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Research Article

Variable susceptibility in populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) to propargite and chlorpyrifos

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Abstract: The two-spotted spider mite, *Tetranychus urticae*, is a major pest of crops and ornamental plants worldwide. It is a highly polyphagous pest that has a notorious reputation for its ability to rapidly develop resistance to commonly used pesticides. Thus, in the current study, the contact toxicity of propargite (EC 57%) and chlorpyrifos (EC 40.8%) was investigated against two populations of *T. urticae* from Iran, Mahallat (MhR) and Karaj (KrS), using a leaf-dip bioassay. Findings showed that the LC_{50} of propargite against MhR and KrS populations was 5337.90 and 116.81 mg a.i/l, respectively. While the LC_{50} of the chlorpyrifos against MhR and KrS populations was 2760.83 and 479.25 mg a.i/l, respectively. Based on the median lethal concentrations (LC_{50}), MhR and KrS populations were considered as resistant and susceptible populations to both pesticides, respectively. MhR population was 5.76-fold and 45.70-fold more resistant to chlorpyrifos and propargite than the KrS population, respectively. Insecticide synergists including triphenyl phosphate (TPP), piperonyl butoxide (PBO), and diethyl maleate (DEM)) revealed the contribution of esterases, glutathione S-transferases (GST), and cytochrome P_{450} monooxygenases (P_{450}s) to resistance. Nonetheless, the involvement of esterases and P_{450}s was more evident against chlorpyrifos and propargite, respectively. The activity of P_{450}s, GSTs, esterases, and acetylcholinesterase (AChE) was measured in susceptible and resistant populations. All enzymes showed significantly higher activity in the resistant population than in the susceptible one. Additionally, zymogram analysis of esterase showed two distinct bands in the MhR population, whereas the stronger band was absent in KrS population. These results indicate that metabolic pathways are associated with chlorpyrifos and propargite resistance in the MhR population.

Keywords: *Tetranychus urticae*, resistance mechanisms, detoxification enzymes

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is the main constraint of crops and ornamental plants worldwide (Bajda *et al.,* 2018). It is a highly polyphagous pest feeding on more than 1200 host plant species in 250 families (Grbić *et al.,* 2011). Although successful biological control of *T. urticae* using predatory mites and insects has been proven in many protected crops (Opit *et al.,* 2004; van Leeuwen *et al.,* 2010), efficient control of this pest relies largely on the use of insecticides and acaricides (van Leeuwen *et al.,* 2015). However, *T. urticae* is well known for its ability to rapidly develop resistance against different classes of pesticides (van Leeuwen *et al.,* 2010). This ability arises from a variety of features including high

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fecundity, short generation time, inbreeding, and haplodiploid sex determination (arrhenotokous reproduction) (van Leeuwen *et al.,* 2010; Grbić *et al.,* 2011 *et al*). Based on Arthropod Pesticide Resistance Database (APRD) (http://www.pesticideresis-tance.com/search.php), 512 cases of *T. urticae* resistance have been reported to 96 active ingredients of pesticides, which is the highest incidence of pesticide resistance among arthropods. Indiscriminate use of pesticides for pest control can lead to broad cross-resistance within and between different classes of pesticides, resulting in resistance to newly developed pesticides within only 2-4 years (Grbić *et al.,* 2011). Control of multi-resistant mites has become increasingly difficult and failures in control of resistant populations have been reported for many compounds including propargite, fenbutatin-oxide, fluvalinate (Goodwin *et al.,* 1995), abamectin (Piraneo *et al.,* 2015; Brown *et al.,* 2017), fenpyroximate (Ay and Kara, 2011), tebufenpyrad (Herron and Rophali, 1998), cyenopyrafen(Khalighi *et al.,* 2015), bifenazate (Chen *et al.,* 2019), etc. (Nauen *et al.,* 2001; Vassiliou and Kitsis, 2013). Generally, farmers tackle this problem using higher doses and frequencies of pesticides to keep resistant pest populations under economic damage thresholds (Denholm *et al.,* 1998; Farahani *et al.,* 2018). Besides increasing the costs of chemical control, this strategy can negatively affect populations of natural enemies and pollinators and leaves higher pesticide residue on products threaten the health of consumers.

Organosulfites and organophosphates are two main classes of pesticides that are widely used against mites in the agricultural ecosystem (Van Leeuwen *et al.,* 2009). From the former group, Propargite was chosen which disrupts oxidative phosphorylation by inhibition of the mitochondrial ATP synthase (Van Leeuwen *et al.,* 2009). From the latter group, Chlorpyrifos was selected that inhibits acetylcholinesterase (AChE), an enzyme hydrolyzing acetylcholine (ACh) at cholinergic synapses. Also, synergist studies were carried out to elucidate their efficacy on the two populations of the mite from two different geographical sites. So the current study aimed to determine the chlorpyrifos and propargite resistance mechanisms in MhR and KrS populations of *T. urticae* using three commercial synergists including Triphenyl Phosphate (TPP), Piperonyl Butoxide (PBO) and Diethyl Maleate (DEM).

Materials and Methods

Chemicals

Bovine Serum Albumin (BSA), PBO, DEM, TPP, Triton X-100, Reduced Glutathione (GSH), 1-choloro-2,4- dinitrobenzene (CDNB), α-Naphthyl Acetate (α-NA) and β-Naphthyl Acetate (β-NA), Ammonium Persulfate (APS), Bromophenol blue, Tris, Acrylamide, N,N´-methylene-bisacrylamide (Bis-acrylamide), Tetramethylethylenediamine (TEMED), Phenol, Chloroform, Ethanol, and Sodium Dodecyl Sulfate (SDS) were purchased from Merck (Merck, Darmstadt, Germany). Fast blue RR salt was obtained from Fluka (Fluka, Buchs, Switzerland). 3, 3', 5, 5'-Tetramethylbenzidine (TMBZ) was purchased from Panreac. All other chemicals were purchased from Sigma-Aldrich. Propargite (EC 57%) and chlorpyrifos (EC 40.8%) were purchased from Golsam Company.

Mites

MhR population of *T. urticae* was collected from infested ornamental greenhouses in Mahallat (Markazi Province, Iran). KrS population was provided from Acarology Laboratory, Plant Protection Department, College of Agriculture and Natural Resources, University of Tehran, where they had never been exposed to any pesticide. After species identification using the key proposed by Zhang (2003), the two populations were transferred to the laboratory, where a stock colony was established on potted kidney bean plants, *Phaseolus vulgaris*. The colonies were maintained in plastic boxes in a growth chamber with a controlled condition (26 ± 1 °C, 60 ± 5% RH, and 16L: 8D photoperiod).

Toxicity bioassays

Commercial formulations of propargite (EC 57%) and chlorpyrifos (EC 40.8%) were used in this study. The toxicity of propargite and chlorpyrifos

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to the KrS and MhR populations of *T. urticae* was studied using the leaf-dip bioassay method (Morin *et al.,* 2002). Based on dose-setting pre-tests, five serial concentrations (propargite concentration in KrS population: 32.16, 62.70, 122.24, 238.36, 464.77; propargite concentration in MhR population: 1611.54, 3000.81, 5587.78, 10404.93, 19374.89; concentration of chlorpyrifos in KrS population: 113.97, 238.11, 497.49, 1025.86, 2122.21; chlorpyrifos concentrations in MhR population: 884.69, 1502.41, 2551.46, 4333, 7358.48 mg a.i. /L), causing the mortality range of 10-90%, and a control treatment (distilled water) were determined for each pesticide. Square leaf discs (3 × 3cm) of kidney bean plants were submerged in pesticide solutions for 30 seconds. After drying under a fume hood for an hour, treated leaves were spread over a layer of wet cotton at the bottom of Petri dishes (9 cm diameter). The edge of the leaf was covered with wet filter paper to prevent mites from escaping. Twenty female adults (< 24-h old) of *T. urticae* were released on each leaf disc. In control, the leaves were submerged in the same volume of distilled water. The Petri dishes were incubated in a growth chamber (26 ± 1 °C, 60 ± 5% RH, and 16L: 8D photoperiod) and mortality was recorded 24 h post-treatment. The mites were considered dead when they did not move their appendages following stimulation with a fine brush. The bioassays were conducted with five replicates with 120 same-aged adult female mites per each replicate.

The percentages of mortality were corrected using Abbott's formula (Abbott, 1925) as follows:

$$Corrected\ mortality = \frac{C-T}{C} \times 100$$

Where T and C are the number of alive mites in treatment and control, respectively (Abbott, 1925). Mortality data were used to estimate the median lethal concentrations (LC_{50}) for propargite and chlorpyrifos.

Synergism assays

The synergistic effects of three inhibitory synergists including piperonyl butoxide (PBO), triphenyl phosphate (TPP), and diethyl maleate (DEM), were assayed using the residual contact vial (RCV) bioassay (Van Leeuwen *et al.,* 2004). Various concentrations of each synergist dissolved in acetone, and 150 µl of each concentration was used to thoroughly cover the internal surface of a 7.2 ml glass vial by rolling the vials under a fume hood for an hour. Adult females (< 24-h old) of *T. urticae* were incubated in each vial for an hour at 25 ºC. The mites were then transferred on leaf discs treated with different concentrations of propargite and chlorpyrifos, and the experiment was continued similar to the toxicity bioassay. Bracketing tests were performed to determine the safe dose of each synergist. The highest dose of each synergist that caused less than 10% mortality was selected for the final bioassay. Final concentrations of synergists used in bioassays were 200 ppm for PBO, 500 ppm for DEM, and 1000 ppm for TPP. The synergism test was done with five replicates with 120 same-aged adult female mites per each replicate.

Biochemical assays

Protein concentration estimation

Protein concentration was quantified as described by Bradford (1976), using bovine serum albumin as a standard.

Determination of esterase activity

The general esterase activity of *T. urticae* in resistant and susceptible populations was measured using α-Na and β-Na as substrates according to Van Asperen (1962) with slight modifications. Briefly, the enzyme sample was prepared by homogenizing the whole body of 100 adult female mites in 500 µl of 0.04 M phosphate buffer (pH 7.0), containing 0.1% (v/v) Triton X-100 on the ice and centrifuging at 10,000 g for 20 min at 4 °C. The total reaction volume per well of a 96-well microplate consisting of 20 µl of enzyme solution, 90 µl of the substrate (30 mM in acetone), and 70 µl of phosphate buffer (0.1 M, pH 7.0) was incubated for 30 min followed by the addition of 90 µl fast blue RR (1 mg/ml in buffer). Optical density was read at 450 and 540 nm for α-Na and β-Na (Respectively) every 2 min for 20 min using a microplate reader (ELX808 Bio-Tek) (Van Asperen, 1962).

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Determination of AChE activity

AChE activity was measured using acetylthiocholine iodide (ACT) as substrate according to Ellman *et al.* (1961) with slight modifications. Briefly, the whole body of 100 female adults of *T. urticae* was homogenized in 400 µl of 0.1 M phosphate buffer, pH 7.0, containing 0.1% (v/v) Triton X-100 on ice. The homogenate was maintained for 20 min on ice, then centrifuged at 10,000 g for 10 min at 4 °C. The total reaction volume per well of a 96-well microplate was 220 µl consisting of 40 µl enzyme solution, 140 µl phosphate buffer and 40 µl substrate (2.5 mM). AChE activity was determined by the change in absorbance as measured every 2 min for 20 min at 405 nm using a microplate reader (ELX808 Bio-Tek).

Determination of GST activity

Glutathione S-transferase (GST) activity was measured using 2, 4-Dinitrochlorobenzene (CDNB) according to Habig *et al.* (1974). The enzyme sample was prepared by homogenizing 100 adult female mites' whole body in 1000 µl phosphate buffer 10 mM (pH7.0) on the ice and centrifuging at 10,000 g for 20 min at 4 °C. The total reaction volume per well of a 96-well microplate was 165 µl, consisting of 15 µl of enzyme solution, 50 µl CDNB (containing 0.1% (v/v) ethanol)), and 100 µl GSH. Enzyme activity was determined by the change in absorbance as measured every 30 s for 5 min at 340 nm using a microplate reader (ELX808 Bio-Tek) (Habig *et al.,* 1974).

MFO Assay

Cytochrome P_{450} activity was measured in terms of general oxidase level using the heme peroxidation method (Brogdon *et al.,* 1997). 3, 3'5, 5'-tetramethylbenzidine (TMBZ) was used as a substrate. The enzyme sample was prepared by homogenizing 50 adult female mites' whole body in 400 µl of 0.1 M phosphate buffer, pH 7.0 on the ice, and centrifuged at 10,000 g for 20 min at 4 °C. The total reaction volume per well of a 96-well microplate was 325 µl, including 20 µl of enzyme solution, 80 µl of 0.625 M potassium phosphate buffer (pH 7.2), 200 µl of TMBZ (0.01 g TMBZ dissolved in 5 ml methanol plus 15 ml of 0.25 M sodium acetate buffer pH 5.0) solution, and 25 µl of hydrogen peroxide (H_2O_2) (3%). The plates were incubated at room temperature for 2 hours, and the optical density was read at 450 nm. A standard curve was constructed to calculate the equivalent units (EU) of cytochrome P_{450} per mg of protein (Brogdon *et al.,* 1997).

Native-PAGE electrophoresis of enzyme carboxylesterase

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was conducted on 7.5% slab gels according to Davis (1964). Electrophoresis was performed at 4 °C. After running, the gel was washed by shaking gently in 1% (V/V) Triton X-100 dissolved in phosphate buffer (pH 7.2). Gel staining was done based on Devonshire (1977). Briefly, the gel was soaked in a solution of sodium phosphate buffer (0.1M and pH 6.0) containing 0.5 ml (30 mM) of α-naphthyl acetate dissolved in acetone with 0.12 % fast blue RR and shook gently for an hour at room temperature. Finally, the gel was washed in distilled water to observe the bands.

Statistical analysis

LC_{50} was determined using probit analysis with the software Polo-PC (LeOra Software 2007). The data on enzyme activities between resistant (MhR) and susceptible (KrS) populations were subjected to Student's t-test in SPSS software (version 22). Significant differences were evaluated using Tukey's post hoc test at $P < 0.05$.

Results

Resistance level in bioassay

Results of probit analysis for estimation of LC_{50} of propargite and chlorpyrifos against two populations of *T. urticae* are shown in Table 1. The LC_{50} values of chlorpyrifos in KrS and MhR populations were 479.25 and 2760.83 mg a. i./l and the LC_{50} values of propargite in KrS and MhR populations were 116.81 and 5337.90 mg a. i. /l, respectively. There were significant differences in LC_{50} values of both chlorpyrifos and propargite against both populations. So, these

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two populations are called resistant (MhR) and susceptible (KrS) populations. The resistance level of MhR and KrS populations to either chlorpyrifos or propargite was determined by comparing the LC_{50} values. The results indicated that the MhR population was 5.76-fold and 45.70-fold more resistant to chlorpyrifos and propargite respectively than the KrS population (Table 1).

Synergism

The LC_{50} values of chlorpyrifos and propargite significantly decreased in both MhR and KrS populations when the pesticides were applied in combination with synergists (Table 1). The order of synergistic effects of the synergists on MhR population was TPP > DEM > PBO and PBO > DEM > TPP, for chlorpyrifos and propargite, respectively. While, the order of synergistic effects after combining pesticides with each synergist in KrS population was TPP > DEM > PBO and DEM > PBO > TPP, for chlorpyrifos and propargite, respectively (Table 1). The results revealed significant synergistic effects of DEM and PBO with propargite. However, the synergistic effect of TPP on propargite was not statistically significant (Table 1). Also, none of the synergists showed a significant synergistic effect on KrS population (Table 1).

Table 1 Results of probit analysis for estimation of chlorpyrifos and propargite LC_{50} on a resistant (MhR) and a susceptible (KrS) population of *Tetranychus urticae* with or without PBO, DEM, and TPP synergists.

Pesticide	Population	Synergist	N[1]	LC_{50} (95% CI)[2] (mg a. i. /l)[3]	Slope ± SE	χ^2 (df)	SR[4]
Chlorpyrifos (EC 40.8%)	KrS	0	600	479.25 (408.53 - 561.40)	1.81 ± 0.153	3.92 (23)	-
		PBO	600	361.37 (300.93 - 433.33)	1.57 ± 0.134	5.77 (23)	1.33
		DEM	600	322.01 (266.47 - 388.79)	1.52 ± 0.128	4.03 (23)	1.49
		TPP	600	294.65 (242.86 - 357.91)	1.48 ± 0.125	4.49 (23)	1.63
	MhR	0	600	2760.83 (2408.50 - 3156.53)	2.32 ± 0.221	7.08 (23)	-
		PBO	600	1881.35 (1637.60 - 2125.42)	2.33 ± 0.205	5.49 (23)	1.47
		DEM	600	1483.68 (1277.79 - 1717.83)	2.06 ± 0.177	4.45 (23)	1.86
		TPP	600	1100.23 (934.73 - 1284.81)	1.99 ± 0.174	5.49 (23)	2.51
Propargite (EC 57%)	KrS	0	600	116.81 (100.04 - 136.23)	1.84 ± 0.163	7.01 (23)	-
		PBO	600	87.70 (84.57 - 115.01)	1.87 ± 0.158	3.94 (23)	1.18
		DEM	600	85.52 (72.83 - 100.26)	1.80 ± 0.152	5.38 (23)	1.37
		TPP	600	102.08 (87.05 - 119.37)	1.80 ± 0.159	6.99 (23)	1.14
	MhR	0	600	5337.90 (4600.11 - 6132.75)	2.36 ± 0.209	7.48 (23)	-
		PBO	600	2474.87 (2043.64 - 2966.05)	1.82 ± 0.163	5.61 (23)	2.16
		DEM	600	3406.37 (2813.23 - 4091.18)	1.70 ± 0.151	4.45 (23)	1.57
		TPP	600	4762.37 (4036.50 - 5558.64)	2.02 ± 0.182	5.72 (23)	1.12

[1] Total number of mites used in the bioassay.
[2] Confidence interval.
[3] Active ingredient per liter.
[4] Synergistic ratio = LC_{50} not synergized insecticide / LC_{50} synergized insecticide.

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Esterase activity

The activity of general esterases was quantified in adult *T. urticae*. Results showed that there was a significant difference in the esterase activity of mites between resistant and susceptible populations ($t = 11.76$, $df = 4$, $P < 0.05$) (Table 2). Using α-NA as the substrate, the enzyme activity was 0.09 ± 0.003 and 0.17 ± 0.007 (µU/mg protein) in KrS and MhR populations, respectively. However, the enzyme activity when β-NA substrate used was 0.047 ± 0.001and 0.052 ± 0.002 (µU/mg protein), for KrS and MhR populations, respectively. Therefore, the esterase activity of MhR population was 1.88- and 1.1 times higher than that of Krs population when α-NA and β-NA were used as the substrates, respectively ($t = 11.40$, $df = 4$, $P < 0.05$).

AChE activity

Results showed a significant difference in the activity of AChE enzyme between resistant and susceptible populations ($t = 6.33$, $df = 4$, $P < 0.05$). It was 0.006 ± 0.001 and 0.005 ± 0.002 (µU/mg protein) in MhR and KrS population respectively. Therefore, the activity of AChE in MhR population was 1.29 times higher than that of KrS population (Table 2).

GST activity

The activity of GSTs was measured using GSH and CDNB as substrates. There was a significant difference in GST activity among MhR (0.079 ± 0.003 µU/mg protein) and KrS (0.057 ± 0.001 µU/mg protein) populations ($t = 20.88$, $df = 4$, $P < 0.05$). GST activity in MhR population was 1.40 times higher than that for KrS population (Table 2).

Monooxygenase contents

The total content of heme-containing protein was measured using heme-peroxidase assay as an indicator of P_{450} activity. The results revealed significant differences in P_{450} content of MhR and KrS populations ($t = 16.52$, $df = 4$, $P < 0.01$), indicating that the P_{450} activity of resistant MhR population (0.061 ± 0.006units of cytochrome P_{450}/mg protein) was 1.49 times higher than that of susceptible KrS population (0.041 ± 0.008 units of cytochrome P_{450}/mg protein) (Table 2).

Table 2 Activity (mean ± SE) of acetylcholinesterase (AChE), general esterase, glutathione S-transferases (GSTs), and monooxygenases P_{450} in a resistant (MhR) and a susceptible (KrS) population of *Tetranychus urticae*.

Enzyme	Substrate	Enzyme activity (± SE)[1]		R/S[a]
		Susceptible (KrS)	Resistant (MhR)	
Esterase (µmol. min^{-1}.mg $protein^{-1}$)	α-NA	0.090 ± 0.003	0.170 ± 0.007*	1.88
	β-NA	0.047 ± 0.001	0.052 ± 0.002*	1.10
GSTs (µmol. min^{-1}.mg $protein^{-1}$)	CDNB	0.057 ± 0.001	0.079 ± 0.003*	1.39
P_{450}(mg. mg^{-1} protein)	TMBZ	0.041 ± 0.008	0.061 ± 0.006*	1.49
AChE (µmol. min^{-1}.mg $protein^{-1}$)	ACT	0.005 ± 0.002	0.006 ± 0.001*	1.29

[1] Enzyme activity in MhR population/enzyme activity in KrS population.
* Indicate significantly different at $P < 0.05$ (t-student test).

Esterase banding pattern

The esterase activity of *T. urticae* populations was visually analyzed using native PAGE. Three distinct bands (E1, E2, and E3) of esterase were detected in the resistant population (MhR), while the susceptible population (KrS) only exhibited one faint band (E1) (Fig. 1).

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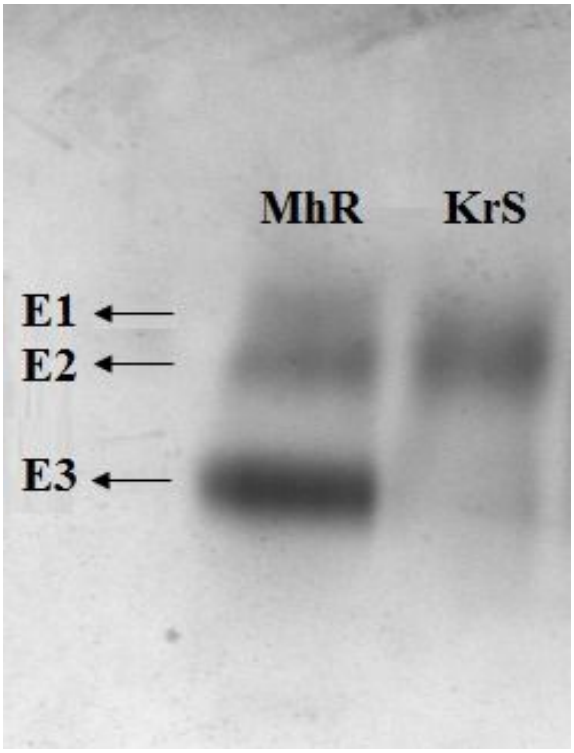

Figure 1 General esterases in susceptible (KrS) and resistant (MhR) populations of *Tetranychus urticae*using α-NA and β-NA substrates.

Discussion

In this study, the toxicity of propargite and chlorpyrifos was investigated against two isolated populations of *T. urticae* with different levels of resistance. Mahallat area is a major place for the production of many ornamental plants in Iran. Many pesticides are annually used in this area for control of different pests on ornamental plants. Failures of spider mites control using convenient acaricides have been repeatedly reported by farmers in this region, indicating this population has probably developed some degree of resistance against a variety of pesticides. The occurrence of resistance may have been accelerated by factors such as inappropriate timing of pesticide application, incorrect spraying techniques, as well as the continuous application of a recommended pesticide without rotation with other compounds (Farahani *et al.,* 2018).

We evaluated the resistance level of Mahallat population to propargite and chlorpyrifos by comparing the LC_{50} of these pesticides with those of an assumed susceptible population with no history of pesticide exposure (KrS). Bioassays showed that MhR population has a low level of resistance (RR = 5.76) to chlorpyrifos, but a moderate level of resistance to propargite (RR = 45.70). Based on the information collected from growers, chlorpyrifos is rarely used for control of spider mites in greenhouses of Mahallat area, whereas the use of propargite is relatively widespread in this area. Therefore, the higher rate of resistance to propargite indicates that *T. urticae* population was under high pressure of this pesticide over time in this area. Since it has been reported that resistance selection in spider mites is directly associated with the frequency of pesticide use (Herron *et al.,* 1998). The findings of this research are consistent with previous studies. Keena and Granett (1990) reported that the LC_{50} values of propargite in resistant populations of *T. pacificus* and *T. urticae* were 22 and 52.51 times higher than the susceptible population in California almond orchards. By contrast, Sökeli *et al.* (2007) reported a high level of resistance of *T. urticae* to chlorpyrifos, besides the very low level of resistance to propargite and abamectin, in populations collected from apple orchards in Turkey. This observation is not surprising, because farmers in this area prefer to use broad-spectrum pesticides such as chlorpyrifos, rather than narrow-spectrum pesticides such as propargite, to simultaneously control a wider range of pests as reported by Sökeli *et al.* (2007). Similarly, Ay (2005) reported higher rates of resistance to chlorpyrifos in *T. urticae* populations collected from regions with a long history of greenhouse vegetable production and pesticide application. Three enzyme inhibitory synergists (PBO, DEM, and TPP) were used to study the susceptibility of both MhR and KrS populations to chlorpyrifos and propargite and also to elucidate possible mechanisms involved in the resistance. The results revealed significant synergistic effects of DEM and PBO

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with propargite. However, the synergistic effect of TPP on propargite was not statistically significant. Also, none of the synergists showed a significant effect on KrS population. Synergists are chemical compounds that do not have pesticide activity, but they can enhance pesticide efficiency (Snoeck *et al.,* 2017). Some of these compounds can inhibit detoxification enzymes preventing the reduction of the concentration of pesticides in the target site (Dehkordi *et al.,* 2017). In agreement with our results, Luo *et al.* (2014) reported strong synergistic effects of DEM, and to a lesser extent PBO, with propargite in resistant populations of *T. cinnabarinus*, indicating that P_{450}s and GSTs -mediated metabolism are probably associated with propargite resistance in this population. The non-overlapping 95% CI at the LC_{50} level suggests that all synergists significantly increased the susceptibility of MhR population to propargite. However, the synergistic effect of TPP was significantly higher than PBO and DEM on chlorpyrifos, indicating that esterase-mediated metabolism may be a potential pathway involved in chlorpyrifos resistance by *T. urticae*. Yang *et al.* (2001) reported a 1.7-fold increase in the toxicity of the organophosphate dimethoate against *T. urticae* when applied in combination with TPP, but not PBO or DEM synergists. Similarly, esterase enzymes were found to play a role in chlorpyrifos resistance of *T. urticae* (Ay and Yorulmaz 2010) and *Panonychus ulmi* (Kumral *et al.,* 2009). Metabolism of pesticides before reaching the target site through quantitative or qualitative changes in major detoxification enzyme (P_{450}s, GSTs, and esterases) is a widespread mechanism of resistance in many species of pests (Van Leeuwen *et al.,* 2010). Synergism studies are the first step to determine the possible metabolic resistance mechanisms of pests (Scott, 1990). The obtained results revealed that the metabolic mechanism was one of the probable mechanisms involved in observed resistance. Also, detoxification enzymes assays were conducted to demonstrate metabolic involvement in the resistance. The enzyme assay results indicated that the levels of esterase, GST and cytochrome P_{450} monooxygenase activity in MhR population were significantly higher than KrS population so that the enzyme activity ratios were 1.91, 1.40, and 1.49, respectively. These results suggest that metabolic degradation is one of the mechanisms of chlorpyriphos and propargite resistance in the MhR population. These findings were supported by the results of synergistic studies. According to the Arthropod Pesticide Resistance Database (APRD), *T. urticae* has developed resistance to more than 30 organophosphate and carbamate pesticides, which target acetylcholinesterase. Although target-site insensitivity of AChE through point mutations seems to be the most common resistance mechanism of mites and ticks to organophosphates, the possible involvement of metabolic detoxification pathways as supporting or major resistance mechanism has also been reported in several studies (Van Leeuwen *et al.,* 2010). Alternatively, enhanced resistance of *T. urticae* to organophosphate pesticides may be achieved through elevating the expression of AChE by gene duplication (Kwon *et al.,* 2010 and 2012). In our study, the activity of AChE enzyme in a resistant population was significantly higher than in the susceptible population. In the gel assays, three bands appeared in MhR population in the gel in which bands E2 and E3 were major bands and E1 was a minor band while in KrS population only E2 was observed confirming that esterase has an important role in the observed resistance in MhR population. Mohammadzadeh *et al.* (2014) showed that there were three bands in the resistant population of *T. urticae* in polyacrylamide gel while they observed only one band in the susceptible population.

In conclusion, this study provided evidence for the resistance of *T. urticae* population to propargite, and a lesser extent chlorpyrifos, in Mahallat area. The elevated resistance possibly resulted from a long history of continuous exposure to these pesticides since the area has been highlighted as a major center for the production of ornamental plants for many years.

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Based on a variety of synergistic, biochemical, and native gel electrophoresis assays, it is postulated that metabolic detoxification pathways contribute to elevated resistance of MhR population to both chlorpyrifos and propargite.

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References

Abbott W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Ay, R. 2005. Determination of susceptibility and resistance of some greenhouse populations of *Tetranychus urticae* Koch to chlorpyrifos (Dursban 4) by the petri dish-Potter tower method. Journal of Pest Science, 78: 139-143.

Ay, R. and Yorulmaz, S. 2010 Inheritance and detoxification enzyme levels in *Tetranychus urticae* Koch (Acari: Tetranychidae) strain selected with chlorpyrifos. Journal of Pest Science, 83: 85-93.

Ay, R. and Kara F. E. 2011. Toxicity, inheritance of fenpyroximate resistance, and detoxification-enzyme levels in a laboratory-selected fenpyroximate-resistant population of *Tetranychus urticae* Koch (Acari: Tetranychidae). Crop Protection, 30(6): 605-610.

Bajda, S., Riga Wybouw, M., Papadaki, N., Ouranou, S., Fotoukkiaii, E., Vontas, S. M. J. and Van Leeuwen, T. 2018. Fitness costs of key point mutations that underlie acaricide target-site resistance in the two-spotted spider mite *Tetranychus urticae*. Evolutionary Applications, 11(9): 1540-1553.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2): 248-254 .

Brogdon, W. G., McAllister, J. C. and Vulule, J. 1997. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. Journal of the American Mosquito Control Association, 13(3): 233-237.

Brown, S., Kerns, D. L., Gore, J., Lorenz, G. and Stewart, S. 2017. Susceptibility of two-spotted spider mites (*Tetranychus urticae*) to abamectin in Midsouth cotton. Crop Protection, 98: 179-183.

Chen, J. C., Gong, Y. J., Shi, P., Wang, Z. H., Cao, L. J., Wang, P. and Wei, S. J. 2019. Field-evolved resistance and cross-resistance of the two-spotted spider mite, *Tetranychus urticae*, to bifenazate, cyenopyrafen and SYP-9625. Experimental and Applied Acarology, 77(4): 545-554.

Davis, B. J. 1964. Disc electrophoresis—ii method and application to human serum proteins. Annals of the New York Academy of Sciences, 121: 404-427 .

Dehkordi, A. S., Abadi, Y. S., Nasirian, H., Hazratian, T., Gorouhi, M. A., Yousefi, S. and Paksa, A. 2017. Synergists action of piperonylbutoxide and S, S, S-tributylphosphorotrithioate on toxicity of carbamate insecticides against *Blattella germanica*. Asian Pacific Journal of Tropical Medicine, 10(10): 981-986.

Devonshire, A. L. 1977. The properties of a carboxylesterase from the peach-potato aphid, *Myzus persicae* (Sulz.), and its role in conferring insecticide resistance. The Biochemical Journal, 167: 675-683.

Denholm. I., A. R. Horowitz, et al. 1998. Management of resistance to novel insecticides. In: Ishaaya, I. and Degheele, D. (Eds.), Insecticides with Novel Modes of Action: Mechanisms and Application. Springer-Verlag, Berlin, pp. 260-282.

Ellman G. L., Courtney K. D., Andres V. and Feather-Stone R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7: 88-95 .

[DOR: 20.1001.1.22519041.2021.10.1.3.7]

Farahani, S., Bandani, A. and Eslami, S. 2018. Comparison of susceptibility of two Iranian populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) to spirodiclofen. Persian Journal of Acarology, 7(3): 279-287.

Goodwin, S., Herron, G., Gough, N., Welliiam, T., Rophail, J. and Parker, R. 1995. Relationship between insecticide-acaricide resistance and field control in *Tetranychus urticae* (Acari: Tetranychidae) infesting roses. Journal of Economic Entomology, 88: 1106-1112.

Grbić, M., Van Leeuwen, T., Clark, R. M., Rombauts, S., Rouzé, P., Grbić, V., Osborne, E. J., Dermauw, W., Ngoc, P. C. T., Ortego, F. and Hernández-Crespo P. 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature, 479: 487-492.

Habig, W. H., Pabst, M. J. and Jakoby, W. B. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249: 7130-7139.

Herron, G. A., Edge, V. E., Wilson, L. J. and Rophail, J. 1998. Organophosphate resistance in spider mites (Acari: Tetranychidae) from cotton in Australia. Experimental and Applied Acarology, 22: 17-30.

Herron, G. A. and Rophali, J. 1998. Tebufenpyrad (Pyranica®) resistance detected in two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) from apples in Western Australia. Experimental and Applied Acarology, 22: 633-641.

Keena, M. A. and Granett, G. 1990. Genetic analysis of propargite resistance in Pacific spider mites and two-spotted spider mites (Acari: Tetranychidae). Journal of Economic Entomology, 83: 655-661.

Khalighi, M., Dermauw, W., Wybouw, N., Bajda, S., Osakabe, M., Tirry, L., Van Leeuwen, T. 2015. Molecular analysis of cyenopyrafen resistance in the two-spotted spider mite *Tetranychus urticae*. Pest Management Science, 32.

Kumral, N. A, Susurluk, H., Gencer, N. S. and Gürkan, M. O. 2009. Resistance to chlorpyrifos and lambda-cyhalothrin along with detoxifying enzyme activities in field-collected female populations of European red mite. Phytoparasitica, 37: 7-15.

Kwon, D. H., Choi, J. Y., Je, Y. H. and Lee, S. H. 2012. The overexpression of acetylcholinesterase compensates for the reduced catalytic activity caused by resistance-conferring mutations in *Tetranychus urticae*. Insect Biochemistry and Molecular Biology, 42: 212-219.

Kwon, D. H., Clark, J. M. and Lee, S. H. 2010. Extensive gene duplication of acetylcholinesterase associated with organophosphate resistance in the two-spotted spider mite. Insect Molecular Biology, 19: 195-204.

LeOra Software. 2007. LeOra Software Company. Available from: < http://www. leorasoftware.com>.

Luo, Y. J., Yang, Z. G., Xie, D. Y., Ding, W., Da, A. S., Ni, J., Chai, J. P., Huang, P., Jiang, X. J. and Li, S. X. 2014. Molecular cloning and expression of glutathione S-transferases involved in Propargite resistance of the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval). Pesticide Biochemistry and Physiology, 114: 44-51.

Mohammadzadeh, M., Bandani, A. R. and Sabahi, Q. 2014. Comparison of susceptibility of two populations of *Tetranychus urticae* Koch to two acaricides, abamectin and propargite.

Archives of Phytopathology and Plant Protection, 47(17): 2112-2123.

Morin, S., Williamson, M. S., Goodson, S. J., Brown, J. K., Tabashnik, B. E. and Dennehy, T. J. 2002. Mutations in the *Bemisia tabaci* para sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. Insect Biochemistry and Molecular Biology, 32(12): 1781-1791 .

Nauen, R., Stumpf, N., Elbert, A., Zebitz, C. P. W. and Kraus, W. 2001. Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Management Science, 57: 253-261.

[DOR: 20.1001.1.22519041.2021.10.1.3.7]

Opit, G. P., Nechols, J. R. and Margolies, D. C. 2004. Biological control of two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseidae) on ivy geranium: Assessment of predator release ratios. Biological Control, 29(3): 445-452.

Piraneo, T. G., Bull, J., Morales, M. A., Lavine, L. C., Walsh, D. B. and Zhu, F. 2015. Molecular mechanisms of *Tetranychus urticae* chemical adaptation in hop fields. Scientific Reports, 5, 17090.

Scott, J. G. 1990. Investigating mechanisms of insecticide resistance: methods, strategies, and pitfalls. In: Roush, R. T., Tabashnik, B. E. (Eds.), Pesticide Resistance in Arthropods Springer, Boston, MA. pp. 39-57.

Snoeck, S., Greenhalgh, R., Tirry, L., Clark, R. M., Van Leeuwen, T. and Dermauw, W. 2017. The effect of insecticide synergist treatment on genome-wide gene expression in a polyphagous pest. Scientific Reports, 7(1): 13440.

Sökeli, E., Ay, R. and Karaca, S. 2007. Determination of the resistance level of two-spotted spider mite (*Tetranychus urticae* Koch) populations in apple orchards in Isparta Province against some pesticides. Tarim Bilimleri Dergisi, 13: 326-330.

Van Asperen, K. 1962. A study of housefly esterases by means of a sensitive colorimetric method. Journal of Insect Physiology, 8(4): 401-416 .

Van Leeuwen, T., Stillatus, V. and Tirry, L. 2004. Genetic analysis and cross-resistance spectrum of a laboratory-selected chlorfenapyr resistant population of two-spotted spider mite (Acari: Tetranychidae). Experimental and Applied Acarology, 32(4): 249-261 .

Van Leeuwen, T., Tirry, L., Yamamoto, A., Nauen, R. and Dermauw, W. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pesticide Biochemistry and Physiology, 121: 12-21.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W. and Tirry, L. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. Insect Biochemistry and Molecular Biology, 40: 563-572.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A. and Tirry, L. 2009. Mechanisms of Acaricide Resistance in the Two-Spotted Spider Mite *Tetranychus urticae*. In: Ishaaya, I. and Horowitz, A. R. (Eds.), Biorational Control of Arthropod Pests. Springer Netherlands, pp. 347-393.

Vassiliou, V. A. and Kitsis, P. 2013. Acaricide Resistance in *Tetranychus urticae* (Acari: Tetranychidae) Populations from Cyprus. Journal of Economic Entomology, 106: 1848-1854.

Yang, X., Zhu, Y. K., Buschman, L. and Margolies, D. 2001. Comparative susceptibility and possible detoxification mechanisms for selected miticides in Banks grass mite and two-spotted spider mite (Acari: Tetranychidae). Experimental and Applied Acarology, 25: 293-299.

Zhang, Z. Q. 2003. Mites of Greenhouses Identification, Biology and Control. CABI. UK. 244 pp.

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حساسیت متغیر جمعیت‌های کنه تارتن دولکه‌ای *Tetranychus urticae* Koch (Acari: Tetranychidae) به پروپارژیت و کلرپایریفوس

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چکیده: کنه تارتن دولکه‌ای *Tetranychus urticae* Koch یکی از آفات مهم گیاهان در فضای آزاد و گلخانه در سراسر جهان است. این آفت با دامنه وسیع میزبانی و تعداد نسل بالا یکی از مهم‌ترین آفاتی است که به‌سرعت به انواع مختلف آفت‌کش‌ها مقاوم می‌شود. بنابراین، در این مطالعه حساسیت کنه تارتن دولکه‌ای به کلرپایرفوس (EC 40.8%) و پروپارژیت (EC 57%) در دو جمعیت جمع‌آوری شده از کرج و محلات به‌روش غوطه‌وری دیسک‌های برگی بررسی شد. نتایج زیست‌سنجی نشان داد غلظت کشنده ۵۰ درصد پروپارژیت در جمعیت‌های محلات و کرج به‌ترتیب ۵۳۳۷/۹ و ۱۱۶/۸۱ میلی‌گرم ماده مؤثر بر لیتر بود. هم‌چنین غلظت کشنده ۵۰ درصد کلرپایریفوس در دو جمعیت محلات و کرج به‌ترتیب ۲۷۶۰/۸۳ و ۴۷۹/۲۵ میلی‌گرم ماده مؤثر بر لیتر به‌دست آمد. بنابراین، با توجه به آنالیز داده‌های زیست‌سنجی جمعیت محلات به‌عنوان جمعیت مقاوم و جمعیت کرج به‌عنوان جمعیت حساس درنظر گرفته شد که نرخ مقاومت جمعیت محلات به کلرپایریفوس و پروپارژیت به‌ترتیب ۵/۷۶ و ۴۵/۷۰ برابر بیش‌تر از جمعیت کرج است. علاوه بر این، نتایج مطالعات سینرژیستی نقش آنزیم‌های استراز، گلوتاتیون اس ترانسفراز و مونواکسیژنازهای P_{450} را نشان داد و مشخص شد که بیش‌ترین تأثیر سینرژیستی در مقاومت جمعیت محلات به کلرپایریفوس و پروپارژیت به‌ترتیب مربوط به سینرژیست‌های TPP و PBO بوده است. هم‌چنین، نتایج آزمون‌های بیوشیمیایی نشان داد که سطح فعالیت هر سه آنزیم مذکور در جمعیت محلات بالاتر از جمعیت کرج است. از طرفی سنجش فعالیت آنزیم استراز در ژل الکتروفورز حاکی از وجود دو باند آنزیمی در جمعیت محلات و یک باند در جمعیت کرج بود. با توجه به نتایج به‌دست آمده از این مطالعه نتیجه‌گیری می‌شود که احتمالاً سازوکار سم‌زدایی متابولیکی نقش اصلی را در مقاومت مشاهده شده داشته باشد.

واژگان کلیدی: *Tetranychus urticae*، سازوکارهای مقاومت، آنزیم‌های سم‌زدا

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