

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

M. Azizi¹ and J. Khajehali^{1*}

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 (LC₅₀) of different populations were estimated by bioassays using a leaf-dip method. The LC₅₀ 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 LC₅₀ 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 LC₅₀ value of abamectin 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.

INTRODUCTION

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

¹ Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Islamic Republic of Iran.

*Corresponding author; e-mail: khajehali@cc.iut.ac.ir



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 indoxacarb (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; Zibae *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 (GluCl_s) (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 $25\pm 2^\circ\text{C}$, $65\pm 5\%$ 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 \times 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 \times 6 \times 6 cm) that had a hole covered (3 \times 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 \times g for 15 minutes at 4 $^\circ\text{C}$. 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 $\text{mg protein}^{-1} \text{ min}^{-1}$.

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 $10,000\times g$ 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 $\text{mg protein}^{-1} \text{ min}^{-1}$.

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 $10,000\times g$ 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 $5.67 \text{ mg ai L}^{-1}$ (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 $143.18 \text{ mg ai L}^{-1}$ 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 | χ 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)= LC₅₀ of resistant populations/LC₅₀ 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 LC₅₀ values was different from that based on the LC₉₅ 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 LC₅₀ 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 | χ 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)= LC₅₀ Abamectin alone/LC₅₀ abamectin+synergists.

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

| Population | GSTs | | ESTs | | CYP450s | |
|---------------------|-------------------|--------------------|-------------------|-------|-------------------|-------|
| | 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 LC₉₅ 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) (Konus, 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 (Konus, 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 GluCl1 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 PxGluCl1 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.

REFERENCES

1. APRD. 2019. Arthropod Pesticide Resistance Database. www.pesticideresistance.org.
2. Argentine, J. A., Clark, J. M. and Lin, H. 1992. Genetics and Biochemical Mechanisms of abamectin Resistance in Two Isogenic Strains of Colorado Potato Beetle. *Pestic. Biochem. Physiol.*, **44**: 191–207.
3. Aynalem, B. 2018. Tomato Leaf Miner [*Tuta absoluta* Meyrick] (Lepidoptera: Gelechiidae) and Its current Ecofriendly Management Strategies: A Review. *J. Agric. Biotech. Sustain. Dev.*, **10**(2): 11-24.
4. Baniameri, V. and Cheraghian, A. 2012. The First Report and Control Strategies of *Tuta absoluta* in Iran. *EPPO Bull.*, **42**(2): 322-324.
5. Biondi, A., Guedes, R. N. C., Wan, F. H. and Desneux, N. 2018. Ecology, Worldwide Spread, and Management of the Invasive South American Tomato Pinworm, *Tuta absoluta*: Past, Present, and Future. *Annu. Rev. Entomol.*, **63**: 239-258.
6. Bradford, M. M. 1976. A rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.*, **72**(1-2): 248-254.
7. Brogdon, W. G., McCallister, J. C. and Vulule, J. 1997. Heme Peroxidase Activity Measured in Single Mosquitoes Identifies Individuals Expressing an Elevated Oxidase

- for Insecticide Resistance. *J. Am. Mosq. Control Assoc. Assoc.*, **13(3)**: 233-237.
8. Çağatay, N. S., Menault, P., Riga, M., Vontas, J. and Ay, R. 2018. Identification and Characterization of Abamectin Resistance in *Tetranychus urticae* Koch Populations from Greenhouses in Turkey. *Crop Prot.*, **112**: 112-117.
 9. Campos, M. R., Silva, T. B., Silva, W. M., Silva, J. E. and Siqueira, H. A. A. 2015. Spinosyn Resistance in the Tomato Borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *J. Pest Sci.*, **88**: 405-412.
 10. Casida, J. E. and Durkin, K. A. 2013. Neuroactive Insecticides: Targets, Selectivity, Resistance, and Secondary Effects. *Annu. Rev. Entomol.*, **58**: 97-117.
 11. Che, W., Huang, J., Guan, F., Wu, Y. and Yang, Y. 2015. Cross-Resistance and Inheritance of Resistance to Emamectin Benzoate in *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, **108(4)**: 2015-2020.
 12. Chen, X., Yuan, L., Du, Y., Zhang, Y. and Wang J. 2011. Cross-Resistance and Biochemical Mechanisms of Abamectin Resistance in the Western Flower Thrips, *Frankliniella occidentalis*. *Pestic. Biochem. Physiol.*, **101(1)**: 34-38.
 13. Clark, J. M., Scott, J. G., Campos F. and Bloomquist J. R. 1995. Resistance to Avermectins: Extent, Mechanisms, and Management Implications. *Annu. Rev. Entomol.*, **40(1)**: 1-30.
 14. Dermauw, W., Ilias, A., Riga, M., Tsagkarakou, A., Grbić, M., Tirry, L., Van Leeuwen, T. and Vontas, J. 2012. The Cys-Loop Ligand-Gated Ion Channel Gene Family of *Tetranychus urticae*: Implications for Acaricide Toxicology and a Novel Mutation Associated with Abamectin Resistance. *Insect Biochem. Mol. Biol.*, **42(7)**: 455-465.
 15. Desneux, N., Wajnberg, E., Wyckhuys, K. A. G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Catalán Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T. and Urbaneja, A. 2010. Biological Invasion of European Tomato Crops by *Tuta absoluta*: Ecology, History of Invasion and Prospects for Biological Control. *J. Pest Sci.*, **83(3)**: 197-215.
 16. Desneux, N., Luna, M. G., Guillemaud, T. and Urbaneja, A. 2011. The Invasive South American Tomato Pinworm, *Tuta absoluta*, Continues to Spread in Afro-Eurasia and Beyond: The New Threat to Tomato World Production. *J. Pest Sci.*, **84(4)**: 403-408.
 17. Dybas, R. A. 1989. Abamectin Use in Crop Protection. In: "Ivermectin and Abamectin", (Ed.): Campbell, W. C. Springer Verlag, New York, PP. 287-310.
 18. Ferguson J. S. 2004. Development and Stability of Insecticide Resistance in the Leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to Cyromazine, Abamectin, and Spinosad. *J. Econ. Entomol.*, **97(1)**: 112-119.
 19. Grant, D. F., Bender, D. M. and Hammock, B. D. 1989. Quantitative Kinetic Assays for Glutathione S-Transferase and General Esterase in Individual Mosquitoes Using an EIA Reader. *Insect Biochem.*, **19(8)**: 741-751.
 20. Guedes, R. N. C., Roditakis, E., Campos, M. R., Haddi, K., Bielza, P., Siqueira, H. A. A., Tsagkarakou, A., Vontas, J. and Nauen, R. 2019. Insecticide Resistance in the Tomato Pinworm *Tuta absoluta*: Patterns, Spread, Mechanisms, Management and Outlook. *J. Pest Sci.*, **92(4)**: 1329-1342. 9
 21. Guedes R. N. C. and Siqueira, H. A. A. 2013. The Tomato Borer *Tuta absoluta*: Insecticide Resistance and Control Failure. *CAB Rev.*, **7**: 1-7.
 22. Habig, W. H., Pabst, M. J. and Jakoby, W. B. 1974. Glutathione S-Transferases the First Enzymatic Step in Mercapturic Acid Formation. *J. Biol. Chem.*, **249(22)**: 7130-7139.
 23. Haddi, K., Berger, M., Bielza, P., Rapisarda, C., Williamson, M. S., Moores, G. and Bass, C. 2017. Mutation in the *ace-1* Gene of the Tomato Leaf Miner (*Tuta absoluta*) Associated with Organophosphates Resistance. *J. Appl. Entomol.*, **141(8)**: 612-619.
 24. Halliday, W. R. and Burnham, K. P. 1990. Choosing the Optimal Diagnostic Dose for Monitoring Insecticide Resistance. *J. Econ. Entomol.*, **83**: 1151-1990.
 25. Huang, J. and Casida, J. E. 1997. Avermectin B1a Binds to High- and Low-Affinity Sites with Dual Effects on the γ -Aminobutyric Acid-Gated Chloride Channel of Cultured Cerebellar Granule Neurons. *J. Pharmacol. Exp. Ther.*, **281(1)**: 261-266.
 26. Ilias, A., Vassiliou, V. A., Vontas, J. and Tsagkarakou, A. 2017. Molecular



- Diagnostics for Detecting Pyrethroid and Abamectin Resistance Mutations in *Tetranychus urticae*. *Pestic. Biochem. Physiol.*, **135**: 9-14.
27. Jing, T. X., Tan, Y., Ding, B. Y., Dou, W., Wei, D. D. and Wang, J. J. 2018. NADPH-Cytochrome P450 Reductase Mediates the Resistance of *Aphis (Toxoptera) citricidus* (Kirkaldy) to Abamectin. *Front. Physiol.*, **10**: 9-986.
28. Kerns, D. L. and Gaylor, M. J. 1992. Insecticide Resistance in Field Populations of the Cotton Aphid (Homoptera: Aphididae). *J. Econ. Entomol.*, **85**(1): 1-8.
29. Konuş, M. 2014. Analysing Resistance of Different *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Strains to Abamectin Insecticide. *Turk. J. Biochem.*, **39**(3): 291-297.
30. Kwon, D. H., Seong, G. M., Kang, T. J. and Lee, S. H. 2010a. Multiple Resistance Mechanisms to Abamectin in the Two-Spotted Spider Mite. *J. Asia-Pac. Entomol.*, **13**: 229-232.
31. Kwon, D. H., Yoon, K. S., Clark, J. M. and Lee, S. H. 2010b. A Point Mutation in a Glutamate-Gated Chloride Channel Confers Abamectin Resistance in the Two-Spotted Spider Mite, *Tetranychus urticae* Koch. *Insect Mol. Biol.*, **19**(4): 583-591.
32. Lasota, J. A. and Dybas, R. A. 1991. Avermectins, a Novel Class of Compounds: Implications for Use in Arthropod Pest Control. *Annu. Rev. Entomol.*, **36**(1): 91-117.
33. Li, X., Schuler, M. A. and Berenbaum, M. R. 2007. Molecular Mechanisms of Metabolic Resistance to Synthetic and Natural Xenobiotics. *Annu. Rev. Entomol.*, **52**: 231-253.
34. Lietti, M. M. M., Botto, E. and Alzogaray, R. A. 2005. Insecticide Resistance in Argentine Populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop. Entomol.*, **34**(1): 113-119.
35. Lin, H., Chuan-hua, X., Jin-jun, W., Ming, L., Wen-cai, L. and Zhi-mo, Z. 2009. Resistance Selection and Biochemical Mechanism of Resistance to Two Acaricides in *Tetranychus cinnabarinus* (Boidval). *Pestic. Biochem. Physiol.*, **93**(1): 47-52.
36. Liu, F., Shi, X., Liang, Y., Wu, Q., Xu, B., Xie, W., Wang, S., Zhang, Y. and Liu, N. 2014. A 36-bp Deletion in the Alpha Subunit of Glutamate-Gated Chloride Channel Contributes to Abamectin Resistance in *Plutella xylostella*. *Entomol. Exp. Appl.*, **153**(2): 85-92.
37. Meyrick, E. 1917. Descriptions of South American Micro-Lepidoptera. *Trans. Ent. Soc. London.*, **65**(1): 1-52.
38. Monteiro, V. B., Gondim Jr, M. G. C., Oliveira, J. E. M., Siqueira, H. A. A. and Sousa, J. M. 2015. Monitoring *Tetranychus urticae* koch (acari: tetranychidae) Resistance to Abamectin in Vineyards in the Lower Middle São Francisco Valley. *Crop Prot.*, **69**: 90-96.
39. Niedmann, L. and Meza-Basso, L. 2006. Evaluation of Native Strains of *Bacillus thuringiensis* as an Alternative of Integrated Management of the Tomato Leaf Miner (*Tuta absoluta* Meyrick; Lepidoptera: Gelechiidae) in Chile. *Agric. Téc.*, **66**(3): 235-246.
40. Nourbakhsh, S. 2019. List of Important Pests, Diseases and Weeds of Major Agricultural Crops, Pesticides and Recommended Methods for their Control. Ministry of Jihad-e- Agriculture, Plant Protection Organization, Iran.
41. Picanço, M. C., Bacci, L., Crespo, A. L. B., Miranda, M. M. M. and Martins, J. C. 2007. Effect of Integrated Pest Management Practices on Tomato Production and Conservation of Natural Enemies. *Agric. For. Entomol.*, **9**(4): 327-335
42. Pu, X., Yang, Y., Wu, S. and Wu, Y. 2010. Characterisation of Abamectin Resistance in a Field-Evolved Multiresistant Population of *Plutella xylostella*. *Pest Manag. Sci.*, **66**(4): 371-378.
43. Putter, I., Mac Connell, J. G., Preiser, F. A., Haidri, A., Ristich, S. S. and Dybas, R. A. 1981. Avermectins: Novel Insecticides, Acaricides and Nematicides from a Soil Microorganism. *Experientia*, **37**: 963-964.
44. Qayyum, M. A., Wakil, W., Arif, M. J., Sahi, S. T., Saeed, N. A. and Russell, D. A. 2015. Multiple Resistances against Formulated Organophosphates, Pyrethroids, and Newer-Chemistry Insecticides in Populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan. *J. Econ. Entomol.*, **108**(1): 286-293.
45. Qian, L., Cao, G., Song, J., Yin, Q. and Han, Z. 2008. Biochemical Mechanisms Conferring Cross-Resistance between Tebufenozide and Abamectin in *Plutella*

- xylostella*. *Pestic. Biochem. Physiol.*, **91(3)**: 175-179.
46. Reyes, M., Rocha, K., Alarcón, L., Siegwart, M. and Sauphanor, B. 2012. Metabolic Mechanisms Involved in the Resistance of Field Populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad. *Pestic. Biochem. Physiol.*, **102(1)**: 45-50.
 47. Riga, M., Tsakireli, D., Ilias, A., Morou, E., Myridakis, A., Stephanou, E. G., Nauen, R., Dermauw, W., Van Leeuwen, T., Paine, M. and Vontas, J. 2014. Abamectin Is Metabolized by CYP392A16, a Cytochrome P450 Associated with High Levels of Acaricide Resistance in *Tetranychus urticae* *Insect Biochem. Mol. Biol.*, **46(1)**: 43-53.
 48. Robertson, J. L., Jones, M. M., Olguin, E. and Alberts, B. 2017. *Bioassays with Arthropods*. CRC press, Boca Raton.
 49. Roditakis, E., Mavridis, K., Riga, M., Vasakis, E., Morou, E., Rison, J. L. and Vontas, J. 2017a. Identification and Detection of Indoxacarb Resistance Mutations in the Para Sodium Channel of the Tomato leafminer, *Tuta absoluta*. *Pest Manag. Sci.*, **73(8)**: 1679-1688.
 50. Roditakis, E., Steinbach, D., Moritz, G., Vasakis, E., Stavrakaki, M., Ilias, A., García-Vidal, L., Martínez-Aguirre, M. D. R., Bielza, P., Morou, E., Silva, J. E., Silva, W. M., Siqueira, H. A. A., Iqbal, S., Troczka, B. J., Williamson, M. S., Bass, C., Tsagkarakou, A., Vontas, J. and Nauen, R. 2017b. Ryanodine Receptor Point Mutations Confer Diamide Insecticide Resistance in Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Insect Biochem. Mol. Biol.*, **80**: 11-20.
 51. Roditakis, E., Vasakis, E., García-Vidal, L., Martínez-Aguirre, M. D. R., Rison, J. L., Haxaire-Lutun, M. O., Nauen, R., Tsagkarakou, A. and Bielza, P. 2018. A Four-Year Survey on Insecticide Resistance and Likelihood of Chemical Control Failure for Tomato Leaf Miner *Tuta absoluta* in the European/Asian Region. *J. Pest Sci.*, **91**: 421-435.
 52. Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M. and Bassi, A. 2015. First Report of *Tuta absoluta* Resistance to Diamide Insecticides. *J. Pest Sci.*, **88**: 9-16.
 53. SAS. 2015. *Base SAS 9.4 Procedures Guide*. SAS Institute. 36. Satar, G., Ulusoy,
 54. Saleem, M., Hussain, D., Ghouse, G., Abbas, M. and Fisher, S. W. 2016. Monitoring of Insecticide Resistance in *Spodoptera litura* (Lepidoptera: Noctuidae) from Four Districts of Punjab, Pakistan to Conventional and New Chemistry Insecticides. *Crop Protec.*, **79**: 177-184.
 55. Santos, V. S., De Siqueira, H. A. A., Da Silva, J. E. and De Farias, M. J. D. C. 2011. Insecticide Resistance in Populations of the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), from the state of Pernambuco, Brazil. *Neotrop. Entomol.*, **40(2)**: 264-270.
 56. Silva, G. A., Picanço, M. C., Bacci, L., Crespo, A. L. B., Rosado, J. F. and Guedes, R. N. C. 2011. Control Failure Likelihood and Spatial Dependence of Insecticide Resistance in the Tomato Pinworm, *Tuta absoluta*. *Pest Manag. Sci.*, **67(8)**: 913-920.
 57. Silva, T. B. M., Silva, W. M., Campos, M. R., Silva, J. E., Ribeiro, L. M. S. and Siqueira, H. A. A. 2016a. Susceptibility Levels of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to Minor Classes of Insecticides in Brazil. *Crop Protec.*, **79**: 80-86.
 58. Silva, W. M., Berger, M., Bass, C., Williamson, M., Moura, D. M. N., Ribeiro, L. M. S. and Siqueira, H. A. A. 2016b. Mutation (G275E) of the Nicotinic Acetylcholine Receptor $\alpha 6$ Subunit Is Associated with High Levels of Resistance to Spinosyns in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Pestic. Biochem. Physiol.*, **131**: 1-8.
 59. Siqueira, H. A. A., Guedes, R. N. C., Fragoso, D. B. and Magalhaes, L. C. 2001. Abamectin Resistance and Synergism in Brazilian Populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Int. J. Pest. Manag.*, **47(4)**: 247-251.
 60. Siqueira, H. A. A., Guedes, R. N. C. and Picanco, M. C. 2000. Insecticide Resistance in Populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agric. Forest Entomol.*, **2(2)**: 147-153.
 61. Software, L. 2002. PoloPlus: Probit and Logit Analysis. In: "*LeOra Software*". Berkeley, CA.
 62. Stumpf, N. and Nauen, R. 2002. Biochemical Markers Linked to Abamectin Resistance in *Tetranychus urticae* (Acari: Tetranychidae). *Pestic. Biochem. Physiol.*, **72(2)**: 111-121.



63. Tonnang, H. E. Z., Mohamed, S. F., Khamis, F. and Ekesi, S. 2015. Identification and Risk Assessment for Worldwide Invasion and Spread of *Tuta absoluta* with a Focus on Sub-Saharan Africa: Implications for Phytosanitary Measures and Management. *PLoS One*, **10(8)**: e0135283.
64. Wang, L. and Wu, Y. 2007. Cross-Resistance and Biochemical Mechanisms of Abamectin Resistance in the B-Type *Bemisia tabaci*. *J. Appl. Entomol.*, **131(2)**: 98-103.
65. Wang, R. and Wu, Y. 2014. Dominant Fitness Costs of Abamectin Resistance in *Plutella xylostella*. *Pest Manag. Sci.*, **70(12)**: 1872-1876.
66. Wang, X., Wang, R., Yang, Y., Wu, S., O'Reilly, A. O. and Wu, Y. 2016. A Point Mutation in the Glutamate-Gated Chloride Channel of *Plutella xylostella* Is Associated with Resistance to Abamectin. *Insect Mol. Biol.*, **25(2)**: 116-125.
67. Wang, X., Puinean, A. M., O'Reilly, A. O., Williamson, M. S., Smelt, C. L. C., Millar, N. S. and Wu, Y. 2017. Mutations on M3 Helix of *Plutella xylostella* Glutamate-Gated Chloride Channel Confer Unequal Resistance to Abamectin by Two Different Mechanisms. *Insect Biochem. Mol. Biol.*, **86**: 50-57.
68. Wei, P., Che, W., Wang, J., Xiao, D., Wang, R. and Luo, C. 2018. RNA Interference of Glutamate-Gated Chloride Channel Decreases Abamectin Susceptibility in *Bemisia tabaci*. *Pestic. Biochem. Physiol.*, **145**: 1-7.
69. Wei, Q. B., Lei, Z. R., Nauen, R., Cai, D. C. and Gao, Y. L. 2015. Abamectin Resistance in Strains of Vegetable Leafminer, *Liriomyza sativae* (Diptera: Agromyzidae) Is Linked to Elevated Glutathione S-Transferase Activity. *Insect Sci.*, **22(2)**: 243-250.
70. Wolstenholme, A. J. and Rogers, A. T. 2005. Glutamate-Gated Chloride Channels and the Mode of Action of the Avermectin/Milbemycin Anthelmintics. *Parasitology*, **131**: S85-S95.
71. Xuan, N., Guo, X., Xie, H. Y., Lou, Q. N., Lu X. B., Liu G. X. and Picimbon, J. F. 2015. Increased Expression of *CSP* and *CYP* Genes in Adult Silkworm Females Exposed to Avermectins. *Insect Sci.*, **22(2)**: 203-219.
72. Yalcin, M., Mermer, S., Kozaci, L. D. and Turgut, C. 2015. Insecticide Resistance in Two Populations of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) from Turkey. *Turk. Entomol. Derg.*, **39(2)**: 137-145.
73. Yin, Q., Qian, L., Song, P., Jian, T. and Han, Z. 2019. Molecular Mechanisms Conferring Asymmetrical Cross-Resistance between Tebufenozide and Abamectin in *Plutella xylostella*. *J. Asia Pac. Entomol.*, **22(1)**: 189-193.
74. Zhang, S., Zhang, X., Shen J., Mao, K., You, H. and Li, J. 2016. Susceptibility of Field Populations of the Diamondback Moth, *Plutella xylostella*, to a Selection of Insecticides in Central China. *Pestic. Biochem. Physiol.*, **132**: 38-46.
75. Zibae, I., Mahmood, K., Esmaily, M., Bandani, A. R. and Kristensen, M. 2018. Organophosphate and Pyrethroid Resistances in the Tomato Leaf Miner *Tuta absoluta* (Lepidoptera: Gelechiidae) from Iran. *J. Appl. Entomol.*, **142(1-2)**: 181-191.

ارزیابی مقاومت جمعیت‌های مینوز گوجه فرنگی *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)، در برابر حشره کش 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 حشره کش آبامکتین ارزیابی گردید. با انجام آزمون‌های زیست‌سنجی به روش غوطه‌وری، دوز کشنده میانگین (LC₅₀) جمعیت‌های مختلف اندازه‌گیری شد. میزان LC₅₀ آبامکتین در جمعیت مرجع دانشگاه صنعتی اصفهان 5/67 میلی‌گرم ماده موثر بر لیتر (mg a.i/L) تخمین زده شد، در حالی که جمعیت شهر ابریشم 1 با LC₅₀ برابر 143/18 (mg a.i/L) در مقایسه با جمعیت مرجع 25 برابر مقاوم بود. پیش‌تیمار جمعیت‌های مختلف با سینرژست DEM (مهارکننده آنزیم‌های گلوکاتایون اس ترانسفراز) سمیت آبامکتین را به‌طور قابل توجهی افزایش داد. هم‌چنین تفاوت معنی‌داری بین میانگین فعالیت گلوکاتایون اس ترانسفرازها در جمعیت‌های مقاوم با جمعیت مرجع مشاهده شد. سینرژست TPP (مهارکننده استرازاها) سمیت آبامکتین را 1/73 تا 3/7 برابر افزایش داد. میزان فعالیت استرازاها در این جمعیت‌ها نیز با جمعیت مرجع تفاوت معنی‌داری نشان داد. علاوه‌براین مهار فعالیت آنزیم‌های سیتوکروم P450 مونواکسیژناز توسط سینرژست PBO سمیت آبامکتین را از 1/3 به 2/9 برابر افزایش داد. بیشترین نسبت سینرژستی برای DEM (5/86)، TPP (3/73) و PBO (2/91) برابر) در جمعیت شهر ابریشم 1 مشاهده شد. به‌نظر می‌رسد که گلوکاتایون اس ترانسفرازها و استرازاها نقش مهم‌تری را در بروز مقاومت به آبامکتین در جمعیت‌های مورد آزمایش ایفا می‌کنند. مقاومت بالای جمعیت‌های جمع‌آوری شده از استان اصفهان به حشره کش 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.