013), chemical control was used, which has adverse effects on natural enemies, causes serious contamination to edible crops and environment and prompts the development of insecticide resistance (Taleh *et al*., 2020). Therefore, bioinsecticides have become a common tactic in pest management strategy (Senthil-Nathan, 2015), such as neem and *Bacillus thuringiensis* formulations, which reduced *T. absoluta* infestation similarly to chemical insecticides without affecting the crop yield (Buragohain *et al*., 2021). Biopesticides, which originate from natural resources, could be useful alternatives to chemical synthetic insecticides and may delay the development of resistance

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(Moustafa *et al.*, 2021). Bala *et al.* (2021) found that the highest resistance was observed in *T. absoluta* with λ-cyhalothrin, propoxur and chlorpyrifos-methyl insecticides (LD_{50} of 7,461.474, 1,023.51 and 106.351 ppm, respectively), while the highest susceptibility was observed from abamectin with LD_{50} of 0.034 ppm.

Belonging to the biopesticides group, *B. thuringiensis* (Bt) is used effectively against several pests and disease vectors from the orders Lepidoptera, Coleoptera, Hymenoptera and Diptera (Pigott and Ellar 2007). *B. thuringiensis* produces more than one hundred kinds of cry toxins that have different specificity and toxicity against target insect pests (Moustafa *et al*., 2013). Active ingredient components of Spinosad are spinosyn A and spinosyn D, which are produced by the soil actinomycete *Saccharopolyspora spinosa* (Salgado, 1998). Spinosad works as an agonist for nicotinic acetylcholine receptor in the central nervous system, causing hyper-excitation, then, involuntary muscle contractions and tremors, resulting in insect paralysis due to neuromuscular fatigue (Orr *et al*., 2009; Salgado and Spark, 2005). Emamectin benzoate is another biopesticide, which is isolated during the fermentation process of *Streptomyces avermitilis* (Liguori *et al*., 2008). It stimulates the release of γ-aminobutyric acid, facilitating flux of chloride ion in nerve axon, causing insect paralysis and death within two to four days (Argentine *et al*., 2002). This product exhibits high efficacy against lepidopteran insect (Gacemi and Guenaoui, 2012).

Identifying and determining the level of trace contaminants in food and environment is critical in protecting human health and environment (Uysal-Pala and Bilisli, 2006). Consequently, several studies have conducted field experiments on tomato fruits to study the dissipation rates of different bioinsecticides (Yoshii *et al.,* 2004; Islam *et al.,* 2009).

Therefore, the objective of this study was to assess the insecticidal activity of different biopesticides including; Bt formulations (Lepinox®, Dipel 2X® and Protecto®), spinosad formulation (Tracer®) and emamectin benzoate formulation (Proclaim®) against *T. absoluta* compared to chemical insecticides (indoxacarb, and chlorpyrifos) under field conditions. Additional objectives included assessment of their adverse effects on two predators, namely, *Nesidiocoris tenuis* and *Macrolophus pygmaeus* Reuter and determination of the dissipation rate of the tested insecticides in tomato fruits.

MATERIALS AND METHODS

Insecticides Tested

Information regarding the tested insecticides is shown in Table 1.

Experimental Design

The field experiments were conducted in two different governorates: Giza "Badrashin Area -Mazghona Village", and Qalyubia "Banha Area - Tersa Village" in October 2018 and 2019, where the temperature ranged from 26°C to 35°C and the relative humidity ranged from 50-80%. Experimental areas were approximately half Fadden (one Fadden equals 4,200 m^2) planted with tomato *Lycorpersicon esculantum* (Mill.) variety "super seeds" tolerant to TYLCV (tomato yellow leaf curl virus- Fareed Gaharaa Company, Egypt).

Experimental areas were divided into equal plot sizes with completely randomized design with three replicates for each treatment. Each plot had three rows with twenty-two plants; the distance between plants within the row was 0.5 m. The recommended packages of practices were followed in establishing plants, except insect pest management.

Field Trial

All treatments were applied using the recommended rates of the tested insecticides, diluted in water, using

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Table 1. Tested insecticides information in the treatments.

Insecticides Common name	Products and formulation used[a]	Recommended rate (g or mL/Fed)	Production company name	Mode of Action[b]
Bacillus thuringiensis	Lepinox 3.75% WP	100 g	Biocont laboratory- Czech Republic	Microbial disruptors of insect midgut membranes
	Dipel 2x 6.4% WP	200 g	Valent Corporation USA	
	Protecto 9.4% WP	300 g	Biocide Production Unit-Plant Protection Research	
Spinosad	Tracer 24% SC	50 mL	Dow AgroSciences, UK	Nicotinic acetylcholine receptor (nAChR) allosteric modulators – Site I
Emamectin benzoate	Proclaim 5% SG	60 g	Syngenta Agro. Switzerland	Glutamate-gated chloride channel (GluCl) allosteric modulators
Chlorpyrifos	Dursban 48% EC	1 L	Dow Agro Sciences, UK	Acetylcholinesterase (AChE) inhibitors
Indoxacarb	Avaunt 15% EC	25 mL	DuPont, France	Voltage Sodium channel blockers

[a] All Bt formulations equivalent to 32,000 IU mg^{-1} of *Bacillus thuringiensis* sp. kurstaki strain. [b] Insecticide Resistance Action Committee (IRAC, 2020).

knapsack sprayer 20 L capacity as foliar application. The control group was formed using water only. The tomato plants infested with *T. absoluta* larvae after one hour post application (zero time), 1, 3, 7, 10 and 14 days were reordered. After 21 days from the first spray, a second spray was performed the same as the previous steps.

Scouting of *T. absoluta* Population

T. absoluta density was determined based on daily sampling program on ten randomized tomato plants by counting the number of active mines with 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae on three plant levels through the upper, middle, and lower leaves until reaching the Economic Threshold (ET), 5 larvae at least/10 plants. The chemical applications had begun according to the pesticides protocol provided by the Egyptian Agricultural Ministry.

Monitoring of the Natural Enemies

Nesidiocoris tenuis and Macrolophus pygmaeus Abundance

Abundance of natural enemies *N. tenuis,* and *M. pygmaeus* were monitored using beating trays and beating cards, which were made of off-white card or yellow paper. They were held under the vegetation with one hand, while the vegetation was shaken ten times using the other hand or a stick (Burts and Retan, 1973). The insects on the plant fell onto the sheet, were counted and collected quickly by hand, using fine forceps or aspirator before they could escape. Abundance was recorded pre- and post-treatment in 1, 3, 5, 7, 10, and 14 days.

The results were interpreted according to the International Organization for Biological Control (IOBC) toxicity categories, in which 1= Harmless (< 25% mortality); 2= Slightly harmful (25–50% mortality); 3= Moderately harmful (51–75% mortality); 4= Harmful (> 75% mortality) (Sterk *et al*., 1999).

Residue Analysis in Tomato Fruits

Sampling

A representative sample (c.a., 1-2 kg) of tomato fruits was collected randomly from sampling plots at interval periods of one hour to 14 days after insecticide applications. Control samples were also collected. The samples were immediately transferred to the laboratory in an icebox (5-11ºC).

Material and Reagents

Four insecticides standards i.e., emamectin benzoate, spinosad, indoxacarb, and chlorpyrifos of 98.2-99.5% purity were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Stock solutions of 100 µg mL^{-1} for each insecticide were dissolved individually with ethyl acetate and acetonitrile according to their polarity and solubility.

Organic solvents (acetone, ethyl acetate, methanol, and acetonitrile), and all residue analysis chemicals were purchased from Sigma- Aldrich (Fluka Analytical) (France).

QuEChERS liquid extraction salt packet was supplied by Interchim, USA, and dispersive kits for cleanup, containing 150 and 25 mg PSA MgSO4, were supplied by Agilent Technologies Inc.

Sample Extraction and Clean Up

The extraction and clean up method used was based on QuEChERS (Anastassiades *et al.,* 2003). Ten g of the frozen tomato fruits homogenate was weighed into 50 mL falcon tubes. Ten mL acetonitrile was added and the tube vigorously shaken using a vortex mixer at maximum speed for 1 minute. A QuEChERS liquid extraction salt packet was added and the whole mixture was centrifuged at 4,000 rpm for 5 minutes at 5 ºC. Upper acetonitrile layer was transmitted to falcon tube (2 mL) and cleanup using 25 mg PSA and 150 mg anhydrous magnesium sulfate, then, the tube was vortexed for 1 min and centrifuged at 4,000 rpm for 5 minutes.

Analytical Technique

The extract was filtered using a 0.22 µm PTFE filter (Millipore, Billerica. MA) and was transferred to a vial for liquid chromatography (HPLC-DAD) to detect the spinosad, emamectin benzoate, and indoxacarb, and gas chromatography (GC-FPD) was used to detect chlorpyrifos.

Data Analysis

The efficacy of the tested insecticides and percentages of the reduction in *T. absoluta* infestation and their side effect on *N. tenuis, and M. pygmaeus* were calculated using the following equation of Henderson and Tilton (1955):

Reduction (%) = [1-(n in Ck before treatment×n in T after treatment)/(n in Ck after treatment×n in T before treatment)]×100

Where, n= Number of mines/plants, T= Treated, and Ck= Control. One-way ANOVA and mean separation tests were performed by Dunnett multiple comparison test with the software of GraphPad prism 9.0.0 (Graph- Pad Software Inc., La Jolla, CA) to compare treatments. The dissipation kinetics of the investigated insecticide residues on tomato fruits was evaluated by calculating the rate of dissipation by comparing the decline of residue concentration against time after treatment. For dissipation of the tested insecticides as above, the following exponential relationships corresponded to the general first-order kinetics equation**:**

$Ct=C0e^{-kt}$

Where, *Ct* means the Concentration of the insecticide residue at the time of *t*, *C0* signifies the initial deposits after treatment

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and k is the constant rate of insecticide dissipation per day. From this equation, the dissipation half-life periods ($t_{1/2}$= ln 2/k) of the studied insecticide were determined (Fantke and Juraske, 2013; Hoskins, 1961).

RESULTS

Efficacy of Tested Insecticides against *T. absoluta* Density

Giza Governorate (Badrashin Area)

The number of newly formed mines by larvae of *T. absoluta* within tomato leaves before spraying and up to 14 days after field application are presented in Table (2). The number of mines/10 plants differs between treatments and control. Throughout the experiments, emamectin benzoate was numerically the best treatment, reaching 1.67 mines/10 plants post treatment. This treatment was numerically followed by indoxacarb, spinosad, Bt formulations (Lepinox®, Dipel 2x® and Protecto®) and chlorpyrifos as 2.06, 2.11, 3.06, 3.28, 3.89, and 4.78 mines/10 plants, respectively, compared with control 8.22 mines (df= 7; F= 7,16; P< 0.001). Considering the infection percentages after 14 days of application, the observed number of mines in emamectin benzoate replicates were 17.22%, followed by indoxacarb, spinosad, Bt formulations (Lepinox®, Dipel 2x® and Protecto®) at 20.56, 24.39, 30.26, 31.11, and 37.22% (df= 7; F= 7,16; P< 0.001), respectively. Thus, all insecticides under field conditions caused a noticeable reduction in *T. absoluta* infestations when compared with chlorpyrifos. Emamectin benzoate was the best treatment, reaching a reduction of 78.05%, followed by indoxacarb, spinosad, Bt formulations (Dipel 2x®, Lepinox®, and Protecto®), and chlorpyrifos at 77.01, 69.65, 60.89, 55.24%, 51.19, and 50.79%, respectively.

Qalyubia Governorate (Banha Area)

All treatments significantly registered a smaller number of larvae compared to untreated check (Table 2), which is similar to the results for Giza governorate. Emamectin benzoate recorded a minimum larvae population of 1.39 mines/10 plants compared to other tested insecticides, proving to be the most effective treatment. The next most effective insecticide was indoxacarb at 2.11 mines/10 plants, followed by spinosad, Bt formulations (Dipel 2x®, Lepinox® and Protecto®), and chlorpyrifos at 2.44, 2.89, 3.22, 3.46, and 4.33 active mines/10 plants, respectively. Conversely, control was 9 mines/10 plants. The emamectin benzoate caused the lowest percentage of infection: 13.89% among treatments after 14 days from application. In contrast, the infestation percentage was 21.11, 24.44, 32.22, 33.89, 39.44, and 39.45% for indoxacarb, spinosad, Bt formulations (Lepinox®, Dipel 2x® and Protecto®), and chlorpyrifos, respectively, compared to 90.00% in the control (F= 7,16; df= 7; P< 0.001). After comparing reduction percentages between each treatment with chlorpyrifos, only emamectin benzoate and spinosad caused significant reduction with 87.11 and 80.44%, respectively.

Side Effect of Tested Insecticides on *N. tenuis* and *M. pygmaeus*

Data in Table (3) show that *N. tenuis* was more sensitive than *M. pygmaeus* (Table 4). Scouting for *N. tenuis* predator in both Governorates, Giza and Qualybia showed that Bt formulations had the lowest impact on reducing the percentage of their populations (Tables 3 and 4). The reduction percentage was 0 and 1.03% at the adult and nymph stages for Dipel 2x®, while Lepinox® had 1.01% for adult and 2.36% for nymph, and for Protecto® it was 4.19% for adult and 5.07% for nymph. In contrast, chlorpyrifos had a negative effect on both stages, ranging from 70 to 80% for adult and nymph,

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Table 2. Mean±SE of *T. absoluta* mines/10 plants, infestation percentage on tomato plant and reduction percentage of *T. absoluta* after 14 days from spraying tomato plants with tested insecticides in Giza and Qalyubia governorates.

Insecticides	Rate of application / Feddan	Giza governorate			Qalyubia governorate		
		Plants±SE	Infestation%±SE	Reduction%±SE	Mean of mines/10 plants±SE	Infestation%±SE	Reduction%±SE
Control		8.22± 0.65	83.06 ± 6.56		9.00 ± 0.72	90.00 ± 7.25	
Lepinox (Bt)	100 g	3.06**** ± 0.99	30.26**** ± 10.11	55.24^{ns} ± 12.69	3.22**** ±1.20	32.22**** ± 12.04	73.33^{ns} ± 9.50
Dipel 2x (Bt)	200 g	3.28**** ± 1.09	31.11**** ± 9.76	60.89^{ns} ± 13.95	2.89****±1.19	33.89**** ± 10.78	74.19^{ns} ± 11.19
Protecto (Bt)	300 g	3.89**** ± 0.59	37.22**** ± 9.64	51.19^{ns} ± 12.43	3.46**** ±1.12	39.44****± 10.55	64.74^{ns} ± 8.84
Spinosad	50 mL	2.11**** ± 0.71	24.39**** ± 7.08	69.65^{ns} ± 1.12	2.44**** ±0.88	24.44**** ± 8.81	80.44* ± 7.07
Emamectin benzoate	60 g	1.67**** ± 0.68	17.22**** ± 8.09	78.05^{ns} ± 11.12	1.39**** ±0.53	13.89**** ± 5.33	87.11* ± 6.05
Chlorpyrifos	1 L	4.78*** ± 1.34	47.78*** ± 13.41	50.79^{ns} ± 12.70	4.33**** ±1.11	39.45**** ± 10.49	52.05^{ns} ± 10.56
Indoxacarb	25 mL	2.06****± 0.62	20.56****± 6.23	77.01^{ns} ± 7.35	2.11**** ±0.76	21.11**** ± 5.33	79.52^{ns} ± 6.78

^{ns} Is no significant different at P> 0.05, * Significant different at P≤ 0.05, ** Significant different at P≤ 0.01, *** Significant different at P≤ 0.001, **** Significant different at P≤ 0.0001.

Table 3. Mean±SE of *Nesidiocoris tennis* population and reduction% after 14 days of insecticides application in Giza and Qalyubia governorates.

Insecticides formulations	Giza governorate				Qualybia governorate			
	Mean of population±SE		Reduction%±SE		Mean of population±SE		Reduction%±SE	
	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph
Control	17.67±0.60	14.01±3.03			14.89 ± 0.41	13.28± 0.60		
Lepinox (Bt)	17.73* ±0.40	22.02**** ±5.49	1.01****±3.03	2.36****±4.55	16.28^{ns} ±0.59	15.61* ± 0.74	5.92***± 4.20	0.00* ± 8.85
Dipel 2x (Bt)	16.45^{ns} ±1.26	28.19****±1.33	0.00**** ±0.00	1.03****±5.80	14.44^{ns} ±0.58	12.89* ± 0.52	9.85** ± 3.16	5.81* ± 3.94
Protecto (Bt)	16.06*±0.61	33.41****±5.81	4.19****±1.33	5.07****±4.82	17.28^{ns} ±0.93	16.61^{ns} ± 1.07	4.22*** ± 6.89	4.86* ± 6.10
Spinosad	12.22***±1.07	10.12**±5.76	33.41****±5.81	36.62****±6.57	10.17** ± 0.89	9.12** ± 1.36	44.64^{ns} ±5.42	25.27^{ns} ± 10.12
Emamectin benzoate	16.06*** ±0.80	17.15**±7.18	10.12****±5.76	15.70****±5.09	15.11^{ns} ±1.01	15.06* ± 0.60	16.85** ± 6.11	10.62^{ns} ± 5.34
Chlorpyrifos	4.94****±1.17	6.19****±3.15	70.15±7.18	72.61±5.86	3.39**** ± 1.22	3.12**** ± 0.78	81.46 ± 7.07	81.33 ± 4.22
Indoxacarb	12.03****±0.65	12.00^{ns}±0.67	36.19****±3.15	30.81****±5.09	11.00** ±1.77	11.34*** ± 1.72	41.85^{ns} ± 9.35	33.83^{ns} ± 9.91

^{ns} Is no significant different at P> 0.05, * Significant different at P≤ 0.05, ** Significant different at P≤ 0.01, *** Significant different at P≤ 0.001, **** Significant different at P≤ 0.0001.

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respectively. Similar results were obtained with *M. pygmaeus* (Table 4), while the reduction percentage was 5.35 and 2.73 at the adult and nymph stages for Protecto®. In contrast, chlorpyrifos had a negative effect as 84.42 and 82.02% for adult and nymph, respectively.

In Qalyubia, the results also show that Bt formulations scored the lowest reduction percentage followed by emamectin benzoate with 10.62% for adults and 16.85% for nymphs. Thus, chlorpyrifos had the most fatal effect by 83.34 and 84.46% for adult and nymph stages, respectively.

Residue of Tested Insecticides in Tomato Fruits under Open Field Conditions

Recovery

Recovery experiments were performed in order to study the accuracy of the analytical method. The system of GC and HPLC was used to measure recoveries of emamectin benzoate, indoxacarb, spinosad and chlorpyrifos from spiked samples. Untreated samples of tomato fruits were spiked at three levels of 0.1, 0.5 and 1.0 µg g^{-1}. The recovery percentage ranged from 88.4% to 105.2%, which was calculated by spiking 10 g of blank samples with standard solution. (Table 5).

Persistence of Emamectin Benzoate, Indoxacarb, Spinosad and Chlorpyrifos Pesticides in Tomato Fruits

The values of the insecticide residues and their loss percentage detected in tomato fruits are shown in Table (6). For emamectin benzoate, the initial deposit was 1.88 ppm, then, the amount of residues decreased sharply three days after application to reach 0.82 ppm with a high loss of 56.38%. These results mean that emamectin benzoate amounts dropped to about half their value after 72 hours from initial application. The rapid degradation continued, reaching 0.15 ppm with 92.02% loss after five days from application. The residues reached 0.006 ppm

Table 4. Mean±SE of *Macrolophus pygmaeus* population and reduction% after 14 days of insecticides application in Giza and Qalyubia governorates.

Insecticides	Giza governorate				Qalyubia governorate			
	Mean of population±SE		Reduction%±SE		Mean of population±SE		Reduction%±SE	
	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph
Control	19.00±1.19	15.89±1.47			14.88±0.41	13.28±0.60		
Lepinox (Bt)	16.55^{**}±0.58	15.72^{ns}±0.99	5.86^{****}±4.13	3.16^{****}±5.57	16.28^{*}±0.59	15.61^{****}±0.74	9.56^{****}±6.52	0.00^{****} ±8.85
Dipel 2x (Bt)	16.39^{***}±0.78	15.22^{ns}±0.92	6.78^{****}±2.06	4.02^{****}±6.28	14.44^{ns}±0.58	12.89^{ns}±0.52	9.76^{****}±3.13	5.81^{****}±3.94
Protecto (Bt)	22.00^{***}±1.2	14.28^{****}±1.95	5.35^{****}±2.02	2.73^{****}±7.17	17.28^{***}±0.93	16.61^{****}±1.07	0.00^{****}±6.77	1.16^{****}±5.65
Spinosad	15.56^{****}±1.65	10.78^{****}±1.53	41.66^{****}±5.19	35.19^{****}±3.89	10.17^{****}±0.9	9.11^{****}±1.36	44.61^{****}±5.4	25.27^{****}±10.12
Emamectin benzoate	15.33^{****}±0.59	14.49^{**}±0.89	12.85^{****}±3.82	4.90^{****}±3.96	15.11^{ns}±1.01	15.06^{***}±0.60	16.85^{****}±6.1	10.62^{****}±5.35
Chlorpyrifos	3.02^{****}±0.75	2.55^{****}±0.92	82.02±4.61	84.42±3.38	3.39^{****}±1.22	3.11^{****}±0.78	84.46±7.07	83.34±4.23
Indoxacarb	9.22^{****}±0.74	10.11^{****}±0.92	46.28^{****}±5.71	26.26^{****}±9.71	11.00^{****}±1.7	11.34^{****}±1.12	41.85^{****}±9.3	33.99^{****}±9.99

^{ns} Is no significant different at P> 0.05, * Significant different at P≤ 0.05, ** Significant different at P≤ 0.01, *** Significant different at P≤ 0.001, **** Significant different at P≤ 0.0001.

Table 5. Fortified recoveries of tested insecticides in tomato fruits.

Insecticides	Spiking level (μg g^{-1})	Average recovery	±RSD
Emamectin benzoate	0.1	92.31	5.23
	0.5	95.14	4.85
	1	98.02	4.12
Spinosad	0.1	88.40	2.99
	0.5	89.65	10.44
	1	91.02	5.11
Indoxacarb	0.1	89.27	3.51
	0.5	96.21	4.09
	1	102.5	2.27
Chlorpyrifos	0.1	91.39	2.18
	0.5	93.27	2.01
	1	105.2	3.01

Table 6. Amounts of insecticides residues in tomato fruits under open field conditions.[a]

DAT	Emamectin benzoate		Spinosad		Indoxacarb		Chlorpyrifos	
	Residue (μg g^{-1})	Loss (%)	Residue (μg g^{-1})	Loss (%)	Residue (μg g^{-1})	Loss (%)	Residue (μg g^{-1})	Loss (%)
0	1.88	0.00	7.75	0.00	4.04	0.00	7.32	0.00
1	0.82	56.38	3.03	60.90	2.17	46.28	4.49	38.66
3	0.30	84.04	1.91	75.35	0.83	79.45	3.42	53.27
5	0.15	92.02	1.02	86.83	0.65	83.91	2.35	67.89
7	0.006	99.68	0.83	89.29	0.04	99.00	1.24	83.06
10	ND	---	ND	---	ND	---	0.57	92.21
14	ND	---	ND	---	ND	---	0.07	99.04
RL_{50} (days)	1.26		1.61		1.57		2.66	
MRL	0.02		0.7		0.5		1	
PHI	7 days		10 days		7 days		10 days	

[a] DAT: Day After Treatment; ND: Not Detected; RL_{50}: Residue half-Life; MRL: Maximum Rsidue Limits (EU, 2016), PHI: Pre Harvest Intervals.

with a very high percentage loss of 99.68% seven days after application. No detectable residue of emamectin benzoate was observed after 10 and 14 days. The maximum residue limit of emamectin benzoate in tomato fruits is 0.02 ppm (European Commission 2019), thus the Pre-Harvest Interval (PHI) was seven days. The obtained Residual half-Life values (RL_{50}) of emamectin benzoate in tomato fruits was 1.26 days. In addition, data in Table (6) show that the initial deposits of spinosad in tomato fruits was 7.75 ppm one hour after application. It decreased rapidly to 3.03 ppm with 60.90% loss within 24 hours and decreased to 1.91, 1.02 and 0.83 ppm after 3, 5 and 7 days from application with 75.35, 86.83, and 89.29 % loss, respectively. No detectable residue of spinosad was observed 10 and 14 days after application. Based on the EU (2016), the Maximum Residue Limit (MRL) for spinosad on tomato is 0.7 ppm, so the corresponding recommended Pre-Harvest Interval (PHI) was 10 days. The obtained Residual half-Life values (RL_{50}) of spinosad in tomato fruits was 1.61 days. The persistence of indoxacarb data set in Table (6) indicates that the amount of indoxacarb

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in tomato fruits sharply decreased within the first 24 hours after application to 2.17 ppm, showing a 46.28% loss. After three days from application, the residues of indoxacarb decreased to 0.83 ppm with 79.45% loss. The rapid degradation continued, reaching 83.91% loss after five days from application. The corresponding value after 7 days was 0.04 ppm with a very high percentage loss of 99.00%. No detectable residues of indoxacarb were observed after 10 and 14 days. The maximum residue limits of indoxcarb in tomato fruits was 0.5 ppm according to EU (2016), thus the Pre-Harvest Interval (PHI) was 7 days. The RL_{50} value of indoxacarb in tomato fruits was 1.57 days.

In the case of chlorpyrifos, the initial deposit was 7.32 ppm, which decreased sharply one day after application to reach 4.49 ppm with 38.66% loss (Table 6). The rapid degradation continued for chlorpyrifos to reach 3.42 ppm with 53.27% loss three days after the initial application. Five days after application, the residual amounts were 2.35 ppm with a loss of 67.89%. The corresponding value after 7 days was 1.24 ppm with a high loss of 83.06%. After 10 and 14 days, residual amounts were 0.57 and 0.07 ppm with a very high percentage loss of 92.21 and 99.04, respectively.

DISCUSSION

The concept of Integrated Pest Management (IPM) is based on using minimum doses of chemical pesticides when urgently needed together with biological controls to control pests with minimum adverse effects on the natural habitation. Therefore, the efficacy and potential risks of pesticides on target insect pests and non-target organisms are major criteria when selecting an IPM program (Peshin and Zhang, 2014**)**. Biopesticides could be used as an environmentally friendly approach in pest control because of their safety to humans and ecosystem (Chandler *et al.,* 2011). However, UV light and sunlight could have a major degradation impact on bioinsecticides (Moustafa *et al*., 2018); therefore, these products should be evaluated before use.

In this study, the results showed that all treatments caused significant reduction percentage of *T. absoluta* under field conditions. Among all the treatments, emamectin benzoate showed the greatest reduction percentage of *T. absoluta* in both locations, followed by indoxacarb in Giza and spinosad in Banha. These results agreed with Soliman (2012), who tested the efficacy of emamectin benzoate and indoxacarb on *T. absoluta* density under field conditions and determined the reduction percentage for each treatment alone, along the mixture of two treatments together. The result was 92.38, 93.30, and 87.85% for, respectively, emamectin benzoate, indoxacarb, and a mixture of emamectin benzoate+indoxacarb. These findings agree with those obtained earlier by Gacemi and Guenaoui (2012), who found that the biopesticide emamectin benzoate caused an acceptable mortality against larvae of *T. absoluta* at 87%. Mortality rate of 90% was observed in larvae of *T. absoluta* on tomato leaves (López *et al*., 2010), while Braham and Hajji (2012) showed that spinosad was insecticidally active in the ratio of 91% of *T. absoluta* larvae. In addition, Gacemi *et al*. (2016) indicated that emamectin benzoate caused a mortality rate of 100% in all larval stages.

In addition**,** Sridhar *et al*. (2016) tested the efficacy of three insecticides, spinotram, spinosad and cyantraniliprole, on reduction of *T. absoluta* fruit damage. The three treatments caused a significant reduction and proved effective up to 7 days after application. Hanafy and El-Sayed (2013) emphasized that spinetoram was the most toxic insecticide in decreasing infestation of *T. absoluta,* followed by spinosad. In addition, the lowest population of *T. absoluta* in tomato leaves and fruits was observed following application of spinosad, emamectin benzoate, and chlorantraniliprole (Bastola, 2020). Additionally, other

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researchers revealed that different insecticides could be used effectively against *T. absoluta,* such as spinosad (Bratu *et al*., 2015; Samir *et al*., 2015), azadirachtin, emamectin benzoate, spinosad, chlorantraniliprole (Eleonora and Vili 2014), indoxacarb and chlorantraniliprole (Roditakis and Seraphides, 2013). Thus, spinosad and indoxacarb have an insecticidal activity against lepidopteran pests (Cisneros *et al*., 2002; Wing *et al*., 2000) with low effect on natural enemies (Williams *et al.,* 2003; Galvan *et al*., 2005). This finding was in line with Kandil *et al.* (2020), who, in a laboratory assessment, found that Lepinox formulation of *Bt* was the most effective against the 2nd instar larvae of *T. absoluta* with 0.01×10^{-2} µg mL^{-1}, followed by spinosad, and emamectin benzoate.

Results revealed that all tested biopesticides were safe to the two predators *N. tenuis* and *M. pygmaeus* according to the IOBC toxicity categories (Sterk *et al*., 1999), that could be included in IPM program. In addition, Sterk *et al*. (2003) and Miles (2006) found that spinosad had no insecticidal effect on adults and nymphs of both predatory mirid species. Thus, Williams *et al.* (2003) classified spinosad as a harmless insecticide to predator species in reviewed data exploring the susceptibility of different predator species.

The results obtained in this study show that all dissipation rates for the tested insecticides follow first order kinetic reaction. Moreover, the results provide important information on the level of residue of insecticides on tomato fruits post application. It is clear that the insecticide dissipated rapidly three days after application. This may be due to volatilization or removal by weathering, heat, decomposition, sunlight and UV radiation (Spynu, 1989). Thus, results indicated that tomato fruits could be consumed safely after 7 and 10 days post application of emamectin benzoate, indoxacab and Spinosad, respectively. These results agree with Urvashi *et al*. (2012), who estimated the indoxacarb residues by QuEChERS method in cabbage samples following different treatments of Avaunt 15% EC. The average of indoxacarb recoveries on cabbage were shown to be 83.93, 89.86, and 95.40%, respectively. The indoxacarb initial deposits on cabbage were 0.18 and 0.39 mg kg^{-1}, respectively, at single and double dosages. Indoxacarb residues were lower than the LOQ of 0.01 mg kg^{-1} after 7 and 10 days, respectively, at single and double dosages. At the field and double field rates, the indoxacarb half-life was 2.88 and 1.92 days, respectively. Madan *et al*. (2018) investigated the dissipation behavior of indoxacarb 4.5% SC in tomato fruits sprayed at two doses (37.13 and 74.26 g ai ha^{-1}). They found that the recovery percentages for tomato samples were 84.50 to 90.58%. The initial deposits at single and double doses were 0.63 and 0.82 mg kg^{-1}, respectively, and the half-life values at respective doses ranged from 2.37 to 2.48 days. In addition, Szpyrka *et al*. (2017) found that the initial residue levels decreased regularly and, at one month, the detected level was 0.01 mg kg^{-1} for chlorpyrifos-methyl. The half-life values for chlorantraniliprole, chlorpyrifos-methyl and indoxacarb were 16-17, 4-6 and 20-24 days, respectively.

CONCLUSIONS

It is clear from the results obtained in this study that biopesticides demonstrate high efficacy against *T. absoluta*, and can be used safely during harvesting time. In addition, data proved that Bt formulations are environmentally friendly and safer for the naturally occurring predator than other tested insecticides.

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سمیت حشره‌کش‌ها علیه مینوز برگ گوجه‌فرنگی، Tuta absoluta ، و شکارگرهای آن و تعیین میزان انتشار بقایای آنها در میوه‌های گوجه‌فرنگی

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چکیده

گوجه فرنگی یکی از سبزیجات مهم در نقاط مختلف جهان است که در تمام طول سال در آنجا کشت می شود. در حال حاضر، مهمترین مشکل پیش روی تولیدکنندگان گوجه فرنگی در جهان، خسارت مخرب ناشی از مینوز مهاجم برگ گوجه فرنگی، *Tuta absoluta* Meyrick (Gelechiidae:Lepidoptera) است. در این پژوهش، اثربخشی سه حشره‌کش زیستی (فرمولاسیون Bacillus thuringiensis، spinosad و emamectin benzoate، و دو حشره‌کش شیمیایی (indoxacarb و chlorpyrifos) علیه *T. absoluta* و اثرات نامطلوب آنها بر شکارگرها در دو استان مختلف مصر بر اساس دزُهای توصیه‌شده حشره کش‌های این آزمایش انجام شد. افزون براین، هدررفت باقی مانده حشره کش های آزمایش شده در میوه های گوجه فرنگی تعیین شد. نتایج نشان داد که emamectin benzoate موثرترین حشره کش بود که بیشترین کاهش تراکم T. absoluta را به ترتیب در استان های Gizaو Qualybia با ۷۸.۰۵% و ۸۷.۱۱% نشان داد و پس از آن ایندوکساکارب در Giza (۷۷.۰۱ %) و spinosadدرQualybia (۸۰/٤٤%) قرار داشت. همچنین، یافته‌های ما ثابت کرد که فرمولاسیون‌های آفت‌کش‌های زیستی آزمایش‌شده، به‌ویژه فرمولاسیون‌های Bt، برای دو مورد از مهم‌ترین شکارگرها در کشت گوجه‌فرنگی یعنی Nesidiocoris tenuis و Macrolophus pygmaeus Reuterدوست‌دار محیط‌زیست هستند. افزون براین، تجزیه و تحلیل بقایای حشره‌کش روی میوه‌های گوجه‌فرنگی نشان داد که بقایای حشره‌کش‌های زیستی سریع‌تر از حشره‌کش‌های معمولی (کلرپیریفوس chlorpyrifos) هدر رفته و پراکنده(dissipated) می‌شوند. نتایج این تحقیق نشان داد که می توان از حشره کش های زیستی برای مدیریت *T. absoluta* در شرایط مزرعه استفاده کرد.

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