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Susceptibility of eggs and larvae of tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to some insecticides

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Abstract

Use of pesticides as an important part of integrated pest management programs has principal role in control of tomato leafminer, *Tuta absoluta* (Meyrick). Therefore, evaluating the efficacy of insecticides and introducing new effective compounds are important for management of this pest. Eggs and 2nd instar larvae of tomato leafminer are the main stages exposed to insecticide sprayings. This study was conducted to determine the susceptibility of eggs and 2nd instar larvae of *T. absoluta* to five insecticides. LC_{50} values obtained for spinosad, chlorantraniliprole, indoxacarb, abamectin, and zeta-cypermethrin for 2nd instar larvae were 0.08, 0.09, 10.8, 0.29 and 232.2 mg ai/L respectively. The eggs were treated with LC_{50}s of these insecticides for 2nd instar larvae to assess their ovi-larvicidal effects. Mortality of the egg/neonates in chlorantraniliprole, spinosad, indoxacarb, abamectin, zeta-cypermethrin treatments and control were 88.8, 76.8, 58.1, 35, 50.5 and 14.6 %, respectively. The results revealed that spinosad and chlorantraniliprole had strong ovicidal and larvicidal activities; and if perform equally well in large scale greenhouse and field conditions, they may be considered in *T. absoluta* management programs.

Key words: chlorantraniliprole, spinosad, zeta-cypermethrin, indoxacarb, abamectin

حساسیت تخم‌ها و لاروهای شب‌پره‌ی مینوز گوجه‌فرنگی *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) به برخی حشره‌کش‌ها

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چکیده

بکارگیری آفت کش‌ها به عنوان بخش مهمی از برنامه‌های مدیریت تلفیقی آفت، نقش اساسی در کنترل شب‌پره‌ی مینوز گوجه‌فرنگی (Meyrick) *Tuta absoluta* دارد. بنابراین ارزیابی اثر آفت‌کش‌ها و معرفی ترکیبات جدید برای مدیریت این آفت مهم است. تخم و لارو سن ۲ شب‌پره‌ی مینوز گوجه‌فرنگی مراحلی هستند که در زمان سم پاشی در معرض حشره‌کش‌ها قرار می‌گیرند. این مطالعه برای تعیین حساسیت تخم‌ها و لاروهای سن ۲ شب‌پره‌ی مینوز گوجه‌فرنگی نسبت به پنج حشره‌کش انجام گرفت. مقادیر LC_{50} برای آفت‌کش‌های اسپینوسد، کلرانترانیلی‌پرول، ایندوکساکارب، ابامکتین و زتا-سایپرمترین که روی لاروهای سن دو آزمایش شدند، به ترتیب ۰/۰۸، ۰/۰۹، ۱۰/۸، ۰/۲۹، ۲۳۲/۲ میلی‌گرم ماده‌ی موثره در لیتر بدست آمدند. برای ارزیابی اثر تخم‌کشی، تخم‌ها با مقادیر LC_{50} این حشره‌کش‌ها تیمار شدند. میزان تلفات تخم/ لارو نئونات در تیمارهای کلرانترانیلی‌پرول، اسپینوسد، ایندوکساکارب، ابامکتین، زتا-سایپرمترین و شاهد به ترتیب ۸۸/۸، ۷۶/۸، ۵۸/۱، ۳۵، ۵۰/۵ و۱۴/۶ درصد بود. در بین آفت‌کش‌های مورد آزمایش کلرانترانیلی‌پرول و اسپینوسد بیشترین اثر تخم‌کشی و لارو کشی را نشان دادند، بنابراین می‌توانند در برنامه‌ی مدیریت شب‌پره‌ی مینوز گوجه‌فرنگی در سطوح بزرگ گلخانه و مزرعه مورد توجه قرار گیرند.

واژه‌های کلیدی: اسپینوسد، ابامکتین، ایندوکساکارب، کلرانترانیلی‌پرول، زتا- سایپرمترین.

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Introduction

Tomatoes are among the popular vegetables consumed all over the world, and tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an important pest of this crop. This insect is native to South America and was introduced to Iran in 2010; and is presently dispersed throughout most of the tomato producing provinces (Anonymous, 2012). Characteristics such as having several generations per year and high fecundity, have made this insect an important pest (Anonymous, 2011). The tomato leafminer feeds on all parts of the tomato plant except roots; and damages them in greenhouses and fields. This pest can cause up to 100% loss in tomato crops if no control measures are applied (Desneux *et al.*, 2010).

Although several control methods are used against tomato leafminer, insecticides play an important role in management of this pest (Roditakis *et al*., 2013b). Diamides, such as chlorantraniliprole and flubendiamide are effective insecticides on lepidopteran pests and have been extensively applied to control *T. absoluta*, (Roditakis *et al*., 2015). Some biopesticides have also been used in managing this pest (El Hajj *et al*., 2017). Resistance of *T. absoluta* to several groups of chemical insecticides has been reported by many researchers (Siqueira *et al.*, 2000; Silva *et al*., 2011; USDA, 2011; Haddi *et al.*, 2012; Roditakis *et al.*, 2013a). Therefore, testing new insecticides and assessing different aspects of their effects, would help in devising appropriate management programs for this pest. Spinosad, chlorantraniliprole, abamectin and indoxacarb are among the commonly used insecticides against *T. absoluta* with varying effects on different populations in various regions (Roditakis *et al.*, 2013b & Campos *et al.*, 2015a). We wanted to assess the susceptibility of Iranian population of this pest to these insecticides. Zeta-cypermethrin is used for controlling coleopteran, hemipteran and lepidopteran pests in different crops (Tomalin, 1994). It has also been reported as an effective insecticide with good ovicidal and residual activity on neonate larvae of *Ostrinia nubilalis* (Hubner) (Rinkleff *et al*. 1995). Since this insecticide has not been used for controlling *T. absoluta*, we were interested in evaluating its potential for controlling this pest.

Choosing the appropriate life stage and using a reliable bioassay method is essential for accurately assessing the effect of different insecticides. The larvae spend most of their lives in the mines and are not exposed to most of the insecticides. Sometimes the 2nd instar larvae come out of their mines and get exposed to pesticides (Fernandez & Montagne, 1990). Since there is a considerable overlap between different life stages of this pest and eggs are laid singly on the surface of foliage (Anonymous, 2011), egg stage can be affected by insecticides as well.

This study was conducted to assess the effects of five insecticides on eggs and 2nd instar larvae of *T. absoluta* using IRAC method with some modifications (Anonymous, 2012).

Materials and methods

Rearing Insects

The study was conducted in the greenhouse of Department of Plant Protection of the University of Tabriz. Rearing insects and bioassays were carried out at 26 ± 2 °C, 60 ± 10% RH, and 16: 8 h (light: dark) photoperiod. The foliage of Urbana tomato cultivar was used for rearing the insects and doing bioassays. Tomato plants were grown in pots (containing 25% vermicompost, 25% cocopeat and 50% perlite). Tap water was used for irrigation and the plants were fertilized once a week. The fertilizer included 1- 4- 2 ratios of N-P-K respectively. The insects used for establishing the colony were obtained from an existing colony of *T. absoluta* in the greenhouse of the Department of Plant Protection. The adults were kept in wooden cages (60×70×60 cm) covered with 80 mesh organdy cloth. They were fed with 10% sucrose solution. The larvae were kept in 10×20 ×30 cm transparent plastic containers and fed with tomato leaves. The insecticides tested and their modes of action (Anonymous, 2019) are presented in Table 1.

Table 1. Characteristics of insecticides used in this study

Active ingredient	Trade name and formulation	Company name and country	Group and mode of action
Spinosad	Laser® 48 SC	Dow Agrosciences, UK,	5 Nicotinic acetylcholine receptor modulator
Indoxacarb	Indoxacarb®15 SC	Tragusa, Spain	22 A Sodium channel blocker
Chlorantraniliprole	Coragen®18.5 SC	DuPont, USA	28 Ryanodine receptor modulator
Abamectin	Vertimec® 1.8 EC	Syngenta, Switzerland	6 Chloride channel modulator
Zeta-cypermethrin	Deltarase® 10 EC	Agrega, Turkey	3 Sodium channel modulator

Bioassays

Larvae

Based on preliminary experiments on 2nd instar larvae, the ranges of concentrations for spinosad, abamectin, indoxacarb, chlorantraniliprole and zeta-cypermethrin were 0.04- 0.14, 0.21- 0.54, 4.5- 22.5, 0.05- 0.22 and 125- 350 mg ai L^{-1}, respectively. Each insecticide treatment consisted of five concentrations and a control. Distilled water was used for dilutions. The non-ionic surfactant Tween 80® at concentration of 500 ppm was used as the wetting agent in all treatments including controls.

Tomato leaves (2×2 cm pieces) were treated with different concentrations of the insecticides using dipping method. Each treated leaf piece was put on a moist cotton ball placed in a glass vial; and one 2nd instar larva was transferred on the treated leaf (Anonymous, 2012). The 2nd instar larvae were synchronized using the method described by Roditakis *et al* (2013a). In short, three potted tomato plants were placed in a cage containing 80-100 moths and the females were let oviposit for 48 h. Then the plants with the eggs on them were transferred to an insect free cage and kept in it until the eggs developed to 2nd instar larvae.

To assess larval mortality, 48 hours after treatment the larvae were carefully removed from the mines. Probing the larvae with a soft hairbrush and watching their movement was the basis for judgment of larval mortality. If the larvae could not move a distance equal to their body length they were recorded as dead. Twenty larvae were used for each concentration; and each treatment was replicated three times at different days.

Eggs

The eggs were treated with LC_{50}s of the insecticides for 2^{nd} instar larvae to assess ovi-larvicidal effects. Twenty-four-hour old eggs were used to evaluate the ovi-larvicidal effect of the insecticides. Since the eggs of *T. absoluta* are glued onto the surface of tomato leaves and removing them damages the eggs, leaf dipping method was used to avoid harming the eggs. The leaflets with *T. absoluta* eggs on them were dipped in the insecticide solutions for five seconds. The treated leaves were let to dry at room temperature and transferred into glass containers (5 cm in height and 8 cm in diameter) and kept for 5 days at rearing conditions until the mines appeared. The number of mines formed by neonate larvae on the treated leaves were considered as the number of live larvae. Ovicidal and larvicidal effects of the insecticides could not be clearly distinguished. The mortality could have been due to toxic effect of the insecticides on the developing embryo (ovicidal effect) or because of toxicity of the chemical residue on egg shell or leaf surface to the newly eclosed larvae. Hence, the ovi-larvicidal effects of the insecticides were assessed and appearance of the mines was used as the endpoint.

The treatments were replicated three times at different days. The number of eggs used in different treatments and the concentration of the insecticides tested are shown in table 3.

Data Analysis

The LC_{50} values were estimated using probit option of SAS 9.0 and dose-response lines were drawn using Excel (ver. 2010). Data of egg experiments were analyzed by SAS 9.0 as well.

Results

Second Instar Larvae

The LC_{50} and LC_{90} values estimated for five insecticides are shown in table 2. The results indicated that spinosad and chlorantraniliprole were the most effective insecticides on 2^{nd} instar larvae of *T. absoluta* followed by abamectin and indoxacarb. Zeta-cypermethrin was the least effective compound among the insecticides tested.

Table 2. Toxicity of the insecticides on 2nd instar larvae of *Tuta absoluta*.

Insecticides	No. of larvae	Slope ± SE	LC_{50} (CL 95%)	LC_{90} (CL 95%)	χ^2 (df)	*P*
Spinosad	300	3.38 ± 0.49	0.08 (0.06-0.09)	0.21 (0.16-0.31)	0.5 (3)	0.910
Chlorantraniliprole	303	2.44 ± 0.380	0.09 (0.07-0.11)	0.33 (0.25-0.56)	0.8 (3)	0.845
Indoxacarb	301	2.19 ± 0.36	10.8 (9.06-13.05)	41.74 (28.99-82.38)	0.6 (3)	0.801
Abamectin	303	3.95 ± 0.58	0.29 (0.26-0.32)	0.62 (0.52-0.82)	0.5 (3)	0.918
Zeta-cypermethrin	302	2.79 ± 0.52	232 (204-269)	666 (485-1274)	0.9 (3)	0.574

Dose – response lines are shown in Fig. 1. Slope of the lines was steep which indicates the tested population was homogenous in terms of susceptibility to these insecticides.

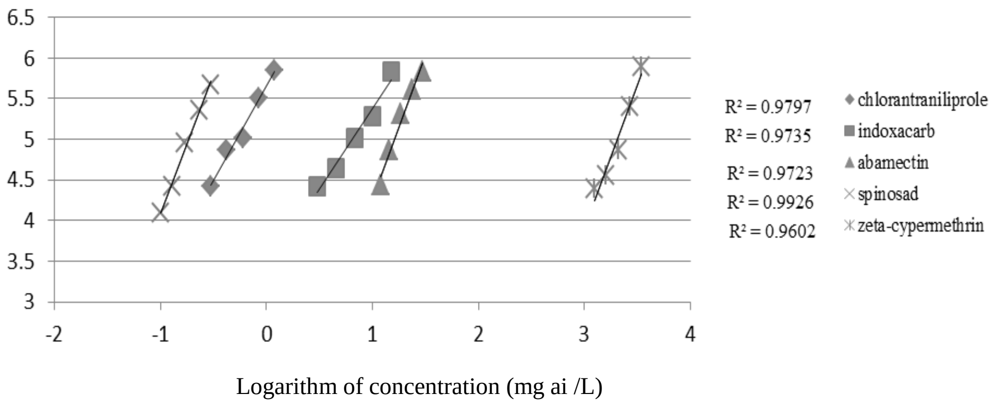

Fig. 1. Dose - response lines of the insecticides on 2nd- instar larvae of *Tuta absoluta*.

Eggs

Comparison of mean ovi-larvicidal effects are presented in Table 3. The results revealed that spinosad and chlorantraniliprole had the highest toxic effects compared with the other three insecticides and control.

Table. 3. Comparison of insecticide effects on *Tuta absoluta* eggs

Treatments	Concentration (mg ai/L)	No. of eggs	No. of mines	Percent mortality of eggs
Control	0	130	111	14.6 e
Zeta-Cypermethrin	232	99	49	50.5 cd
Indoxacarb	10.8	153	64	58.1bc
Abamectin	0.29	137	89	35.0 d
Chlorantraniliprole	0.09	152	17	88.8 a
Spinosad	0.08	134	31	76.8 a

Discussion

In the present study, the LC_{50} value of chlorantraniliprole for 2nd instar larvae was 0.09 mg ai/L, which confirms the strong larvicidal effect of this insecticide on tomato leafminer. On the other hand, reduction in efficacy of chlorantraniliprole has also been reported due to development of resistance in Brazilian population of *T. absoluta* (Silva *et al.*, 2016). Ugurlu Karaagac (2012), tested chlorantraniliprole on two populations of *T. absoluta* from Turkey and reported LC_{50} values 6.35 and 0.46 mg ai/L for them. Her results were different from the LC_{50} value estimated for chlorantraniliprole in our study. Since the bioassay method and larval stage tested were similar in both studies, the difference may be due to difference in susceptibility, or the insecticide exposure history of the populations studied.

Campos *et al*. (2015b) studied the effect of chlorantraniliprole (SC, soluble concentrate) on *T. absoluta* and estimated LC_{50} values ranging from 3.17 - 29.64 mg ai/L for this insecticide. In this study LC_{50} value for the 2nd instar larvae was 0.09 mg ai/L which was considerably less than those reported by Campos *et al*. (2015b). The differences in bioassay methods and pesticide formulations as well as the difference in susceptibility of the populations may have caused different results in the two studies. Variation in toxicity to *T. absoluta* due to difference in formulations of sulphur (WP vs. DP) has also been reported by (Zappala *et al.*, 2012).

The results obtained for spinosad in this study were in agreement with those reported by Deleva & Harizanova (2014), who assessed the effect of some insecticides on second instar larvae of *T. absoluta* using IRAC method. Campos *et al.* (2015a), studied the susceptibility of eight populations of *T. absoluta* in Brazilian tomato fields to spinosad and reported the LC_{50} values in the range of 0.007 - 0.626 mg ai/L. In their study the LC_{50} reported for (Paulı´nia—SP) population (0.06 mg ai/L) was fairly close to the LC_{50} value of this insecticide in our study (0.08 mg ai/L). Contrary to our results, spinosad was ineffective on some Brazilian field populations because of development of resistance to this insecticide (Campos *et al.* 2014).

The estimated LC_{50} value for abamectin in the present study (0.29 mg ai/L) was somewhat different from the LC_{50} value (0.43 ai/L) reported by Ugurlu Karaagac (2012) in spite of the fact that the bioassay method and life stage tested were similar in both studies.

LC_{50} value of zeta-cypermethrin on 2nd instar larvae was 232 mg ai/L in this study. Zeta-cypermethrin was less toxic than the other tested insecticides. Resistance of this pest to pyrethroids has been reported by several researchers (Roditakis *et al*., 2013b, Siqueira *et al* 2000, Haddi *et al.*, 2012, Silva *et al*., 2011). The lower toxicity of zeta-cypermethrin in this study may also be due to resistance; but further studies would be needed to confirm this possibility. Roditakis *et al.* (2013a & b) assessed the effects of indoxacarb and chlorantraniliprole on 2nd instar *T. absoluta.* Their results were close to the results obtained from our study, possibly due to similarity in the bioassay method and life-stage tested.

Silva *et al*. (2016), investigated the effects of abamectin and indoxacarb on 2nd instar larvae of eight populations of *T. absoluta.* The ranges of estimated LC_{50} values were 0.86 - 2.89 mg ai/L and 0.54 - 3.38 mg ai/L for indoxacarb and abamectin, respectively. Difference in the results can be due to the differences in the bioassay methods, natural variabilities and formulations of the insecticides. Lietti *et al*. (2005) have related development of resistance of an Argentinian field population of *T. absoluta* to abamectin to frequency of using this insecticide. Studied *T. absoluta* population is currently quite susceptible to abamectin. Hence, in order to maintain usefulness of this insecticide in *T. absoluta* management programs, it would be necessary to avoid frequent use of this biorational insecticide and use it in rotation with other effective insecticides.

In our study indoxacarb was effective on this pest (LC_{50} = 10.8 mg ai/L), while Yalcin *et al*. (2015) reported much lower toxicity of this insecticide to Turkish population of *T. absoluta* (LC_{50} =215.26 mg ai/L). This much of difference in susceptibilities of the two populations could in part be justified by shorter history and less frequent use of indoxacarb in Iran.

Kok *et al*. (2012) reported that cypermethrin had better ovicidal effect on *Metisa plana* compared with chlorantraniliprole. On the contrary, in this study chlorantraniliprole had a greater ovi-larvicidal effect on tomato leafminer compared with zeta-cypermethrin. This can be due to isomeric difference between cypermethrin and zeta-cypermethrin on one hand, and possible difference in susceptibility of the tested insects on the other hand. Although the difference in bioassay methods used cannot be discarded. In another study, Rinkleff *et al*. (1995) reported considerable ovi-larvicidal activity of zeta-cypermethrin on European corn borer. The difference between the two results could well be due to species difference. Our results were in agreement with the results obtained by Nozad-Bonab *et al.* (2017) who reported that spinosad and chlorantraniliprole were effective on eggs of *T. absoluta.* The results revealed that spinosad had more ovi-larvicidal effect on *T. absoluta* eggs than indoxacarb. Boiteau & Noronha (2007), also reported that spinosad was more toxic than indoxacarb on *Ostrinia nubilalis* Hübnerour eggs. In present study, zeta-cypermethrin and abamectin had similar effects on the eggs of *T. absoluta*, while cypermethrin was 14- fold more toxic than avermectin B_1 on eggs of *Plutella xylostella* (Lepidoptera: Plutellidae) (Abro *et al*. 1988). Difference in species on one hand, and isomerism on the other hand maybe accounted for the difference in toxicities.

Conclusion

Excellent activity along with novel mode of action makes chlorantraniliprole useful in *T. absoluta* management programs and a suitable candidate for insecticide resistance management as well. Spinosad is relatively safe on non-target organisms and may suit for IPM programs. Chlorantraniliprole and spinosad were most effective on 2^{nd} instar larvae and eggs of *T. absoluta* followed by abamectin and indoxacarb. If they perform equally well in commercial scale in field and greenhouse, they would be suitable candidates to be used in alternation in integrated tomato pest management programs along with other non-chemical control methods.

Since zeta-cypermethrin did not have considerable larvicidal effect on tomato leafminer, probably it would not be a suitable compound for controlling this pest.

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