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Monitoring the susceptibility of different populations of tomato leaf miner, *Tuta absoluta* to indoxacarb and its combination with azadirachtin

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Abstract

Tomato leaf miner, *Tuta absoluta*, is one of the most detrimental biological agents in tomato cultivation, and there have been many reports of its worldwide resistance to various insecticides. This study aimed to evaluate the resistance of six *T. absoluta* populations collected from four provinces of Iran, including Ardabil, Tehran, Alborz, and Khuzestan to the widely-used insecticide indoxacarb. Leaf-dipping method bioassay was performed against the second-instar larvae. Estimated 72 h-LC_{50} values had not any overlapping between their 95% confidence intervals in all populations, indicating the presence of significant resistance in six populations compared to the susceptible one. The highest to lowest resistance ratios were obtained for the populations of Ziba Shahr (25.83), Mohammad Shahr (13.08), Parsabad Moghan (8.68), Safiabad (8.52), Ardabil (4.23), and Benoot-e Bala (2.19), respectively. The indoxacarb mixed with azadirachtin at the LC_{10}: LC_{10} ratio showed an additive effect, while the LC_{25}: LC_{25} ratio showed a synergistic effect. Also, the larval mortality caused by a mixture of LC_{25} values of indoxacarb and azadirachtin was significantly higher than mortality due to their separately used LC_{50} values in the susceptible population. In conclusion, some indoxacarb-resistant levels were documented in field second-instar larvae of *T. absoluta*. However, adding azadirachtin enhanced insecticidal efficiency of indoxacarb, which may be applicable in the suitable and safe management of this detrimental insect pest.

Keywords: Azadirachtin, Indoxacarb, Resistance ratio, Susceptible population, Tomato leaf miner

پایش حساسیت جمعیت‌های مختلف شب‌پره مینوز گوجه‌فرنگی *Tuta absoluta* به ایندوکساکارب و اختلاط این حشره‌کش با آزادیراکتین

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چکیده

شب‌پره مینوز گوجه‌فرنگی *Tuta absoluta* یکی از آفات مهم گوجه‌فرنگی می‌باشد که گزارش‌های بسیاری از مقاومت این آفت به حشره‌کش‌های مختلف از سراسر جهان شده است. این مطالعه به منظور بررسی احتمال مقاومت شش جمعیت *T. absoluta* جمع آوری شده از چهار استان ایران، شامل اردبیل، تهران، البرز و خوزستان به حشره‌کش ایندوکساکارب انجام شد. زیست سنجی روی مرحله لارو سن دو با روش غوطه‌ورسازی برگ در شرایط آزمایشگاهی صورت گرفت. مقادیر LC_{50} برآورد شده پس از گذشت ۷۲ ساعت در محدوده اطمینان ۹۵ درصد در تمام جمعیت‌های مناطق مختلف با جمعیت حساس همپوشانی نداشت که نشانگر وجود مقاومت معنی‌دار در این جمعیت‌ها در مقایسه با جمعیت حساس می‌باشد. نرخ مقاومت به ترتیب از بیشترین به کمترین برای جمعیت زیباشهر (۲۵/۸۳)، محمدشهر (۱۳/۰۸)، پارس‌آباد مغان (۸/۶۸)، صفی‌آباد (۸/۵۲)، اردبیل (۴/۲۳) و بنوت بالا (۲/۱۹) به دست آمد. اختلاط ایندوکساکارب و آزادیراکتین با نسبت LC_{10}: LC_{10} تقابل افزایشی و با نسبت LC_{25}: LC_{25} تقابل سینرژیستی نشان داد. مرگ و میر لاروهای تیمار شده با مخلوط حشره‌کش‌های ایندوکساکارب و آزادیراکتین با نسبت LC_{25}: LC_{25} به طور معنی‌داری بالاتر از تیمار جداگانه حشرات با LC_{50} این ترکیبات در جمعیت حساس بود. در نهایت، سطوحی از مقاومت لاروهای سن دو مینوز گوجه‌فرنگی مناطق مختلف ثبت گردید و از طرف دیگر، حشره‌کش آزادیراکتین باعث افزایش سمیت ایندوکساکارب شد که می‌تواند منجر به مدیریت مناسب و ایمن این آفت شود.

کلمات کلیدی: آزادیراکتین، ایندوکساکارب، نسبت مقاومت، جمعیت حساس، شب‌پره مینوز گوجه‌فرنگی

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Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is one of the most harmful pests of tomato (*Solanum lycopersicum* L.) worldwide. The larvae cause significant function loss by affecting different parts of the plant (Picanço *et al*. 2007; Tropea *et al*. 2012). The center of the origin of *T. absoluta* is South America; however, tomato leaf miner quickly spread to European countries and gradually to almost all Central and Southwest Asian, African countries, and Iran (FERA 2009; Desneux *et al*. 2010; Baniameri & Cheraghian 2012; Guillemaud *et al*. 2015; Biondi *et al*. 2018).

Chemical insecticides are commonly used to control harmful insect pests such as tomato leaf miner (Desneux *et al*. 2010; Sheikhigarjan *et al*. 2018; Ashtari 2021). However, insecticide resistance in tomato leaf miner has been reported in various groups of insecticides including organophosphates (OPs), pyrethroids, diamides, and oxadiazines, and even bio-rational pesticides abamectin and spinosad (Silva *et al*. 2011; Guedes & Picanço 2012; Gontijo *et al*. 2013; Campos *et al*. 2014; Silva *et al*. 2015; Silva *et al*. 2016; Roditakis *et al*. 2015, 2016, 2018; Guedes *et al*. 2019). The development of insecticide resistance is due to the dominance of resistant genes and the selection pressure, which leads to the ineffectiveness of insecticides on pest populations (Hemingway & Ranson 2000). Identifying the resistant and or susceptible populations is essential to the management of insect pests. The recommendation of ineffective pesticides to control resistant populations should also be avoided in order to prevent environmental pollution and to reduce production costs.

Indoxacarb belongs to the oxadiazine group. This chemical is effective against a wide range of insect pests, including moths, beetles, leafhoppers, weevils, and flies (McCann *et al*. 2001; Silver *et al*. 2010). However, several reports about the resistance of insect pests to indoxacarb can be found; in the study of Shono *et al*. (2004), the housefly (*Musca domestica* L.) was 13-fold resistance to indoxacarb and showed up to 118-fold resistance by small laboratory selection. Pang *et al*. (2012) reported that LC_{50} of indoxacarb for oriental tobacco budworm (*Helicoverpa assulta* Guenee) increased by 4.19-fold in laboratory resistance selection, in which carboxylesterase (CarE) and glutathione-S-transferases (GSTs) may be involved. Indoxacarb resistance has also been found in the tobacco caterpillar (*Spodoptera litura* (F.)), where resistance levels increased to 95-fold in a field population after laboratory selection (Sayyed *et al*. 2008). Some recent studies have also reported the resistance of *T. absoluta* to indoxacarb in populations from Brazil (Silva *et al*. 2011), Italy (Roditakis *et al*. 2012a), Greece (Roditakis *et al*. 2012b), and Turkey (Yalcin *et al*. 2015). The reduced susceptibility to indoxacarb in *T. absoluta* populations was also identified from Greece and Italy by Roditakis *et al*. (2016). However, there are limited reports addressing the resistance of tomato leaf miner to indoxacarb in Iran (Nazeri *et al*. 2014; Barati *et al*. 2018).

In this study, the susceptibility of six populations of *T. absoluta* from different geographical regions of Iran and one susceptible population to the indoxacarb was evaluated. Mixing insecticides can be considered as an efficient method to increase the susceptibility of insect pests (Ahmad 2009). Therefore, enhancing the toxicity of indoxacarb mixed with azadirachtin, a bio-rational pesticide derived from the neem tree (Boursier *et al*. 2011), against *T. absoluta* larvae was the main objective of this study.

Materials and methods

Host plant rearing

Seeds of tomato (*S. lycopersicum* var. Super Strain B; Unigen company, Spain) were taken from the Agricultural Research, Education and Extension Organization (AREEO) (Ardabil, Iran) and cultivated regularly in plastic pots (20-cm diameter and 19-cm height; 15 pots per week) containing soil, sand, and perlite (70: 15: 15 ratios respectively) under greenhouse conditions (20 ± 3°C, relative humidity of 55 ± 10%, and natural photoperiod). Plants were irrigated every 3 days and placed inside wooden shelves (50 × 50 × 80 cm) covered with netting to protect against other pests. At a height of about 30 cm, the plants were relocated to the growth chamber for infection with *T. absoluta* at 25 ± 2 °C, 65 ± 5% relative humidity, and a photoperiod of 16: 8 (L: D).

Insects

The tomato leaves infected with leaf miner larvae were collected from tomato farms in different areas of Iran, including Ardabil (38.2514° N, 48.2973° E) and Parsabad Moghan (39.6208° N, 47.9051° E) in Ardebil province, Mohammad Shahr (35.7499° N, 50.9029° E) in Alborz province, Ziba Shahr (35.4287° N, 51.5754° E) in Tehran province, Benoot-e Bala (32.2258° N, 48.4900° E) and Safiabad (32.2632° N, 48.4163° E) in Khuzestan province, and were transferred to the growth chambers inside the plastic containers with the net lid. The mentioned fields were not sprayed with any pesticides. Collected insects were maintained in growth chambers on tomato plants at 25 ± 2 °C, 65 ± 5% relative humidity, and a photoperiod of 16: 8 (L: D) for three generations before bioassays at the Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran. Water-honey solution (10%) was used for feeding adults (Krechemer & Foerster 2015). Farmers confirmed the low efficiency of indoxacarb for control of *T. absoluta* in all six locations within three years ago.

Insecticides

Commercial formulations of the following insecticides were used: indoxacarb (Indoxacarb® 15% SC; Ariashimi, Iran) and azadirachtin (Neem Azal® 1% EC; Trifolio, Germany).

Bioassays

Bioassays were performed on 2nd-instar larvae of *T. absoluta* by leaf dipping method in the toxic solutions (IRAC No: 022). Five concentrations of indoxacarb were prepared to investigate larval mortality and estimate LC (Lethal Concentration) values. In order to determine the main concentrations, several preliminary experiments were performed to obtain concentrations causing 25 - 75% mortality (Robertson & Preisler 2007). The susceptible population was obtained from population raised over several years in the laboratory of the Department of Plant Protection at the University of Mohaghegh Ardabili. Finally, the main experiments using five concentrations of indoxacarb were performed for the susceptible (2, 3, 6, 10, and 19 mg a.i./l), Benoot-e Bala (3, 6, 12, 25, and 52 mg a.i./l), Ardabil (10, 17, 29, 50, and 82 mg a.i./l), Parsabad Moghan (19, 29, 46, 75, and 120 mg a.i./l), Safiabad (15, 26, 45, 79, and 135 mg a.i./l), Mohammad Shahr (37, 52, 77, 114, and 165 mg a.i./l), and Ziba Shahr (75, 101, 147, 212, and 300 mg a.i./l) populations. The resistance ratios were calculated via dividing the LC_{50} of different populations by the LC_{50} of the susceptible one (Roditakis *et al*. 2016). Tomato leaves were immersed in the prepared concentrations for 15 seconds and kept at room temperature for 1 h to dry. Tween 20 (0.05 %) was used in all treatments and control groups as a solvent. Larvae obtained from 24-hour eggs were homogenized based on the length of the larval period (three-day-old larvae). Twenty larvae (less than 24 h old) were placed on the treated leaves in a plastic Petri dish (9 cm in diameter) for each concentration. The petioles of the leaves were placed inside wet cotton covered with aluminum foil. Three replications were assigned for each treatment. To exchange the air inside the Petri dishes, a hole was made in their caps and blocked with a net. Mortality was counted after 72 hours and larvae that did not respond to mechanical stimuli were scored as dead.

Combination of indoxacarb and azadirachtin

In this experiment, a combination of sublethal concentrations of indoxacarb and azadirachtin were evaluated on 2nd instar larvae in the susceptible population based on the Zhu *et al.* (2017) study. In this research, LC values of azadirachtin obtained from our previous study (Taleh *et al*., 2021) were used and the susceptible population is the same as the population studied in the Taleh *et al*. (2021). Considered concentrations, in respect for indoxacarb and azadirachtin, were LC_{10}: LC_{10} (0.55: 0.78 mg a.i./l) and LC_{25}: LC_{25} (1.65:1.92 mg a.i./l) along with separate LC_{10} (0.55 and 0.78 mg a.i./l), LC_{25} (1.65 and 1.92 mg a.i./l), and LC_{50} (5.6 and 5.19 mg a.i./l). The LC values of indoxacarb were estimated according to the results of above-mentioned bioassay. The larvae were treated similarly to the method described above. Twenty homogenous larvae were placed on the treated leaves in a Petri dish, three replications were assigned for each treatment, and the mortality was calculated after 72 hours.

Data analysis

Abbott's formula (Abbott, 1925) was used to correct the mortality of *T. absoluta* larvae in the control groups. Lethal concentrations (LC) with a 95% confidence interval were calculated using probit analysis in SPSS software (ver.24). The expected mortality (M_E) for the mixture of indoxacarb with azadirachtin was calculated using the formula $M_E = M_B + M_A (1 - M_B)$, in which M_B is the observed mortality caused by azadirachtin and M_A is the observed mortality caused by indoxacarb. Calculated chi-square values through $\chi 2 = (M_{AB} - M_E)^2/M_E$, in which M_{AB} is the observed mortality for the mixture of indoxacarb with azadirachtin, used to compare with corresponding values in the chi-square table. If the calculated chi-square value overstepped the corresponding value in the table (df = 1), it would be an additive effect. The $M_{AB} - M_E > 0$ indicated synergism; and the $M_{AB} - M_E < 0$ indicated antagonism (Koppenhofer & Kaya 1996; Wu *et al*. 2017). The data of mortality of 2nd-instar larvae of *T. absoluta* were subjected to ANOVA. Means of the mortality of 2nd-instar larvae of *T. absoluta* exposed to different concentrations of indoxacarb and combination of this insecticide with azadirachtin in the susceptible population were compared by Tukey's test with SPSS software (version 24) at P = 0.05 and significant differences were recorded.

Results

LC_{50} values of indoxacarb calculated for all tested populations are given in Table 1. The LC_{50} values for all populations were significantly higher than the corresponding value in the susceptible population (5.6 mg a.i./l). Benoot-e Bala (12.28 mg a.i./l) and Ziba Shahr (144.68 mg a.i./l) populations had the lowest and highest LC_{50} values, respectively.

Table 1. Estimates of the 72 h-LC_{50} values and resistance ratios of indoxacarb against 2nd-instar larvae of different populations of *Tuta absoluta* from main tomato cultivation regions of Iran.

Population	LC_{50} (95% Confidence Limits) (mg a.i./L)	RR [A]	χ^2 (df = 3)	*P* value	Slope ± SE	Number[B]
Susceptible [C]	5.6 (4.52-6.93) [f]	1	0.52	0.91	1.27±0.16	360
Benoot-e Bala	12.28 (9.7-15.56) [e]	2.19	0.55	0.9	1.15±0.13	360
Ardabil	23.67 (19.15-28.48) [d]	4.22	1.04	0.79	1.42±0.18	360
Safiabad	47.76 (39.21-58.58) [c]	8.52	1.21	0.74	1.35±0.17	360
Parsabad Moghan	48.64 (41.22-57.67) [c]	8.68	0.26	0.96	1.62±0.2	360
Mohammad Shahr	73.26 (63.74-83.65) [b]	13.08	0.71	0.87	2.00±0.26	360
Ziba Shahr	144.68 (129.39-161.5) [a]	25.83	0.33	0.95	2.46±0.28	360

[A] RR (resistance ratio) = LC_{50} of each population/LC_{50} of most susceptible population

[B] Number of test insects in each population

[C] Susceptible population

Different letters indicate significant differences among LC_{50} for each population, according to not overlapping between 95% confidence intervals of LC_{50} values.

Based on the overlapping between 95% confidence intervals of LC_{50} values, Parsabad Moghan and Safiabad populations had no significant difference. However, significant differences were found among other populations. Resistance ratios, from highest to lowest, were also represented for the populations of Ziba Shahr (25.83), Mohammad Shahr (13.08), Parsabad Moghan (8.68), Safiabad (8.52), Ardabil (4.23), and Benoot-e Bala (2.19) in Table 1.

Dose-response curves for populations in different regions are shown in Figure 1. The population of Ziba Shahr has the highest line slope compared with other populations. On the other hand, the population of Benoot-e Bala has the lowest line slope.

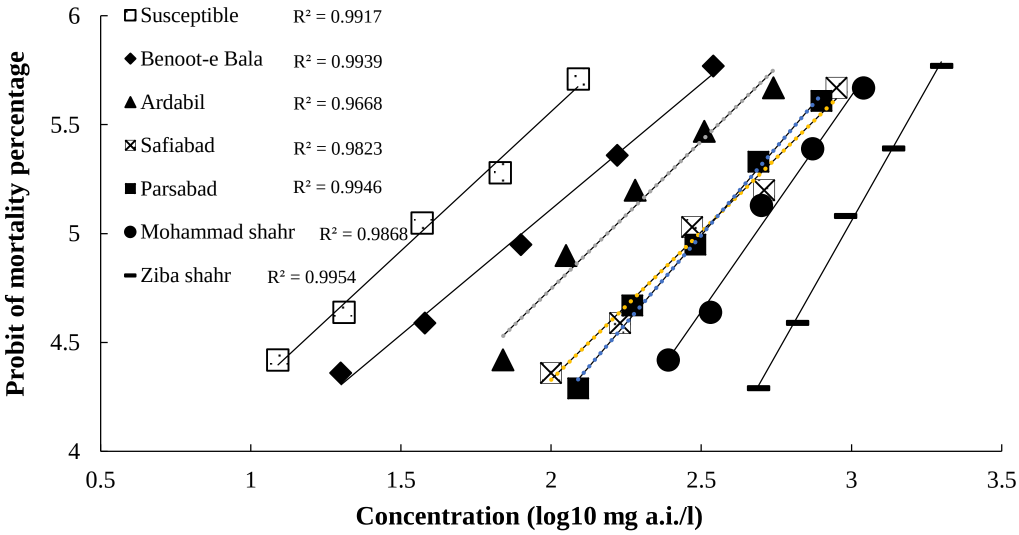

Figure 1. Dose-response lines of indoxacarb for 2nd-instar larvae of *T. absoluta* in different regions.

According to the Taleh *et al*. (2021) study, the LC_{50} value of azadirachtin was 5.19 (4.35 - 6.17) mg a.i./l, which has no significant difference from the corresponding value for indoxacarb (5.6 (4.52 - 6.93) mg a.i./l) on the susceptible population. The indoxacarb mixed with azadirachtin at the LC_{10}: LC_{10} ratio showed an additive effect, while a synergistic effect was found by the LC_{25}:LC_{25} ratio. In other words, mixture's mortality rate was higher than their individual mortality rates (Figure 2). The combination of indoxacarb with azadirachtin (LC_{25}: LC_{25}) was enhanced the toxicity and caused 68% larval mortality in comparison with 51.6% and 48.3% mortality for LC_{50} values of indoxacarb and azadirachtin, respectively, in the susceptible population (F = 67.49, df = 7,16; P = 0.05) (Figure 2).

Discussion

Based on the high damage of *T. absoluta* and its resistance to some conventional insecticides (Nazeri *et al*. 2014; Zibaee *et al*. 2017; Barati *et al*. 2018) search for more effective agents and strategies in the management of this pest is necessary. Indoxacarb has been registered to use against *T. absoluta* in Iran (Baniameri & Cheraghian 2012). Excessive use of this insecticide and its high selection pressure in the agricultural areas will lead to the development of resistance in the Iranian populations of *T. absoluta*. In the present study, the presence of resistance in some Iranian populations of *T. absoluta* was confirmed based on the high slope in dose-response curves. Indeed, using higher doses of the insecticide may be resulted in significant increases in pest mortality and a potential increase in selection pressure leading to the development of resistance (Moadeli *et al*. 2014).

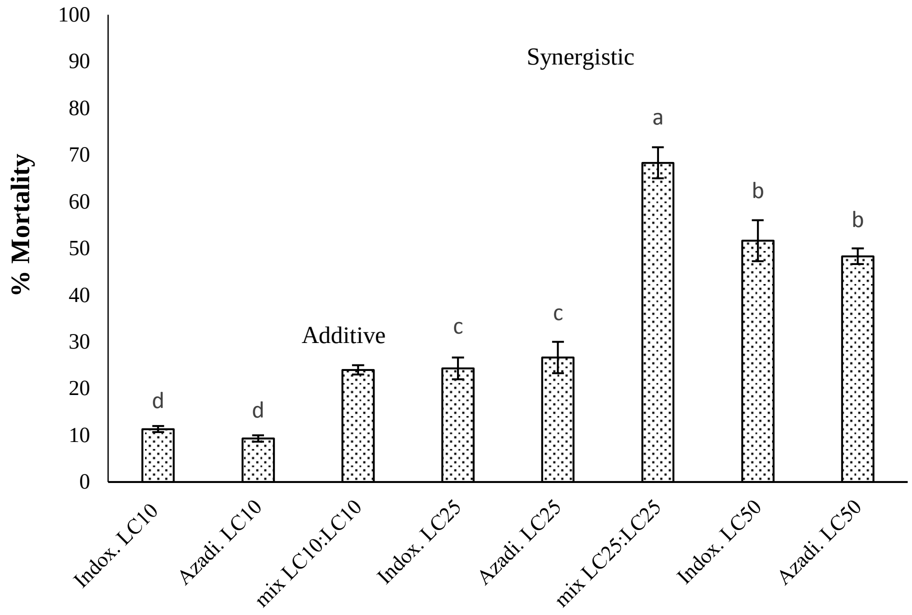

Figure 2. The mortality of 2nd instar larvae of *T. absoluta* exposed to different concentrations of indoxacarb and its combination with azadirachtin in the susceptible population and its type of interaction (synergistic, additive or antagonistic) (Koppenhofer & Kaya 1996; Wu *et al*. 2017). Indox.: indoxacarb; Azadi.: azadirachtin; mix: mixture of indoxacarb and azadirachtin.

Ziba Shahr population with the highest line slope is significantly different from other populations in terms of indoxacarb resistance. Farm population of Ziba Shahr showed more resistance to indoxacarb in all populations (with a resistance rate of 25.83-fold that of a sensitive population), which was higher in comparison with the populations from Italy (12-fold) (Roditakis *et al*. 2012a), Greece (10-fold) (Roditakis *et al*. 2012b), and Turkey (8-fold) (Yalcin *et al*. 2015), and near to the populations from Brazil (27-fold) (Silva *et al*. 2011). Excessive use of this insecticide in tomato fields can be a cause for create resistance. The coefficient of the determination of dose–response lines (R^2) represented an appropriate correlation between indoxacarb concentrations and response of the populations (Figure 1), denoting that the experiment populations were homogenized (Moadeli *et al*. 2014).

High potential of azadirachtin in insect pest management particularly in organic farming has been approved by the previous studies (Santos *et al*. 2015; Chaudhary *et al*. 2017; Zhong *et al*. 2017). For example, the susceptibility of *T. absoluta* to different formulations of azadirachtin was reported (Tomé *et al*. 2013; Amizadeh *et al*. 2015; Nazarpour *et al*. 2016; Hosseinzadeh *et al*. 2019; Taleh *et al*. 2021). Furthermore, multiple modes of actions of azadirachtin, from direct toxicity to the egg-laying and larval movement deterrence effects, to Brazilian populations of *T. absoluta* was documented (Tomé *et al*. 2013).

In the other study, toxicity of azadirachtin on the second-instar larvae of *T. absoluta* with 24 h-LC_{50} of 53.53 mg ai/l along with its additive interaction with *Bacillus thuringiensis* var. *kurstaki* (Berliner) was found by Amizadeh *et al*. (2015). In the current study, azadirachtin mixed with indoxacarb to reduce or delay resistance, as this combination enhanced the mortality of *T. absoluta*, which may be related to multiple modes of action of azadirachtin.

Iranian tomato growers should consider the existence of *T. absoluta* population resistance exposed to the conventional insecticide indoxacarb. Its frequent application will increase costs and probably the resistance of insect pests.

According to the present findings, mixing indoxacarb with azadirachtin resulted in additive and synergistic phenomena, the mortality of 2nd-instar larvae of *T. absoluta* in susceptible population was increased compared to the separate uses. In general, the combination of two insecticides can reduce insect pest resistance and the toxicity can be augmented (Attique *et al*. 2006; Ullah *et al*. 2017; Yu & Ting 2019; Taleh *et al*. 2021). If the results are confirmed by performing field studies, mixing indoxacarb with azadirachtin can be considered a viable option for the effective management of *T. absoluta*.

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