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Management of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) Using Biopesticides on Tomato Crop under Greenhouse Conditions

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Abstract

Tuta absoluta is the major insect invading tomato crop under greenhouse and open field conditions in Lebanon. Farmers mainly depend on chemical control to reduce damage caused by the larva. The hazard use of chemical agents can lead to resistance accumulation. The objective of this study is to investigate alternative agents like biopesticides to control this pest. Two field trials were conducted at the Lebanese Agricultural Research Institute (LARI) for two years under greenhouse conditions. In 2014, the first trial was conducted in two greenhouses: 1-control greenhouse without insect proof net (CG); 2-double door Greenhouse with insect proof net (DDG). In 2015, the second trial was conducted only in control greenhouse.Four treatments and control (not treated plot) were adopted in both trials. The biopesticides used in this study were Neem azal and *Bacillus thuringiensis*. Results of the first trial showed that using insect proof net reduced the captured adults on the water trap as compared with control greenhouse and thus reducing the damaged caused by the larva of tomato leaf miner on leaves and fruits. The adopted treatments have shown significant differences in the number of mines/leaf, live larva/leaf and percent of damaged fruits in both trials compared to the control. Applying Bacillius thuringiensis and neem azal separately and mixing them together have shown a promising alternative method to chemical control.

1. Introduction

Tomato leafminer (*Tuta absoluta*, Meyrick Lepidoptera: Gelechiidae) is currently recognized as a major threat to tomato production in both greenhouse and open field conditions. The moth originates from South America and was spread to Europe, North Africa, Asia and recently in the Middle East over the past ten years (Desneux, 2011; FAO, 2016; Alberto Urbaneja, 2012). Their larvae damage leaves, fruits, and stems (Savino et al., 2012), causing considerable losses on tomato yield. Losses of 50-100% have been reported on tomato (EPPO, 2005; Gebrelebanos, 2015). Tomato leafminer has a high reproductive potential (Cuthbertson et al., 2013). In favorable conditions, the insect can complete about 10-12 generations in a year (IRAC, 2011; NAPPO, 2012). Tomato leafminer is difficult to control because the larvae feed inside the plant (Guedes et al., 2012; Gebrelebanos, 2015) and their capability to develop resistance to synthetic insecticides (IRAC, 2011; Guedes et al., 2012; Siqueira et al., 2010). Resistance to pyrethroid (Siqueira et al., 2010), abamectin (Siqueira et al., 2001; Guedes et al., 2011; Lietti et al., 2005), cartap (Siqueira et al., 2001; Siqueira et al., 2000), permethrin, methamidophos (Siqueira et al., 2001; Lietti et al., 2005) and spinosad (Campos et al., 2014) used to control tomato leaf miner has been reported.

In Lebanon, control of tomato leaf miner mainly depends on chemical insecticides. Mass trapping is a viable alternative method (Lobos et al., 2013), however, Megido et al. (2013) reported that sex pheromone-based strategy is sometimes more expensive than conventional chemical control. Therefore, the development of control strategies based on the use of bioinsecticides as alternatives to synthetic insecticides should be encouraged due to their potent effects on insect pests, low mammalian toxicity, low persistence in the environment, and biodegradability (EPPO, 2005; Khater, 2012; Koul et al., 2004). Thus, organic molecules of botanical origin may offer a safe source of compounds for pest management, being environmentally friendly, and an excellent alternative to persistent synthetic insecticides (Biondi et al., 2006). Neem seed extract active ingredient azadiractin 1%, acts as antifeeding, deterrent and growth inhibitor against a number of insects and mites including tomato leaf miner (Marčić et al., 2009; Koul et al., 2004).

Bacillus thuringiensis (Bt) is used as a biopesticide agent against tomato leaf miner by producing a toxic crystal protein that affects larval stage of many Lepidoptera species. Bt was mentioned in (Gonzalez-Cabrera, J et al.,2011) work to be effective against tomato leaf miner .Mixing Neem Azal % with Bt was reported to have a high selective toxicity on *T. absoluta* and low negative effect on their predators (Nazarpour1 et al., 2016).

Some biopesticides were reported not to cause harm to natural enemies (Shabozoi et al., 2011), but the sublethal effect of azadirachtin on predator should not be ignored (Judit et al., 2011). Applying Bt as a biopesticide proved to be safe for generalist parasitoid of *Tuta absoluta* (Biondi, 2013).

The objective of this study is to investigate the effect of neem azal and Bt on *T. absoluta* under greenhouse condition in Lebanon.

2. Materials and Methods

Two trials were conducted at the Lebanese Agricultural Research Institute (LARI) in Lebaa station (33°32.681′ North, 35°27.088′ East, 354 m a.s.l.), south Lebanon, province of Jizzin, under greenhouse conditions during the 2014-2015 spring seasons. The Newton variety was used in both trials. In 2014, the first trial was performed in two greenhouses in order to compare the effect of maintenance type of the greenhouse on the insect density. The first one is a double door greenhouse closed tightly with an insect proof net (DDG). The second greenhouse was kept without insect proof net (Control greenhouse-CG). Both greenhouses were with the same area (256 m^2). In 2015, the second trial was performed only in control greenhouse (CG). In both trials, the selected bioinsecticides (Neem Azal (1%) and *Bacillus thuringiensis* var. Kurstaki, 32 MIU g^{-1} [millions of International Units per gram]) were assigned to 4 treatments as follow:

Treatment I (NA10): Neem Azal sprayed at 10 days frequency (60 ml/20 L water);

Treatment II (NA15): Neem Azal sprayed at 15 days frequency (60 ml/20 L water);

Treatment III (BT10): Bacillus Thuringiensis sprayed at 10 days frequency (20 g/20 L water);

Treatment IV (NABT): Neem Azal mixed with Bacillus Thuringiensis sprayed at 10 days frequency with the same rate as other treatment;

Treatment V (Ctrl): Kept as untreated control.

A water trap baited with sex pheromone was placed in the center of the greenhouse to monitor the male activity of *T. absoluta*.

2.1 Data Recording

Three complete leaves were collected randomly from each experimental plot at each sampling date, and brought to the lab to record the average number of mines and larva/leaf. Four plants were chosen randomly from each experimental plot and the % of damaged fruits was assessed as follow: (Number of damaged fruits)/(Total number of fruits) × 100.

To evaluate the effect of treatments as compared with control on yield parameters, total fruit/plant, fruit weight, diameter and height were recored from each experimental plot. In the second trial (2015), in addition to these parameters, soluble solids and acid fruit (Total acidity was expressed as percent of citric acid equivalent) content were measured by the means of refractometer and titration.Fruits were divided into four categories: < 70 g (I); 70-120 g (II); 121-200 g (III); > 200 g (IV) and the percent of each fruit category was recorded.

2.2 Experimental Design and Statistical Analysis

The two trials were conducted in randomized complete block design (RCBD) with four blocks. The experimental unit consisted of 20 plants. Repeated analysis of variance was performed for testing the effect of treatments, time and their interaction on the number of mines and larva/leaf and % of damaged fruits by using SAS for windows V8. A post hoc Tukey's test ($p < 0.05$) was used to discriminate among means of treatments. The normality assumption was not met for the number of captured adults of *T. absoluta*, number of mines/leaf and % of damaged fruits of control plots in both greenhouses, therefore a Wilcoxon test was conducted instead of two sample T test. The sphericity assumption for repeated analysis was not also met, therefore the results of univariate were reported instead.

3. Results and Discussion

3.1 First Trial-2014

The number of captured adults of tomato leafminer were significantly greater in the control greenhouse (Mdn = 88) than in the double door greenhouse (Mdn = 8), (Wilcoxon test, Z = 3.87, p = .0001, r = $.45^{ns}$). They ranged from (19 to 390) and (4.2 to 86) over 13 weeks (Figure 1). Since the same treatments were used in both greenhouses, these differences in control plots are likely due to the use of insect proof net. The number of mines/leaf in control plots of the control greenhouse (CG, Mdn = 6.3) differed significantly from the control plots of the double door greenhouse (DDG, Mdn = 0.35), Z = 4.35, p < .0001, r = $0.36^{(p = 0.0175)}$. Regarding the % of damaged fruits, a Wilcoxon test showed a significant difference between control plots of the (CG, Mdn = 60) and (DDG, Mdn = 6), Z = 5.3, p < .0001, r = $.51^{(p = 0.01)}$. These differences are also likely due to the use of insect proof net. These data agree with the previous findings obtained by (Cherif et al., 2013).

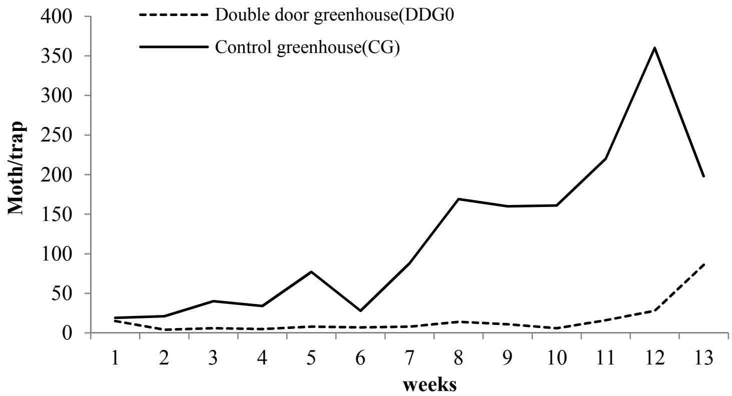

Figure 1. Dynamics of *Tuta absoluta* during spring season in both double door and control greenhouses in Lebaa Station (2014)

A repeated measures of anova was conducted to compare the effect of treatments, time and their interaction on the number of mines and larva/leaf and % of damaged fruits in both control and double door greenhouses. Mauchly's test indicated that the assumption of sphericity was not met for all tested variables in both greenhouses and therefore, a Greenhouse-Geisser correction was used for time and time × treatment interaction. There was a significant effect of time on the number of mines and larva/leaf and % of damaged fruits in both greenhouses (Table 1). An interaction of time and treatment showed a significant effect on the three variables meaning that the effect of time differ by treatments (Table 1). Effect size of treatments was relatively higher than the effect size of time and time -treatment interaction.

Table 1. Results of repeated measures analysis (Anova) (df, F, P, η^2) for the first trial (2014)

	Mine/Leaf				Larva/Leaf				% Damaged Fruits			
	df	*F*	*P*	η^2	*df*	*F*	*P*	η^2	*df*	*F*	*P*	η^2
Control greenhouse(CG)												
Trt	4,15	17.5	< .0001	.82	4,15	16.9	< .0001	.82	4,15	196.3	< .0001	.98
Time	10,150	38.7	< .0001	.72	10,150	17.9	< .0001	.54	5,75	34.6	< .0001	.70
Trt × Time	40,150	5.9	< .0001	.61	40,150	4.2	< .0001	.53	20,75	9.4	< .0001	.71
Double door greenhouse(DDG)												
Trt	4,15	17.2	< .0001	.82	4,15	19.9	< .0001	.84	4,15	27.7	< .0001	.88
Time	10,150	24.4	< .0001	.62	10,150	9.8	0.0005	.40	5,75	25.7	< .0001	.63
Trt × Time	40,150	4.9	0.0005	.57	40,150	4.97	0.0005	.57	20,75	5.7	< .0001	.60

Effect of treatments on the studied variables in both greenhouses was significant and therefore, a post hoc tukey test was conducted. The test showed a significant effect of treatments as compared with control on the number of mines and larva/leaf and the % of damaged fruits in both control and double door greenhouses. The results in the Table 2 show no significant differences between treatments themselves except for the % of damaged fruits in the control greenhouse where the significant was observed between Bt and NA15 treatments.

Table 2. Means and standard deviation of mines/leaf (M/L), larva/leaf (L/L) and percent of damaged fruits (%DF) as affected by treatments in both DDG and CG greenhouses of the first trial (2014)

Treatments	Control greenhouse			Double door greenhouse		
	M/L	L/L	%DF	M/L	L/L	%DF
NA10	3.9 ±0.7b	0.6±0.1b	9.3±0.5bc	0.5±0.4b	0.03±0.1b	2.0±0.6b
NA15	1.7±0.8b	0.2±0.1b	4.0±1.0c	0.4±0.1b	0.06±0.1b	1.3±0.2b
NABT	2.4±0.3b	0.3±0.1b	7.5±0.9bc	0.5±0.2b	0.13±0.2b	1.7±0.3b
BT	1.9±0.7b	0.3±0.1b	9.8±2.5b	0.6±0.2b	0.1±0.1b	1.9±0.4b
Control	10.9±4.8a	1.7±0.4a	45.6±4.6a	3.2±1.0a	0.9±0.2a	6.4±1.6a

Note. Columns with different letters are significantly different (Tukey's test, $p < 0.05$).

3.2 Second Trial-2015

Figure 2 shows the dynamics of tomato leafminer adults through the spring growing season on tamoato crop in the CG greenhouse. The counted moths ranged from 37 to 428 moth/trap/week. The Maximum captures were observed at the first week of June.

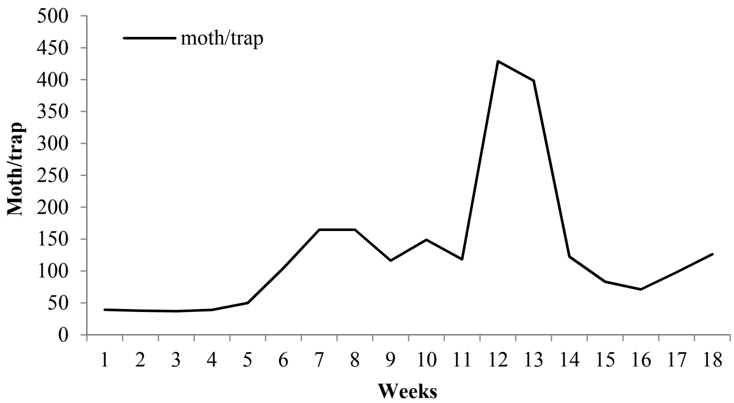

Figure 2. Dynamics of *Tuta absoluta* during the spring growing season of tomato crop planted in control geenhouse in Lebaa Station, 2015

As in the first trial of 2014, nearly the same patern of effect of treatments, time and their interaction on the studied variables was observed. Repeated analysis of variance showed a relatively low effect size of time on the number of larva/leaf and % of damaged fruits (Table 3). Effect of treatments, time and their interaction on the number of mines and larva/leaf and % of damaged fruits was significant. A post hoc tukey test showed a significant effect of treatments as compared with control on the three studied parameters. No significant differences were indicated between treatments themselves in regard to these parameters (Table 3).

Table 3. Results of repeated measures analysis (Anova) (*df*, *F*, *P*, η^2) of the effect of time, treatment (Trt) and their interaction on the number of mine and larva/leaf and % of damaged fruit in the second trial (2015)

	Mine/leaf				Larva/leaf				% damaged fruits			
	df	*F*	*P*	η^2	*df*	*F*	*P*	η^2	*df*	*F*	*P*	η^2
Trt	4,15	78.4	< .0001	.95	4,15	20.4	< .0001	.84	4,15	40	< .0001	.91
Time	16,240	41.9	< .0001	.74	16,240	4.6	0.0042	.24	8,120	3.7	0.037	.20
Trt*Time	64,240	10.6	< .0001	.74	64,240	1.8	0.578	.33	32,120	2.7	0.0242	.42

Table 4. Average values of mines/leaf (M/L), larva/leaf (L/L) and percent of damaged fruits (%DF) as affected by treatments in CG greenhouse of the second trial (2015)

Treatments	M/L	L/L	%DF
NA10	4 ±1.4b	0.3±0.1b	1.3±1.5b
NA15	3.5±0.8b	0.2±0.3b	1.2±0.5b
NABT	1.6±0.2b	0.2±0.1b	0.4±0.6b
BT	4.1±1.3b	0.3±0.1b	1.8±2.2b
Control	14.9±1.6a	0.9±0.3a	19.6±4.6a

Note. Columns with different letters are significantly different (Tukey's test, $p < 0.05$).

3.3 Agronomic Traits

The results showed no significant differences between treatments and control regarding total number of fruits/plant in both DDG and CG greenhouses of the first trial respectively ($F(4,15) = 0.98$, $p = 0.448$, $\eta^2 = .12$; $F(4,15) = 0.41$, $p = 0.8$, $\eta^2 = .09$).

In the second trial (2015), The results showed no significant differences between treatments and control regarding total number of fruits/plant ,acidity and brix degree respectively ($F(4,15) = 1.59$, $p = 0.227$, $\eta^2 = .30$; $F(4,15) = 0.82$, $p = 0.53$, $\eta^2 = .18$; $F(4,15) = 0.66$, $p = 0.63$, $\eta^2 = .15$). Most of the fruits occurred between the second and third categories which accounted for about 70-80%. This reflects variety attributes.

All treatments significantly reduced the number of mines, larva and damaged fruits as compared with control. There was not significant diffirence between NA10 and NA15. Applying NA at 15 days frequency kept the damaged caused by tomato leafminer at low levels. The advantage of applying NA at 15 days frequency was to reduce the number of sprays per season as compared with NA10. These results agree with the result obtained by Yankova (2014) reported that the highest effectiveness of neem Azal was recorded after 15 days. Our results of mixing Neem azal with Bt agree with the findings obtained by (Mohammed, 2002; Abbas, 2011) reported in their work the high efficacy of mixing BT with other biopesticides.Using BT alone also showed a significant difference as compared with control for the same studied parameters.

4. Conclusions

This study is the first assessment of the performance of two biopesticides in Lebanon on *Tuta absoluta*. Both two biopesticides were effective in reducing the damage made by the larva on fruit and leaves. The results obtained are consistent for two successive years. Mixing the two biopesticides has shown a significant effect in reducing the number of mines and the % of damaged fruits. Using neem azal in a 15-day frequency could be recommended instead of the 10 days frequency as no significant differences between them were observed. Number of mines/leaf and % of damaged fruit were affected by the maintenance type of the greenhouse. Crop yield parameters were not affected by the adopted treatments.

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