

## Research Article

Variable susceptibility in populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) to propargite and chlorpyrifosSaeed Farahani<sup>1</sup>, Ali Reza Bandani<sup>1\*</sup> and Azam Amiri<sup>2</sup>

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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<sub>50</sub> of propargite against MhR and KrS populations was 5337.90 and 116.81 mg a.i./l, respectively. While the LC<sub>50</sub> 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<sub>50</sub>), 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<sub>450</sub> monooxygenases (P<sub>450</sub>s) to resistance. Nonetheless, the involvement of esterases and P<sub>450</sub>s was more evident against chlorpyrifos and propargite, respectively. The activity of P<sub>450</sub>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.pesticideresistance.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), bifentazate (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-chloro-2,4-dinitrobenzene (CDNB),  $\alpha$ -Naphthyl Acetate ( $\alpha$ -NA) and  $\beta$ -Naphthyl Acetate ( $\beta$ -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 ( $26 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  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

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:

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

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<sub>50</sub>) 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 °C. 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).

**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<sub>450</sub> 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<sub>2</sub>O<sub>2</sub>) (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<sub>450</sub> 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  $\alpha$ -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<sub>50</sub> 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<sub>50</sub> of propargite and chlorpyrifos against two populations of *T. urticae* are shown in Table 1. The LC<sub>50</sub> values of chlorpyrifos in KrS and MhR populations were 479.25 and 2760.83 mg a. i./l and the LC<sub>50</sub> 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<sub>50</sub> values of both chlorpyrifos and propargite against both populations. So, these

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

<sup>1</sup> Total number of mites used in the bioassay.

<sup>2</sup> Confidence interval.

<sup>3</sup> Active ingredient per liter.

<sup>4</sup> Synergistic ratio =  $LC_{50}$  not synergized insecticide /  $LC_{50}$  synergized insecticide.

**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  $\alpha$ -NA as the substrate, the enzyme activity was  $0.09 \pm 0.003$  and  $0.17 \pm 0.007$  ( $\mu\text{U}/\text{mg}$  protein) in KrS and MhR populations, respectively. However, the enzyme activity when  $\beta$ -NA substrate used was  $0.047 \pm 0.001$  and  $0.052 \pm 0.002$  ( $\mu\text{U}/\text{mg}$  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  $\alpha$ -NA and  $\beta$ -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  $0.006 \pm 0.001$  and  $0.005 \pm 0.002$  ( $\mu\text{U}/\text{mg}$  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 ( $0.079 \pm 0.003$   $\mu\text{U}/\text{mg}$  protein) and KrS ( $0.057 \pm 0.001$   $\mu\text{U}/\text{mg}$  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 ( $0.061 \pm 0.006$  units of cytochrome  $P_{450}/\text{mg}$  protein) was 1.49 times higher than that of susceptible KrS population ( $0.041 \pm 0.008$  units of cytochrome  $P_{450}/\text{mg}$  protein) (Table 2).

**Table 2** Activity (mean  $\pm$  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 ( $\pm$ SE) <sup>1</sup> |                     |                  |
|---------------------------------------------------------------|--------------|------------------------------------------|---------------------|------------------|
|                                                               |              | Susceptible (KrS)                        | Resistant (MhR)     | R/S <sup>a</sup> |
| Esterase ( $\mu\text{mol. min}^{-1}.\text{mg protein}^{-1}$ ) | $\alpha$ -NA | $0.090 \pm 0.003$                        | $0.170 \pm 0.007^*$ | 1.88             |
|                                                               | $\beta$ -NA  | $0.047 \pm 0.001$                        | $0.052 \pm 0.002^*$ | 1.10             |
| GSTs ( $\mu\text{mol. min}^{-1}.\text{mg protein}^{-1}$ )     | CDNB         | $0.057 \pm 0.001$                        | $0.079 \pm 0.003^*$ | 1.39             |
| $P_{450}$ ( $\text{mg. mg}^{-1}$ protein)                     | TMBZ         | $0.041 \pm 0.008$                        | $0.061 \pm 0.006^*$ | 1.49             |
| AChE ( $\mu\text{mol. min}^{-1}.\text{mg protein}^{-1}$ )     | ACT          | $0.005 \pm 0.002$                        | $0.006 \pm 0.001^*$ | 1.29             |

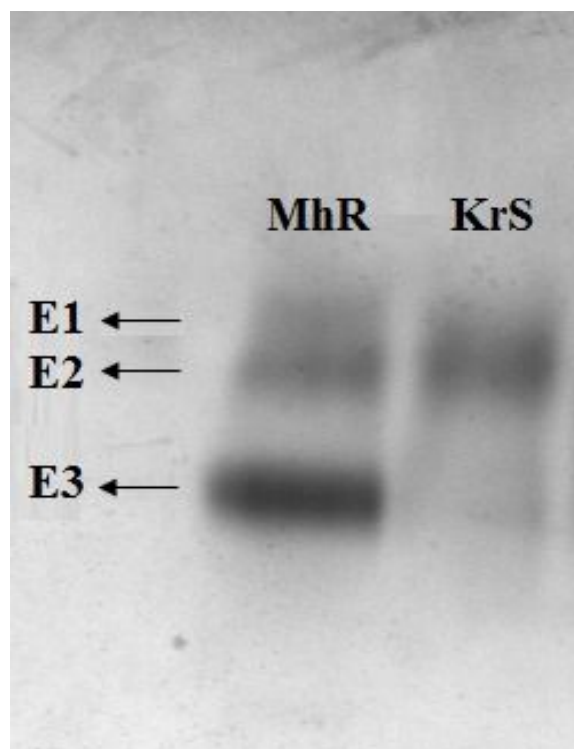
<sup>1</sup> Enzyme activity in MhR population/enzyme activity in KrS population.

\* 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).



**Figure 1** General esterases in susceptible (KrS) and resistant (MhR) populations of *Tetranychus urticae* using  $\alpha$ -NA and  $\beta$ -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

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<sub>450</sub>S and GSTs-mediated metabolism are probably associated with propargite resistance in this population. The non-overlapping 95% CI at the LC<sub>50</sub> 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<sub>450</sub>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<sub>450</sub> 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 chlorpyrifos 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.

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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## حساسیت متغیر جمعیت‌های کنه تارتن دولک‌های (*Tetranychus urticae* Koch (Acari: حساسیت متغیر جمعیت‌های کنه تارتن دولک‌های (*Tetranychidae*) به پروپارزیت و کلرپایرِفوس

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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 آفات است که به سرعت به انواع مختلف آفت‌کش‌ها مقاوم می‌شود. بنابراین، در این مطالعه حساسیت کنه تارتن دولک‌های به کلرپایرِفوس (EC 40.8%) و پروپارزیت (EC 57%) در دو جمعیت جمع‌آوری شده از کرج و محلات به روش غوطه‌وری دیسک‌های برگ‌ی بررسی شد. نتایج زیست‌سنجی نشان داد غلظت کشنده ۵۰ درصد پروپارزیت در جمعیت‌های محلات و کرج به ترتیب ۵۳۳۷/۹ و ۱۱۶/۸۱ میلی گرم ماده مؤثر بر لیتر بود. همچنین غلظت کشنده ۵۰ درصد کلرپایرِفوس در دو جمعیت محلات و کرج به ترتیب ۲۷۶۰/۸۳ و ۴۷۹/۲۵ میلی گرم ماده مؤثر بر لیتر به دست آمد. بنابراین، با توجه به آنالیز داده‌های زیست‌سنجی جمعیت محلات به عنوان جمعیت مقاوم و جمعیت کرج به عنوان جمعیت حساس در نظر گرفته شد که نرخ مقاومت جمعیت محلات به کلرپایرِفوس و پروپارزیت به ترتیب ۵/۷۶ و ۴۵/۷۰ برابر بیش‌تر از جمعیت کرج است. علاوه بر این، نتایج مطالعات سینرژیستی نقش آنزیم‌های استراز، گلوکاتایون اس ترانسفراز و مونواکسیژنازهای P<sub>450</sub> را نشان داد و مشخص شد که بیش‌ترین تأثیر سینرژیستی در مقاومت جمعیت محلات به کلرپایرِفوس و پروپارزیت به ترتیب 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 به دست 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