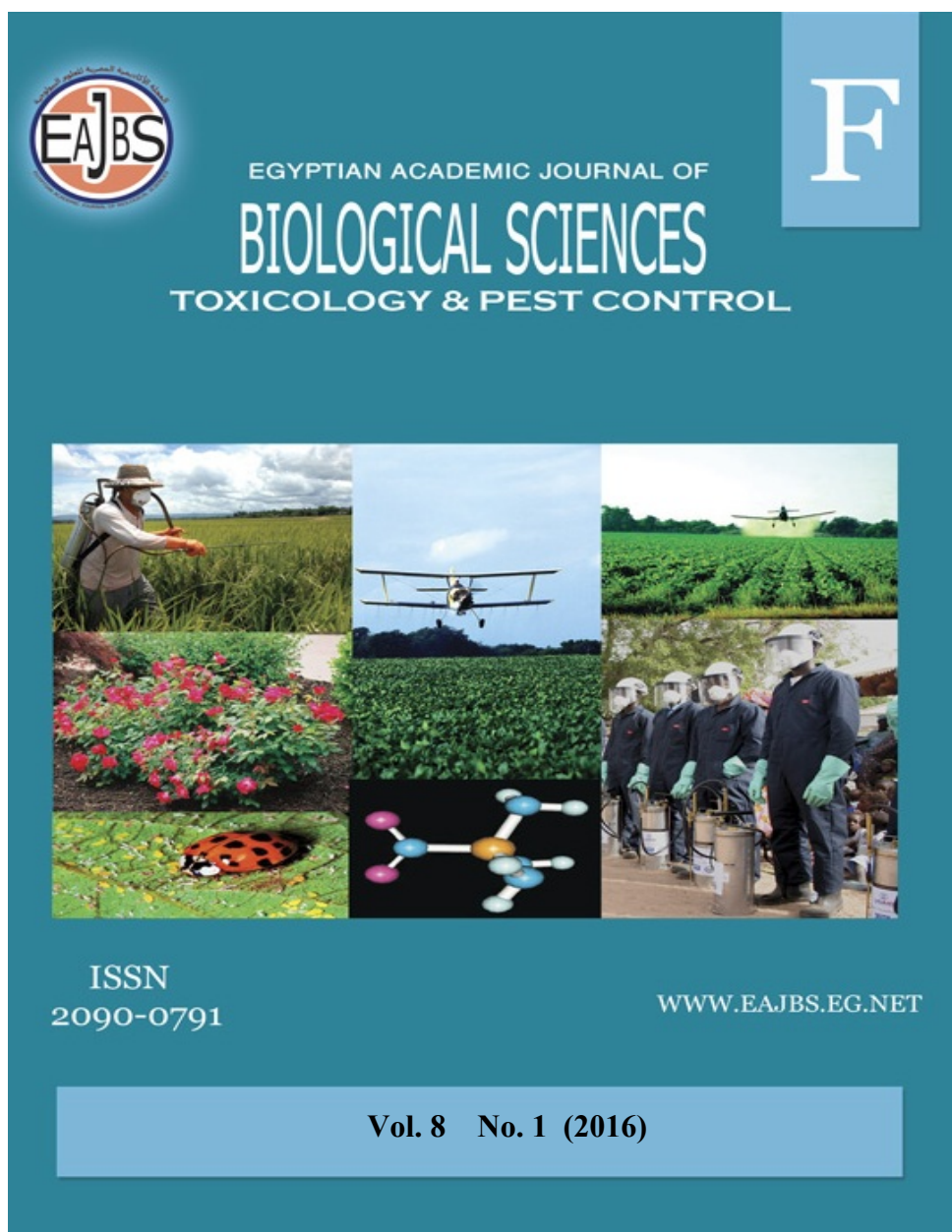


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**Biological Control of the Tomato Leafminer, *Tuta absoluta* Meyrick**

(Lepidoptera, gelechiidae)

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**ABSTRACT**

Experiments were conducted to evaluate the efficacy applied biological program "Spinosad+Trichogramma+ Pheromone" against the tomato leafminer, *Tuta absoluta* (Meyrick), (Lepidoptera: Gelechiidae) on tomato plants under open-field conditions on two tomato varieties (Supper stream and Casle rock) and its effect on the productivity of the crop in both two varieties under study. Results indicated the efficacy biological control program on mean number male moths and number larvae of *T. absoluta* in Supper stream was higher than Casle rock, which gave low numbers 15.8, 1.5 for Supper stream comparing with Casle rock (21.9 and 1.9), respectively. Also, showed the effect of biological control program on number of natural enemies after use yellow sticky traps and sweeping net traps, had little effect on two tomato varieties comparison untreated plot. The results indicated that the weight of 100 fruits was high in the two varieties but on Supper stream was higher than Casle rock. The use of biological control program reduced percentage reduction in both two varieties but it was in Casle rock recorded a decrease less than Supper stream. The current study recorded that the mean number fruits/plant was higher in two varieties, but Supper stream more than Casle rock. But yield production after applied biological program on Supper stream was more than Casle rock, comparison untreated plot. Residue of Spinosad was determined after 1, 3, 7, 10 and 15 days from application, the concentration of Spinosad 2 h after treatment was 0.223 mg/kg. The residues amount decreased to 0.118 mg/kg within the first 24 h after application following that period residues decreased to 0.092, 0.074, and 0.015 mg/kg, at 3, 7, and 10 days after treatment, respectively, but after 15 days from treatment contained no detectable amount of spinosad.

**INTRODUCTION**

Tomato *Lycopersicon esculentum* Mill is one of the most important crops in many parts of the world particularly in Egypt. It is consumed as a fresh table tomato and as an essential raw material for a variety of food processing industries. Tomatoes are grown in both greenhouses and in open fields. The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a serious pest of both open fields and greenhouses. It is a South America species (Giordano & Silva, 1999) and recently became an alime species in Europe subsequently spread throughout the Mediterranean Basin (EPPO, 2011).

This pest was first recorded from eastern Spain in late 2006 (Urbaneja *et al.*, 2007) then Morocco, Algeria, France, Greece, Malta, Egypt and other countries (Roditakis *et al.*, 2010). *T. absoluta* has invaded Egypt through Marsa Matruh Governorate near the Libyan border in 2009. In 2010, it was reached Giza, and then well established in all Governorates of Egypt (Temerak, 2011). Tomato trade and active flight or passive movement by wind current are the main mechanisms of the current spread of this pest (Desneux *et al.*, 2010). Adults of *T. absoluta* usually lay eggs on the underside of leaves and on stems. After hatching, young larvae penetrate leaves, aerial fruits or stems, on which they feed and develop, thus creating conspicuous mines which may be invaded later by secondary pathogens, leading to fruit rot (EPPO, 2005) and thereby directly reducing crop value and causing significant yield losses of up to 100% if it is not controlled. So, *Tuta absoluta* is an economically significant pest of tomatoes that is currently undergoing a rapid expansion in its geographical distribution (Desneux *et al.*, 2010). It is very essential to control *T. absoluta* that attacks tomato plant starting from seedling till fruiting stages in order to prevent potential huge damage caused by such pest. Chemical control has been the main method of control (Frañca *et al.*, 2000). The effectiveness of insecticides alone might be sometimes impaired because of the mine-feeding behavior of larvae or deficient spraying technology (Lietti *et al.*, 2005). The appeal of spinosad, a fermentation product of the soil actinomycete *Saccharopolyspora spinosa* (Mertz and Yao), includes its safety profile and acceptable use in organically produced tomatoes (Puinean *et al.*, 2013 & Racke, 2006).

The objective of our study is evaluating the efficacy of bioinsecticides spinosad against the tomato

leafminer, *T. absoluta* on tomato plants under open-field conditions in two tomato crop varieties and its effect on the productivity of the crop in two varieties.

## MATERIALS AND METHODS

### Study area:

Two tomato varieties were used Supper 55 (Supper stream) and Supper 550 (Castle rock); these varieties are commonly cultivated in Egypt for local consumption. The experiments were carried out in Etay, El-Beheira Governorate during 2014 summer season. This study was begun from June 29 to September 16. In this respect area of about 700 m<sup>2</sup> was chosen to be cultivated by two varieties. Experimental area was divided into 12 plots, and arranged in completely randomized blocks design, and each variety was replicated three times. The samples were kept in paper bags and transferred to the laboratory for inspection using the stereomicroscope. All plots received normal agriculture practices and were kept free of any insecticides treatments.

### Biological control program:

The biological control program used was (Spinosad + Trichogramma + Pheromone), the area was planted with 750 tomato seedlings (10000 plants/feddan). Usage rates were 35 ml Spinosad/Feddan + three Trichogramma cards + Pheromone and control.

### Pheromone used:

3E, 8E, 11Z -14 AC (C16 H26 O2), (E, Z, Z) -3, 8, 11 -Tetradecatrienyl acetate was obtained from Plant Protection Research Institute, Agriculture Research Center, Cairo, Egypt.

### Bioinsecticide:

Spinosad was used in its commercial formulation registered for use in tomato fields against the tomato borer (Tracer 24) with rate of application (35 ml/Feddan). It was obtained from Plant Protection Research Institute,

Agriculture Research Center, Giza, Egypt.

**Egg parasitoid of *Trichogramma*:**

Approximately 70 to 75 adult/m<sup>2</sup> were implemented according to (Gaffar, 2013 & Cabello *et al.*, 2009). The rate of release of each treatment was (136000 individuals/release/182 m<sup>2</sup>, divided into 5 paper cards; each card contained almost 3000 individual) use 3 replicates and the distance between the cards 6 meters was obtained from Plant Protection Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

**Insects:**

**Leafminer, *T. absoluta*:**

For assessing the infestation by larvae of the leafminer, *T. absoluta* on two tomato varieties "Supper 55 and Supper 550", 25 tomato plants per treatment were collected after 2, 5, 7 and 10 days from application from each plot (Oliveira *et al.*, 2009).

**Predators:**

The different species of predators were collected and placed into killing jar containing ethyl acetate for 10-15 minutes then transferred to plastic bags.

**Traps used:**

**Yellow stick traps** (15 × 20 cm) were coated with a special sticky on T-shaped sticks at 20 cm heights in the tomato fields.

**Sweep net size** (38 cm diameter, mesh) were used by swinging the net through the plant at 180 along a 100 m transect. The traps were positioned in the same rows of plots. The crop rows and traps were aligned in an east to west direction.

**Use pheromone in traps:**

The population abundance of *T. absoluta* males was recorded in both control and treated fields using delta sticky trap baited with one capsule containing pheromone. Baited traps were hung at the top level of the plant canopy and the baits were renewed at intervals of 30 days, throughout the sampling period. The sticky plate was changed every 7

days, checked and the numbers of captured adult males were counted and recorded.

**Yield of tomato crop and infestation:**

Tomato yield was determined by collecting tomato fruits from each plot and weighed then recorded in Kg/plot and calculated per feddane. Concerning of fruits infestation, 100 fruits from each plot were collected and examined, numbers of larvae were recorded and the percentage of reduction fruits were calculated according to the equation of Henderson and Tilton equation (1955).

**Determination of residues:**

Spinosad residues were determined in tomato fruits after 1, 3, 7, 10 and 15 days from treatment. The residues were determined by Central Agricultural Pesticides Laboratory, Agriculture Research Center, Giza, Egypt.

**Statistical analysis:**

The mean number of *T. absoluta* was analyzed statistically using a one way analysis of variance. When ANOVA indicates that significant differences were found, (P<0.05) means were separated by a Least Significant Difference Test (LSD) followed by Duncan's test for comparison between different treatment using SAS program (SAS Institute 1988).

**RESULTS AND DISCUSSION**

**Effects of biological program on *T. absoluta* and its natural enemies in two tomato varieties:**

**Effect on number of male moths of *T. absoluta*:**

Data in table (1) show mean number of male moths/trap/week. The results indicated that the efficacy of applied biological control program "Spinosad + Trichogramma + Pheromone" on Supper stream variety was more effective than Casle rock variety, Mean number of males/trap/week was in Supper stream and 21.9 in Casle rock.

Statistical analysis in Table (1) indicated highly significant difference between the biological control program and untreated plots in catching male

moths of *T. obsoluta* in Supper stream and Casle rock varieties (LSD = 0.13 and 0.20, respectively).

Table 1: Effect of biological control program on the male numbers of the leafminer, *Totaabsoluta* in two varieties of tomato plants.

Inspection Date	Mean No. of male moths/trap/week			
	Tomato varieties			
	Supper stream		Casle rock	
	Biological program	Control	Biological program	Control
29/6/2014	29	182	31	212
6/7	24	186	28	214
13/7	18	184	27	221
20/7	17	183	16	213
27/7	16	191	18	221
4/8	14	196	19	219
11/8	13	203	20	220
18/8	10	209	19	221
25/8	12	213	20	222
2/9	13	216	23	220
9/9	12	219	20	224
16/9	12	223	22	226
Total	190	2405	263	2633
Mean	15.8	200.4	21.9	219.4
LSD 0.05	0.13		0.20	

- Biological program (Spinosad + Trichogramma + Pheromone)

### Effect of biological control program in reduction the leafminer, *Tuta absoluta* larvae infesting tomato plants:

Data in Table (2) showed that the effect of biological control program on reduction of the larvae of *T. absoluta* after 2, 5, 7 and 10 days. Results showed that the percent reduction of larval infestation after 1<sup>st</sup> application indicated that 0.0mean No. of larvae/25 plants and percent reduction 100% in the tomato varieties "Supper stream and Casle rock", respectively. After the 2<sup>nd</sup> application data shows the percent reduction of larval infestation was 89.5% with mean No. 0.75 larvae/25 plants in Supper stream variety, whereas, reduction% was 95, with mean No. 1.7 larvae/25 plants in Casle rock variety. In case the 3<sup>rd</sup> application data indicated that the percent reduction of larval infestation was 78.9% , with mean No. 2.0 larvae/25 plants, in Supper stream variety, whereas, was 80.9% reduction, with mean No. 2.3 larvae/25 plants in Casle rock. In 4<sup>th</sup> application data indicated that the percent reduction of larval infestation was 89.5%

average reduction with mean No. 2.3 larvae/25 plants in Supper stream variety, whereas, reached 85.4% reduction, with mean No. 3.3 larvae/25 plants in Casle rock. In case the 5<sup>th</sup> application data indicated that the percent reduction of larval infestation was 92.7% , with mean No. 2.5 larvae/25 plants, in Supper stream variety, whereas, was 94.1% reduction, with mean No. 2.3 larvae/25 plants in Casle rock. The results showed that efficacy of applied biological program on Supper streamann was higher than Casle rock were 1.5 and 1.9 as a general mean, respectively. This results agreement with (Terzidis, 2011) found Spinosad has been mentioned previously and is a popular bio-insecticide used against *T. absoluta* and other tomato pests. Statistical analysis showed highly significant difference between the biological control program and untreated plots in larval reduction of *T. absolutain* Supper stream and Casle rock varieties LSD = 0.01 and 0.09, respectively.

Table 2: Effect of applied biological control program on numbers of larvae/25 plants and larval reduction% of the leafminer, *Tuta absoluta* on two varieties of tomato plants.

Application (days)	Number of larvae/25 plants and larval reduction (%)					
	Tomato varieties					
	Supper stream			Casle rock		
	Biological program		Untreated	Biological program		Untreated
	No. of larvae/25 plants	Red.%	No. of larvae/25 plants	No. of larvae/25 plants	Red.%	No. of larvae/25 plants
1 <sup>st</sup> application						
2	•	100	•	•	100	•
5	•	100	•	•	100	•
7	•	100	•	•	100	•
10	•	100	2	•	100	•
Mean	•	100	0.5	•	100	•
2 <sup>nd</sup> application						
2	•	100	5	6	100	6
5	2	71.0	7	1	83.3	6
7	•	100	6	•	100	8
10	1	87.0	8	•	100	9
Mean	0.5	89.5	6.5	1.7	95	7.2
3 <sup>rd</sup> application						
2	•	73.0	6	1	85.7	7
5	1	88.9	9	•	100	15
7	3	70.0	10	4	66.7	12
10	4	69.2	13	4	71.4	14
Mean	2	78.9	9.5	2.3	80.9	11.5
4 <sup>th</sup> application						
2	3	84.2	19	3	84.2	19
5	2	90.5	21	4	82.3	23
7	1	95.7	23	5	81.5	27
10	3	87.5	24	2	93.5	31
Mean	2.25	89.5	21.8	3.3	85.4	25
5 <sup>th</sup> application						
2	2	93.5	31	3	90.9	33
5	3	90.6	32	2	94.6	37
7	2	94.4	36	1	97.6	41
10	3	92.3	39	3	93.33	45
Mean	2.5	92.7	34.5	2.3	94.1	39
General Mean	1.5	90.1	14.6	1.9	91.2	16.5
LSD0.05	0.01		0.09			

- Biological program (Spinosad + Trichogramma + Pheromone)

#### Efficacy of traps to catches natural enemies in tomato fields during applied biological control program:

Data in Table (3) show weekly numbers of natural enemies (Coccinellidae, Aphid lion, Syrphidae, *Hemianax ephippige* and *Nesidiocoris tenuis*) caught by yellow sticky traps on tomato plants during summer season. The results show that the numbers of caught natural enemies in summer season reach 1.9, 5.0, 2.5, 5.6 and 1.2 /trap/week for

Coccinellidae, Aphid lion, Syrphidae, *Hemianax ephippige* and *Nesidiocoris tenuis*, respectively in Supper stream variety. In Casle rock variety the respective catches were 3.4, 5.9, 2.9, 4.2 and 0.7. Predators catches by seeping net traps on tomato plants, the numbers of catches averaged 6.5, 5.3, 4.1, 5.9 and 3.2 /trap/week for Coccinellidae, Aphid lion, Syrphidae, *Hemianax ephippige* and *Nesidiocoris tenuis*, respectively in Supper stream variety. In Casle rock

variety the respective values were 8.5, 6.1, 4.7, 7.5 and 1.3. Statistical analysis showed highly significant difference between the two tomato varieties in catches natural enemies using yellow sticky traps (LSD = 0.09). Also found that

highly significant difference between the two tomato varieties in catches natural enemies using sweeping net traps (LSD = 0.05). The obtained results agree with those obtained with results indicate by that (Walker *et al.*, 2012).

Table 3: Effect of applied biological program on mean number of natural enemies by catching with yellow sticky trap and sweeping net in two varieties of tomato plants.

Natural enemies	Mean number of natural enemies one trap/week							
	Yellow sticky				Sweep net			
	Tomato varieties				Tomato varieties			
	Supper stream		Casle rock		Supper stream		Casle rock	
	Biological program	control	Biological program	control	Biological program	control	Biological program	control
Coccinellidae	1.9	3.2	3.42	5.41	6.51	11.53	8.52	12.55
Aphid lion	5.0	6.5	5.94	8.33	5.32	7.42	6.11	7.57
Syrphidae	2.5	3.7	2.92	4.35	4.12	4.73	4.72	7.81
<i>Hemianax ephippige</i>	5.6	7.1	4.21	5.83	5.94	8.31	7.51	10.54
<i>Nesidiocoris tenuis</i>	1.2	2.1	0.73	1.52	3.22	4.42	1.32	5.70
LSD0.05	0.09		0.04		0.03		0.05	

#### Effect of biological control program on productivity of two tomato varieties Effect on infestation %:

Results in Table (4) indicated reductions in percentage of infestation with *T. absolutin* 100 fruits in two tomato varieties. The results indicate that the biological control program reduced the percentages of infestation in 100 fruits for the two varieties. Percentages

of reduction were 7.4 and 7.0% in Supper stream and Casle rock, respectively. Statistical analysis showed highly significant difference between the biological control program and the untreated in percentage of infestation by *T. absolutin* Supper stream and Casle rock varieties LSD = 0.015 and 0.023, respectively.

Table 4: Effect of biological control program on 100 fruits infestation with the leafminer, *Tuta absolutin* two tomato varieties.

Harvest	Reduction% in 100 fruits infected			
	Tomato varieties			
	Supper stream		Casle rock	
	Biological program	Control	Biological program	Control
First	11.2	29.6	8.2	31.16
Second	10.5	33.9	10.4	34.89
Third	6.8	32.3	5.21	37.34
Fourth	3.5	36.0	6.67	39.50
Fifth	4.8	28.7	4.34	43.23
Mean	7.4	32.1	6.97	37.22
LSD0.05	0.015		0.023	

#### Effect on 100 fruits weight:

The results indicated that the weight of 100 fruits was high in the two varieties Supper stream and Casle rock, recorded 5170.6 g and 4903 g,

respectively. On the other hand untreated (control) recorded 3947 and 3833.4 for supper stream and casle rock respectively, this shown in Table (5). Statistical analysis showed that highly

significant difference between the biological control program and untreated in catching male moths of *T. absoluta* in Supper stream and Casle rock varieties during summer season LSD = 0.054 and 0.092, respectively.

Table 5: Effect of biological control program on weight of 100 fruits in two tomato varieties after infestation with the leafminer, *Tuta absoluta* male moth.

Harvest	Reduction% in 100 fruits infected			
	Tomato varieties			
	Supper stream		Casle rock	
	Biological program	Control	Biological program	Control
First	5198	4004	4983	3990
Second	5017	4011	5011	4117
Third	5234	3912	4972	3698
Fourth	5189	3894	5017	3711
Fifth	5215	3914	4536	3651
Mean	5170.6	3947.0	4903.0	3833.4
LSD0.05	0.045		0.092	

### Effect on yield production

Results in Table (6) shows highest yield production of the two tomato varieties of tomato plants, Supper stream and Casle rock comparing with the untreated (control). From these results the yield production after treatment of biological control program on Supper

stream was more than Casle rock variety, it gave 18810 Kg/Feddand and 17569 Kg/Feddand, respectively. On the other hand, yield production in the untreated gave 2548 Kg/Feddand and 3009 Kg/Feddand for Supper stream and Casle rock, respectively.

Table 6: Effect of biological control program on yield production in two tomato varieties after infestation with the leafminer, *Tuta absoluta* male moth.

Harvest	Yield production (Kg/Feddand)			
	Tomato varieties			
	Supper stream		Casle rock	
	Biological program	Control	Biological program	Control
First	4221	1041	3793	1016
Second	3843	573	3612	571
Third	4174	592	3785	543
Fourth	3116	465	3122	482
Fifth	3478	417	3257	397
Mean	18832	2548	17569	3009
LSD0.05	0.090		0.031	

Mass trapping using tomato leafminer's sex pheromone (Hassan and Alzaidi, 2009), application of a pheromone-based mating disruption technique (Cocco *et al.* 2013), and biological control using *Trichogramma* parasitoids (Cabello *et al.* 2012; Chailleux *et al.* 2012) may provide environmentally safe and adequate control of this pest these agree with the current study.

Determination of Spinosad residues:

The dissipation trends of Spinosad rapidly after application in tomato fruit were shown in Table (7). The concentration of Spinosad 2 h after treatment was 0.223 mg/kg. The residues amount decreased to 0.118 mg/kg within the first 24 h after application following that period residues decreased to 0.092, 0.074, and 0.015 mg/kg, at 3, 7, and 10 days after treatment, respectively. But after 15 days no detectable amount of Spinosad. The half-life of Spinosad calculated at recommended dose was



2.25 day shown in Table (7). The dissipation of the pesticide residues in/on crops depends on environmental condition, type of application, plant species, dosage, and interval between application, the relation between the treated surface and its weight and living state of the plant surface, in addition to harvest time (Tomkinset *al.*,1999). While the FAO/WHO has not established

maximum residue limits (MRLs) for Spinosad, European Union MRL for Spinosad in tomato is 0.3 mg/kg. It can thus be concluded that the preharvest interval (PHI) of spinosad on tomato was 1-days after the last treatment. Recoveries and relative standard deviation (RSD) of spinosad in tomato at various fortification level shown in Table (8).

Table 7: Residues of Spinosad on/in tomato fruits.

Time interval (days)	Residues of Spinosad		
	Residues	Loss <sup>c</sup>	Persistence% <sup>d</sup>
	(ppm)	(%)	(%)
Initial <sup>a</sup>	0.223±0.03	0.00	100
1	0.118±0.009	47.08	52.92
3	0.092 ±0.01	58.74	41.26
7	0.074 ±0.08	66.81	33.19
10	0.015±0.007	93.27	6.73
15	ND <sup>b</sup>	100	0
T <sub>1/2</sub> <sup>e</sup> (hours)	2.25		
MRL <sup>f</sup> (ppm)	0.3		

The values were corrected according to the recoveries percent. a =Samples were taken one hour after application. b= (Not detectable). c = [(initial residue-residues found at different time) /initial residue] x100. d=100-% loss. e = (Half-life). f= the maximum residue limits according to (EU 2010).

Table 8: Recoveries and relative standard deviation (RSD) of spinosad in tomato at various fortification level.

Matrix	Fortified level (mg kg <sup>-1</sup> ) (n*=5)	Recovery (%)	RSD (%)
Tomato	0.01	90.7	10
	0.05	96.4	5
	0.1	99.6	8

We can concluded that use biological control program “(Spinosad + Trichogramma + Pheromone” against the leafminer *Tuta absoluta* in two tomato varieties because of its high efficiency, also safety on natural enemies and cause increase yield production within these treatments under study. Also, residual in/on tomato fruits after 2 h. from application. The residues amount of spinosad decreased to 0.118 mg/kg within the first 24 h

after application this was less than maximum residue limits (MRL).

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## ARABIC SUMMERY

### المكافحة الحيوية لنافقات نبات الطماطم التوتا ابلويوتا (ميركل) رتبة حرشفية الاجنحة

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تهدف الدراسة لتقييم كفاءة تطبيق برنامج مكافحة بالمركب Spinosad مع طفيل التريكوجراما والفرمونات الجاذبة ضد حافرة اوراق الطماطم *Tuta absoluta* رتبة Lepidoptera علي نباتات الطماطم تحت الظروف الحقلية المفتوحة علي صنفين من الطماطم (Supper strean and Casle rock) وتأثير البرنامج علي إنتاجية المحصول في كلا الصنفين. أوضحت النتائج كفاءة البرنامج علي متوسط عدد الذكور/المصيصة وعدد اليرقات لحشرة *Tuta absoluta* في صنف Supper strean أعلى من صنف Caslerock. أظهرت لايوجد تأثير علي اعداد الأعداء الحيوية بعد تطبيق البرنامج باستخدام مصائد yellow sticky sweet net في كلا الصنفين مقارنة بالكنترول. النتائج أوضحت أن وزن ١٠٠ ثمرة كان اعلى في Supper strean من Casle rock. واستخدام البرنامج أدى إلي تقليل النسبة المئوية للاصابة باليرقات في كلا الصنفين ولكن في صنف Casle rock سجل إنخفاض أقل من صنف Supper strean. الدراسة أظهرت أن متوسط عدد الثمار لكل نبات كان اعلى في كلا الصنفين ولكن صنف Supper strean كان اعلى من صنف Casle rock. اشارتالنتائج ارتفاع إنتاجية صنف الـ Supper الذي سجل ١٨٨١٠ كجم/فدان و ١٧٥٦٩ كجم/فدان لصنف Casle rock مقارنة بالكنترول ٣٠٧٠ كجم/فدان. أظهرت نتائج تقدير الاثر المتبقي لـ Spinosad على الثمار بعد ساعتين و ١ و ٣ و ٧ و ١٠ و ١٥ يوم من التطبيق. اولا: بعد ساعتين من المعاملة ٠.٢٢٣ ملجم/كجم، و إنخفضت إلي ٠.١١٨ ملجم/كجم بعد ايوام من المعاملة ثم انخفض إلي ٠.٠٩٢ و ٠.٠٧٤ و ٠.٠١٥ ملجم/كجم بعد ٣ و ٧ و ١٠ يوم من المعاملة علي التوالي، ولكن بعد ١٥ يوم من المعاملة لم يلاحظ أي متبقي لـ Spinosad امان المركب امن على الثمار بعد تطبيق البرنامج.