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Evaluation of Resistance to Abamectin in the Populations of *Tuta absoluta* (Lepidoptera: Gelechiidae), Collected from Isfahan Province, Iran

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ABSTRACT

The tomato leafminer (*Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae) is one of the most important pests of tomato worldwide. In this study, resistance of different populations of the tomato leaf miner from Isfahan Province was evaluated against abamectin. The median Lethal Concentrations (LC_{50}) of different populations were estimated by bioassays using a leaf-dip method. The LC_{50} value of abamectin in the reference population of Isfahan University of Technology (IUT) was estimated as 5.67 mg ai L^{-1}, while the population of Shahre-e-Abrisham 1 showed the highest (25-fold) resistance, with an LC_{50} value of 143.18 mg ai L^{-1}. Pre-treatment of different populations with diethyl maleate (DEM) synergist, an inhibitor of glutathione-S-transferases (GSTs), increased significantly abamectin toxicity. GST activity was also found significantly different between resistant and reference populations. Triphenyl phosphate (TPP), an inhibitor of esterases (ESTs), reduced the LC_{50} value of abamctin in the populations as much as 1.73- to 3.73-fold. The activity of ESTs in these populations was also significantly different. Furthermore, inhibition of cytochrome P450 monooxygenases (CYP450s) by piperonyl butoxide (PBO) increased abamectin toxicity between 1.3- to 2.9-fold in tested populations. The highest ratios of synergism for DEM (5.86), TPP (3.73-fold), and PBO (2.91-fold) were observed in Shahre-e-Abrisham 1. It seems that GSTs and ESTs play a more important role in the resistance development against abamectin in the studied populations. High levels of resistance to abamectin in the collected populations from Isfahan Province shows the importance of insecticide resistance management based on the early detection of resistance and alternative use of insecticides.

Keywords: Enzyme activity, Insecticide resistance management, Insecticide resistance mechanisms, Synergist.

INTRODUCTON

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most important tomato pests in many parts of the world, including Iran (Desneux *et al*., 2010; Baniameri and Cheraghian, 2012; Guedes and Siqueira, 2013). Larvae of *T. absoluta* mine the leaves, flowers, shoots, apical buds and fruits so that the non-control results in yield losses between 80% - 100% (Picanço *et al*., 2007; Desneux *et al*., 2010).

The tomato leaf miner is native to South America (Meyrick, 1917) and then spread to Africa, Europe and the Middle East (Desneux *et al*., 2010, 2011; Tonnang *et al*., 2015). In 2010, *T. absoluta* was reported for the first time in Iran (Baniameri and Cheraghian, 2012). To manage *T. absoluta*, combining biological control methods, using resistant host plants, biopesticides, and chemical control are recommended. Insecticides are less effective in controlling this pest due to larval feeding site into leaf mesophyll and lack of direct exposure to

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insecticides (Aynalem, 2018; Biondi *et al*., 2018). Also, widespread use of pesticides has led to the development of resistance to pesticides such as organophosphates, carbamates, pyrethroids, diamides, spinosyns, abamectin, and indixacarb (Siqueira *et al*., 2000, 2001; Lietti *et al*., 2005; Reyes *et al*., 2012; Konuş, 2014; Campos *et al*., 2015; Silva *et al*., 2016a, b; Haddi *et al*., 2017; Roditakis *et al*., 2015, 2017a, b, 2018; Zibaee *et al*., 2018; Guedes *et al*., 2019).

Abamectin belongs to the avermectin subfamily of macrocyclic lactone compounds and is the result of fermentation of the soil bacterium *Streptomyces avermitilis* (Dybas, 1989; Lasota and Dybas, 1991). Abamectin is an acaricide-insecticide that has exhibited high potencies for a broad spectrum of invertebrate pests (Putter *et al*., 1981). The mode of action of abamectin is acting on γ-Aminobutyric Acid (GABA) receptors and Glutamate-gated Chloride channels (GluCls) (Casida and Durkin, 2013).

Resistance to abamectin has been reported in some arthropod pests such as *Plutella xylostella* (Santos *et al*., 2011), *Frankliniella occidentalis* (Chen *et al*., 2011), *Spodoptera litura* (Saleem *et al*., 2016), *S. exigua* (Che *et al*., 2015), *Helicoverpa armigera* (Qayyum *et al*., 2015), *Liriomyza trifolii* (Ferguson, 2004), *L. sativae* (Wei *et al*., 2015), *Bemisia tabaci* (Wang and Wu, 2007), *Tetranychus cinnabarinus* (Lin *et al*., 2009), and *T. urticae* (Çağatay *et al*., 2018). Despite several studies on abamectin resistance in *T. absoluta* using bioassays, mechanism of resistance has not been fully characterized (Siqueira *et al*., 2000, 2001; Lietti *et al*., 2005; Silva *et al*., 2011, 2016a; Konuş, 2014; Guedes *et al*., 2019). One of the main mechanisms of abamectin resistance in *T. absoluta* is the increasing insecticide metabolism by means of some enzymatic systems such as CYP450s, ESTs and GSTs (Konuş, 2014). Due to the interference of these enzyme systems in resistance to abamectin, the trait in tomato leaf miner populations may be oligo or polygenic (Siqueira *et al*., 2001). Altered target site sensitivity is also a major abamectin resistance mechanism reported in *P. xylostella* and *T. urticae* (Dermauw *et al*., 2012; Wang *et al*., 2017).

Although abamectin is not one of the registered insecticides to control tomato leaf miner, it has been used to control other greenhouse pests in Iran (Nourbakhsh, 2019).

In this study, we collected nine *T. absoluta* populations from Isfahan Province and aimed to determine their resistance levels to abamectin. Additionally, we aimed to perform synergism and biochemical assays to investigate metabolic mechanisms of resistance to abamectin in this pest.

MATERIALS AND METHODS

Insect Populations

Nine different populations of tomato leaf miner from greenhouse tomato crops in Isfahan Province were collected in 2017-2018. These collection sites were Shahre-e-Abrisham (two populations), Falavarjan, Ruran, Hasseh, IUT, Karchegan, Kondelan and Mourche Khort (Table 1). Larvae of different developmental stages were obtained from various parts of plants, including leaves, stems and fruits. They were transferred to the laboratory and kept until the emergence of the adults. Then, adults were released in cages (50×50×80 cm) containing tomato plants and colony of

Table 1. The geographical location of the sampling sites of *T. absoluta* populations in Isfahan Province.

Region	Location
Shahre-e-Abrisham	32°33′18″N 51°34′23″E
Falavarjan	32°33′19″N 51°30′35″E
Karchegan	32°24′37″N 51°08′09″E
Ruran	32°28′32″N 51°53′38″E
Hasseh	32°42′24″N 51°45′20″E
Mourche Khort	33°05′24″N 51°28′44″E
Kondelan	32°24′00″N 52°01′00″E
IUT	32°42′01″N 51°31′16″E

each population was kept in the greenhouse conditions at 25±2°C, 65±5% RH, and a photoperiod of approximately 16:8 (L: D) hour without any exposure to insecticides (Yalcin *et al*., 2015). Also, adults were fed with 10% sucrose solution. Second instar larvae were used in the bioassays. Developmentally synchronous larvae of tomato leaf miner were selected by isolating about 40 adult moths from each colony and transferring them to transparent plastic containers (25 cm high× 15 cm diameter) containing the tomato leaves. To keep the leaves fresh, a piece of moistened cotton pad was placed in the base of leaf petioles (Niedmann and Meza-Basso, 2006). Adults were allowed to oviposit on insect-free tomato leaves for 24–48 hours. These leaves were incubated until emergence of the second instar larvae, which was determined by the width of head capsule and body size (Yalcin *et al*., 2015).

Abamectin Bioassays

A leaf-dip bioassay method was used to evaluate resistance to abamectin (Vertimec® 1.8% EC, Syngenta, Switzerland) (Reyes *et al*., 2012). There were three replicates in each concentration and 4 to 5 insecticide concentrations with mortality between 10-90% (Halliday and Burnham, 1990). Tomato leaves were individually dipped in fresh solutions of the insecticide for 10 s, so that the entire surface was equally covered. Distilled water was used for the control. The leaves were air dried for 1 hour. Also, a piece of moistened cotton pad was put near leaf petioles to prevent leaf wilting (Niedmann and Meza-Basso, 2006). After the surface of the leaves became completely dry, insecticide-treated tomato leaves were placed in plastic boxes (9×6×6 cm) that had a hole covered (3×3 cm) with organdy cloth in the cap. Each treatment was replicated 5 times and in each replicate 10 second-instar larvae of tomato leaf miner were used and maintained in the same conditions as for rearing. Larval mortality was assessed 48h after treatment with abamectin.

Synergism Bioassays

After determining the resistance levels, synergism assays were performed to detect the involvement of detoxifying enzymes in resistance to abamectin. DEM, TPP and PBO synergists, inhibitors of GSTs, ESTs and CYP450s, respectively, were used for synergism assays. Each synergist was dissolved in acetone and then diluted in distilled water to the desired concentrations and used at the highest sublethal dose with less than 10% mortality, based on the preliminary tests. Final concentrations were 200 ppm for PBO and DEM and 1,000 ppm for TPP. The synergism assays were performed similar to toxicity bioassays. However, the larvae first exposed to leaves had been treated with each synergist for 4 h and then transferred to leaves treated with different concentrations of the insecticide. After 48 hours, the larval mortality rate was monitored and recorded.

Biochemical Assays

To determine the activities of GSTs, ESTs, and CYP450s, the second instar larvae were used. The Bradford method was used for the samples protein quantification with Bovine Serum Albumin (BSA) as standard (Bradford, 1976).

GST Activity

To measure the activity of GSTs, three larvae were homogenized on ice in 500 µL sodium phosphate buffer (0.2 M, pH 7) and then centrifuged at 12,000×g for 15 minutes at 4°C. The supernatant was used as the enzyme source. The GST activity was determined according to Habig *et al*. (1974). 1-Chloro-2, 4-Dinitrobenzene (CDNB) was used as a substrate in the presence of

reduced Glutathione (GSH). The total reaction volume was 430 µL, consisting of 200 µL CDNB, 200 µL GSH, 30 µL supernatant and absorbance was read at 340 nm every 30 s for 5 min by spectrophotometer (UNICO, Dayton, USA). GST activity was calculated using the extinction coefficient of 9.6 mM cm^{-1} and the results were given as nmol of CDNB conjugated mg $protein^{-1}$ min^{-1}.

EST Activity

Three larvae were homogenized on ice in 500 µL sodium phosphate buffer 0.1 M (pH 7.5) containing 0.1% (w/v) Triton X-100. Homogenized solution was centrifuged at 10,000×g for 15 minutes at 4°C. The supernatant was separated and used to measure the activity of ESTs based on the Grant *et al*. (1989) with slight modifications. Substrate solution included 15 mg of Fast Blue RR salt solved in 25 mL of 0.2 M sodium phosphate buffer (pH 6.0) and 250 µL of 100 mM^{-1} α-Naphthyl Acetate (α-NA) in acetone. Final reaction volume was 500 µL consisted of 50 µL of 0.2 M sodium phosphate buffer, pH 6.0, 400 µL of substrate solution and 50 µL enzymatic extract. Change in absorbance was measured continuously at 450 nm every 30 seconds for 5 minutes by spectrophotometer (UNICO, Dayton, USA). EST activity was expressed as nmol α-naphthol mg $protein^{-1}$ min^{-1}.

CYP450 Activity

To determine CYP450 activities, three larvae were homogenized on ice in 500 µL of potassium phosphate buffer (0.1 M, pH 7) and centrifuged at 10,000×g for 20 minutes at 4°C. The supernatant was used as the enzyme source. CYP450 activity was assayed based on the method of Brogdon *et al.* (1997) and using 3, 3', 5, 5'-Tetramethylbenzidine (TMBZ) as substrate. Reactions were carried out by mixing 400 µL TMBZ, 50 µL hydrogen peroxide 3%, 160 µL potassium phosphate buffer (0.625M, pH 7.2) and 40 µL enzymatic extract. The mixture was incubated in the dark at room temperature for 2 h. Finally, an endpoint absorbance reading was performed at 450 nm by spectrophotometer (UNICO, Dayton, USA) and the activity was reported as unit mg $protein^{-1}$.

Data Analysis

The LC_{50} and LC_{95} values and their 95% Fiducial Limits (FL) were calculated using the Polo-Plus 2.0 program (Software, 2002). The Resistance Ratios (RR) were calculated using LC_{50} values of the resistant populations to LC_{50} of the reference population. Synergism Ratios (SR) were estimated by dividing the LC_{50} values of abamectin alone to LC_{50} values of abamectin+synergists (Robertson *et al*., 2017). Tukey's test (HSD) $P< 0.05$ was used to compare means by SAS program (SAS Institute, 2015).

RESULTS

Abamectin Resistance Levels

The susceptibility of nine populations of tomato leaf miner collected from Isfahan Province was evaluated against abamectin and the results of bioassay are presented in Table 2. The lowest LC_{50} value was observed in the population of IUT and its value was 5.67 mg ai L^{-1} (considered as the reference population). LC_{50} values in other populations were estimated between 10.92-143.18 mg ai L^{-1}, in which the population of the Shahre-e-Abrisham 1 with LC_{50} value of 143.18 mg ai L^{-1} and the resistance ratio of 25.25-fold was recognized as the most resistant population. After that, Falavarjan, Karchegan and Ruran populations were 20.11, 19.63 and 16.66-times more resistant to abamectin than the reference population of IUT, respectively.

Table 2. Log-dose probit-mortality data for the insecticide abamectin against second instar larvae of different *T. absoluta* populations.

Population	N[a]	LC_{50}[b]	FL 95%[c]	RR (FL 95%)[d]	LC_{95}	Slope (±SE)[e]	$\chi 2$ (df)[f]
IUT	250	5.67	3.91-7.42	-	55.64	1.65 ± 0.3	0.22 (3)
Shahre-e-Abrisham 2	260	10.92	8.65-13.40	1.92 (1.33-2.78)	62.65	2.16 ± 0.31	0.15 (3)
Kondelan	250	13.77	10.47-17.34	2.42 (1.65-3.57)	104.22	1.87 ± 0.3	1.77 (3)
Mourche Khort	260	24.27	18.84-29.85	4.28 (2.94-6.22)	140.95	2.15 ± 0.31	0.73 (3)
Hasseh	275	25.52	20.66-30.79	4.50 (3.14-6.45)	141.89	2.20 ± 0.25	2.78 (4)
Ruran	250	94.50	65.20-123.72	16.66 (10.89-25.50)	927.44	1.65 ± 0.3	0.22 (3)
Karchegan	250	111.32	89.38-135.18	19.63 (13.67-28.19)	600.49	2.24 ± 0.33	1.78 (3)
Falavarjan	250	114.04	92.71-137.16	20.11 (14.07-28.73)	481.49	2.63 ± 0.63	1.91 (3)
Shahre-e-Abrisham 1	270	143.18	78.59-266.29	25.25 (17.46-36.52)	1087.23	1.86 ± 0.27	2.08 (3)

[a] Total number of insects bioassayed; [b] Milligrams of active ingredient per liter water (mg ai L^{-1}); [c] 95% Fiducial Limits; [d] RR (Resistance Ratio)= LC_{50} of resistant populations/LC_{50} of reference population; [e] Standard Error, [f] Chi-square and degree of freedom.

Significant variations were observed in the slopes of the regression lines, which ranged from 1.65 to 2.63. Due to this significant variation, the order of resistance of the populations based on the LC_{50} values was different from that based on the LC_{95} values.

Synergism Bioassays

In order to determine the role of enzymatic detoxification in the abamectin resistance, synergists DEM, TPP, and PBO were used and the results are summarized in Table 3. In the bioassays using DEM, the population of Shahre-e-Abrisham 1 showed the highest synergistic ratio and LC_{50} decreased from 143.18 to 24.42 mg ai L^{-1} (SR= 5.86). After that, the synergistic ratio in the populations of Karchegan, Kondelan, IUT and Falavarjan populations was 3.71, 3.18, 2.74 and 2.66-folds, respectively. EST inhibitor TPP decreased the resistance levels of abamectin 3.73, 3.43, 2.77 and 2.65-fold in Shahre-e-Abrisham 1, Ruran, Kondelan and Hasseh populations, respectively. PBO also affected abamectin sensitivity with synergistic ratios of 2.91 and 2.31-fold in Shahre-e-Abrisham 1 and Ruran populations, respectively. The synergism assays suggest that GSTs and ESTs were the main enzymes involved in abamectin resistance in the studied populations.

Detoxification Enzyme Activities

In vitro GST activity assays revealed a significant difference between the GST activities in the population of Karchegan with other populations. This activity was 5.49, 2.29 and 2.11-fold greater in Karchegan, Shahre-e-Abrisham 1 and Kondelan populations compared to the reference IUT population, respectively (Table 4). There were also significant increases in the activity of ESTs, 4-fold higher in Hasseh, Ruran and Shahre-e-Abrisham 1 populations, compared to the reference population (Table 4). A 1.08-fold higher, but not significant, CYP450 activity was observed in Hasseh population compared with IUT reference population (Table 4).

DISCUSSION

So far, at least 20 invertebrate species have developed high levels of resistance to abamectin throughout the world. Of these, *P. xylostella* and *T. urticae* are among the most resistant species to abamectin in terms of reported cases, with 71 and 53 resistance cases, respectively (APRD, 2019). In *P.*

Table 3. The effect of DEM, TPP and PBO synergists on the toxicity of abamectin in *T. absoluta* populations.

Population	N[a]	LC_{50} (FL 95%)[b]	Slope (±SE)[c]	$\chi 2$ (df)[d]	SR (FL 95%)[e]
IUT	250	5.67 (3.91-7.42)	1.65 ± 0.30	0.22 (3)	-
+ DEM	265	2.06 (1.002-2.98)	1.40 ± 0.29	0.52 (3)	2.74 (1.56-4.38)
+ TPP	260	2.70 (1.40-3.84)	1.30 ± 0.28	0.11 (3)	2.09 (1.22-3.57)
+ PBO	265	3.08 (2.01-4.07)	1.45 ± 0.26	0.54 (3)	1.83 (1.17-2.85)
Shahre-e-Abrisham 2	260	10.92 (8.65-13.40)	2.16 ± 0.31	0.15 (3)	-
+ DEM	245	4.22 (1.96-6.17)	1.35 ± 0.30	0.33 (3)	2.58 (1.50-4.43)
+ TPP	250	5.68 (3.11-7.97)	1.32 ± 0.28	0.44 (3)	1.92 (1.20-3.06)
+ PBO	245	8.45 (5.54-11.60)	1.31 ± 0.28	0.58 (3)	1.29 (0.86-1.92)
Kondelan	250	13.77 (10.47-17.34)	1.87 ± 0.30	1.77 (3)	-
+ DEM	252	4.33 (2.35-6.04)	1.58 ± 0.31	0.43 (3)	3.18 (1.95-5.18)
+ TPP	275	4.96 (2.60-7.03)	1.35 ± 0.28	0.27 (3)	2.77 (1.67-4.58)
+ PBO	250	7.15 (4.53-9.67)	1.43 ± 0.29	0.67 (3)	1.92 (1.26-2.93)
Mourche Khort	260	24.27 (18.84-29.85)	2.15 ± 0.31	0.73 (3)	-
+ DEM	260	9.35 (4.47-13.57)	1.51 ± 0.31	0.68 (3)	2.59 (1.51-4.44)
+ TPP	260	13.96 (7.36-19.79)	1.28 ± 0.28	0.89 (3)	1.73 (1.06-2.83)
+ PBO	260	18.52 (11.11-25.81)	1.30 ± 0.29	0.37 (3)	1.31 (0.84-2.03)
Hasseh	275	25.25 (20.66-30.79)	2.20 ± 0.25	2.78 (4)	-
+ DEM	250	15.77 (12.24-19.24)	2.27 ± 0.37	0.78 (3)	1.61 (1.20-2.17)
+ TPP	260	9.62 (4.85-13.76)	1.45 ± 0.29	0.31 (3)	2.65 (1.60-4.39)
+ PBO	270	16.54 (10.26-22.42)	1.41 ± 0.28	1.22 (3)	1.54 (1.02-2.32)
Ruran	250	94.50 (65.20-123.72)	1.65 ± 0.30	0.22 (3)	-
+ DEM	253	37.23 (24.63-48.83)	1.57 ± 0.27	0.11 (3)	2.53 (1.63-3.93)
+ TPP	260	27.50 (15.24-38.17)	1.57 ± 0.30	0.20 (3)	3.43 (2.05-5.73)
+ PBO	255	40.79 (25.18-55.27)	1.44 ± 0.28	0.37 (3)	2.31 (1.45-3.70)
Karchegan	250	111.32 (89.38-135.18)	2.24 ± 0.33	1.78 (3)	-
+ DEM	253	29.94 (19.27-39.41)	1.74 ± 0.29	0.33 (3)	3.71 (2.51-5.49)
+ TPP	253	46.97 (35.05-58.77)	2.01 ± 0.31	0.07 (3)	2.37 (1.72-3.26)
+ PBO	255	54.56 (39.37-70.60)	1.66 ± 0.29	0.55 (3)	2.04 (1.44-2.87)
Falavarjan	250	114.04 (92.71-137.16)	2.63 ± 0.63	1.91 (3)	-
+ DEM	270	42.84 (32.40-52.95)	2.11 ± 0.31	0.25 (3)	2.66 (1.96-3.61)
+ TPP	277	45.72 (33.27-57.97)	1.77 ± 0.28	0.48 (3)	2.49 (1.79-3.46)
+ PBO	270	54.22 (41.22-67.89)	1.88 ± 0.29	0.23 (3)	2.10 (1.54-2.86)
Shahre-e-Abrisham 1	270	143.18 (78.59-266.29)	1.86 ± 0.27	2.08 (3)	-
+ DEM	262	24.42 (19.40-30.04)	2.18 ± 0.32	0.46 (3)	5.86 (4.33-7.92)
+ TPP	265	38.30 (26.25-50.35)	1.54 ± 0.29	0.38 (3)	3.73 (2.57-5.42)
+ PBO	275	49.16 (38.70-65.55)	1.80 ± 0.30	1.19 (3)	2.91 (2.09-4.05)

[a] Total number of insects bioassayed, [b] Milligrams of active ingredient per liter water (mg ai L^{-1}) and 95% Fiducial Limits; [c] Standard Error; [d] Chi-square and degree of freedom, [e] SR (Synergism Ratio)= LC_{50} Abamectin alone/LC_{50} abamectin+synergists.

Table 4. Mean activity of detoxification enzymes in different populations of *T. absoluta*.

	GSTs		ESTs		CYP450s	
Population	CDNB[a]	Ratio[b]	α-NA[c]	Ratio	TMBZ[d]	Ratio
IUT	430.8 ± 46.09 bcd	-	176.7 ± 17.98 d	-	43.4 ± 6.30 ab	-
Shahre-e-Abrisham 2	329.04 ± 112.97 d	0.76	279.2 ± 25.59 cd	1.58	16.9 ± 1.60 c	0.38
Kondelan	911.7 ± 32.81 bc	2.11	273.2 ± 113.33 cd	1.54	24.0 ± 2.99 bc	0.55
Mourche Khort	449.6 ± 56.19 bcd	1.04	334.1 ± 10.64 cd	1.89	16.1 ± 2.16 b	0.37
Hasseh	328.2 ± 66.95 d	0.76	917.4 ± 48.30 a	5.19	47.3 ± 8.70 a	1.08
Ruran	400.8 ± 83.91 cd	0.93	759.2 ± 77.94 ab	4.29	38.3 ± 1.49 ab	0.88
Karchegan	2367.2 ± 281.5 a	5.49	515.9 ± 12.10 bc	2.91	18.3 ± 1.38 c	0.42
Falavarjan	443.5 ± 33.52 bcd	1.02	477.6 ± 46.14 c	2.50	7.5 ± 0.91 c	0.17
Shahre-e-Abrisham 1	987.0 ± 111.98 b	2.29	776.0 ± 11.10 a	4.3	25.7 ± 2.28 bc	0.59

[a] nmol mg $protein^{-1}$ min^{-1} (CDNB= 1-Chloro-2,4-Dinitrobenzene); [b] Enzyme activity resistant populations/Enzyme activity reference IUT population; [c] nmol mg $protein^{-1}$ min^{-1} (α-NA= α-Naphthyl Acetate), [d] U mg $protein^{-1}$ (3,3', 5,5'-Tetramethylbenzidine).

xylostella and *T. urticae*, the highest reported resistance ratios against abamectin are 23670 and 8272-folds, respectively (Pu *et al*., 2010; Monteiro *et al*., 2015). Our study showed a decrease in susceptibility to abamectin in all tested tomato leaf miner populations, considering the lowest estimated LC_{95} value was 55.64 mg ai L^{-1}, while the recommended dose of abamectin is 0.5 mL L^{-1} (9 mg ai L^{-1}). The populations of *T. absoluta* collected from Isfahan Province were 1.92- to 25.25-times resistant to abamectin compared to the IUT reference population. The difference in resistance levels is probably due to differences in the use of abamectin and other pesticides such as indixacarb and pyrethroid insecticides in areas where *T. absoluta* populations were collected. In addition, genetic diversity among tomato leaf miner populations may affect the value of resistance (Kerns and Gaylor, 1992). *T. absoluta* populations from Brazil were 5.2 to 9.4-folds (Siqueira *et al*., 2001), while tomato leaf miner populations from Turkey exhibited low levels of resistance to abamectin (2.3-3.03 folds) (Konuş, 2014).

Increased activity of detoxification enzymes (GSTs, ESTs and CYP450s) is one of the common mechanisms of resistance to insecticides (Li *et al*., 2007). Synergistic studies confirmed that GSTs, ESTs and CYP450s were associated with cross-resistance between abamectin and tebufenozide in *P. xylostella* (Qian *et al*., 2008). Synergism data presented in Table 3 shows that GSTs and ESTs have a more important role in detoxification of abamectin than the CYP450s. The inhibition of GST activity by the synergist DEM increased abamectin toxicity in the populations of Shahre-e-Abrisham 1, Karchegan and Kondelan and there was a significant difference in the synergistic ratio of these three populations with that of IUT reference population. Furthermore, *in vitro* assay of GST activity demonstrated an increased activity of this enzyme in abamectin resistant populations of Shahre-e-Abrisham 1, Karchegan and Kondelan. It has been previously shown that in *B. tabaci* and *T. urticae*, GSTs and CYP450s are involved in resistance to abamectin (Stumpf and Nauen, 2002; Wang and Wu, 2007). It has been also reported that PBO and DEM increased 3.9 and 1.4-times abamectin toxicity in NJ-Abm strain of *B. tabaci*, respectively (Wang and Wu, 2007). Also, involvement of GSTs in conferring resistance to abamectin in *T. cinnabarinus* and *L. sativae* has been suggested (Lin *et al*., 2009; Wei *et al*., 2015).

After inhibiting the activity of ESTs by TPP synergist, abamectin toxicity increased in Shahre-e-Abrisham 1, Ruran, Kondelan and Hasseh populations. Besides, there was a significant difference between the mean of EST activity in Hasseh, Shahre-e-Abrisham 1 and Ruran populations compared with IUT reference population. However, the study of Wang and Wu (2007) on *B. tabaci* did not confirm the involvement of this enzyme system in abamectin resistance. Enhanced esterase activity has been suggested in abamectin resistance in other pests such as *T. urticae* and Colorado potato beetle. However, it is unknown if the esterases linked to abamectin resistance function as sequestration proteins or if they hydrolyze abamectin (Argentine *et al*., 1992; Kwon *et al*., 2010a). Lin *et al* (2009) study on the resistance mechanism of *T. cinnabarinus* showed that the activity of ESTs in resistant strain was 2.7 times higher than that of susceptible strain and *T. cinnabarinus* resistance to abamectin was correlated with the change in carboxylesterase activity. Siqueira *et al*. (2001) showed that, in *T. absoluta* populations of Brazil, increased EST activity is the main cause of resistance to abamectin. However, ESTs did not play a significant role in the resistance to abamectin in *T. absoluta* populations from Turkey, while CYP450s could make a major contribution to the resistance development (Konuş, 2014). Inhibition of CYP450 activity by PBO (Table 3) did not significantly increase abamectin toxicity, compared to DEM and TPP. The CYP450 genes have been reported to be involved in

the abamectin resistance in *P. xylostella* and *Aphis citricidus* populations (Pu *et al*., 2010; Jing *et al*., 2018). Moreover, increased expression of CYP genes by abamectin has been documented in adults of *Bombyx mori* (Xuan *et al*., 2015). Using *T. urticae* resistant to abamectin, Riga *et al.* (2014) demonstrated that CYP392A16 catalyzes hydroxylation of abamectin that results in a less-toxic compound. Also, CYP450 was reported to play a main role in the cross-resistance between tebufenozide and abamectin in *P. xylostella* (Yin *et al*., 2019).

In addition to metabolic mechanisms, target site resistance has also been reported in abamectin resistant arthropod populations (Liu *et al*., 2014; Ilias *et al*., 2017). Several mutations in the arthropod GluCls were proposed to be associated with target site resistance to abamectin, including: A309V in *P. xylostella*, G323D (GluCl1) and G326E (GluCl3) in *T. urticae* (Kwon *et al*., 2010b; Dermauw *et al*., 2012; Wang *et al*., 2016). The A309V mutation in PxGluCl resulted in 11000-fold resistance to abamectin in Roth-Abm strain of *P. xylostella* and its frequency in this strain was 94.7% (Wang *et al*., 2016). Resistance to abamectin is often mediated by a multigenic system in a number of pests (Clark *et al*., 1995; Pu *et al*., 2010; Dermauw *et al*., 2012; Zhang *et al*., 2016). Genetic analysis showed that resistance to abamectin is associated with significant adaptation costs, and these costs were autosomal and dominant. Therefore, abamectin rotation with other insecticides without cross-resistance could be considered as an effective insecticide resistance management (Wang and Wu, 2014).

In conclusion, our study demonstrated the widespread resistance to abamectin in populations of *T. absoluta* collected from Isfahan Province. Synergistic and biochemical studies showed higher synergistic ratios of DEM and TPP than PBO and higher GST and esterase activities compared to the monooxygenases, which might suggest glutathione-S-transferases and esterases have a more important role in resistance to abamectin than cytochrome P450 monooxygenases. However, future molecular studies are required to determine the exact mechanisms of resistance to abamectin in *T. absoluta*. An in-depth knowledge of molecular mechanisms of abamectin resistance is important in early resistance detection and designing strategies to prevent or delay resistance development. Thus, further studies using molecular methods such as *in vitro* expression systems, genome wide sequencing, and silencing candidate genes are necessary to clarify the involved resistance mechanisms in this species.

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ارزیابی مقاومت جمعیت‌های مینوز گوجه فرنگی *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)، در برابر حشره‌کش آبامکتین در استان اصفهان، ایران

م. عزیزی و ج. خواجه‌علی

چکیده

مینوز برگ گوجه فرنگی، *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)، یکی از آفات مهم گوجه فرنگی در سراسر دنیا به شمار می‌آید. در این مطالعه، مقاومت جمعیت‌های مختلف مینوز برگ گوجه فرنگی جمع‌آوری شده از استان اصفهان در برابر حشره‌کش آبامکتین ارزیابی گردید. با انجام آزمون‌های زیست‌سنجی به روش غوطه‌وری، دوز کشنده میانگین(LC_{50}) جمعیت‌های مختلف اندازه‌گیری شد. میزان LC_{50} آبامکتین در جمعیت مرجع دانشگاه صنعتی اصفهان 5/67 میلی‌گرم ماده موثر بر لیتر (mg a.i/L) تخمین زده شد، درحالی‌که جمعیت شهر ابریشم1 با LC_{50} برابر (mg a.i/L) 143/18 در مقایسه با جمعیت مرجع 25 برابر مقاوم بود. پیش‌تیمار جمعیت-های مختلف با سینرژیست DEM (مهارکننده آنزیم‌های گلوتاتیون اس ترانسفراز) سمیت آبامکتین را به‌طور قابل توجهی افزایش داد. هم‌چنین تفاوت معنی‌داری بین میانگین فعالیت گلوتاتیون‌اس‌ترانسفرازها در جمعیت‌های مقاوم با جمعیت مرجع مشاهده شد. سینرژیست TPP (مهارکننده استرازها) سمیت آبامکتین را 1/73 تا 3/7 برابر افزایش داد. میزان فعالیت استرازها در این جمعیت‌ها نیز با جمعیت مرجع تفاوت معنی‌داری نشان داد. علاوه‌براین مهار فعالیت آنزیم‌های سیتوکروم P450 مونواکسیژناز توسط سینرژیست PBO سمیت آبامکتین را از 1/3 به 2/9 برابر افزایش داد. بیشترین نسبت سینرژیستی برای DEM (5/86)، TPP (3/73) و PBO (2/91 برابر) در جمعیت شهر ابریشم1 مشاهده شد. به‌نظر می‌رسد که گلوتاتیون اس ترانسفرازها و استرازها نقش مهم‌تری را در بروز مقاومت به آبامکتین در جمعیت‌های مورد آزمایش ایفا می‌کنند. مقاومت بالای جمعیت‌های جمع‌آوری شده از استان اصفهان به حشره‌کش آبامکتین نشان می‌دهد که لازم است برنامه‌های مدیریت مقاومت به حشره‌کش‌ها بر پایه ارزیابی زودهنگام مقاومت و تناوب در استفاده از حشره‌کش‌های متفاوت در کنترل مینوز برگ گوجه فرنگی بکار گرفته شود.