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Research Article

Residual toxicity of some insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) larvae under laboratory conditions

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Abstract
Tomato leaf miner (TLM), *Tuta absoluta* (Meyrick), is a destructive insect pest in greenhouses and fields on tomato plants. In this study, the efficacy of seven insecticides: spinosad (Laser®), chlorantraniliprole (Coragen®), indoxacarb (Steward®), abamectin (Vertimec®), metaflumizone (Alverde®), azadirachtin (NeemAzal®), and *Bacillus thuringiensis* was evaluated against *T. absoluta* during 30 days from spraying. Tomato plants were sprayed by half and a quarter of the recommended field concentrations of the insecticides. Thirty 2nd instar larvae were placed on the treated plants, 2, 4, 7, 10, 13, 17, 22, 26, and 30 days after treatment, and mortality was recorded 48 hours later. The results showed that spinosad and chlorantraniliprole in the half of the field recommended concentration caused maximum efficacy on *T. absoluta* larvae, 100 and 80 % mortality at the end of 30 days, respectively. On the other hand, indoxacarb caused 57 % mortality on the 22nd day after exposure to the quarter of the field recommended concentration. In the quarter field-recommended concentration, abamectin and metaflumizone also had moderate mortality (52 and 54 % on the 10th day). The application of these three compounds can be considered suitable for preventing an increasing pest population from reaching the economic injury level. *Bacillus thuringiensis var. kurstaki* and azadirachtin insecticides had slight mortality and short persistence on this pest. Experts and farmers can select the appropriate compounds to control this pest based on the pest population status according to the obtained results.
Key words: Residual effects, Tomato leaf miner, Bioinsecticide, Protection time

تأثیر باقیمـاندۀ چند حشـره‌کـش روی لاروهای *Tuta absoluta* (Lepidoptear: Gelechiidae) در شرایط آزمایشگاهی

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چکیده
مینوز گوجه فرنگی *Tuta absoluta* (Meyrick) یک آفت مخرب روی بوته‌های گوجه فرنگی در گلخانه‌ها و مزارع است. در این مطالعه اثربخشی ۷ حشره‌کش اسپینوساد، کلرانترانیلی‌پرول، ایندوکساکارب، آبامکتین، متافلومیزون، آزادیراکتین و *Bacillus thuringiensis* در مدت ۳۰ روز پس از سمپاشی روی مینوز گوجه فرنگی مورد بررسی قرار گرفت. بوته‌های گوجه فرنگی با نصف و یک چهارم غلظت توصیه شدۀ مزرعه‌ای حشره کش‌ها سمپاشی شدند. تعداد ۳۰ عدد لارو سن دوم در ۲، ٤، ۷، ۱۰، ۱۳، ۱۷، ۲۲، ۲٦ و ۳۰ روز پس از تیمار، روی گیاهان تیمار شده قرار گرفتند و ٤۸ ساعت بعد تلفات آنها ثبت شد. نتایج نشان داد که اسپینوساد و کلرانترانیلی-پرول در نیم غلظت توصیه شدۀ مزرعه‌ای بیشترین کارایی را روی لاروهای مینوز گوجه فرنگی داشتند و به ترتیب ۱۰۰ و ۸۰ درصد تلفات در پایان ۳۰ روز ایجاد کردند. از سوی دیگر، ایندوکساکارب باعث تلفات ۵۷ درصد در روز ۲۲ام پس از تیمار با غلظت یک چهارم توصیه شدۀ مزرعه‌ای شد. در غلظت یک چهارم توصیه شدۀ مزرعه‌ای، آبامکتین و متافلومیزون کارایی مناسبی داشتند (۵۲ و ٥٤ درصد در روز دهم). بر این اساس بکارگیری این سه ترکیب را می‌توان برای جلوگیری از رسیدن جمعیت آفت به سطح آسیب اقتصادی مناسب دانست. حشره‌کش‌های *Bt* و آزادیراکتین تلفات جزئی و ماندگاری کوتاهی بر روی این آفت داشتند. با توجه به نتایج به‌دست‌آمده و بر اساس وضعیت جمعیت آفت، کارشناسان و کشاورزان می‌توانند ترکیبات مناسب را برای کنترل این آفت انتخاب کنند.
واژه‌های کلیدی: اثرات باقیمانده، مینوز گوجه فرنگی، حشره‌کش‌های زیستی، زمان پیشگیری

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Introduction

Tomato, *Solanum lycopersicum* L. is an important vegetable crop in Iran used as fresh and processed products (Aboutalebi *et al.*, 2012). Tomato leaf miner (TLM), *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a key pest of Solanaceae in many regions throughout the world. This pest originates from South America and subsequently extended to North America in the late 1960s (Miranda *et al.,* 1998; Torres *et al.,* 2001; Guedes & Picanço, 2012). *T. absoluta* invaded Iran fields in 2010 apparently by natural extending from northwestern borders between west Azarbaijan province and neighboring countries, either Turkey or Iraq (Baniameri & Cheraghian, 2012). This pest rapidly expanded and was detected in all major tomato-growing areas in Iran (Shekhi Gorjan *et al.,* 2018).

Characters such as high fecundity rate, numerous annual generations, and ability to feed on other Solanaceous plants such as pepper, tobacco, eggplant, potato, etc., make this insect a serious tomato pest throughout the world (Anonymous, 2011). The larvae can dig mines in leaf tissues and sometimes destroy the whole plant if no control measure is adopted (Desneux *et al.,* 2010; Shahini *et al.,* 2021). Chemical control is the major control method, whether in Iran or abroad (Baniameri & Cheraghian, 2012; Kumar *et al.,* 2020). The endophytic nature of larvae, and the high potential of resistance selection against insecticides, caused the insecticide applications have be insufficient (Haddi *et al.,* 2012; Roditakis *et al.,* 2013; Guedes *et al.,* 2019). Nevertheless, the insecticides are still the main component of an IPM program of this pest. Only a few insecticides are available against *T. absoluta* (Kumar *et al.,* 2020). In some areas, insecticide application against *T. absoluta* reaches 30 times in 60 days. Consequently, finding effective pesticides and obtaining comprehensive information such as half-life, side effects, relative selectivity, and safety about them is necessary to avoid wasting time and undesirable effects (Picanço *et al.,* 2007; Soares *et al.,* 2019).

Chlorantraniliprole is an anthranilic diamide insecticide introduced in 2006, with a new mode of action that is effective on many lepidopteran pests in low concentrations (Lahm *et al.,* 2009). On the other hand, spinosad is produced by fermentation of soil actinomycete, *Saccharopolyspora spinosa*. This bioinsecticide controls lepidopteran and dipteran pests with low side effects on the environment and beneficial insects (Yee, 2018). Indoxacarb is also an effective pesticide on *T. absoluta*, belonging to the oxadiazine group that affects some pests (Oxborough *et al.,* 2015). The other biorational pesticide used against *T. absoluta* is abamectin produced by a soil bacterium, *Streptomyces avermitilis* (ex Burg, Kim, and Goodfellow), which has nematicidal, acaricidal, and insecticidal activity (Lasota & Dybas, 1991). Metaflumizone belongs to semicarbazone insecticides, is the other option in the chemical control of *T. absoluta* discovered in 1990 and showed high efficacy on Lepidoptera, Coleoptera, Diptera, and Hymenoptera pest species (Takagi *et al.,* 2007). On the other hand, a limonoid compound extracted from the Neem tree, *Azadirachta indica A.Juss,* i.e., azadirachtin, affects feeding, growth, molting, and reproduction of insects (Mordue & Blackwell, 1993). The other biorational insecticide is *Bacillus thuringiensis* Berliner that produces various protein-based toxins against pests of the order Lepidoptera, Diptera, Coleoptera, and so on (Agaisse & Lereclus, 1995). Having enough information about the residual effects of pesticides with particular emphasis on bio-insecticides helps us manage pests and produce safe crops by avoiding unnecessary pesticides applications. This study evaluated the residual toxicity of four biorational (abamectin, spinosad, *Bt*, and azadirachtin) and three conventional (metaflumizone, indoxacarb, and chlorantraniliprole) insecticides on *T. absoluta*.

Materials and methods

Rearing *T. absoluta*

Different larval stages of *T. absoluta* were collected from a tomato field in Bilasuvar County, Ardabil Province (39° 39′ 31.37″N 48 ° 34′ 67.06″E) and moved to a greenhouse section of the Department of Plant Protection, University of Tabriz, Tabriz, Iran. The larvae were kept at 27 ± 2 °C, 50 ± 10 % RH, and 16: 8 h (L: D) photoperiod. The larvae were fed with greenhouse grown tomato plants (variety Supercheaf) and reared until adulthood. Tomato plants were planted in plastic pots height 15 cm and 12 in diameter, containing 25 % vermicompost, 25 % cocopeat, and 50 % perlite, and were irrigated every other day with 50 cc of tap water. The adults were transferred to 80 × 70 × 60 cm wooden cages covered by an organdy cloth net (80 mesh) containing two or three potted tomato plants (height 20 – 30 cm) and let them mate and lay eggs on the plants for 24 hours. The adults were fed with a 10 % sugar solution (changed every three days). To obtain first instar larvae of the same age in experiments, tomato plants were replaced 24 hours later. After the egg hatched, the plants were cut and placed in a plastic container (10 × 20 × 30 cm) with a stem enveloped by a piece of wet cotton. To provide proper ventilation, a hole covered by a fine mesh was created on the lid of the containers. The second instar larvae were used in bioassays.

Bioassay

Potted tomato plants (30 cm high) were sprayed with a hand sprayer until runoff stage by half and a quarter of the field recommended concentration (HFR and QFR, respectively) of chlorantraniliprole, indoxacarb, metaflumizone, and some biorational compounds like abamectin, spinosad, *Bt* and azadirachtin. The concentrations of insecticides used in this study are mentioned in Table 1 (Noorbakhsh, 2018). Tween-80 was used as a surfactant at a concentration of 0.05 % (v/v) in all insecticide treatments. In control, the plants were sprayed with distilled water and Tween-80. These plants were transferred to the insect-free section of the greenhouse and used in experiments 2, 4, 7, 10, 13, 17, 22, 26, and 30 days after spraying. At each time interval, treated tomato plants were cut and put into a rectangular transparent plastic container (15 × 10 × 8 cm). Thirty 2nd instar larvae were introduced, and mortality was recorded 48 hours later. Those larvae which were able to move when were touched by a soft brush were assumed to be alive. The tests were repeated three times on different days. The plants were maintained at 27 ± 2 °C, 50 ± 10 % RH, and 16: 8 h (L: D) photoperiod.

Table 1. Insecticides and concentrations used in experiments against *Tuta absoluta* larvae

Insecticide	Active ingredient	Company	Field rate (ppm)	Half concentrations of Field rate (ppm)	Quarter concentrations of Field rate (ppm)
Coragen 18.5 SC	Chlorantraniliprole	Dupont	500	250	125
Vertimec 18 EC	Abamectin	Syngenta	1200	600	300
Laser 48 SC	Spinosad	Dow AgroSciences	250	125	63
Steward 30 WG	Indoxacarb	Dupont	600	300	150
Bt	*Bacillus thuringiensis*	Intrachem Bio	500	250	125
Alverde 240 SC	Metaflumizone	BASF AgProducts	1000	500	250
Neem Azal -T/S 1 EC	Azadirachtin	Trifolio	10000	5000	2500

Statistical analysis

Mortality rates were subjected to a three-way ANOVA (factorial analysis). This analysis of the variance model included the main effects of insecticides including, concentration and exposure time as main effects and the interaction of the main effects. The mortalities were corrected using Abbott's formula (Abbott, 1925), and SAS version 9.2 (SAS, 2008) was used for statistical analyses. The graphs were plotted using Excel software (Microsoft Office 2013).

Results and discussion

Statistical analysis showed that expectedly, mortality of *T. absoluta* larvae in HFR was more than QFR concentration independent of the insecticide used ($F = 32.70$; $df_{t,\,e} = 1, 143$; $P < 0.0001$). In addition, it was different depending on time of exposure ($F = 57.79$; $df_{t,\,e} = 8, 143$; $P < 0.0001$), and kind of the insecticide used ($F = 558$; $df_{t,\,e} = 7, 143$; $P < 0.0001$). The interaction between insecticide and time of exposure also was significant ($F = 3.06$; $df_{t,\,e} = 56, 143$; $P < 0.0001$), which may imply a difference in the half-life of the insecticides. The other interactions were non-significant ($P = 0.0729$ for insecticide × concentration, $P = 0.6024$ for concentration × exposure time, and $P = 0.9914$ for three-way interaction of insecticide × exposure time × concentration) (Table 2).

Table 2. Three - way ANOVA statistics representing effects of insecticides, two concentrations and time intervals between spraying and exposure of *Tuta absoluta* larvae to insecticides.

Source of variation	Type III Sum of Squares	df	Mean Square	F	*P*-value
Corrected Model	98.811[a]	143	0.691	32.340	<0.001
Intercept	262.340	1	262.340	12278.142	<0.001
Time	9.879	8	1.235	57.792*	<0.001
concentration	0.699	1	0.699	32.701*	<0.001
Insecticide	83.457	7	11.922	557.999*	<0.001
Time * concentration	0.137	8	0.017	0.801[ns]	0.602
Time * Insecticide	3.658	56	0.065	3.057*	<0.001
concentration * Insecticide	0.281	7	0.040	1.878[ns]	0.073
Time*concentration* Insecticide	0.701	56	0.013	0.586[ns]	0.991
Error	6.154	288	0.021		
Total	367.305	432			
Corrected Total	104.965	431			

* Significant difference; [ns] non-significant difference

The highest survival rate during the experiment was observed in control, while in chlorantraniliprole and spinosad, the highest mortality rate was observed throughout the experiment (Figs. 1 and 2). The other insecticides displayed an increasing trend of the survival rate by coming to the end of the experiment. Their initial survival rates ranged between 10 to 60 %, which was at >75 % at 1-month residues. The high efficiency of chlorantraniliprole and spinosad can be due to their unique mode of action. These two compounds have a high level of efficacy for lepidopteran pests compared to other pests (Wei *et al.,* 2020; Santos & Pereira 2020). Chlorantraniliprole causes the unregulated release of internal calcium stores, leading to Ca^{2+} depletion, feeding cessation, lethargy, muscle paralysis, and insect death (Wei *et al*., 2020). Meanwhile, through stimulation of nAChR and γ-aminobutyric acid (GABA) receptors, spinosad induces rapid excitation of the organism's nervous system, producing paralysis and death (Santos & Pereira 2020). The results of Greaves *et al.* (1994) agreed with our findings in delayed effects. Leskey *et al.* (2014) showed that contact to three- and seven-day-old residues of five insecticides caused higher mortality on adult *Halyomorpha halys* (Stal) (Hemiptera: Pentatomidae) than the newly treated surface. Insect species, the physical condition, and differences in insecticides of the experiment may be a source of variation between the two studies. On the other hand, residual contacts to pesticides caused behavioral anomalies and long-term abundance decline in some agrobiont spider species as non-target organisms (Pekar & Benes, 2008). Long-time effects of spinosad in our study were confirmed by dos Santos *et al.* (2011), which showed persistency of spinosad in greenhouse conditions against *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) for 50 days from application. In the present study, both spinosad and chlorantraniliprole protected tomato plants very well for 30 days. HFR concentration of spinosad residue caused 100 % mortality on *T. absoluta* after 22-days, while its half-life was reported 1.6–16 days in photolysis condition (Saunders & Bret, 1997). Only a few larvae survived in spinosad and chlorantraniliprole treatments after 30 days. In another study, 1-h, 7-d, and 14-d old residues of chlorantraniliprole were classified as harmless to *Orius* *laevigatus* (Fieber) (Hemiptera: Anthocoridae) as a generalist predator (Biondi *et al.*, 2012). The difference in the results of this study with the present research may be due to differences in the physical condition and the insect target, indicating that *T. absoluta* is more susceptible than a predator.

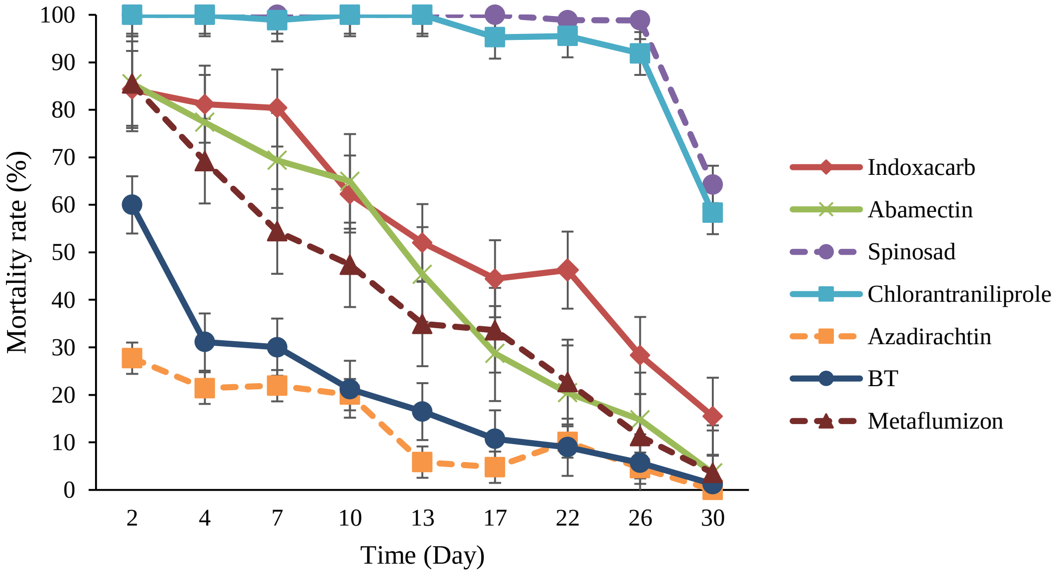

Fig. 1. The mean mortality rate ± SE of *Tuta absoluta* larvae exposed to 2d- to 30d-old residuals of HFR concentration of different compounds

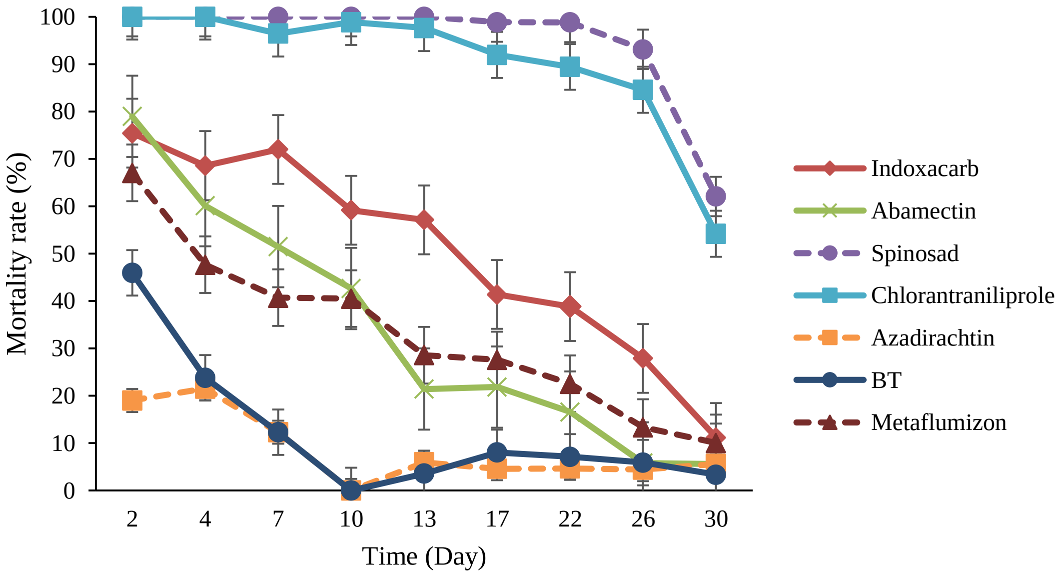

Fig. 2. The mortality rate of *Tuta absoluta* larvae exposed to 2d- to 30d-old residuals of QFR concentration of different compounds

Indoxacarb, abamectin, and metaflumizone had similar residual activity on *T. absoluta* larvae. In a similar study, abamectin and spinosad residues prohibited pupa development and showed severe larvicidal activity against *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), after 35^{th} day of application (Saryazdi *et al.*, 2012). The long-term activity of abamectin has also been reported by some other researchers (Lasota & Dybas, 1991; Van de Veire *et al.*, 2002; Saryazdi *et al.*, 2012). Our results are also in line with Sohrabi *et al.* (2015), who reported that indoxacarb and abamectin had suitable efficiency on the larvae of *T. absoluta*. Seven-d and 14-d old residues of metaflumizone were categorized as moderately harmful and harmless for the general predator (*O. laevigatus*) of *T. absoluta* (Biondi *et al.*, 2012). Also, the 4.5-day half-life of metaflumizone has been reported in rice straw (Chatterjee *et al.*, 2013). In our study, this insecticide caused above 50% mortality in HFR concentration ten days after spraying, which implies a long persistence. In contrast to our results, indoxacarb showed a longer protective duration. The 13-d-old residue of indoxacarb was highly toxic against eggs and larvae of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and 5-d old residue caused 100 % mortality in the larval stage (Liu *et al.*, 2002). The moderate efficiency of these pesticides can be due to the resistance of *T. absoluta*. In the previous studies, the resistance of *T. absoluta* to indoxacarb (Silva, *et al.*, 2011; Dağlı *et al.*, 2012; Yalçin *et al.*, 2015) and abamectin (Siqueira *et al.*, 2001) was demonstrated. Insect species, the physical condition of the experiment, and differences in products of different companies may be a source of variation between our study and those of the above studies. Among the tested insecticides, *Bt* and azadirachtin had the lowest effect and stability compared with the other insecticides. No detectable effect was measurable after two weeks. Previous studies demonstrated that the efficiency of *Bt* depended on time and the age of larvae. Alirezaei & Talaei-Hassanloui (2016) have shown the method and timing of *Bt* application can alter the effectiveness of this bioinsecticide on *T. absoluta* larvae. On the other hand, Gonzalez-Cabrera *et al.* (2011) reported that *Bt* had efficacy on neonatal larvae mortality, although its effectiveness decreases by the age of larvae. Behle *et al.* (1991) reported different half-lives for different formulations of *Bt* (ranged from 4.3 to 7.1-d) against neonate larvae of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Seven-d and 14-d old residues of these insecticides were classified as harmless to *O. laevigatus* (Biondi *et al.*, 2012). Azadirachtin had poor residual activity. It was categorized as a short-lived insecticide by Sundaram & Curry (1994). In fir and oak foliar application, they reported LT_{50} values from 17 to 22 h. The persistence of pesticides residues depends on numerous factors; for example, the persistence of neonicotinoid insecticides was different in wet and dry seasons in tea plants (Gupta & Shanker, 2008). As long as the persistence of pesticides is desirable to protect plants against dangerous pests like tomato leafminer, it may be harmful to beneficial organisms such as *O. Laevigatus*; a natural enemy of this pest.

Conclusion

Among the tested insecticides, chlorantraniliprole and spinosad were the most effective insecticides. These insecticides are perfect choices for prompt situations requiring a sudden population decline. These insecticides provide relative control and will be a good choice in multifactor cases. Finally, *Bt* and azadirachtin may be good choices near harvest, and short persistence is advantageous. These results need confirmation by field studies. Effects on non-target organisms, primarily natural enemies of *T. absoluta* itself also, must be known.

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