



EVALUATION OF SOME INSECTICIDES AGAINST TOMATO LEAF MINOR (*Tuta absoluta*) AND DETERMINATION OF THEIR RESIDUES IN TOMATO FRUITS

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ABSTRACT

Recently tomato leaf miner, *Tuta absoluta*, has become one of the most devastating pests of tomato in Egypt, and caused serious damages to tomato production in invaded areas. For this reason, the present study was undertaken to determine the efficacy of 15 insecticides representing different pesticide groups against this insect and also to study their dissipation in tomato fruits. The laboratory experimental results revealed that profenofos, cyfluthrin, lufenuron, chlorpyrifos-methyl and indoxacarb were the most toxic insecticides as compared to other chemicals. These insecticides, 1 day after application, under field conditions caused 84.1 to 73.5% reduction in infestation. With time the bio-residual activity of these compounds gradually decreased with increase in the number of infested fruits. The initial deposits of profenofos, cyfluthrin, lufenuron and chlorpyrifos-methyl ranged from 28.6 to 6.3 ppm, depending on the rate of insecticide application. The loss rate of these amounted to 91.7 to 97.57%, 15 days after treatment. In conclusion, all tested insecticides proved effective against *T. absoluta* and might be used to control this pest. Further, the bioresidual activity of these compounds did not exceed more than two weeks for the best cases.

Keywords: Insecticide efficacy, leaf miner, residues, tomato, *Tuta absoluta*

INTRODUCTION

Tomato, *Solanum lycopersicum* (*Lycopersicon esculentum* (Mill.)) is one the most important economic vegetables in Egypt so far as the area under vegetables and commercial value of the crop is concerned. The tomato is considered as one of the few cultures for which pests and diseases are equally important, being a host plant to about 200 species of arthropods (Anonymous, 2001). Recently, tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), has assumed the status of most important pest in Egypt (Medeiros *et al.*, 2005). It is reported to be one of the most devastating pests of tomato in South America (Miranda *et al.*, 1998). It was later reported from eastern Spain in late 2006 and has subsequently spread throughout the Mediterranean Basin and Europe (Potting, 2009; Desneux *et al.*, 2010). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas, and currently considered a key agricultural threat to European and North African tomato production. This pest is present throughout the crop duration. Larvae can infest leaves, flower, stem and fruit, causing important losses in tomatoes (Molla *et al.*, 2011). The leafminer injury to the host plant is manifested in the form of reduction of leaf surface area responsible for photosynthetic activity. It

prevents newly emerged leaves from fully expanding and causes leaves to be curled, twisted and eventually necrotic (Knapp *et al.*, 1994). Therefore, both yield and fruit quality can significantly be reduced by direct feeding of *T. absoluta* and pave way for the entry of secondary pathogens through the wounds made by the insect (Vargas, 1970; FernándeZ and Montagne, 1990; Colomo *et al.*, 2002). Tomato crop is treated with pesticides in order to control pest infestation. Residues after pesticide application on vegetable crops need to be determined and the waiting period between application and harvest accordingly be recommended to ensure that the produce is free from residues before it reaches market (Shady *et al.*, 2000). Therefore, present study was undertaken with the objective to evaluate the efficacy of 15 insecticides from diverse pesticide groups against *T. absoluta*; and also to determine their dissipation in tomato fruits to establish the pre-harvest interval (PHI) that should be followed before marketing the produce in order to minimize health hazards.

MATERIALS AND METHODS

Insecticides used

The 15 insecticides tested were profenofos 72EC (Selecron), malathion 57EC, chlorpyrifos-methyl 50EC (Reldan), cyfluthrin 10EC, λ -cyhalothrin 5EC, acetamiprid 20G (Mospilan), lufenuron 5EC (Match), chlorflazuron 5EC (Atabron), indoxacarb 15SC (Avaunt), spinosad 24SC (Tracer), chlorpyrifos [47.5%] + lufenuron [2.5%] 50EC (Feroban), flufenoxuron [3%] + α -cypermethrin [7%] 10EC (Cygron), chlorpyrifos [24%] + cypermethrin [5%] 29EC (Chlorosan), thiamethoxam [14.1%] + λ -cyhalothrin [10.6%] 24.7EC (Engeo) and deltamethrin [3%] + fenitrothion [33%] 33EC (Deltathion).

Insecticidal test against Tuta absoluta larvae under laboratory conditions

Ten larvae were used in insecticidal test. Aqueous dispersions of commercial insecticide formulations were used in a leaf dip bioassay (Cahill *et al.*, 1995). The leaf having the larvae was dipped for 5 sec. in aqueous solutions of recommended insecticide concentrations while the untreated check was dipped in water. Each treatment was replicated thrice and mortality recorded 24 h after treatment.

Field evaluation of tested insecticides on tomato leaf miner

The field experiment was carried out in El-Mahmodia village, Dekernis district, Dakahlyia Governorate, Egypt. Experimental area was planted with tomato *Lycopersicon esculentum* (Mill.) variety 'Super Strain B' after seeded in a greenhouse and then transferred to the field during summer cultivation season of 2011, under normal field and agricultural practices. The experiment block design was randomized with each treatment replicated four times. Each plot had five rows with 25 plants row⁻¹. The 15 plants of medium row were used for data collection. The plant space was 0.5 x 1.0 m. The insecticides, profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl were applied as foliar spray on June 15, 2011 at the recommended rates using a knapsack sprayer. The 6th treatment was left untreated to serve as control. The evaluations against leaf minor were conducted on 3 plants (Gonzalez-Cabrera *et al.*, 2011) were randomly collected from each replicate before spraying as well as 1, 3, 5, 7, 10 and 15 days after spraying. The outer plants were never sampled in order to avoid border effects. Alive larvae were counted using a binocular microscope. Percent reduction in infestation was calculated using Henderson and Tilton equation (1955). To assess the effect of tested insecticides on infested tomato fruits: the infested tomato fruits from 10 plants were randomly counted from each replicate before spraying as well as 1, 3, 5, 7, 10 and 15 days after application. The percent of infested tomato fruits in different treatment was calculated.

Determination of tested insecticide residues in tomato fruits

Representative samples of tomato fruits were collected from the treated plots after 1 h (initial deposits) and 1, 3, 5, 7, 10 and 15 days after spraying. Clean polyethylene bags were used for preserving the collected samples. The samples were stored at -20 °C in deep freezer until analysis.

Ethyl acetate observed to be the best solvent for extracting tested insecticides residues from tomato fruits according to Anonymous (1985). Chromatographing the extract was through activated florisil using elution (50% methylene chloride - 1.5% acetonitrile - 48.5% hexane), according to Mills *et al.* (1972). Five ml of n-hexane were added to extracts and concentrated to 0.5 ml for GLC analysis. A Pye Unicam 4500 GC equipped with flame photometric detector operated in phosphorus mode (529 nm filter) was used for determination of profenofos and chlorpyriphos-methyl insecticides. A Pyrex glass column (1.5 x 4 mm) was packed with 3% OV225 on gas chromosorb Q (80 - 100 mesh). Temperatures maintained in column, detector and injector were 230, 250 and 240°C, respectively. Gas flow rate was 30 ml min⁻¹ for nitrogen, hydrogen and air. The residual half life (RL₅₀) periods for the tested insecticides were calculated as per the equation of Moye *et al.* (1987). Statistical significance of data was assessed by Duncan and Tukey test at P_{0.05} and P_{0.01} (Snedecor and Cochran, 1980)

RESULTS AND DISCUSSION

Insecticidal test against T. absoluta larvae under laboratory conditions

The potency of fifteen compounds representing different chemical groups was evaluated against the tomato leafminer *T. absoluta* larvae (Table 1). The results revealed that profenofos, cyfluthrin, lufenuron, chlorpyriphos - methyl and indoxacarb were the most toxic as compared to other chemicals. This was expressed by the percentages mortalities 88.5, 80.0, 75.2, 75.0 and 74.8%, respectively, while spinosad and malathion were the lowest toxic insecticides, both recorded very low mortality (22.2 and 21.8 %). The levels of mortality for other compounds ranged from 31.5 to 63.5%.

Table 1: Insecticidal test against tomato leafminer (*Tuta absoluta*) larvae under laboratory conditions

Insecticide	Rate of application (ha ⁻¹)	Mean of alive larvae before treatment	Mean of alive larvae after treatment	Mortality (%)
Profenofos	750 ml	11.3 ^f	1.3 ^f	88.5
Cyfluthrin	250 ml	10.0 ^h	2.0 ^{ef}	80.0
Lufenuron	200 ml	13.7 ^c	3.4 ^{de}	75.2
Chlorpyriphos-methyl	1 L	12.0 ^e	3.0 ^{def}	75.0
Indoxacarb	53.6 ml	14.7 ^{ab}	3.7 ^{de}	74.8
Feroban	500 ml	13.7 ^c	5.0 ^{cd}	63.5
Chlorfluazuron	400 ml	11.7 ^{ef}	4.7 ^{cd}	59.8
Cygron	125 ml	14.7 ^{ab}	6.0 ^c	59.2
Chlorosan	375 ml	12.0 ^e	5.0 ^{cd}	58.3
Deltathion	500 ml	12.3 ^{de}	6.3 ^c	48.8
Engeo	80 ml	11.7 ^{ef}	6.4 ^c	45.3
λ-cyhalothrin	500 ml	14.3 ^b	9.3 ^b	35.0
Acetamiprid	100 g	12.7 ^d	8.7 ^b	31.5
Spinosad	60 mg	15.0 ^a	11.7 ^a	22.2
Malathion	1 L	10.7 ^g	8.4 ^b	21.8
LSD _{0.05}	-	0.54	1.93	-

The figures superscripted with same alphabets in the same columns do not significantly differ from each other as per Duncan's multiple range test.

The effect of insecticides on tomato leaf miner under field condition

The efficiency of the most toxic insecticides (profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyriphos-methyl) against *T. absoluta* larvae under field conditions revealed that the chemical treatments were able to suppress the levels of infestation to different degrees in comparison to that of untreated plants (Table 2). The suppression, however, greatly varied according to the nature of the

tested compounds, the rate of use and the time elapsed after spraying. Per cent reduction of infestation was 84.1, 73.3, 73.7, 78.9 and 73.5% in treated plants by profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl, respectively, after one day of spraying. Fifteen days later, the reduction in infestation dropped to 52.5, 42.4, 43.7, 36.8 and 46.1%, respectively. The average reduction percentages were 61.4, 52.0, 51.0, 50.8 and 49.6%, respectively. Similar results were obtained by Santos *et al.* (2011) who reported that indoxacarb and chlorfenapyr caused 96.1 and 91.4% reduction of *T. absoluta* infestation at 3 days after application, respectively, and 93.6 and 93.3%, 7 days after application. Also, Barbosa *et al.* (2011) reported that the tomato plants treated with watery leaf extracts of *Ruta graveolens*, alcoholic leaf extracts of *Copaifera langsdorffii* and *Chenopodium ambrosioides* in the concentration of 5% caused reduction in *T. absoluta* and *B. tabaci* population under field conditions.

Table 2: Per cent reduction of leaf miner (*Tuta absoluta*) on tomato plants treated with various insecticides at different time intervals

Insecticides	Pretreat- ment larval count /10 leaves	Days after insecticide treatment												Overall <u>mean</u>	
		1		3		5		7		10		15			
		*n.	**%o	n.	%	n.	%	n.	%	n.	%	n.	%	n.	%
		larv.	reduc.	larv.	reduc.	larv.	reduc.	larv.	reduc.	larv.	reduc.	larv.	reduc.	larv.	reduc.
Profenofos	13.7 ^{ab}	2.3 ^c	84.1	4.7 ^c	65.4	4.3 ^{cd}	61.4	5.7 ^{cd}	54.1	6.3 ^d	50.8	8.7 _c	52.5	5.33 _c	61.4
Lufenuron	11.7 ^c	3.3 ^b	73.3	4.3 ^d	59.5	4.7 ^c	50.6	5.3 ^d	50.0	7.0 ^c	36.0	9.0 ^{bc}	42.4	5.6 ^c	52.0
Indoxacarb	13.3 ^b	3.7 ^b	73.7	3.7 ^e	69.3	6.3 ^b	41.7	6.7 ^c	44.4	8.3 ^b	33.2	10.0 ^{bc}	43.7	6.45 ^b	51.0
Cyfluthrin	10.3 ^d	2.3 ^c	78.9	3.7 ^e	60.4	3.7 ^d	55.8	6.3 ^{cd}	35.1	6.0 ^d	37.7	8.7 _c	36.8	5.12 ^c	50.8
Chlorpyrifos- methyl	14.3 ^a	4.0 ^b	73.5	5.7 ^b	56.0	5.7 ^b	51.0	8.3 ^b	36.0	8.7 ^b	35.0	10.3 ^b	46.1	7.12 ^b	49.6
Control	10.7 ^d	11.3 ^a		9.7 ^a		8.7 ^a		9.7 ^a		10.0 ^a		14.3 ^a		10.6 ^a	
LSD _{0.05}	0.85	0.68		0.30		0.90		0.98		0.44		1.28		0.72	

*n. larv. = Number of larvae; **%o reduc. = Percent reduction; The figures superscripted with same alphabets in the same columns do not significantly differ from each other as per Duncan's multiple range test

The effect of tested insecticides on tomato fruits infestation

The infestation of fruits by *T. absoluta*, before any insecticidal treatment, ranged from 8.0 to 10.3 fruits. The chemical treatments were able to suppress the levels of infestation to different degrees in comparison to that untreated plants (Table 3). The suppression, however, greatly varied according to the nature of the tested compound and the time elapsed after spraying. Our results revealed that the infested fruits were increased 1 day after treatment, except in case of profenofos (7.2% below the pretreatment count). However, the tomato fruit infestation increased as the time elapsed and reached 30.9, 41.0, 42.7, 37.9 and 53.8%, 15 days after treatment in profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl treatments, respectively, while this amount reached 92.8% in untreated plants at in the same period in comparison to pretreatment infested fruits. As an average of the infestation percent, profenofos was the most effective compound (14.4%), followed by lufenuron and cyfluthrin (21.7 and 24.8%, respectively) and then indoxacarb and chlorpyrifos-methyl (26.4 and 28.75%). Also, obtained data indicated that larvae of this pest can caused significant loss in the yield. In the same respect, the bioresidual activity of these compounds, however, gradually decreased with increasing the number of infested fruits. These results are agreement with those obtained by many investigators (Colomo *et al.* 2002; Molla *et al.*, 2011) who reported that *T. absoluta* larvae can damage tomato plants during all growth stages, producing large galleries in their leaves, burrowing stalks, apical buds, green and ripe fruits. Also, It can cause important yield loss in different production regions and under diverse production systems.

Dissipation behaviour of tested insecticide in tomato fruits

Under defined conditions the retention time for profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl was 6.13, 11.37, 7.12, 4.88 and 4.26 min., respectively (Table 3). In addition, the

Table 3: Leaf miner (*Tuta absoluta*) infested tomato fruit count before and after insecticide treatment

Insecticide	Pre-treatment infested fruit count/10 plants	Infested fruits count (No. plant ⁻¹) days after insecticide treatment						Overall average
		1	3	5	7	10	15	
Profenofos 72EC	9.7 ^{ab}	9.0 ^c (-7.2)	10.3 ^b (6.2)	11.3 ^c (16.5)	11.0 ^c (13.4)	12.3 ^{bc} (26.8)	12.7 ^c (30.9)	11.1 ^c (14.4)
Lufenuron 5EC	8.3 ^c	8.7 ^c (4.8)	8.6 ^{cd} (4.0)	9.7 ^d (16.9)	10.7 ^c (28.9)	11.3 ^d (36.1)	11.7 ^d (41.0)	10.1 ^c (21.7)
Cyfluthrin 10EC	10.3 ^a	11.7 ^a (13.6)	11.7 ^a (13.6)	12.7 ^b (23.3)	13.3 ^b (29.1)	13.0 ^b (26.2)	14.7 ^b (42.7)	12.8 ^b (24.8)
Indoxacarb 15SC	8.7 ^{bc}	10.7 ^b (23.0)	9.7 ^{bc} (11.5)	10.7 ^c (23.0)	11.3 ^c (30.0)	11.7 ^{cd} (34.5)	12.0 ^{cd} (37.9)	11.0 ^c (26.4)
Chlorpyrifos-methyl 50EC	8.0 ^c	8.7 ^c (8.8)	8.3 ^d (3.8)	9.7 ^d (21.3)	11.0 ^c (37.5)	11.7 ^{cd} (46.3)	12.3 ^{cd} (53.8)	10.3 ^c (28.7)
Control	9.7 ^{ab}	10.3 ^b (6.2)	12.3 ^a (26.8)	13.7 ^a (41.2)	14.3 ^a (47.4)	15.7 ^a (61.9)	18.7 ^a (92.8)	14.2 ^a (46.4)
LSD _{0.05}	1.12	0.62	1.097	0.7	0.998	0.844	0.904	0.970

The figures superscripted with same alphabets in the same column do not significantly differ from each other as per Duncan test. *The values in parenthesis are the percent content in comparison to the respective control.

results were corrected according to the rates of recovery, which were determined in fortified untreated samples. The average rates of recovery for these insecticides in tomato fruits were 87.7, 91.6, 80.3, 84.3 and 90.3%, respectively. Data presented in Table 5 demonstrate the residual behavior of profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl residues in tomato fruits. The initial deposits for prementioned insecticides were 23.8, 9.16, 6.3, 10.7 and 28.6 ppm, respectively. These values decreased to 17.9, 7.46, 5.02, 8.58 and 19.9 ppm indicating the rate of loss 24.7, 18.6, 20.16, 19.8 and 30.4% 1 day after application. The results also revealed that the residues decreased to different degrees with the time elapsed after spraying. Thus loss rate amounted to 93.4, 91.7, 92.5, 97.57 and 95.6%, respectively, 15 days after treatment. The amount of residues recorded during the experimental period varied for each insecticide to another. These levels depended on the rate of use, the initial deposits, the rate of exposure of the fruits to the environmental factors and the reaction between the treated surface and the chemical applied (Solliman *et al.*, 2005). Also, Stevens *et al.* (1988) demonstrated that uptake of pesticides on plant surface is affected by the chemical structure, formulation as well as the rate of used insecticide, the nature of the recipient surface, the used spraying equipment and the climatic conditions, especially the ambient temperature during pesticide application. Concerning health hazards, the maximum residue limits (MRL) for profenofos, cyfluthrin and chlorpyrifos-methyl on tomato fruits established by Codex Alimentarius Committee for pesticide residues (Anonymous, 2003) are 2.0, 0.5 and 0.5 ppm, respectively. Accordingly, the pre-harvest intervals (PHI= safety period) for tomato fruits were 15 days for treated plants by profenofos and cyfluthrin after spraying, while this period was more than fifteen days in the case of plants treated by chlorpyrifos – methyl. On the other hand, the values of maximum residue limits for lufenuron and indoxacarb insecticides not available. Bates (1979) mentioned that short periods in treated crops are required for the premarket registration of pesticides and setting maximum residue limits (toxicological acceptable level) to protect the consumer against the possible health hazards of exposure to pesticides.

Table 4: Retention time and recovery rates of tests insecticides in tomato fruits

Insecticide	Retention time (min.)	Recovery rate (%)
Profenofos	6.13	87.7
Lufenuron	11.37	91.6
Indoxacarb	7.12	80.3
Cyfluthrin	4.88	84.3
Chlorpyrifos-methyl	4.26	90.3

Table 5: Dissipation rate of tested insecticides in tomato fruits

Days after spray	Profenofos		Lufenuron		Indoxacarb		Cyfluthrin		Chlorpyrifos- methyl	
	ppm	Loss (%)	Ppm	Loss (%)	ppm	Loss (%)	ppm	Loss (%)	ppm	Loss (%)
0*	23.80	0.0	9.16	0.0	6.30	0.00	10.70	0.0	28.60	0.0
1	17.90	24.7	7.46	18.6	5.02	20.16	8.58	19.8	19.90	30.4
3	12.60	47.1	6.36	30.6	3.80	39.80	6.39	40.3	14.84	48.1
5	7.70	67.6	3.56	61.1	2.57	59.20	4.00	62.6	8.75	69.4
7	5.26	77.9	2.33	72.4	2.00	68.25	2.80	73.8	5.60	80.4
10	2.52	89.4	1.50	83.6	1.28	79.70	1.35	87.4	2.66	90.7
15	1.57	93.4	0.76	91.7	0.47	92.50	0.26	97.6	1.26	95.6

MRL: maximum residue limit; *One hour after application; RL_{50} : the residual half lives periods

Our results revealed also, the half lives (RL_{50}) values in tomato fruits were 74.5, 94.7, 93.6, 77.9 and 66.9 h for profenofos, lufenuron, indoxacarb, cyfluthrin and chlorpyrifos-methyl, respectively. These amounts indicated that lufenuron and indoxacarb had high stability (or had lower degradation rate) in tomato fruits followed by profenofos and cyfluthrin, while chlorpyrifos-methyl more degradation when compared with other compounds under the field conditions.

Finally, our results have shown that profenofos presented greater effect against *T. absoluta* followed by lufenuron and indoxacarb then cyfluthrin while, chlorpyrifos-methyl have the lowest effect. In conclusion, the results show that all tested insecticides were effective to control *T. absoluta* and might be used to control pest outbreaks, but the bioresidual activity of these compounds did not exceed more than two weeks for the best cases. Thus, the frequent application would be necessary. In the same respect, many investigators (Shalaby, 2006; Farrag and Shalaby, 2008) and the World Health Organization classification reported profenofos to be more toxic than lufenuron and indoxacarb on animals and humans (WHO, 2005). So, our study recommended that we can use lufenuron and indoxacarb to control this pest on tomato cultivation during fruit period, but other tested insecticides may be used during vegetation stage.

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