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Research Article

Effects of different insecticides on egg, larva and adult of tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Abstract: Tomato leaf miner, *Tuta absoluta* (Meyrick), is a serious pest of the greenhouse in Iran. Chemical control is the main method in high infestation conditions to deal with this pest. In this study, the efficacy of five insecticides from different groups consisting of abamectin, spinosad, imidacloprid, indoxacarb and cypermethrin was examined on the egg, larvae and adult stages of *T. absoluta* in laboratory conditions. The LC_{50} values of the insecticides were estimated at the larval stage. The results showed that abamectin had the most and imidacloprid had the least ovicidal effect. None of the tested insecticides had a satisfactory effect on the adult stage. The maximum adulticide impact was 40% for spinosad. Abamectin with 0.45 mg ai.l^{-1} of LC_{50} value had the most toxicity and imidacloprid with 980 mg ai.l^{-1} of LC_{50} value had the least toxicity on the third instar larvae. According to the findings, abamectin and spinosad had the highest toxicity against three developmental stages (egg, larvae and adults) of the pest and can be used in infested tomato fields, when all life stages of *T. absoluta* are present at the same time. Meanwhile, it is recommended to use selective insecticides such as indoxacarb, when natural enemies are very active or the population of the tomato leaf miner is mostly at the larval stage.

Keywords: abamectin, cypermethrin, imidacloprid, indoxacarb and spinosad

Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) attacks tomato, eggplants, sweet peppers, potatoes and the weeds from Solanaceae family like *Solanum nigrum* L., *Datura* spp. It can destroy crop up to 100% (Bloem and Spaltenstein, 2011). The major portion of the pest's life cycle is passing inside the host plant's tissues, which makes its chemical control difficult (El-Ghany *et al*., 2016). Although it is not long since its diagnosis in Iran, it threatens host plants in vast areas of the country (Sohrabi *et al*., 2015). Depending on the temperature and favorable conditions, it can have 10 to 12 generations per year. The minimum temperature for activity is 9 degrees Celsius. As long as food is available, the larval stages do not go into diapause. The female lays about 250 to 260 eggs in her lifetime. The eggs hatch after 4-5 days. The young larvae make tunnels inside of leaf, stem or fruit to feed on plant tissues. Larval stages last about 13-14 days. During molting, the larvae are temporarily seen outside the feeding canal. The pupal stage is 9-11 days. The moths are active during the night but they hide among the leaves in daytime (FERA, 2009).

The history of chemical control of *T. absoluta* is relatively long. In the 1970s and 1980s,

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organophosphorus and pyrethroid insecticides were used, respectively. Since 1990, new insecticides such as abamectin, spinosad, tebufenozide, chlorfenapyr have been used (Lietti *et al.*, 2005; Guedes and Siqueira, 2013). Salazar and Araya (2001) reported that tomato leaf miner shows resistance to phosphorous and pyrethroid insecticides. However, newer insecticides had an acceptable control.

Nowadays, More than 260 insecticides, classified in 32 groups with different mode of action, are being used against pests. IRAC has recommended 13 insecticide groups for tomato leaf miner. Spinosad, indoxacarb, deltamethrin, *Bacillus thuringiensis* (Berliner), *Beauveria bassiana* (Balsamo), entomopathogenic nematode such as *Steinernema feltiae* (Filipjev) and *Heterorhabditis bacteriophora* Poinar insecticides have been tested for control tomato leaf miner's larvae in the world (FERA, 2009; Abootorabi, 2014; El-Ghany *et al.*, 2016; Allegrucci *et al.*, 2017). Pyrethrin and chlorpyrifos have been applied against this pest (Fredon-Corse, 2009), cartap, chlorfenapyr, phentoate, metamidofos, spinosad, indoxacarb were used to control the *T. absoluta* (IRAC, 2007); and then, Bt and triflumuron in addition to parasitoids were used in an integrated management. Chlorantraniliprole, flubendiamide, emamectin and metaflumizone insecticides have been recommended for a 120-day term in Spain (Bloem and Spaltenstein, 2011). The ovicidal, larvicidal, pupicidal and adulticidal effects of conventional insecticides were tested in some countries infested with *T. absoluta* (Tomé *et al.*, 2012; Sohrabi *et al.*, 2015). One of the ways to postpone insecticide resistance is decreasing the consecutive usage of insecticides with the same mode of action. Currently, there are 33 groups of insecticides in the world, 13 groups of which are used to control *T. absoluta* (IRAC, 2018). Though only 8 years have passed since the arrival of the first tomato leaf miner in Iran (Abootorabi, 2014), it is necessary to increase the diversity of registered insecticide groups in Iran in addition to evaluating their effectiveness on the egg, larvae and the mature insects.

In this study, the effect of five insecticides from different groups was investigated on different developmental stages of *T. absoluta* including the egg, larvae and adult under laboratory conditions. Moreover, the toxicity of different insecticides on each developmental stages of the pest was compared.

Materials and Methods

All experiments were conducted on the tomato seed variety of Falat Karun in Kermanshah province, Iran. The seeds were planted in a seedling specific tray (40 × 70 cm) with 112 chambers. They were kept at 27 ± 1 ºC temperature, RH of 60% and photoperiod of 16: 8 (L: D) h in the research growth room of Dashte Sabze Gharb Agriculture and Industry Corporation (RojinTaak Agro-industry Complex). After one month, when the seedlings reached 12 cm height, they were transferred to pots of 15 cm diameter and 30 cm height. Also, the host plants were replanted every 15 days in order to have them available all throughout the experiment.

Rearing the moths of *T. absoluta*

In order to rear the moths, tomato leaves that were infected with *T. absoluta* were collected from the tomato fields (34.77º N latitude and 46.8º E longitude) in Kermanshah province and moved to cages that contained tomato pots. After a generation and making sure that the colony was not contaminated with natural enemies and insects' pathogens, the new-generation of moths were transferred to the other cage which was sized 2 × 2 × 2 m for oviposition. In the infection cage, the moths were fed on diluted solution of (10%) honey. In order to cohort the growth stages of *T. absoluta*, the tomato pots were put in the infestation cage for 24 h so that the oviposition could be made on them. Then, the plants were moved out of the cage and kept in the incubator.

Tested insecticides

In this study, abamectin, imidacloprid, indoxacarb, chlorpyrifos, cypermethrin and spinosad were used for bioassay tests on different life stages of the *T. absoluta*. Their formulation and rate of the active ingredients are shown in Table 1.

Table 1 Specifications of registered insecticides tested for bioassay against different life stages of the *Tuta absoluta*.

Common name (Trade name)	Formulation (a.i. %)	Company	Recommended against vegetable pest
Abamectin	EC 1.8	Gyah	Vegetable leaf miner
Spinosad (Tracer®)	SC 24	DOW	Tomato fruit borer
Imidacloprid	SC 35	Arya Shimi	Sucking pests of vegetable
Indoxacarb	SC 15	Ningbo Ferry	Tomato fruit borer
Cypermethrin	EC 40	Mahan	Sucking and chewing pests of vegetable

Bioassay of different growth stages of the *T. absoluta*

Egg stage

Two-day old eggs were placed on the tomato leaves immersed in insecticide solutions for 5 seconds, and were then kept under laboratory conditions to dry for 3 h. All of the treated leaves and the eggs were moved to 9-cm Petri dishes.

To avoid the leaves getting dry, their petioles were put in a micro tube containing water. For each insecticide, 30 eggs of *T. absoluta* were considered with an untreated control. The test was repeated three times. To evaluate the ovicidal effect, eggs with no growth or with dead embryos were considered as ovicidal effect, on the 6th day after treatments.

Larval stage

The leaf dip method was used for bioassay of larvae. After some pre-tests for each insecticide, at least 6 concentrations between 20% to 80% mortality rates were selected. Then, the leaves were immersed for 5 seconds in the insecticide solutions and after drying, they were placed into 9-cm Petri dishes and ten larvae of third instar (one-day old) were released in them. The larvae mortality rates were recorded until 48 h after treatment.

Adult stage

Filter paper method was used to test contact effect of the insecticides on the one-day old moths of *T. absoluta*. First, the 9-cm Whatman filter paper was placed in the Petri and soaked with 1 ml of each insecticide solution. After drying the filter paper, the 10 cohort moths were released in each Petri dish. To avoid mortality of the moths, a piece of cotton wetted in 10% honey was placed in each Petri. On the upper part of each Petri dish, a net with a diameter of 2 cm was considered for ventilation. The mortality rates of all treatments were recorded 48 h after exposure. The tested concentration of the insecticides was carried out in 3 replicates and for each replication at least 30 adult insects were used.

Analysis

All the collected data were analyzed by SAS with proc ANOVA and proc probit commands. The LC_{50} values and their confidence intervals were estimated at 95%. For each insecticide, log-probit lines, LC50 values, 95% fiducial limits (FL) and pearson χ^2 of goodness of fit were determined. The last two parameters indicate a goodness of fit for the bioassay models explaining the relationship between concentration levels and mortality and fitted probabilities. To compare the bioassay lines and LC_{50} values, the overlapping confidence intervals method was applied. Analysis of variance and comparison of means based on Duncan multiple range test in the form of proc ANOVA command were used to assess treatment ovicidal and adulticidal effects.

Results

Ovicidal and larvicidal effects of the tested insecticides and the susceptibly of *T. absoluta* to them were separately explicated. Moreover, sensitivity of the 3rd larvae of *T. absoluta* against five insecticides was

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presented based on the average concentration of 50% mortality (Table 2).

The ovicidal effect

Among the 5 insecticides tested, only abamectin showed ovicidal effects on the tomato leaf miner's egg. At 1000 $mg.l^{-1}$ concentration, it caused 84% mortality on the eggs while cypermethrin and imidacloprid showed the least ovicidal effects among the treatments with 10% mortality at 1000 $mg.l^{-1}$. In other treatments, the ovicidal effect was less than 20%. It should be noted that a lot of the treated eggs had high mortality (80-100%) in neonates even in very low concentrations at just after hatching, thus the mortality rate of first instar larvae was not recorded as a real dead embryo or ovicide toxicity. Results of the mean comparison of the ovicidal effect of the tested insecticides are reported in Table 2.

The larvicidal effect

In order to describe sensitivity of the third instar larvae of tomato leaf miner against the tested insecticides, LC_{50} and their confidence intervals were used. LC_{50} values of insecticides are presented in Table 3. According to the findings, abamectin with 0.45 mg $ai.l^{-1}$ was the most efficient compound tested on larvae while imidacloprid (980 mg $ai.l^{-1}$) was the least effective insecticide for the larvae in this study. Comparing the insecticides' LC_{50} values and their confidence intervals through overlapping method showed that larvicidal effectiveness of all the insecticides are significantly different and they can be classified in 5 individual groups. Spinosad (7.06 mg $ai.l^{-1}$), indoxacarb (24.39 mg $ai.l^{-1}$) and cypermethrin (423.6 mg $ai.l^{-1}$) respectively take the second to fourth positions (Table 3).

The adulticidal effect

Analyses showed that none of the insecticides in concentrations of 1000 $mg.l^{-1}$ from commercial formulation had enough impact on the adults and their maximum mortality rates were less than 50%. Abamectin (30%) and spinosad (40%) had the highest mortality rates on the moths. Also, imidacloprid and cypermethrin with 9-10% had the lowest and finally, indoxacarb showed 20% mortality on the adult insect (Table 2).

Table 2 Ovicidal and adulticidal effects of tested insecticides against eggs and moths of tomato *Tuta absoluta* at 1000 $mg.l^{-1}$ of their traditional formulation.

Common name	Concentration of formulation (a. i.) $mg.l^{-1}$	Ovicidal effect (%)	Adulticide effect (%)
Abamectin	1000 (18)	84.4 ± 2.6^{a}	30 ± 10^{ab}
Spinosad	1000 (240)	17.3 ± 0.45^{b}	40 ± 10^{a}
Imidacloprid	1000 (350)	8.23 ± 2.3^{c}	10 ± 5^{c}
Indoxacarb	1000 (150)	18.03 ± 1.7^{b}	20 ± 5^{bc}
Cypermethrin	700 (280)	10 ± 2^{c}	9 ± 2.6^{c}

Means in a column with the same letter(s) are not significantly different (Duncan's test, $P < 0.05$).

Table 3 Bioassay lines (concentration-mortality) of tested insecticides on the 3rd instar larvae of tomato *Tuta absoluta* and determining LC_{50} values with 95% confidence intervals by leaf dip bioassay method.

Insecticides	No. of insects	n[1]	Slope ± SE	LC_{50} (mg $ai.l^{-1}$) (CL95%)	Chi Squre[2]	P-value[3]
Spinosad	220	6	0.37 ± 2.1	7.06 (5.18-10.66)	0.65	0.98
Abamectin	280	8	0.18 ± 1.2	0.45 (0.31-0.66)	1.71	0.63
Indoxacarb	228	8	0.23 ± 1.15	24.39 (16.03-42.55)	1.68	0.79
Imidacloprid	268	7	0.63 ± 3.1	980 (790.6-1574.3)	3.29	0.19
Cypermethrin	300	7	0.31 ± 1.7	423.6 (318.9-582)	2.0	0.73

1: No. of Concentration.
2: Pearson chi-square value of Goodness-of-Fit test.
3: p-values for the Goodness-of-Fit tests.

Discussion

Among different life stages, the oval and adult stages were tolerant to the tested insecticides whereas the larval stages proved to be susceptible. Moreover, comparing LC_{50} values over all stages showed that spinosad and abamectin are the most toxic insecticides on tomato leaf miner. Ovicidal and adulticidal effects of tested insecticides were less than their larvicidal effects (Terrier, 1984; Sohrabi *et al.*, 2015).

The results also showed that the ovicidal effect of these four insecticides (except for abamectin) is less than 20%. In other words, imidacloprid, indoxacarb and cypermethrin were unable to destroy the egg cell or the embryo inside the egg, although most of the neonate larvae of treated eggs were dead at the time of hatching. Among the tested insecticides, abamectin had the real ovicidal effect (killing the embryo). According to Sohrabi *et al.* (2015) and Mahmoudvand *et al.* (2011), abamectin is an insecticide and acaricide with an acceptable ovicidal effect. Another study found that Rynaxypyr was the most ovicidal insecticide in comparison with indoxacarb, methoxyfenozid, spinosad, emamectin benzoate, spinetoram and pyridalyl against the 1-day old eggs of *Spodoptera littoralis* (Boisd.) (Hanan *et al.*, 2014). Eggs were less susceptible to insecticides. It is related to the developmental rate in egg cell and nervous system of the embryo. Eggs at the early development are more tolerant to insecticides than at near to hatching (Wang et al., 2003) and todays, pesticide companies greatly tend to produce insecticides that affect the harmful growth stage of the pest. For these reason, insecticides with mode of action on nervous and muscular systems of pest make 85% of total insecticide sales in the world (Spark and Nauen, 2015). The ovicidal effect becomes more important in controlling the pest population whose larvae feed on the inner parts of plant (i.e. fruit, leaf and stem). This effect occurs as a result of disturbance in the behavior of the larvae inside the egg or death of the oocyte. Applying compounds with ovicidal effects can reduce survival rate of the neonate larvae up to 50 percent. In addition, it can be very effective in decreasing the vast infestation of tomato leaf miner (Tomé *et al.*, 2012).

The larvae of *T. absoluta* were the most susceptible stage to the tested insecticides; abamectin was the most toxic while imidacloprid was the least against *T. absoluta* larvae, which is in accordance with the report from Sallam *et al.* (2015). Other researchers reported the high efficacy of spinosad and the less contact effect of imidacloprid on the *T. absoluta* larvae (Nilahyan *et al.*, 2012; Bratu *et al.*, 2015; El-Ghany *et al.*, 2016).

Indoxacarb is efficient in controlling *T. absoluta* in the larval stage; however, its ovicidal and adulticidal effects are less promising (Moussa *et al.*, 2013; Sohrabi *et al.*, 2015). Other researchers affirm this notion by widely recommending indoxacarb as the insecticide for *T. absoluta* (IRAC, 2007). The tomato leaf miner resistance to the pyrethroids is because of mutations in the voltage-gated sodium channel (Haddi *et al.*, 2012) and detoxification enzymes. However, the mechanisms of pyrethroids resistance are not precisely clear (IRAC, 2007).

Spinosad had more adulticidal effect (40% >) among the tested insecticides. Lower susceptibility of adult stage in comparison with larval stage of *Tuta absoluta* is due to differences in physiology and behavior. Mobility and mouthpart type of tomato leaf miner adults has caused less mortality of adults than larval stage in the conventional insecticide application. Because the larvae intake more quantity of insecticide by stomach and contact in comparison with adult (Onstad, 2014). Also the low mortality rate of the adults in this study can be due to the method of the experiment where only half of the contact surface was impregnated with insecticide but the upper part of the Petri was not. So the moth could selectively act and avoid sitting on the treated surfaces. The low efficacy of abamectin against adult stage of *T. absoluta 24 hours after the treatment* has been reported by Sohrabi *et al.*

(2015). Based on the results, it can be concluded that in the fields and greenhouses where *T. absoluta* has all the different life stages at the same time, it is better to use spinosad and abamectin. While in fields with more natural enemies and a large extent of the pest population including the larvae, specific insecticides like indoxacarb is advised. Overall, with regard to short life cycle of the *T. absoluta* and its high resistance potential against the pesticides and its fast and vast spreading, it seems necessary to pay more attention to the dominant developmental stage of the pest population in application time and to continue laboratory and field studies continuously to advise new insecticides so that they could be interchangeably used in the integrated pest management (IPM) of *T. absoluta.*

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اثر تخم‌کشی، لاروکشی و بالغ‌کشی چندحشره‌کش روی بید گوجه‌فرنگی ***Tuta absoluta*** **(Lepidoptera: Gelechiidae)**

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چکیده: بید گوجه‌فرنگی یکی از آفات مهم گوجه‌فرنگی در ایران است. کنترل شیمیایی یکی از رایج‌ترین روش کنترل آفت در شرایط طغیانی است. در این تحقیق اثر پنج حشره‌کش از گروه‌های مختلف شامل آبامکتین، اسپینوساد، ایمیداکلوپراید، ایندوکساکارب و سایپرمترین در سطح آزمایشگاه روی مراحل زیستی مختلف بید گوجه‌فرنگی بررسی و مورد مقایسه قرار گرفتند. از شاخص LC_{50} برای تعیین سمّیت روی لاروهای بید گوجه‌فرنگی استفاده شد. نتایج نشان داد که حشره‌کش آبامکتین و ایمیداکلوپراید به‌ترتیب بیش‌ترین و کم‌ترین خاصیت تخم‌کشی را در میان تیمارها دارند و از لحاظ تلفات روی شب پره‌ها هیچ‌کدام از حشره‌کش‌ها روی حشرات بالغ مؤثر نیستند و بیش‌ترین تلفات در شب‌پره مربوط به حشره‌کش اسپینوساد (۴۰٪) است. براساس LC_{50} به‌دست آمده از زیست‌سنجی لاروهای سن سوم بید گوجه‌فرنگی، حشره‌کش‌های آبامکتین ($0/45\ mg\ ai.l^{-1}$) و ایمیداکلوپراید ($980\ mg\ ai.l^{-1}$) به‌ترتیب قوی‌ترین و ضعیف‌ترین اثر سمّی را برای لارو بید گوجه‌فرنگی داشتند. براساس نتایج به‌دست آمده در مزارع آلوده گوجه‌فرنگی، که در یک زمان همه مراحل مختلف رشدی آفت مشاهده می‌شود می‌توان از حشره‌کش‌های اسپنوساد و آبامکتین استفاده کرد ولی در مزارعی که فعالیت دشمنان طبیعی بیش‌تر است و جمعیت غالب بید گوجه‌فرنگی را مرحله لاروی تشکیل می‌دهد کاربرد حشره‌کش‌های انتخابی مانند ایندوکساکارب توصیه می‌شود.

واژگان کلیدی: آبامکتین،اسپینوزاد ،ایمیداکلوپراید، ایندوکساکارب، سایپرمترین