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Efficacy and Residual Analysis of Three Insecticides against *Spodoptera littoralis* (Boisd.) on Field-grown Tomatoes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Spodoptera littoralis (Boisd.) is among the most destructive insect pests in Egypt, the field study was conducted at El-Beheira Governorate, Egypt to evaluate the toxicity of three insecticides against 2nd and 4th larval instars of these insect and residual effect in tomato fruit into open field application. The outcomes demonstrated that spinosad, methoxyfenzide and diflubenzuron proved to be very toxic. Semi-field application showed that spinosad was the most effective with 91.38% and 100% insect pest mortalities at initial and residual effects against second instar larvae, respectively. The initial effect manifested higher morality (90.25%) for fourth instar larvae when treated with diflubenzuron followed by spinosad (86.50%), then methoxyfenzide (84.25%), while the residual effects of all

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tested insecticides were 100% mortality. The findings additionally demonstrated that the pre-harvest intervals (PHI) for diflubenzuron, spinosad, and methoxyfenzide were 3,7 and 10 days, respectively, for tomato fruits. Following this PHI, the products are considered safe for export and local consumption, as the residue level satisfies the maximum residue limit, which is the level documented by the European Union.

Keywords: Insecticides; Spodeptera littoralis; tomato fruits; residual effect.

1. INTRODUCTION

Spodoptera littoralis (Boisd.) (Leopedeptera: Noctuuidae) is among of the most destructive insect pests in Egypt. Cotton, vegetables, and ornamentals are all at risk. The cotton leaf worm, *S. littoralis*, is one of the most harmful phytophagous insect pests in Egypt, which reproduces quickly and causes large crop losses. According to EU. [1], this extremely polyphagous species attack about 87 host plants belonging to about 40 families. Several growers use methomyl; a carbamate, and the organophosphorus pesticides; chlorpyrifos, profenofos to control this pest, which is quite harmful to human-beings Tomlin [2].

Since it infests a wide variety of host plants, it is regarded as a major pest with significant economic in many countries. Bio-pesticide causes paralysis by promoting the release of amino butyric acid, an inhibitory neurotransmitter Raslan *et al.,* [3]. The conventional insecticide methomyl was used by Abdel-Rahim [4] to inhibit lepidopterous pests. Emamectin benzoate is a novel bio-insecticide that is developed by the fermentation of *Streptomyces* choice for use in integrated pest management (IPM) systems. It is quite safe for fish, livestock and human -beings. In addition, it is safe on pollinating insects Remove act predators Dahi *et al*. [5].

Lufenuron and abamectin may be used frequently against cotton leafworm (Freitas and Bueno [6]. The tomato (*Lycopersicon esculentum* Mill.) is one of the most important solanaceous vegetable crop rown in Egypt. There are numerous harmful insects infest tomato plants Palumbo, S.C.and E.T. Natwick [20] Between 2000 and 2002, the QuEChERS method was created as a new sample-preparation technique for pesticide multiresidue analysis *Barrania et al.,* [7]. Pre-Harvest Interval (PHI) of tomato treated with tested pesticides was to be determined.

This work aimed to study the dissipation rate as well as residue levels of spinosad, methoxyfenzide and diflubezuron insecticide in tomato fruits under Egyptian field condition. As well as to provide some insights on how well the spinosad, methoxyfenzide and diflubenzuron insecticides work against the pest. Also, to determine the pre-Harvest intervals (PHI's) to minimize health risks.

2. MATERIALS AND METHODS

2.1 Field Trials and Sample Collection

The field trials were achieved at El-Beheira Governorate, Egypt and the experiment (during summer 2023 growing season) was laid out in completely randomized block design, with four replicates for each three treatments and control. Thus, the experimental area was divided into 16 plots (4treatment x 4 replicates) with 21 m^2 for each plot (1x4 m for each replicate). The tomato plants were grown with a distance of 0.5 m between the plants and another, the first plot treatment by spinosad (Sbanfk 10% SC) with rate at the of 60 ml/ 10 L, the second plot for treatment by methoxyfenzide (Mabuzid 24 % SC) with rate at the of 37.5 ml /100 L water, third, plot for treatment by deiflubenzuron (Bistmalin 48% SC) with rate 65 ml /100 L. and the fourth for control. After application two kilograms of tomato fruits were collected randomly from control and applied plots, one hour after application during 1, 3, 7, 10 and 15 days, respectively. Fruit samples were stored in a freezer at -20ºC and extraction daily.

2.2 Insect Rearing

Eggs masses of *S. littoralis* field strain were obtained from cotton fields at Etay-El-Baroud, Beheira Governorate, that had not been treated with insecticides prior to the egg mass collection. These masses were moved to the laboratory and maintained under 25 ± 2 ^{o} C, 65 ± 5 RH and 10:14, L: D, photoperiod till the development of 4^{th} instar larvae; then used in the test. The larvae were fed on fresh leaves of castor bean, *Ricinus communis*, as described by El-Defrawi et al. [8].

2.3 Laboratory Experiments

Experiments were performed under laboratory conditions of 25 ± 2^{o} C, 70 % ± 5 RH and 10:14, L: D, photoperiod. Five *S. littoralis* 4^{th} instar larvae were put in a 500 ml plastic jar covered with muslin cloth, representing one replication. For every feeding date, ten replications of each treatment were created. The sprayed tomato leaves were picked up immediately after an hour from spray (zero time), and then after 1-, 2-, 4-, and 6-days post spray and transferred directly to the laboratory for feeding the selected larvae. Following one day of feeding on treated leaves, the survived larvae were transmitted to new and clean 500 ml plastic jar and fed on untreated cotton leaves till pupation. Number of dead larvae and percentage of mortality were recorded after 3 ,5 ,7 and 10 days post treatment. It is supposed that the larva is dead if no movement was observed when it was touched with a small brush. Larval duration, percentages of normal and deformed pupae, and percentages of normal and malformed emerging adults were determined. The mortality of larvae was counted and recorded 24 hrs after feeding and corrected for natural mortality using Abbot's formula [9].

2.4 Standards and Reagents

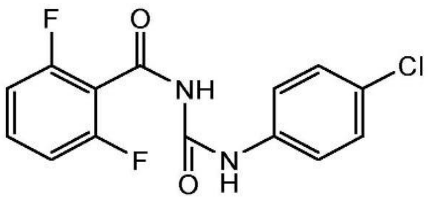
Spinosad, methoxyfenzide and diflubenzuron reference standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), with 99% purity (Fig. 1).

All other reagents and solvents were obtained from Sigma Aldrich and were HPLC grade. Stock solutions of tested pesticides were prepared at a concentration of 100 µg/ml in acetonitrile and kept in a refrigerator (4°C). Calibration standard and working solutions concentrations (ranging from 0.01 to 5.0 µg /ml) were prepared by serial dilution of the stock solutions.

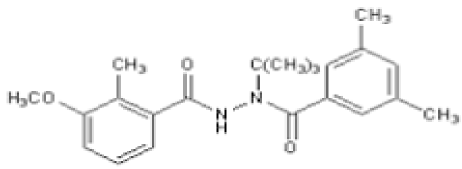
QuEChERS salts 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate, and d-SPE salts were purchased from Agilent Technologies (Wilmington, DE, USA).

2.5 Sample Processing

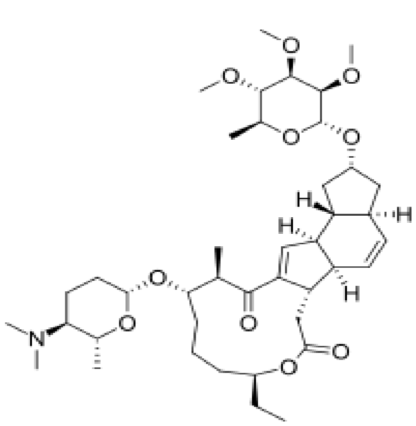
The extraction and cleaning method, and the steps of the analytical process were as 10g of tomato sample is placed into a 50 ml falcon tube, followed by the addition of 10 mL of acetonitrile and the salts of the QuEChERS extract, centrifugation at 4000 rpm for 5 min, and the transfer of 1 mL of the acetonitrile extract to a 15-mL centrifuge tube containing 25 mg of primary



Diflubenzuron



Methoxyfenzide



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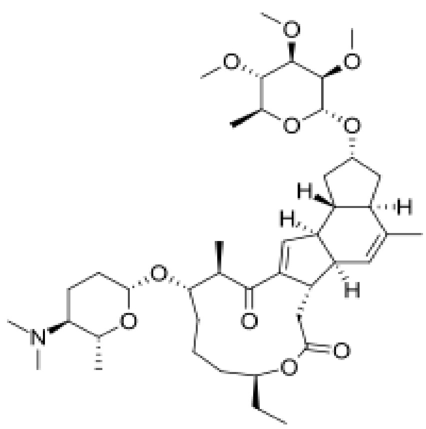

D

Spinosad

Fig. 1. Structures of tested pesticides

secondary amine (PSA) and 150 mg of anhydrous $MgSo_4$ After one minute of shaking, the tube underwent 5 min of centrifugation at 4000 rpm. The supernatants were filtered through a Millipore, Billerica, Massachusetts, 0.2 µm PTFE filter, before being put into auto sampling vials for HPLC-DAD quantity Anastassiades et al., [10].

2.6 Instrument and Apparatus

The chromatographic quantity was conducted using an Agilent 1100 series HPLC system, quadruple pump, variable wavelength diode array detector (DAD), and analytical column Nucleosil [11] (30 mm by 4.6 mm ID, 5 mm). For Spinosad, methoxyfenzide and diflubenzuron, the injection volume was 20 µl acetonitrile 70% + water 30%, acetonitrile 80% + water 20% and acetonitrile 90% + water 10%, respectively and the mobile phase flow rate was 1 mL/min. The detection wavelength was 254, 230 and 210 nm, respectively. The retention time for spinosad, was 7.12 and 13.24 min., retention time for methoxyfenzide was 5.63 min and diflubezuron was 4.07 min.

3. RESULTS AND DISCUSSION

3.1 Impact of the Evaluated Pesticides on Cotton Leafworm under Semifield Conditions

Semi-field studies were carried out to evaluate initial effect (3 and 5 days after spraying with the tested insecticides) and residual effect (7 and 10 days) after spraying against the second and fourth larval instars of cotton leafworm, *Spodoptera littoralis* and corrected larval mortality percentages were calculated (Tables 1 & 2).

Data in Table (1) show the initial and residual effects of insecticides on second instar larvae of cotton leaf worm spinosad insecticide induced the highest mortality (average 91.38%) followed by methoxyfenzide and diflobezuron with 88.50 % and 89.63% initial kill, respectively. The average residual effects of the three above-mentioned insecticides were 100.00 % to mortality for each one.

As observed in Table (2) the initial effect manifested the highest mortality (90.25%) for fourth instar larvae when treated with diflubenzuron followed by spinosad (86.50%) and then, methoxyfenzide (84.25%) while the residuals effect of all the tested insecticides were 100% mortality for each one. These results agree with the results of Barrania *et al.,* [7] who reported that the average mortalities percentages of deaths (initial kill) resulting from novaluron and chlorpyrifos-methyl were 84.80 % and 91.2 % for *S. littoralis* larvae in its second instar, and 77.20 % and 89.9% for fourth instar larvae.

In contrast, the average percentage of mortality (residual toxicity) were 70.50 and 71.90 % for larvae of second instar, and 61.90 and 67.60 % for larvae of fourth instar.

Abo El-Ghar *et al*. [12] reported that the initial deposition levels of tested pesticides on tomato fruits differ mainly due to the surface area to mass ratio and the nature of the treated surface. El-Dewy [13] reported that the durability of residuals of the evaluated pesticides in cotton leaves treated with Lt_{50} emamectin-benzoate and chlorfluazuron were 5.59 and 5.56 days, respectively. Therefore, it could be concluded that, chlorfenapyr, emamectin benzoate and lufenuron caused high toxicity against *S. littoralis* and these insecticides had the shortest persistence residues in tomato fruits. Therefore, integrated pest management (IPM) programs can use these chemical insecticides.

3.2 Dissipation of Pesticide Residues in and on Tomato Fruits

3.2.1 Dissipation of diflubenzuron residues

The investigation of diflubenzuron treatment on tomato fruits under field conditions was carried out utilizing novel ethodology (Table 3 and Fig. 2).

Table 1. Effect of the tested insecticides on the corrected mortality percentages of second instar larvae of cotton leafworm under semi-field conditions at El Bheira Governorate

Treatment	% Corrected mortality					
	Initial effect			Residual effect		
	After		Average	After		Average
	3days	5days		7dys	10days	
Spinosad	92.25	90.50	91.38	100.00	100.00	100.00
Methoxyfenzide	88.00	89.25	88.50	100.00	100.00	100.00
Diflubenzuron	88.75	90.50	89.63	100.00	100.00	100.00

Table 2. Effect of the tested insecticides on the corrected mortality percentages of fourth instar larvae of cotton leaf worm under semi-field conditions in El-Bheira Governorate

Treatment	% Corrected mortality					
	Initial effect			Residual effect		
	After		Average	After		Average
	3days	5days		7dys	10days	
Spinosad	88.00	85.00	86.50	100.00	100.00	100.00
Methoxyfenzide	88.00	80.50	84.25	100.00	100.00	100.00
Diflubenzuron	90.50	90.00	90.25	100.00	100.00	100.00

One hour after treatment, the residue of diflubenzuron in tomato fruits was 1.03 mg/kg, at zero-time, 0.59 mg/kg after1 day, and 0.13, 0.05, and 0.01 mg/kg at 3, 7 and 10 days of treatment, respectively. Fifteen days following application, diflubenzuron lingering levels were beneath the limits for discovery. Ten days prior to the recommended dose application, the EU [14] MRL was used to estimate the PHI value. The half-life of diflubenzuron was 1.17 days.

3.2.2 Dissipation of spinosad residues

The results in Table 4 and Fig. 2 cleared that the residues of spinosad in and on tomato fruits under field conditions. One hour after application, was 0.93 mg/kg, then, at that point, 0.51 mg/kg after one day, and 0.24 and 0.10 mg/kg following 3 and 7 days of treatment, separately. Ten days following application, spinosad lingering levels were beneath the limits for discovery. Three days prior to the recommended dose application, the EU [15] MRL was used to estimate the PHI value. The half-life of spinosad was 1.31 days.

3.2.3 Dissipation of methoxyfenzide residues

Data in Table 5 and Fig. 2 cleared the residues of methoxyfenzide in and on tomato fruits under field conditions one hour after treatment, was

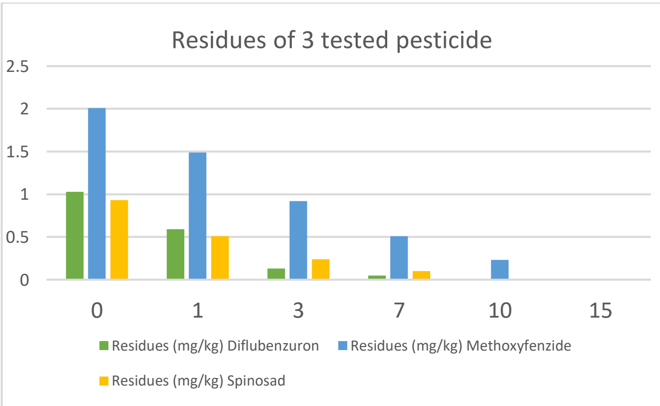

Fig. 2. Residues effect of Spinosad, methoxyfenzide and diflobezuron on tomato fruits during 15 days

Table 3. Dissipation of diflubenzuron residues in and on tomato fruits

Days after application	Residues (mg/kg)	Loss %	Persistence %
0	1.03	0.00	100
1	0.59	42.71	57.29
3	0.13	87.37	12.63
7	0.05	95.14	4.86
10	0.01	99.02	0.98
15	ND	0.00	0.00
MRL (EU [14])	0.01		
RL50 (days)	1.17		
PHI (days)	10.00		

Rl_{50}: Half-life period. MRL:Maximum residue level. PHI: Pre-harvest interval. ND: Not detected

Table 4. Dissipation of spinosad residues in and on tomato fruit

Days after application	Residues (mg/kg)	% Loss	% presistance
0	0.93	0.00	100
1	0.51	38.16	61.84
3	0.24	74.19	25.81
7	0.10	89.24	10.76
10	ND	0.00	0.00
15	ND	0.00	0.00
MRL (EU 2022)	0.70		
RL50 (days)	1.31		
PHI (days)	3.00		

Rl_{50}: Half-life period. MRL: Maximum residue level. PHI: Pre-harvest interval. ND: Not detected

Table 5. Dissipation of methoxyfenzide residues in and on tomato fruits

Time after application (days)	Residues (mg/kg)	% loss	% presistance
0	2.01	0.00	100
1	1.49	25.87	74.13
3	0.92	54.22	45.78
7	0.51	74.62	25.38
10	0.23	88.55	11.45
15	ND	0.00	0.00
MRL (EU [1])	0.60		
RL50 (days)	2.76		
PHI (days)	7.00		

Rl50: Half-life period. MRL: Maximum residue level. PHI: Pre-harvest interval. ND: Not detected

2.01 mg/kg, then, at that point, 1.49 mg/kg after1 day, and 0.92, 0.51 and 0.23 mg/kg following 3,7 and 10 days of treatment, separately. Fifteen days following application, methoxyfenzide lingering levels were beneath the limits for discovery. Seven days prior to the recommended dose application, the EU [1] MRL was used to estimate the PHI value. The half-life of methoxyfenzide was 2.76 days.

Our results agree with those of Adak and Mukherjee [16] who observed that spinosad residues were below the determination limit in/on tomato fruits after 15 days of application case of recommended dose (51 g a.i. ha^{-1}). The half-life of Spinosad was between 3.18 and 3.74 days for the recommended dose. Based on the CODEX-MRL of spinosad (0.3 mg kg^{-1}), pre-harvest interval (PHI) was 7.54 days for the recommended dose of spray. Also, Kashyap *et al.* [11] showed that the half-life values of spinosad were determined to be 1.20 and 1.60 days at recommended and double the recommended dosage, respectively. The safety interval for spinosad sprayed tomato fruit was determined to be 1.92 and 3.88 days at application rate of 15 and 30 g a.i. ha^{-1}, respectively.

Abdelfatah *et al.* [17] reported that the dissipation half-life time of spinosad residues in tomato fruits was 0.36 days. Depending on the maximum residue limits (MRL), the pre-harvest interval (PHI) of spinosad was one day after the application. Ramadan et al*.,* [18] indicated that tomato fruits could be safely consumed after less one day of application at the recommended rate for spinosad, according to the recommended EU maximum residue limits (MRLs).

Kashyap et al*.,* [11,19,20] reported that the estimated $t_{1/2}$ values were 3.3 and 8.5 days for methoxyfenozide in fruits and leaves of grapes, respectively. Pre-harvest interval (PHI) value was 10 days after application of methoxyfenozide to fruits and leaves of the grape. Also, Alhamami et al*.* [21-23] showed that the residue concentrations of diflubenzuron were below the EU-MRL value of 0.01 mg.kg^{-1} at 21 days after application.

4. CONCLUSION

This study evaluated the toxicity of three insecticides against *S. littoralis's* second and fourth larval instars in tomatoes on open field application. The outcomes demonstrated that spinosad, methoxyfenzide and diflubezuron proved to be very toxic. However, results of semi-field application showed that spinosad was the most effective causing 91% and 100% mortalities at initial and residual effect against second instar and fourth larvae, respectively. The initial effect manifested higher (91% mortality) for fourth instar larvae when treated with diflubenzuron, followed by spinosad (86.5%) then methoxyfenzide (84%) while the residual effect of all the tested insecticides, caused 100% mortality.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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