Effect of Some Insecticides and Their Field Persistence on Tomato Against Spodoptera littoralis (Boisd.)

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Abstract: This study evaluated the toxicity of three insecticides belonging to different groups of insecticides, emamectin benzoate (Tomaguard 5% SG), lufenuron (Grand 5% EC) and chlorfenapyr (Lepifena 24%SC) against 2nd and 4th larval instars of S. littoralis under semi-field condition in tomato field Qalyubia Governorate, Egypt during 2021 season and also for the analysis of pesticide residues in tomatoes after open field application. The results showed that emamectin benzoate and chlorfenapyr were found to be proved very toxic (LC₅₀ = 0.0005 & 0.0009 ppm for second instar) and (LC₅₀ = 0.0011 &0.0018 ppm for fourth instar), respectively, Whereas the toxicity scores of the insecticide lufenuron exhibited lower LC₅₀ values, 0.1569 and 0.4378ppm for second and fourth instars S. littoralis, respectively and results of semi-field application showed, chlorfenapyr was the most effect caused 96.05% and 100% mortalities at initial and residual effect, respectively for second instar larvae while for fourth instar larvae the initial effect manifested higher (was 88.46% mortality) when treated with chlorfenapyr followed by lufenuron (80.85%) then emamectin benzoate (60.25%). Persistence of chlorfenapyr, emamectin benzoate and lufenuron were 100% at the initial time (one hour after application), but this value begins to decrease (were 57.5%, 20.74% and 71.2%, respectively) after 3 days after application from spraying where were lower than the USA EPA's MRL. Therefore, a harvest interval should be more than 10, 7, 5 days, which could be considered as safe for human beings and animals. The results also showed that, pre-harvest period (PHI) for chlorfenapyr, emamectin benzoate, and lufenuron were 10, 7, and 5 days, respectively, in tomatoes, which are safe for human consumption and export after this period of application, as the residue level was equal to the maximum residue limit, which is the level recorded by the European Union.

KEYWORDS: Insecticides, Residues, Tomato, Spodoptera, littoralis.

1.INTRODUCTION:

Cotton leaf worm, *Spodoptera litoralis* attacks many agricultural crops in Egypt and many other countries and causes high damage, so it must be control, whether by biological methods or by using traditional or non-traditional insecticides. With regard to the management of the cotton leafworm or other pest on edible crops, it is important to consider the pesticide's efficacy in killing the pest, environmental contamination, and food safety (**El-Geddawy** *et al.* **2014**). Pesticides are among the many hazardous chemicals that humans and animals encounter daily, and are intentionally introduced into the environment to enhance agricultural production, reduce pest damage to crops. (**Krol et al. 2000**).

Emamectin Benzoate has excellent insecticidal activity and is a non-systemic insecticide that penetrates leaf tissue and paralyzes Lepidoptera, which stops feeding within hours of feeding which leads to death larvae within 3 or 4 days (Grafton-cardwell *et al.* 2005) and Dahi *et al.*, (2017) found that, highly significant increase in larval duration and decrease in egg production and affect egg fertility when treated larva with emamectin benzoate and also obvious reduction in infestation percentages in the open field (Lotfy and Embaby 2020). Lufenuron acts mostly by ingestion where larvae are cease feeding and unable to moult. Also acts transovarially, reducing fecundity and egg hatch (Abdel-Hamid *et al.*, 2021). The use of insect growth regulators

(IGRs) in insect control has shown good and effective results against lepidopteran insects **Farag**, (2001), Abdel-Aal, (2003) and (Abd El-Aziz, et al., 2017). Also, the mixing of lufenuron & emamectin benzoate (Heater 3% SC) increases the efficiency of these insecticides (Ahmed 2020) and (Abdel Aziz 2019). Chlorfenapyr is an insecticide and acaricide with stomach and contact action and limited systemic activity in plants and is classified as a slightly hazardous insecticide as per WHO criterion (**Raghavendra** *et al.*, 2011). Estimating pre-harvest intervals (PHIs) and residues of pesticides used on treated crops are essential requirements for registering a new pesticide and for appropriately setting maximum residue limits (MRLs) to protect the consumer from pesticide risks.

This work aims to the efficacy of three insecticides; emamectin benzoate, lufenuron and chlorfenapyr belonging to different groups of insecticides were evaluated for their effect on *S. littoralis* larvae and its magnitude residues in Tomato.

2.MATERIALS AND METHODS: 2.1.Insecticides used:

The efficacy of three insecticides belonging to different groups of insecticides were evaluated for their effect on larvae of *S. littoralis* and its residues in Tomato.

Insecticides	Emamectin benzoate	Lufenuron	Chlorfenapyr		
Trade Name	Tomaguard 5% SG	Grand 5% EC	Lepifena 24%SC		
Mode of action	Glutamate-gated chloride channel (Glucl) allosteric modulator	Inhibitor of chitin biosynthesis	uncouples oxidative phosphorylation at the mitochondria		
The chemical structure	$CH_{3}O + CH_{3}O + CH_{$	CF3CHFCF20-CI-NHCONHCO F	F_3C CN CN CN CN CN CN $CH_2OCH_2CH_3$		
Supplier	Magico Group, Egypt	Sand Valley, Egypt	Puretech Import & Export, Egypt		
Field recommended dose	40 cm ³ /100L water	$50 \text{ cm}^3/100 \text{L}$ water	$60 \text{ cm}^3/100\text{L}$ water		

Table (1): List of insecticides selected for this study.

2.2. Evaluation of the efficacy of selected insecticides against S. littoralis: 2.2.1. Rearing of Spodoptera littoralis:

The laboratory strain was obtained from the Cotton Leafworm Research Department at the Plant Protection Research Institute and was cultured under laboratory conditions according to El-Defrawi et al. (1964).

2.2.2.Bioassay Tests:

Chosen new moulting of 2nd and 4th larval instars of S. littoralis and starved about 3 hours before treatment. Serial concentrations of each insecticide were prepared. By using leaf dipping technique castor bean leaves dipped in each concentration for 5 second according to Abo El-Ghar et al., (1994), while the untreated treatment was only dipped in water and all leaves were left to dry under room conditions then placed after complete dry in glass jars (500 ml capacity). Larva fed for 72 or 24 hrs when treated leaves with bio insecticide & (IGR) or chemical (tradition) insecticide, respectively after that, feed with untreated leaves for five days. All treatments of insecticides included three replicates (twenty larvae for each) and three replicates contains larvae fed on untreated leaves as a control. All treatments were kept at 26±2°C and 65±5% R.H. Mortality rates are recorded and corrected daily according to the Abbott equation (Abbott, **1925**) then calculated LC_{50} values by using probit- analysis method of Finney (1971)

2.2.3.Semi Field studies:

The field experiments were constructed in tomato fields at Banha District, Qalyubia Governorate, Egypt during seasons of 2021. 1/2 feddan used for field application in a completely randomized block design. Each treatment is applied to an area of approximately 175 m², three plots per treatment additionally untreated (control) area sprayed with water only. Application of the tested insecticides were applied with the recommended rates using knapsack sprayer. Randomly samples of tomato leaves collected after treatment about one hour and continue for ten days. Then transferred the treated and untreated of leaves which collected to the laboratory to feeding separate groups of second or fourth larval instars of cotton leafworm. Percentage mortalities were calculated after 1, 7 and 10 days of chemical insecticide and after 3, 5, 7 and 10 days of bio and IGR insecticides treatments and the mortality scores were corrected using Abbott's formula (Abbott, 1925).

Acetonitrile was purchased from Merck (Darmstadt, Germany) in HPLC grade quality. Ultra-pure water was prepared by a Millipore system.

Anhydrous magnesium sulfate was obtained from Merck (Darmstadt, Germany). Anhydrous magnesium sulphate was activated by heating at 200°C for 8 hours in a muffle furnace, then cooled and kept in desiccators. Primary secondary amine (PSA, 40 µm Bondesil) and graphite carbon black (GCB, 40 µm) sorbents were obtained from Agilent Technologies (Santa Clara, CA). Sodium chloride and sodium sulphate in analytical grade were purchased from El Nasr Pharmaceutical Chemicals Company (Cairo, Egypt).

Reference standards of chlorfenapyr, emamectin benzoate and lufenuron were obtained from Dr. Ehrensdorfer (Augsburg, Germany), with purities >98%.

2.3. Apparatus and equipments:-

The equipment's; PTFE 50 ml and 15 ml with screw cap tubes, vials 2 ml with screw top, blender HGB55E, vortex shaker, desiccator, analytical balances, rotary evaporator, syringe filters PTFE and high-speed centrifuge were used in this study.

High Performance Liquid Chromatography (HPLC):- Agilent Model 1260 (Agilent Technology, Waldbronn, Germany), with quaternary pump, auto sampler injector, thermostat compartment for the column and photodiode array detector.

2.4.Preparation of standard solution

The stock solution was prepared using acetonitrile as solvent containing 1,000 μ gmL⁻¹ of analytic. The standard solutions were prepared by serial dilution and stored at 4°C until used. Standard calibration curve of chlorfenapyr, emamectin benzoate and lufenuron was constructed by plotting analytic concentrations versus peak area

2.5.Analytical procedures for insecticides residues:

2.5.1.Sampling of Tomatoes:

After spray of the tested insecticides, samples of tomatoes were taken randomly from each replicate at intervals of zero time (2h after application), 3, 5, 7, 10 and 15 days, and stored at -20 °C until using for analysis.

2.5.2.Extraction and Clean-up:-

- Chlorfenapyr, Emamectin benzoate and Lufenuron
- 10 g was weighed into a 50 ml centrifuge tube (with • screw cap)
- 10 ml of acetonitrile was added •
- Shake vigorously for 1 minute (first extraction step).
- Add 4 g of MgSO₄, 1 g of NaCl, 1 g of Na₃Citrate dihydrate and 0,5 g of Na₂HCitrat sesquihydrate shake each tube directly after the salt addition shortly



- Centrifuge for 5 minutes at >3000g.
- Transfer 1 ml of the extracts into a PP single use centrifugation tomatoe, which 25 mg of PSA and 150 mg of MgSO₄, Centrifuge for 5 min at >3000 g.
- The samples are transferred into autosampler vials to be used for the multi-residue determination by HPLC techniques

2.5.3.Recovery value:

Recovery of the efficiency of the chromatographic analysis for determination of chlorfenapyr, emamectin benzoate and lufenuron residues in tomatoes were run by adding known amount of each insecticide as alone to untreated tomatoes samples which then put through the extraction and residue determination. The recovery values were calculated as the following formula:

Recovery value = $(\mu g \text{ insecticide } / g \text{ sample found}) / (\mu g)$ insecticide /g sample added) X100

The average recovery values of tomatoes samples were used to correct all obtained values of chlorfenapyr, emamectin benzoate and lufenuron residues.

2.5.4.Insecticide residue calculation:

0.99893

10.48789

The residuals are calculated as the following equation Malhouf (1975):

> Residue $(ng/\mu l) = Ps.B \cdot V/Pst.G \cdot C$ Where:

Ps = sample peak area, B = amount of standardsolution injected (ng), V = volume of sample solution final (ml), Pst. = standard peak area, G = weight of sample (g) and C = amount of sample solution injected.



Calibration Curves



Fig (1): Calibration curve for standard of chlorfenapyr, emamectin benzoate and lufenuron

Pesticides	Mobile phase	Flow rate (ml/min)	Detector wavelength (nm)	R. Time
Chlorfenapyr	Acetonitrile: Water 80:20	0.8	265	4.925
Emamectin benzoate	Acetonitrile: Water 50:50	1	265	7.931
Lufenuron	Acetonitrile: Water: Methanol 50:45:5	0.8	255	8.216

DAD: Diode Array Detection

3.RESULTS AND DISCUSSION

3.1.Effect of selected insecticides against *S. littoralis:*

3.1.1. Toxicity of insecticides to cotton leaf worm, *S. littoralis*:

The toxicity insecticides were tested against second and fourth instar larvae of *S. littoralis* to obtain their relevant median lethal concentrations (LC₅₀). The results were displayed in Table (3) showed that, emamectin benzoate was found to be highly toxic (LC₅₀=0.0005 & 0.0011ppm) against second and fourth instar *S. littoralis*, respectively and chlorfenapyr also proved very toxic against second and fourth *S. littoralis* where LC_{50} values were 0.0009 and 0.0018ppm, respectively. Whereas the toxicity scores of the insecticide lufenuron exhibited lower LC_{50} values, 0.1569 and 0.4378ppm for second and fourth instars *S. littoralis*, respectively. The results agree with (**Khan**, **Arshad** *et al.* **2021**) reported that, emamectin benzoate was found to be highly toxic with an LC_{50} value of 2.97 mg/l against third instar larvae of *S. littura*, while the lufenuron, methoxyfenozide and novaluron were the least toxic with an LC_{50} value of 7.85, 21.06 and 29.56 mg/l, respectively. **Ezz El-Din** *et al.*, (2009) and **Abdu-Allah** (2010) also found that emamectin benzoate is a highly effective insecticide against *S. littoralis* larvae.

Table (3): Toxicity of the tested insecticides against 2nd and 4th inster larvae of cotton leaf worm, S. littoralis.

Treatments	Instar	LC50	Lower limit	Upper limit	Slope
emamectin benzoate	Second	0.0005	0.004	0.001	1.497 ± 0.310
emamectin benzoate	Fourth	0.0011	0.001	0.002	1.593 ± 0.340
1	Secound	0.1569	0.066	0.237	1.658 ± 0.337
lufenuron	Fourth	0.4378	0.251	0.599	1.803 ± 0.320
- 1 , 1 , 6 ,	Secound	0.0009	0.004	0.001	1.331 ± 0.279
chlorfenapyr	Fourth	0.0018	0.001	0.002	1.483 ± 0.026
	routui	0.0018	0.001	0.002	1.403 ± 0.020

3.1.2.Effect of the tested insecticides on the cotton leafworm under the semifield condations:

Semi-field studies were carried to evaluate initial effect (24 hours after spraying with chlorfenapyr) or (3 and 5 days after spraying with emamectin benzoate and lufenuron) and residual effect (7 and 10 days after spraying with all the tested insecticides) against second and fourth larval instars of cotton leafworm and corrected larval mortality percentage were calculated Tables (4 & 5).

Data in Table (4) showed the efficacies of tested compounds against second instar larvae, where chlorfenapyr was the most effect caused 96.05% and 100% mortalities at initial and residual effect, respectively. While, the initial effect when treated second instar larval with emamectin benzoate and lufenuron were 80.9 and 82.8% mortalities and the residual effect were 98.2 and 96.75% mortalities, respectively.

Table 4: Effect of the tested insecticides on the corrected mortality percentages of second instar larvae of cotton leaf
worm under semi-field conditions at Qalyubia Governorate.

	% Corrected mortality							
Treatments		Initial	effect	Residual effect				
11000000	after	after	after		After	after		
	1 days	3 days	5 days	mean	7 days	10 days	mean	
emamectin benzoate	-	67.10	94.70	80.90	96.40	100.00	98.20	
lufenuron	-	82.40	83.20	82.80	93.50	100.00	96.75	
chlorfenapyr	96.05	-	-	96.05	100.00	100.00	100.00	

As observed in Table (5) the initial effect manifested higher (was 88.46% mortality) for fourth instar larvae when treated with chlorfenapyr followed by lufenuron (80.85%) then emamectin benzoate (60.25%) while the residual effect of the tested insecticides, emamectin benzoate was the highest efficiency resulting 98.7% mortality followed by chlorfenapyr (94%) then lufenuron (92.45%). These results agree with results **Barrania** *et al.*, (2012) reported that the % mortalities average (initial kill) caused by novaluron and chlorpyrifos-methyl were 84.8 and 91.2 %, respectively against 2nd instar of *S. littoralis* larvae, and were 77.2 and 89.9 %, respectively against 4th instar larvae, while

% mortality average (residual toxicity) were 70.5 and 71.9 %, respectively against 2nd instar of larvae, and were 61.9 and

67.6 %, respectively against 4th instar larvae.

	% Corrected mortality							
Tuesday	Initial effect				Residual effect			
Treatments	after	after	after	mean	after	After	mean	
	1 days	3 days	5 days		7 days	10 days		
emamectin benzoate	-	44.90	75.60	60.25	98.70	98.70	98.70	
Lufenuron	-	80.40	81.30	80.85	90.40	94.50	92.45	
Chlorfenapyr	88.46	-	-	88.46	92.40	95.60	94.00	

Table (5): Effect of the tested insecticides on the corrected mortality percentages of fourth instar larvae of cotton leaf worm under semi-field conditions in Qalyubia Governorate.

3.2. Residues of the insecticides in tomato:

The dissipation rate of chlorfenapyr, emamectin benzoate and lufenuron in tomatoes were exhibited first order kinetics. The regression equations and half-life value are mentioned in Table (6) and Figure (2).

Table (6) showed the residue of chlorfenapyr, emamectin benzoate and lufenuron in tomatoes over the testing time period initial time, 1, 3, 7 and 10 days after treatment. The data showed that the residues in the initial for chlorfenapyr, emamectin benzoate and lufenuron in tomatoes were 4 ± 0.114 , 0.27 ± 0.007 and 0.66 ± 0.018 respectively, one hour after application. The value of residues dropped to 3.2 ± 0.110 , 0.16 ± 0.006 and 0.58 ± 0.021 respectively, this value gave the rate of loss 20%, 40.74% and 12.12% respectively. The values of the residues decreased to 2.3 \pm $0.082, 0.056 \pm 0.002$ and 0.47 ± 0.016 respectively after 3 days after of application, after 7 days the residues reduced to 0.63 ± 0.017 , 0.01 ± 0.001 and 0.21 ± 0.006 ppm respectively. At the 10 days after treatment the decreased of the values were reached to 0.21 ± 0.016 , 0.27 ± 0.007 and 0.1 ± 0.004 ppm, respectively.

Persistence of chlorfenapyr, emamectin benzoate and lufenuron were 100% at the initial time (one hour after application), but this value decreased at after one-day spray to 80%, 59.25% and 87.87%, respectively, the persistence were 57.5%, 20.74% and 71.2% after 3 days after application from spraying were lower than the USA EPA's MRL. Therefore, a harvest interval should be more than 10, 7, 5 days, which is safe for humans and animals. The data showed that the Half-life values (t1/2) were calculated mathematically chlorfenapyr, emamectin benzoate and lufenuron in tomatoes were 4.07, 2.37 and 5.35 days. The differences in the recorded half-life values may be due to differences in the plants grown, temperature, or climate changes during spraying.

The pre-harvest interval (PHI) of chlorfenapyr, emamectin benzoate and lufenuron were 10, 7 and 5 days respectively in tomatoes This observation become orange safe for human consumption and export after this period of application, as the residue level was equal to the maximum residue limit, which is the level recorded by by **EU** (2005a).

Abo El-Ghar and Ramadan (1962) reported that, The initial deposition levels of both tested pesticides on tomato fruits differ mainly due to the surface area to mass ratio and the nature of the treated surface. (El-Dewy, 2013) reported that, the persistence of the tested insecticides residues on cotton foliar with Lt50, emamectin-benzoate and chlorfluazuron with Lt₅₀ (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 longest persistence residues and high initial effect in field tomatos. Therefore, these chemical insecticides can be used in the integrated pest management (IPM) programmers. El-Zahi (2015) reported that the type of plant treated is effective in the toxicological properties of the tested insecticides, and this may be useful in designing bio-evaluation experiments.

Time after	Chlorfenapyr			Ema	amectin be	enzoate	Lufenuron		
treatment (days)	Residues of (ppm) ± SE	Loss%	persistence	Residues of (ppm) ± SE	Loss%	persistence	Residues of (ppm) ± SE	Loss%	Persistence
Initial *	4 ± 0.114	0	100	$\begin{array}{c} 0.27 \pm \\ 0.007 \end{array}$	0	100	0.66 ± 0.018	0	100
1	3.2 ± 0.110	20	80	$\begin{array}{c} 0.16 \pm \\ 0.006 \end{array}$	40.74	59.25	$\begin{array}{c} 0.58 \pm \\ 0.021 \end{array}$	12.12	87.87
3	2.3 ± 0.082	42.5	57.5	0.056 ± 0.002	79.25	20.74	0.47 ± 0.016	28.78	71.21
5	1.06 ± 0.034	73.5	26.5	0.028 ± 0.002	89.62	10.37	0.31 ± 0.011	53.03	46.96
7	0.63 ± 0.017	80.31	19.68	$\begin{array}{c} 0.01 \pm \\ 0.001 \end{array}$	93.75	6.25	0.21 ± 0.006	63.71	36.20
10	0.21 ± 0.016	90.22	9.88	$\begin{array}{c} 0.27 \pm \\ 0.007 \end{array}$	0	101	0.1 ± 0.004	80.77	19.33
MRL		0.4			0.02			0.4	
Half life		4.07			2.37			5.35	
PHI		10			7			5	

 Table (6): Behavior, %Loss, Persistence, Half-life and PHI residues ± SE of chlorfenapyr, emamectin benzoate and lufenuron

Initial *: Two hour after application, MRL: Maximum Residue Limited



Fig (2): Behavior, Half-life and PHI residues of chlorfenapyr, emamectin benzoate and lufenuron

Conclusion:

This study evaluated the toxicity of three insecticides against 2nd and 4th larval instars of *S. littoralis* and also for the analysis of pesticide residues in tomatoes after open field application. The results showed that emamectin benzoate and

chlorfenapyr were found to be proved very toxic, Whereas the toxicity scores of the insecticide lufenuron exhibited lower LC_{50} values for second and fourth instars *S. littoralis* and results of semi-field application showed, chlorfenapyr was the most effect at initial and residual effect.Persistence of chlorfenapyr, emamectin benzoate and lufenuron were

100% at the initial time (one hour after application), but this value begins to decrease after 3 days after application from spraying where were lower than the USA EPA's MRL. Therefore, a harvest interval should be more than 10, 7, 5 days, which could be considered as safe for human beings and animals. Results also showed that, pre-harvest interval (PHI) of chlorfenapyr, emamectin benzoate and lufenuron were 10, 7 and 5 days respectively in tomatoes where safe for human consumption and export after this period of application, as the residue level was equal to the maximum residue limit, which is the level recorded by the European Union

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