

Evaluation the Efficacy of Certain Bio-Rational Insecticides on *Tuta Absoluta* (Lepidoptera, Gelechiidae) on Tomatoes under Laboratory and Field Conditions

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Abstract: The tomato leaf miner (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an exotic pest on tomato plants either in open field or under protected cultivations at Ismailia Governorate and elsewhere. The efficacy of five different commercial bio-rational products viz., Coragen, Dipel 2X, Match, Proclaim and Spinosad in their recommended dose was evaluated against TLM under laboratory and field conditions. Data indicated that under laboratory conditions, the tested insecticides showed various toxicity to first and fourth instar larvae of TLM. Proclaim caused the highest accumulated mortality in TLM larvae, followed by Coragen and Spinosad 10 days post treatment. Dipel 2X and Match were slightly toxic to first instar, while Match didn't show any toxicity towards fourth instar larvae. Under field condition, the lowest number of mines (2.4 mines/plant) was recorded in Coragen treatment after 10 days of application, followed by Proclaim, Spintor, Dipel and Match compared to control at 2.7, 4, 4.1, 9.1 and 9 mines/plant, respectively. The highest percentage of reduction in infestation of TLM was recorded in Coragen, Spintor and Proclaim treatments at 68.33, 61.97 and 54.89%, respectively. Dipel had moderate effectiveness in reduction of infestation at 42.78%, while the lowest percentage of reduction in infestation was recorded in Match treatment at 27.51%.

Keywords: *Tuta absoluta* – bioinsecticides – Tomato – mortality – infestation.

INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), in only a few years, has become a serious threat to global tomato production. It is now considered to be one of the most damaging invasive pests of tomatoes in the Mediterranean Basin countries such as Egypt, Tunisia, Libya, Morocco and Algeria (Caparros *et al.*, 2012). Potential yield loss in tomatoes (quantity and quality) is significant and can reach up to 100% if the pest is not managed properly (Desneux *et al.*, 2010). The control of TLM relies heavily on chemical control, but decreasing efficacy has been attributed to the development of insecticide resistance (Haddi, 2011). Therefore, modern integrated pest management (IPM) recommends effective pesticides that have low mammalian toxicity, low persistence in the environment, and high degree of selectivity and delay the development of resistance to other classical insecticides (Braham and Hajji, 2012). Fortunately, in Egypt, some bio-rational pesticides began to be used against this pest. The insecticide, "Emamectin benzoate" (Proclaim®) is a new macrocyclic lactone insecticide derived from the natural avermectin family produced by fermentation of a soil microorganism *Streptomyces avermitilis* (Schallman *et al.*, 1987). The insecticide acts on the GABA receptor activating the chloride channel (Aliferis and Jabaji, 2011). This product has been developed for the control of Lepidopterous pests including TLM on a variety of vegetable crops worldwide (Liguori *et al.*, 2008).

Chlorantraniliprole (Coragen®) is a new compound belonging to a new class of selective insecticides (anthranilic diamides) featuring a novel mode of action. By activating the insect ryanodine receptors (RyRs), it stimulates the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells, causing impaired muscle regulation,

paralysis and ultimately death of sensitive species (Cordova *et al.*, 2006). It has very low toxicity for mammals (both acute and chronic), high intrinsic activity on target pests, strong ovicidal and larvicidal properties, long lasting crop protection and no cross-resistance with any existing insecticide (Bassi *et al.*, 2009). It has very high biological activity on several caterpillar species; including *Carpocapsa pomonella* and *Leptinotarsa decemlineata* (Bassi *et al.*, 2009) with outstanding performance on TLM and an extremely low mammalian toxicity profile (Bassi *et al.*, 2012).

Spinosad (Spintor®) is a biopesticide produced through the fermentation of the soil actinomycete, *Saccharopolyspora spinosa* Mertz & Yao (Thompson and Hutchins, 1999). Its active ingredient consists of a mixture of spinosyn A and spinosyn D (Salgado, 1998). Because of its unique mode of action, involving the postsynaptic nicotinic acetylcholine and GABA receptors, spinosad has strong insecticidal activity against insects (Salgado, 1998) especially Lepidoptera (Wang *et al.*, 2009).

Lufenuron (Match®) is an acylurea insect growth regulator belonging to the group of benzoyl-phenylureas (BPUs) and is classified as an insect development inhibitor (IDI). Like all BPU's, lufenuron interferes with chitin biosynthesis acts mainly by ingestion (Arora *et al.*, 2011). Insect growth regulators like lufenuron are claimed to be safe and have little impact on beneficial arthropods as compared to conventional insecticides (Whiting *et al.*, 2000).

Dipel 2X® is *Bacillus thuringiensis v. kurstaki* based insecticides (Lambert and Peferoen, 1992). The insecticidal activity of *B. thuringiensis* is attributed to the parasporal crystals, known as delta endotoxins or insecticidal crystal proteins (ICP) (Xavier *et al.*, 2007), which are toxic to the larval stage of the insects belonging to Lepidoptera, Diptera, and Coleoptera

(Schnepp *et al.*, 1998). Bt-based insecticides are harmless to most wildlife and beneficial insects (De Maagd *et al.*, 2001).

Therefore, the current research aimed to determine the efficacy of Proclaim, Coragen, Spinosad, Dipel 2X and Match against TLM under laboratory and field conditions.

MATERIALS AND METHODS

Maintenance of TLM:

Tomato leaves bearing eggs and different larval instars of tomato leaf miner moth (TLM), *Tuta absoluta* were periodically collected from the TLM infested tomato fields in the Experimental Farm, Faculty of Agriculture, Suez Canal University. Larvae were reared on tomato seedlings (30 cm long) grown in plastic pots (15 cm diameter). Tomato seedlings were placed in a rearing cage (40 cm in width × 60 cm in length × 80 cm in height), three of its sides covered by organza and one covered with glass. These cages were kept under the laboratory conditions of 25± 2°C; 60± 10% RH and photoperiod of 14/10 (L/D) h. After the completion of the immature development, TLM pupae were collected, kept in clean Petri dishes and placed in clean glass jars (30 × 15 cm) until moth emergence and fed on sucrose solution (10%). Upon emergence, adults of TLM were collected, transferred to other rearing cage as previously mentioned for rearing of TLM larvae, and provided with tomato seedlings as an ovipositional substrate to maintain the culture. This cycle was repeated for at least 3 generations before being used in the intended experiments.

Tested Bio-rational Insecticides

The list of commercial formulations of the certain insecticides tested against the first and fourth instars larvae of TLM in this study are presented in (Table 1). These bio-rational insecticides were Coragen (20% SC Chlorantraniliprole), Dipel 2x 7.5 (Bacillus thuringiensis var. kurstaki 32000 IU/mg), Match (50% EC Lufenuron), Proclaim (5% EC Emamectin benzoate), and Spintor 24% SC (spinosyns A and D, *Saccharopolyspora spinosa*). Solutions of all tested compounds were prepared in distilled water at the recommended field rate (FR), (Coragen 60 mg/l a.i., Dipel 2x 128 mg/l a.i., Match 200 mg/l a.i., Proclaim 30 mg/l a.i. and Spintor 60 mg/l a.i.). The tested concentrations of all tested compounds in the present study were freshly prepared one hour prior to experiments.

Laboratory bioassay

The effect of the field rates of the tested bio-rational insecticides were studied against *T. absoluta* first and fourth instar larvae. The treatments were performed using tomato seedlings (cv. GS-12) transplanted in small plastic pots (15 cm diameter) filled with peat moss and watered when needed. The tomato plants were maintained free of TLM infestation in the laboratory until use. One day before the assay, plants (having 4 - 6 true leaves) were artificially infested with 10 larvae / plant. The tested insecticides were sprayed using a small handle sprayer until run off. Treated plants were left for one hour to dry out, then they were kept in a glasshouse

under conditions of 25± 2°C and 60± 10% R.H. Four replicates (plants) and an untreated plant was used as a control for each treatment. Data were recorded 1, 3, 5, 7 and 10 days after insecticide application in terms of dead and live larvae. The mortality rates in each treatment were calculated and corrected using Abbott's formula (Abbott, 1925) as follow:

$$\text{Corrected mortality (\%)} = [(Ck - Ta) / Ck] \times 100$$

where Ck = number of insects in control after application, Ta = number of insects in the treatment after application.

Field bioassay:

The field experiments were conducted to evaluate the field efficiency of tested bio-rational insecticides against the infestation of TLM using tomato plants variety GS grown at the Experimental Farm of Agriculture Faculty, Suez Canal University. The required agricultural practices were applied except for the use of insecticide. The experiment was carried out as a randomized complete block design consisting of 6 treatments. Each treatment contained 3 plots of tomato plants (5 × 5 m). Each plot consisted of 5 rows with a total of 25 plants for each experimental plot. In each treatment, ten plants represented ten replicates were selected randomly and labeled for periodical inspection and data recording. The bio-rational insecticide treatments included Match at 0.4 cm/l, Spintor at 0.25 cm/l, Dipel 2x 7.5 at 2.0 g/l, Proclaim at 0.6 g/l and Coragen 0.3 cm/l. These insecticides were applied using a backpack sprayer in a broadcast application on tomato plants. Applications were preferred when tomato seedlings were at the age of about 1.5 months after transplanting, on 24 April 2013 at 9.00 am. The numbers of newly mines of TLM/10 complete leaflets/plant was recorded before and after application. Data were recorded 1, 3, 5, 7 and 10 days after application on ten selected plants of each treatment. To determine the effectiveness of the tested bio-rational insecticides, the percent of reductions in infestation with TLM throughout the inspection periods were estimated using Henderson and Tilton (1955) equation as follow:

$$\text{Efficiency (\%)} = [1 - (n \text{ in } Ck \text{ before treatment} * n \text{ in } T \text{ after treatment}) / (n \text{ in } Ck \text{ after treatment} * n \text{ in } T \text{ before treatment})] \times 100$$

where: n = number of mines/plant, T = treated, and Ck = control

Statistical analysis:

Normality of data was tested by the Shapiro-Wilks test before further analysis. When a Shapiro-Wilk test indicated that data were normally distributed, data were analyzed by parametric analysis of variance (ANOVA) and then the Holm-Sidak or Student-Newman-Keuls Methods were used for all pairwise multiple comparisons. When data were not normally distributed, a nonparametric Kruskal-Wallis ANOVA on ranks (H test) was used and Tukey's or Dunn's tests were used to compare treatment means at a 0.05 level of

significance. Data were analyzed using SigmaPlot 12.3 (Systat Software, Inc. 2011).

RESULTS

Laboratory bioassay:

The obtained data demonstrated various toxicity of the tested bio-rational insecticides at their field rates to larvae of TLM (Tables 1 and 2). It is also, confirmed the insecticidal potency of emamectin benzoate represented as Proclaim, on both TLM larval instars at 1, 3, 5, 7 and 10 days post treatment compared to the rest of tested bio-rational insecticides. Clearly, Proclaim caused the highest accumulated mortality in TLM larvae, followed by Coragen, Spinosad, Match and Dipel 2x at 10 days post treatment.

Concerning the first instar larvae of TLM, there were significant differences between the tested bio-rational insecticides at their tested recommended dose in the mortality rates in first instar cohorts 1 day ($H_5=16.53$; $P=0.005$), 3 days ($F_{5,54}=12.28$; $P=0.001$), 5 days ($F_{5,54}=16.10$; $P=0.001$), 7 days ($F_{5,54}=16.62$; $P=0.001$), and 10 days ($F_{5,54}=16.62$; $P=0.001$) post treatment. No significant differences were observed between Match and Dipel 2x treatments with control treatment (Table 1).

Pertaining to the fourth instar larvae of TLM, data indicated that mortality in fourth instar larvae is in the order of Proclaim > Coragen > Spintor > Dipel 2x > Match. Significant differences existed among the tested bio-rational insecticides in the mortality rates in 4th larval instar cohorts 1 day ($H_5=14.05$; $P=0.015$), 3

days ($F_{5,54}=23.89$; $P=0.001$), 5 days ($F_{5,54}=17.53$; $P=0.001$), 7 days ($F_{5,54}=21.71$; $P=0.001$), and 10 days ($F_{5,54}=21.71$; $P=0.001$) post treatment (Table 2).

Field Experiment:

The number of newly formed mines by larvae of TLM within tomato leaves before spraying and up to 10 days after field application are presented in (Table 3). Generally, the number of mines/plant differed among different treatments and control irrespective of interval of inspection. The lowest number of mines (2.4 mines/plant) was recorded in Coragen treatment after 10 days of treatment, followed by Proclaim, Spintor, Dipel 2x and Match compared to control at 2.7, 4, 4.1, 9.1 and 9 mines/plant, respectively.

Statistical analyses revealed that the tested bio-rational insecticides yielded significant decrease in the number of TLM mines/plant under field conditions 1 day ($F_{5,54}=6.83$; $P=0.036$), 3 days ($F_{5,54}=7.97$; $P=0.001$), 5 days ($F_{5,54}=8.40$; $P=0.001$), 7 days ($F_{5,54}=13.12$; $P=0.001$) and 10 days ($F_{5,54}=12.84$; $P=0.001$) post treatment (Table 3).

Data presented in Table (4) indicated that all bio-rational insecticides under field conditions caused noticeably reduction in TLM infestations. The highest percent of reduction was recorded in Coragen, Spintor and Proclaim at 68.33, 61.97 and 54.89%, respectively. Dipel 2x had moderate effectiveness in reduction of infestation at 42.78%. Obviously, the lowest percentage of reduction in TLM infestation was recorded in Match treatment as 27.51%.

Table (1): Mortality (%± SE) in TLM first instar larvae fed on tomato seedlings treated with the recommended field rates of certain bio-rational insecticides at different intervals post application

Treatment	Concentration mg/l a.i.	% Mortality in first instar larvae after,				
		1 day	3 days	5 days	7 days	10 days
Control	---	2.50±2.50 ^{bc}	15.00±5.00 ^b	22.50±6.29 ^b	25.00±6.45 ^b	15.00±6.45 ^b
Coragen	60 mg/l	25.00±10.41 ^{ab}	57.50±2.50 ^a	80.00±7.07 ^a	87.50±7.50 ^a	87.50±7.50 ^a
Dipel 2x	128 mg/l	0 ^c	7.50±4.78 ^b	15.00±6.45 ^b	15.00±6.45 ^b	15.00±6.45 ^b
Match	200 mg/l	2.50±2.50 ^{bc}	12.50±4.78 ^b	20.00±5.77 ^b	25.00±6.45 ^b	25.00±6.45 ^b
Proclaim	30 mg/l	27.50±4.78 ^a	55.00±6.45 ^a	90.00±4.08 ^a	97.50±2.50 ^a	97.50±2.50 ^a
Spintor	60 mg/l	35.00±6.45 ^a	52.50±6.29 ^a	65.00±6.45 ^a	65.00±6.45 ^a	65.00±6.45 ^a
P		0.005	0.001	0.001	0.001	0.001

Means within a column followed by different letters are significantly different: $P<0.05$; Tukey Test (1 day post application); or Holm-Sidak Method (3, 5, 7 and 10 days post application).

Table (2): Mortality (%± SE) in TLM fourth instar larvae fed on tomato seedlings treated with the recommended field rates of certain bio-rational insecticides at different intervals post application

Treatment	Concentration mg/l a.i.	% Mortality in fourth instar larvae after,				
		1 day	3 days	5 days	7 days	10 days
Control	---	0 ^a	2.50±2.50 ^b	7.50±4.79 ^c	7.50±4.79 ^d	7.50±4.79 ^d
Coragen	60 mg/l	17.50±8.54 ^a	55.00±5.00 ^a	67.50±7.50 ^{ab}	75.00±6.46 ^{ac}	75.00±6.46 ^{ac}
Dipel 2x	128 mg/l	17.50±4.78 ^a	30.00±9.13 ^a	32.50±8.54 ^b	37.50±7.50 ^{bc}	37.50±7.50 ^{bc}
Match	200 mg/l	0 ^a	0 ^b	2.50±2.50 ^c	2.50±2.50 ^d	2.50±2.50 ^d
Proclaim	30 mg/l	7.50±2.50 ^a	45.00±6.46 ^a	77.50±4.79 ^a	82.50±2.50 ^a	82.50±2.50 ^a
Spintor	60 mg/l	7.50±2.50 ^a	30.00±8.17 ^a	30.00±8.17 ^b	32.50±8.54 ^b	32.50±8.54 ^b
P		0.015	0.001	0.001	0.001	0.001

Means within a column followed by different letters are significantly different: $P<0.05$; Tukey Test (1 day post application); or Holm-Sidak Method (3, 5, 7 and 10 days post application).

Table (3): Mean (\pm SE) of newly formed mines/tomato plant of TLM before and after different intervals post treatment with recommended field rate of certain bio-rational insecticides

Treatment	Field rates	Mean (\pm SE) of newly formed mines/plant					
		Before treatment	After treatment				
			1 day	3 days	5 days	7 days	10 days
Control	---	8.90 \pm 1.74 ^{ab}	10.30 \pm 1.34 ^{ac}	8.70 \pm 1.18 ^a	4.10 \pm 0.62 ^a	8.50 \pm 0.96 ^a	9.00 \pm 1.17 ^a
Coragen	0.30 cm/l	10.50 \pm 1.17 ^{ab}	6.20 \pm 0.80 ^{bc}	4.00 \pm 0.63 ^{bc}	1.20 \pm 0.44 ^b	2.10 \pm 0.50 ^b	2.40 \pm 0.81 ^b
Dipel 2x	2.00 g/l	10.30 \pm 1.47 ^{ab}	7.80 \pm 0.74 ^{bc}	7.50 \pm 0.89 ^{ab}	2.80 \pm 0.55 ^{ab}	4.70 \pm 0.91 ^{bc}	4.10 \pm 0.64 ^b
Match	0.40 cm/l	12.60 \pm 1.67 ^a	12.70 \pm 1.55 ^a	8.60 \pm 1.43 ^a	4.60 \pm 0.69 ^a	6.60 \pm 0.82 ^{ac}	9.10 \pm 0.90 ^a
Proclaim	0.60 g/l	6.30 \pm 0.75 ^b	4.70 \pm 0.70 ^b	2.60 \pm 0.64 ^c	1.40 \pm 0.49 ^b	1.70 \pm 0.39 ^b	2.70 \pm 0.73 ^b
Spintor	0.25 cm/l	10.80 \pm 1.94 ^{ab}	8.90 \pm 1.17 ^{ab}	3.70 \pm 0.72 ^{bc}	1.00 \pm 0.37 ^b	2.80 \pm 0.74 ^b	4.00 \pm 0.75 ^b
<i>P</i>		0.036	0.001	0.001	0.001	0.001	0.001

Means within a column followed by different letters are significantly different: $P < 0.05$; Tukey Test (before treatment); or Holm-Sidak Method (1, 3, 5, 7 and 10 days after treatment).

Table (4): Percentages of reduction* in TLM infestation in tomato plants after different intervals post treatment with certain bio-rational insecticides under field conditions.

Treatment	Field rates	% Reduction of infestation after *					% Effectiveness
		1 day	3 days	5 days	7 days	10 days	
Coragen	0.3 cm/l	48.98	61.03	75.19	79.06	77.39	68.33
Dipel 2x	2.0 g/l	34.56	25.51	40.99	52.22	60.64	42.78
Match	0.4 cm/l	12.91	30.18	20.75	45.15	28.58	27.51
Proclaim	0.6 g/l	35.54	57.78	51.76	71.74	57.62	54.89
Spintor	0.25 cm/l	28.79	64.95	79.90	72.85	63.37	61.97

* Percent reduction in infestation was estimated according to Henderson and Tilton (1955)

DISCUSSION

The current management of TLM in the Mediterranean Basin is mainly based on treatments with chemical insecticides (Gonzalez-Cabrera *et al.*, 2011). Nevertheless, few active ingredients are effective against TLM and selective to beneficial insects at the same time. The obtained results from laboratory and field trials showed that the Emamectin benzoate-based-insecticides Proclaim[®], had the greatest efficacy with mortality rates of 97.50 and 82.50% against first and fourth instar larvae, respectively. These findings are in conformity with those reported earlier by Gacemi and Guenaoui (2012), who found that the biopesticide Emamectin benzoate caused an acceptable mortality against larvae of TLM at 87%. Also, López *et al.* (2010a) observed a mortality rate of 90% in larvae of TLM on tomato leaves. Likewise, Emamectin benzoate was found to be effective against other pests such as *Helicoverpa zea* Boddie (López *et al.*, 2010b) and *Spodoptera littoralis* Bois (Abou-Taleb *et al.*, 2008).

A high toxicity of Chlorantraniliprole (Coragen[®]) was observed against both tested larval instars of TLM, with outstanding performance under field conditions. Early results were promising, even on insecticide-resistant populations with extremely low mammalian toxicity profile (Dinter *et al.*, 2008). Coragen has also demonstrated outstanding *C. pomonella* control either alone or in an alternation program with Steward[®] (Indoxacarb) (Bassi *et al.*, 2009).

Spinosad (Spintor) showed high toxicity on first compared to fourth instar larvae of TLM in laboratory; also high efficiency was confirmed in terms of reduction in TLM infestation under field conditions. These

findings are in agreement with those reported earlier by Nannini *et al.* (2011) who mentioned that Spinosad proved to be highly effective against TLM larvae with considerably decreased pest infestation under semi-field conditions as compared to Indoxacarb, metaflumizone, azadirachtin, and abamectin. Furthermore, Spinosad also showed high efficacy against other lepidoptrous pests, such as *Palpita unionalis* (Hübner) (Mandour *et al.*, 2008), *Agrotis ipsilon* Mahmoud (2004) and *S. littoralis* (Aydin and Gurkan, 2005).

With respect to *B. thuringiensis* (Dipel 2 \times [®]), the obtained data showed low toxicity to both tested TLM larval instars under laboratory conditions. This conclusion was in agreement with that reported by Nannini *et al.* (2011). They stated that products of *B. thuringiensis* subsp. *Aizawai* were less active in glasshouse applications compared to laboratory conditions. However, this conclusion was inconsistent with those obtained by Gonzalez-Cabrera *et al.* (2011) who mentioned that *B. thuringiensis*-based insecticide was highly efficient in controlling TLM in laboratory, greenhouse, and open-field experiments and first instar larvae were the most susceptible, while lower susceptibility was observed in second and third instar larvae. Also, Giustolin *et al.* (2001) found that Btk can cause high mortality in all TLM instars and that the use of Btk has synergistic or additive effects when applied to tomato resistant genotypes.

Lufenuron (Match[®]) showed very low toxicity against the first instar larvae of TLM, but no toxicity was observed against fourth instar larvae with subsequent lower reduction in TLM infestation under field conditions. These results are in disagreement with those reported by Osman and Mahmoud (2008) who

demonstrated that Match[®] gave 100% mortality of 5th instar larvae of *S. littoralis*.

In conclusion, the five tested bio-rational insecticides had various toxic effects on first and fourth instar larvae of TLM under laboratory condition. Also, these insecticides showed dissimilar levels of reduction in infestation of TLM under field condition. Emamectin benzoate was the most toxic to larvae of TLM. Chlorantraniliprole demonstrated strong activity, Spinosad had high toxic effect especially against earlier instar larvae, greatly decreased the infestation rate. *Bacillus thuringiensis* seemed to have moderate effect since it has been able to reduce somewhat the pest infestation. Lufenuron didn't able to manage the insect infestation. The results showed that all tested insecticide, except Match, were effective to control TLM and might be used to control this pest outbreaks. The results gave an acceptable efficacy of the biopesticide Emamectin-benzoate, Chlorantraniliprole and Spinosad against the tomato leafminer TLM Meyrick (Lepidoptera: Gelechiidae). Hence, such these bioinsecticide will continue to be an integral component of pest management programs due mainly to their effectiveness and selectivity.

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