

## Lethal and sublethal effects of emamectin benzoate on the tomato leafminer, *Tuta absoluta*

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### Abstract

*Tuta absoluta* is one of the most important pests of tomato in many countries. Emamectin benzoate (EB) is widely used in the control of lepidopteran pests, but there is sparse information available regarding its sublethal effects on *T. absoluta*. In this research, the lethal and sublethal effects of EB were investigated on *T. absoluta* under laboratory conditions. Acute toxicity bioassay was carried out on 2<sup>nd</sup> instar larvae using residue contact method. The LC<sub>50</sub> value was 14.50 mg a.i. L<sup>-1</sup>, for 24 h exposure time. LC<sub>10</sub> and LC<sub>30</sub> of the insecticide was used for sublethal effect study. The insecticide significantly extended the larval developmental time at LC<sub>30</sub>. Pupal weight, fecundity and longevity of female adults were decreased at both tested concentrations. However, the LC<sub>10</sub> and LC<sub>30</sub> of the insecticide had no significant effects on pupal period, egg hatchability, and male adults' longevity at sublethal effect study. These results suggest that the sublethal effects of EB might affect pest population dynamics significantly by delaying its development, and decreasing its reproduction. Hence, this bioinsecticide can be used in integrated pest management program of *T. absoluta* if its efficacy proven under the field and greenhouse conditions.

**Keywords:** Insecticide, bioassay, IPM, biological traits, toxicity

## اثرات کشندگی و زیرکشندگی امامکتین بنزوات روی *Tuta absoluta*

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### چکیده

شب‌پره مینوز گوجه‌فرنگی، *Tuta absoluta*، یکی از آفات مهم گوجه‌فرنگی در بسیاری از کشورها می‌باشد. حشره کش امامکتین بنزوات به طور گسترده برای کنترل آفات بالپولکدار بکار برده می‌شود ولی اطلاعات کمی درباره اثرات زیرکشندگی آن روی *T. absoluta* موجود است. در این مطالعه، اثرات کشندگی و زیرکشندگی امامکتین بنزوات روی *T. absoluta* تحت شرایط آزمایشگاهی بررسی شد. زیست‌سنجی سمیت حاد روی لاروهای سن دوم *T. absoluta* با روش تماس با باقیمانده سم انجام شد. مقدار LC<sub>50</sub> بدست آمده امامکتین بنزوات ۱۴/۵۰ میلی‌گرم ماده موثره بر لیتر پس از ۲۴ ساعت در معرض قراردهی بود. LC<sub>10</sub> و LC<sub>30</sub> حشره‌کش برای بررسی اثرات زیرکشندگی استفاده شد. حشره‌کش به طور معنی‌دار طول دوره لاری را در غلظت LC<sub>30</sub> افزایش داد. وزن شفیرگی، باروری و طول عمر بالغین ماده‌ها در هر دو غلظت زیرکشنده کاهش یافت. با این حال، در بررسی اثرات زیرکشندگی، LC<sub>10</sub> و LC<sub>30</sub> حشره‌کش اثر معنی‌داری روی طول دوره شفیرگی، درصد تفریح تخم و طول عمر بالغین نر نداشتند. این نتایج نشان می‌دهد که غلظت‌های زیرکشنده امامکتین بنزوات می‌توانند با به تعویق انداختن دوره رشدی و کاهش تولیدمثل، اثرات منفی بر دینامیک جمعیت *T. absoluta* داشته باشند. بنابراین، این حشره‌کش زیستی در صورت ثابت شدن کارایی در شرایط مزرعه و گلخانه می‌تواند در برنامه‌های مدیریت تلفیقی علیه *T. absoluta* بکار رود.

**کلمات کلیدی:** حشره‌کش، زیست‌سنجی، مدیریت تلفیقی آفات، خصوصیات زیستی، سمیت

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## Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the destructive pests affecting tomato in many parts of the world including Iran (Desneux *et al.* 2011; Baniameri & Cheraghian 2011). The larvae of the pest cause damage on the leaf by mining the mesophyll and also attack tomato flowers, stems, and especially fruits (EPPO 2005; Desneux *et al.* 2010). Chemical control is one of the main control techniques used against *T. absoluta*. The intensive application of broad-spectrum insecticides against this pest has led to the development of resistance to some registered insecticides (Siqueira *et al.* 2000; Lietti *et al.* 2005; Silva *et al.* 2011). Biorational insecticides based on natural products, with novel modes of action, could be useful alternative for effective control of leafminer in tomato plants (Gacemi & Guenaoui 2012; Tomé *et al.* 2013).

Emamectin benzoate (EB) belongs to the avermectin group of chemicals produced by the soil-dwelling actinomycete, *Streptomyces avermitilis* (ex Burg *et al.* 1979) Kim and Goodfellow 2002 strain MA-4680 (NRRL 8165) (Ishaaya *et al.* 2002). This compound acts as a chloride channel activator in the nervous system of insects, resulting in suppression of muscle contraction which ultimately results in death (Roditakis *et al.* 2013). EB has remarkable activity against lepidopterans (Argentine *et al.* 2002; Parsaeyan *et al.* 2013; El-Sheikh 2015). Due to the ecological selectivity of EB to a wide range of beneficial arthropods, its application in integrated pest management (IPM) program could be considered as an important pest control option (Lopez *et al.* 2011). Insecticides have lethal and sublethal effects on target and non-target insect species. Sublethal doses/concentrations cause physiological and/or behavioral changes on insect population that survive after exposure to a pesticide and other toxicants at sublethal concentration/dose (Desneux *et al.* 2007). Sublethal effects may be appeared as reductions in life span, developmental time, fecundity, fertility, changes in sex ratio and/or changes in behavior (Stark & Banks 2003, Alyokhin

*et al.* 2008; Wang *et al.* 2008, 2009; Sohrabi *et al.* 2011; Saber *et al.* 2020).

Particular efficacy of EB against *T. absoluta* larvae has been shown in previous studies (Gacemi & Guenaoui 2012; Roditakis *et al.* 2013; Hanafy & El-Sayed 2013; Deleva & Harizanova 2014). However, there is sparse information available regarding the sublethal effects of EB on *T. absoluta* (Esmaily *et al.* 2015). Therefore, this study was carried out to investigate the sublethal effects of this insecticide on larval and pupal developmental time, adult longevity, fecundity, hatchability, and pupal weight of *T. absoluta*. This research will provide information for more effective use of EB in management programs for *T. absoluta* and provide effective pest control strategies.

## Materials and methods

### *Insect rearing*

A laboratory colony of the tomato leafminer, *T. absoluta* was established using larvae collected from infested tomato fields in the Bondaroz, Borazjan region, Bushehr Province (Southern Iran) (29°12'47.4" N, 51°13'56" E) in March 2017. They were reared on tomato plants (cv. Sunside) in a muslin-walled cage (120 × 60 × 60 cm) at 16-25 °C, 40-50% RH, and a photoperiod of 16:8 (L:D) h. The population was maintained in the laboratory for one year without exposure to any insecticide. Tomato plants were placed in the cage weekly for feeding the insects and allowed to mate and lay eggs on the plants.

### *Acute toxicity bioassay*

The toxicity of EB (Proclaim® 5% SG, Syngenta Co., Switzerland) was assessed on 2<sup>nd</sup> instar larvae of *T. absoluta*. The larval instars were staged by the measurement of body length and head capsule width as described previously (Bajracharya & Bhat 2018). The larvae were exposed to the insecticide by residue contact method (Parsaeyan *et al.* 2013). Preliminary experiments were carried out to determine the appropriate concentration range for testing. Five insecticide concentrations including 1, 5, 40, 100 and 1000 mg a.i. L<sup>-1</sup> were used in this assay plus a control treatment where only distilled water was used. Three replicates with 10 individuals

per replication were used for each insecticide concentration and the bioassay test was replicated three times. Three milliliters of either concentration were applied on glass Petri dishes (9 cm diameter by 3 cm height), air-dried for 2 h and then larvae were transferred into glass Petri dishes using a thin brush. The Petri dishes were then kept in an incubator set at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 (L: D) h. Larval mortality was recorded after 24 h exposure by prodding the larva with a fine hair brush.

#### Sublethal effects

Sublethal effects of EB were evaluated on 2<sup>nd</sup> instar larvae of *T. absoluta*. In this study, 100 second instar larvae of tomato leafminer were exposed to LC<sub>10</sub> (0.07 mg a.i. L<sup>-1</sup>) or LC<sub>30</sub> (1.61 mg a.i. L<sup>-1</sup>) of EB using the method previously described. There was a control group treated only with distilled water. After 24 hours, the surviving larvae were individually transferred to untreated tomato leaves placed in Petri dishes (5 cm diameter). Fresh tomato leaves were used to feed the larvae until they transformed into pupae. The Petri dishes were maintained in the laboratory conditions as described above. Pupae were placed individually in plastic containers (52 mm tall, 40 mm diameter) until adults emerged. Larvae and pupal developmental times were estimated for 20 randomly selected live 2<sup>nd</sup> instar larvae from each treatment and placed individually on tomato leaves in Petri dishes. Also, 20 (two-day-old<) pupae in each treatment were randomly selected and weighed individually. The sublethal effects of LC<sub>10</sub> and LC<sub>30</sub> on fecundity, longevity and egg hatching rate were also assessed. For the fecundity study, newly emerged adults were kept in 50 × 30 × 30 cm cages to mate for about 48 h after emergence, and supplied

with a 10% honey solution. Then, 20 healthy looking pairs were picked randomly, and each pair transferred to a smaller plastic cage (10 × 6 × 3 cm) on tomato leaves. The numbers of eggs laid were counted daily until all females died. Leaf replacement continued daily until the death of the females. The mating cages were provided with 10% honey solution dispensed on cotton balls that were replaced every day. The longevity of adult male and female was recorded as well. To evaluate fertility, thirty eggs in six replicates were taken randomly from each pair of adult moths, and the numbers of hatching eggs were recorded. Number of eggs hatching was assessed after 7 d when the egg hatch was completed in the control. All experiments were conducted at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 (L:D) h.

#### Statistical analysis

To estimate LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> values, the data were analyzed by probit analysis using SAS software (SAS Institute 2003). Data of sublethal study experiments were analyzed using one-way analysis of variance (ANOVA) (SAS Institute 2003) after checking for normality. The percentage of hatched egg was arcsin-transformed while the data of male longevity and larval period were square-root transformed for the intended analyses. Means were separated by the least significant differences (LSD) tests (P = 0.05) using SAS software (SAS Institute, 2003).

#### Results

##### Acute Toxicity of Emamectin benzoate

The estimated LC<sub>50</sub> of the residue contact bioassay on 2<sup>nd</sup> instar larvae of *T. absoluta* after 24 h was 14.50 mg a.i. L<sup>-1</sup>. LC<sub>10</sub> and LC<sub>30</sub> values were 0.07 and 1.61 mg a.i. L<sup>-1</sup>, respectively (Table 1).

**Table 1.** Toxicity of emamectin benzoate on second instar larvae of tomato leafminer (n = 450).

Lethal concentrations (mg a.i. L <sup>-1</sup> )			$\chi^2$ (df = 3)	Slope ± SE
LC <sub>10</sub> (95%FL)	LC <sub>30</sub> (95%FL)	LC <sub>50</sub> (95%FL)		
0.07 (0.01 -1.15)	1.61 (0.01 -9.98)	14.50 (0.50-120.35)	12.50	0.55 ± 0.13

FL: Fiducial limit.

### Sublethal effects

The sublethal effects of EB on pre-adult developmental time and pupal weight are shown in Table 2. Developmental time of larvae was significantly extended when exposed to the LC<sub>30</sub> value ( $F = 6.45$ ;  $df = 2, 57$ ;  $P = 0.003$ ); however, the development of pupae was not affected by the LC<sub>10</sub> and LC<sub>30</sub> values of EB ( $F = 2.47$ ;  $df = 2, 57$ ;  $P = 0.094$ ). Pupal weight was significantly decreased by LC<sub>10</sub> and LC<sub>30</sub>; however, no significant difference in pupal weight was observed between LC<sub>10</sub> and LC<sub>30</sub> treatments ( $F = 11.85$ ;  $df = 2, 57$ ;  $P < 0.0001$ ). The sublethal effects of EB on fecundity, the percentage

of egg hatchability, and the adult longevity of *T. absoluta* were shown in Table 3. EB in sublethal study significantly reduced fecundity of *T. absoluta* ( $F = 17.59$ ;  $df = 2, 57$ ;  $P < 0.0001$ ); however, EB had no effect on the hatching of the eggs ( $F = 1.00$ ;  $df = 2, 15$ ;  $P = 0.3911$ ). The LC<sub>10</sub> and LC<sub>30</sub> of EB significantly reduced female longevity (10.40 and 9.15 days, respectively) compared with the control (13.15 days) ( $F = 29.49$ ;  $df = 2, 57$ ;  $P < 0.0001$ ), while male longevity was not affected by the tested concentrations of EB ( $F = 0.49$ ;  $df = 2, 57$ ;  $P = 0.6136$ ) (Table 3).

**Table 2.** Sublethal effects of emamectine benzoate on larval and pupal stages of *Tuta absoluta*.

Treatment	Larval period (days)	Pupal period (days)	Pupal weight (mg)
Control	9.40 ± 0.43b	7.15 ± 0.17ab	3.10 ± 0.12a
LC <sub>10</sub>	10.00 ± 0.23ab	7.60 ± 0.27a	2.05 ± 0.15b
LC <sub>30</sub>	10.80 ± 0.09a	6.85 ± 0.26b	2.30 ± 0.19b

Means followed by the same letter in each column are not significantly different using LSD test ( $P > 0.05$ , LSD, SAS Institute 2003).

**Table 3.** Sublethal effects of emamectine benzoate on *Tuta absoluta* adults.

Treatment	Number of eggs per female	Egg hatch (%)	Male longevity (days)	Female longevity (days)
Control	101.15 ± 3.50a	98.88 ± 1.11a	10.20 ± 0.45a	13.15 ± 0.32a
LC <sub>10</sub>	72.10 ± 7.13b	100 ± 0a	11.00 ± 0.68a	10.40 ± 0.48b
LC <sub>30</sub>	48.85 ± 7.35c	100 ± 0a	10.71 ± 0.39a	9.15 ± 0.30c

Means followed by the same letter in each column are not significantly different at using LSD test ( $P > 0.05$ , LSD, SAS Institute 2003).

### Discussion

Emamectin benzoate showed high effectiveness on tomato leaf miner in this study. Good performance of EB against the tomato leafminer has been also reported in the previous studies. Gacemi & Guenaoui (2012) found that EB caused an acceptable larval mortality rate of 86.7% on this pest. Also, López *et al.* (2010) observed a mortality by 90% on *T. absoluta* larvae on tomato leaves caused by EB. Roditakis *et al.* (2013) tested the effect of emamectin benzoate on different populations of *T. absoluta*. The LC<sub>50</sub> values on 2<sup>nd</sup> instar larvae ranged from 0.03 to 0.12 mg L<sup>-1</sup>, which was lower compared to that of reported in this study. In the study conducted by Esmaily *et al.* (2015),

the LC<sub>50</sub> values of EB on 2<sup>nd</sup> instar larvae of *T. absoluta* was 0.11 mg L<sup>-1</sup>. Toxicity bioassays on third instar larvae of the pest showed the LC<sub>50</sub> of EB was 0.42 mg L<sup>-1</sup> (Taha and Al-Hadek, 2016). This variation in the LC<sub>50</sub> values may be due to the geographical variation of *T. absoluta* populations, or difference in the bioassay method, formulation of the insecticide, and the larval stage tested in these experiments.

The use of insecticides may result in multiple sublethal effects on insect pests with detrimental impacts on some physiological or behavioural process in the surviving insects. In this study, LC<sub>30</sub> concentration of EB increased the duration of *T. absoluta* larval stage. Similarly, Esmaily *et al.*

(2015) reported that larval period was increased in *T. absoluta* larvae treated with the LC<sub>30</sub> of EB. Similar results were found in the cabbage moth, *Mamestra brassicae* L. (Moustafa *et al.*, 2016), *Spodoptera littoralis* Boisid (El-Sheikh, 2015) and *Helicoverpa armigera* (Hübner) (Lixia *et al.*, 2011; Parsaeyan *et al.*, 2013) which showed that EB prolonged larval development time compared with control. Contrary to the present results, Reda *et al.* (2012) reported that EB applied to 1<sup>st</sup> instar larvae of *Pectinophora gossypiella* (Saunders) caused a decrease in larval duration.

In addition to the direct interference of EB with the development of the *T. absoluta* larvae, post-exposure effects of EB on this pest were also observed. The tested insecticide significantly reduced pupal weight of *T. absoluta*. Similar results were found in *T. absoluta* and *H. armigera*, which showed that the pupal weight was significantly decreased when exposed to the sublethal concentration of EB (Parsaeyan *et al.*, 2013; Esmaily *et al.* 2015). However, in another study, *H. armigera*, did not show reduction of pupal weight following exposure to EB (Lixia *et al.*, 2011). It was also found that EB treatment in the larval stage shortened female longevity, so that the longevity was 13.15 days in the control group and decreased to 10.40 and 9.15 days after exposure to the LC<sub>10</sub> and LC<sub>30</sub> of EB, respectively. Reducing female longevity led to a reduction in reproductive capacity. Therefore, egg production was reduced by 28.7% in adults resulted from larvae treated with LC<sub>10</sub> concentrations of EB and rose to 51.7% when exposed to LC<sub>30</sub> value. Similarly, Moustafa *et al.* (2016) reported that EB negatively affected reproductive activity of the cabbage moth, *Mamestra brassicae* L. Similar results were found in *H. armigera*, *P. gossypiella*, and *T. absoluta* which showed that the longevity and fecundity of adults were significantly decreased when exposed to the sublethal concentration of EB (Reda *et al.*, 2012; Parsaeyan *et al.*, 2013; Esmaily *et al.* 2015). In the present study, the fertility (proportionate egg hatch) was not affected when larvae were exposed to EB at both tested concentrations. Contrary to the present results, Parsaeyan *et al.* (2013) and Esmaily *et al.* (2015) observed that the LC<sub>30</sub> of EB significantly reduced fertility of *H. armigera* and *T.*

*absoluta* by 62.06% and 76.41%, respectively. The discrepancies of some effects of EB on different insects as shown in different studies indicate that EB may exert influences in a species-specific manner, revealing the selective insecticidal trait of the compound.

In summary, this study presents both lethal and sublethal effects of EB on *T. absoluta*. Emamectin benzoate effects on larval development time, pupal weight, longevity, and reproductive activity may affect the population density of the subsequent generation. Accordingly, EB has a great potential to be applied as a chemical control agent in integrated pest management of *T. absoluta*. The sublethal effects of EB could have a negative influence on the dynamics of this pest. Therefore, it can be suggested that not only lethal effects but also sublethal effects of EB should be taken into consideration when pest control strategies are developed. These laboratory findings need to be confirmed with some trials to investigate the sublethal effect of this bioinsecticide on populations of this pest in field conditions. Furthermore, it is critical to test the compatibility of EB with major biological control agents of *T. absoluta* to recommend its use for IPM programs in tomato fields.

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