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Fenpyroximate resistance in Iranian populations of the European red mite *Panonychus ulmi* (Acari: Tetranychidae)

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Abstract
The European red mite, *Panonychus ulmi* (Koch), is one of the most important apple orchard pests worldwide. Fenpyroximate, a mitochondrial electron transport inhibitor of complex I (METI-I), is a commonly used acaricide to control this pest. In this study, we determined fenpyroximate resistance levels for 11 *P. ulmi* populations from Iran and a spirodiclofen-resistant strain from Germany (PSR-TK). The LC_{50} values ranged between 121.8 and 5713.9 mg a.i. L^{-1} and the highest resistance ratio (RR) was 47-fold for the Padena population. PBO, TPP and DEM synergist ratios (SRs) were the highest for the PSR-TK (SR = 6.7), Shahin Dej (SR = 6.1) and Semirom3 (SR = 3.6) populations, respectively. In vitro enzyme activity measurements also showed that there was a higher glutathione *S*-transferases (GSTs) activity in the PSR-TK and Shahin Dej population compared to the most susceptible populations, whereas the esterase and P450 monooxygenase activity were not significantly higher in the resistant populations. Last, we screened all populations for the presence of two mutations previously associated with METI-I resistance in spider mites but none of these mutations could be detected. To conclude, moderate to high levels of fenpyroximate resistance were observed in *P. ulmi* populations from Iran, with increased detoxification most likely underlying fenpyroximate resistance.

Keywords *Panonychus ulmi* · Fenpyroximate resistance · Detoxification enzymes · METI-I · PSST

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Introduction

The European red mite, *Panonychus ulmi* (Koch), is a major destructive pest in fruit tree orchards worldwide (Jeppson et al. 1975; Arbabi et al. 2004; Van Leeuwen et al. 2015). Synthetic acaricides are mainly used for the control of *P. ulmi* and related spider mites such as *Panonychus citri* (McGregor) and *Tetranychus urticae* Koch (Van Leeuwen et al. 2013; Leeuwen et al. 2015; Alavijeh et al. 2020). However, the frequent application of acaricides, combined with the short life cycle, arrhenotokous reproduction, and high fecundity rate of spider mites, has resulted in resistance of spider mites against acaricides (Van Leeuwen et al. 2010).

Pyridaben, fenpyroximate, tebufenpyrad and fenazaquin (IRAC Group 21; Sparks and Nauen 2015) are acaricides that inhibit Complex I (NADH-coenzyme Q oxidoreductase) of the oxidative phosphorylation pathway. They are also known as METI-Is (mitochondrial electron transport inhibitors of complex I) and were launched in the 1990s (Konno et al. 1990; Hirata et al. 1995). However, shortly after their introduction, the first cases of METI-I resistance were reported and METI-I resistance continues to be a growing problem (Devine et al. 2001; Stumpf and Nauen 2001; Kim et al. 2004; Sato et al. 2004; Bajda et al. 2017; Alavijeh et al. 2020). Recently, cross-resistance between METI-Is and acaricides that target Complex II of the oxidative phosphorylation pathway (METI-IIs, mitochondrial election transport inhibitors of complex II) has been described (Khalighi et al. 2014, 2016; Sugimoto and Osakabe 2014). The first reports on investigation of METI-I resistance mechanisms were based on synergism and detoxification enzyme assays, and concluded that METI-I resistance was associated with increased activity of P450s (Ozawa 1994; Herron and Rophail 1998; Devine et al. 2001; Van Pottelberge et al. 2009). In addition, enhanced P450 activity was shown to contribute to cross-resistance between METI-Is (Stumpf and Nauen 2001; Kim et al. 2004; Van Pottelberge et al. 2009) and between METI-Is and METI-IIs (Khalighi et al. 2014; Sugimoto and Osakabe 2014). However, in 2015, Bajda et al. identified a mutation in the PSST subunit of Complex I (H92R, *Yarrowia lipolytica* numbering) that was strongly associated with *T. urticae* resistance against METI-Is (Bajda et al. 2017). More recently, the H92R mutation and another PSST mutation, A94V, has been detected in fenpyroximate resistant *P. citri* populations (Alavijeh et al. 2020). The role of both PSST mutations in METI-I resistance was assessed by the introduction of these mutations into a mite strain with susceptible background by marker-assisted back-crossing. Both mutations could only explain a part of the total resistance phenotype, and possibly additive or synergistic action of both target-site resistance and increased detoxification is needed to attain high resistance levels (Bajda et al. 2017; Alavijeh et al. 2020). Noteworthy, genome editing of A94V with CRISPR/Cas9 in fruit flies did not result in altered fenpyroximate susceptibility levels (Alavijeh et al. 2020). The H92R mutation, on the other hand, caused lethality in CRISPR/Cas9 modified *Drosophila* lines and recently its role in METI-I resistance was confirmed by a QTL mapping approach (Snoeck et al. 2019).

In this study we aimed to elucidate the status and mechanisms of fenpyroximate resistance in several Iranian populations of *P. ulmi* by performing toxicity and synergism assays, measuring detoxification enzyme activities and PCR screening of PSST mutations, H92R and A94V.

Materials and methods

Field populations of European red mites, and chemicals

During spring and summer 2016–2019, several populations of *P. ulmi* were collected from major apple-producing areas in Iran where acaricide use was the main control measure, including East Azarbaijan, West Azarbaijan and Isfahan Provinces as described earlier (Badieinia et al. 2020), and also three new populations: Urmia2 (West Azarbaijan), Semirom3 and Padena (Isfahan Province) (Fig. 1). In addition, we also included a spirodiclofen-selected laboratory strain of *P. ulmi* (PSR-TK) (Kramer and Nauen 2011). Upon arrival in the laboratory, all collected populations were reared on apple leaf discs (*Malus domestica* var. Fuji) at 25 ± 1 °C, L16:D8 h photoperiod, and 60 ± 10% RH.

A commercial formulation of fenpyroximate (50 g a.i. L^{-1} SC, Ortus) was used in toxicity bioassays. Synergism assays were performed using piperonyl butoxide (PBO), diethyl maleate (DEM) (Sigma-Aldrich, Bornem, Belgium), and triphenyl phosphate (TPP) (Merck, Darmstadt, Germany).

Bioassay and synergism assay

The susceptibility of *P. ulmi* adult females to fenpyroximate was determined by the method of Van Leeuwen et al. (2004). Briefly, using a Potter spray tower (Burkard Scientific, Uxbridge, UK) the upper side of square apple leaf discs (3.5 × 3.5 cm) was treated with different concentrations of fenpyroximate (1.5 mL, 1 bar pressure, 1.46 ± 0.05 mg spray fluid deposit cm^{-2}), then 10–20 young female mites were transferred onto each leaf disc. Each concentration was replicated 3–4 × on separate days. The treated discs were put in a climatically controlled room at 25 ± 1 °C, 60% RH and L16:D8 h photoperiod. Mortality (defined as unable to move after being prodded with a fine brush) was assessed 24 h after

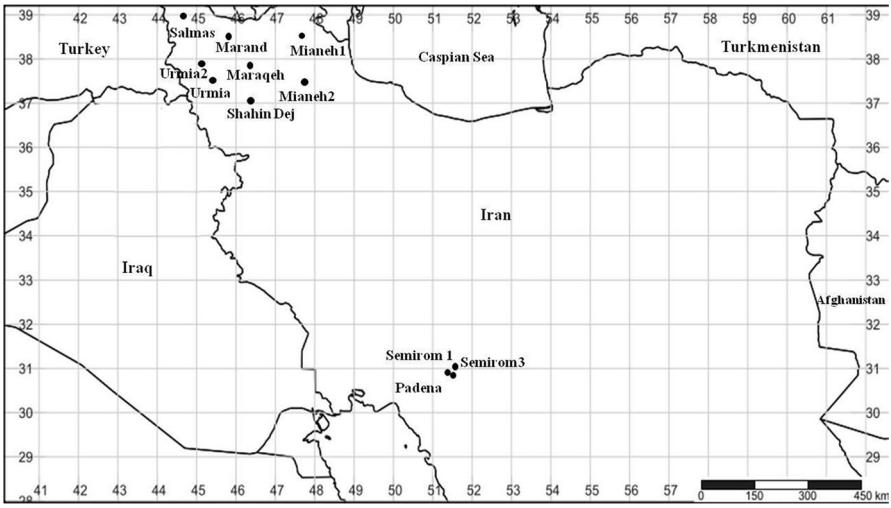

Fig. 1 Collection sites of *Panonychus ulmi* populations from commercial apple orchards in Iran

treatment. Distilled water was used as control and recorded mortality in control assays was always less than 10%.

For synergism experiments, apple leaf discs were treated with 1000, 500, and 1500 mg L^{-1} concentrations of PBO, DEM, and TPP, respectively, 4 h before performing fenpyroximate toxicity tests as described above. Before use, synergists (PBO, DEM, and TPP) were dissolved in acetone and diluted in distilled water. Mites pretreated with distilled water + acetone sprayed on leaf discs were used as control (control mortality never exceeded 10% mortality). Based on the preliminary tests, synergist concentrations of PBO 1000 mg L^{-1}, DEM 500 mg L^{-1} and TPP 1500 mg L^{-1} caused less than 15% mortality. Although it is critical to use a validated reference strain with base-line susceptibility to evaluate resistance levels, such a strain was not available and therefore in this study the most susceptible strain was used to estimate resistance levels and synergistic ratios. LC_{50} values, slopes, resistance ratios (RR: by dividing LC_{50} values from resistant population to the most susceptible strain), synergistic ratios (SR: by dividing LC_{50} values from experiments with and without synergists) and 95% confidence intervals (95% CIs) were determined by probit analysis using POLO-Plus in comparison to the least resistant strain (LeOra Software, Berkeley, CA, USA). If the 95% CIs for LC_{50} ratios included 1 the LC_{50}'s were considered not significantly different (Robertson et al. 2017).

Biochemical assays

Carboxylesterase (CarE) and glutathione *S*-transferases (GSTs) activity was measured using the substrates α-naphthyl acetate (α-NPA) and 1-chloro-2,4-dinitrobenzene (CDNB), respectively (Van Leeuwen et al. 2006; Habig et al. 1974), whereas P450 monooxygenase (P450) activity was indirectly measured by quantification of heme peroxidase activity using 3,3′5,5′-tetramethylbenzidine (TMBZ) (William and Janet 1997), with some modifications. The enzyme sources for CarEs, GSTs and P450s assays were prepared by homogenizing 50 adult female mites of *P. ulmi* in sodium phosphate buffer (300 µL, 0.1 M, pH 7.4) containing 0.1% Triton X-100, sodium phosphate buffer (300 µL, 0.1 M, pH 7.4), and sodium potassium phosphate buffer (400 µL, 0.1 M, pH 7.1), respectively. Next, homogenates were centrifuged at 12,000 × *g* (CarEs and GSTs) and 10,000 × *g* (P450s) for 15 min at 4 °C. Absorbance was recorded with a Unico 1200 Spectrophotometer (UNICO, Dayton, NJ, USA) at 450 nm (CarEs and P450s) and 340 nm (GSTs) at room temperature (25 °C). Total protein concentrations were determined using the Bradford method (1976), with bovine serum albumin as standard. All enzymatic assays and measurement of protein concentrations were repeated at least 3 ×. Significant differences in detoxifying enzymes levels were determined by analysis of variance (ANOVA) followed by least significant difference (LSD) mean separation ($\alpha = 0.05$) with SAS v.9.4 (SAS Institute, Cary, NC, USA).

Molecular assays

Genomic DNA was extracted from 100 to 150 adult female mites of *P. ulmi*, according to Murray and Thompson (1980). For the detection of PSST mutations, we used the previously described primer pair PSST_A94V_F (5′-AAC GAT GAA CAC AAT AGG T-3′) and PSST_A94V_R (5′-GCA ACA GAG TAA GAA TAA TGA-3′), amplifying a partial PSST gene fragment (Alavijeh et al. 2020). PCR reactions were performed in 20 µL final volume with 10 µL Master mix (Ampliqon, Odense, Denmark), 1 µL of each primer, 2 µL template DNA, with the following cycling conditions: 2 min at 94 °C,

followed by 40 cycles of 30 s at 94 °C, 25 s at 48 °C, 45 s at 72 °C and 5 min of final extension at 72 °C. Reactions were carried out by using Bio-Rad thermocycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR products of Padena, Semirom3, PSR-TK, and Marand populations were sequenced by Microsynth (Balgach, Switzerland) using Sanger sequencing technology, with the same primers as used in the amplification. Sequencing data were analyzed using BioEdit v.7.0.1 software (Hall 1999).

Results

Fenpyroximate toxicity in *Panonychus ulmi* populations and synergism assays

Probit analysis showed that fenpyroximate toxicity varied significantly across *P. ulmi* populations, with LC_{50}'s ranging between 121.8 and 5713.9 mg a.i. L^{-1}. Fenpyroximate was most toxic to the Urmia population and, hence, this population was considered as the most susceptible population to calculate resistance ratios (RRs). The highest RRs were found for the Padena, Urmia2, Mianeh2, Semirom3, Shahin Dej, Mianeh1 and PSR-TK populations (14.3- to 46.9-fold) whereas moderate RRs were detected for the Maraqeh and Semirom1 populations (7.66- and 6.1-fold, respectively) (Table 1).

Effects of PBO, TPP and DEM pre-treatment on fenpyroximate toxicity in the Urmia, Salmas, PSR-TK, Shahin Dej and Semirom3 populations are shown in Table 3. PBO enhanced fenpyroximate toxicity 1.7- and 6.7-fold in the Semirom3 and PSR-TK populations, respectively. TPP synergized the toxicity by 6.1-fold in the Shahin Dej population and caused lower synergism ratios (SRs) in Semirom3 and PSR-TK (2.3- and 2.7-fold, respectively). DEM also significantly synergized fenpyroximate toxicity in Semirom3 (3.6-fold) and PSR-TK (2.2-fold) (Table 2).

Table 1 Log-dose probit-mortality data for fenpyroximate tested against female *Panonychus ulmi* adults from various field populations of Iran

Population	n[a]	LC_{50} (mg a.i. L^{-1}) [95% CI]	Slope ± SE	χ^2 (df)	RR (95% CI)
Urmia	230	121.8 [61.61–193.66]	1.0 ± 0.16	0.6 (4)	–
Salmas	217	154.6 [101.60–233.53]	0.9 ± 0.14	2.8 (3)	1.3 (0.56–2.88)
Marand	221	183.6 [50.83–453.20]	0.8 ± 0.13	5.1 (3)	1.6 (0.62–3.67)
Semirom1	196	743.6 [479.28–1106.20]	1.0 ± 0.16	1.4 (3)	6.1 (2.70–13.84)
Maraqeh	184	934.0 [583.96–1932.93]	0.8 ± 0.22	0.8 (3)	7.7 (3.10–19.13)
PSR-TK	195	1748.2 [958.00–3465.45]	1.0 ± 0.17	3.2 (3)	14.3 (6.32–36.36)
Mianeh1	275	2078.0 [583.96–1932.93]	0.6 ± 0.14	1.3 (3)	17.0 (5.12–56.83)
Shahin Dej	132	2539.8 [1645.70–4296.48]	1.9 ± 0.30	1.2 (3)	20.5 (8.95–48.50)
Semirom3	198	2591.3 [1493.20–4161.43]	1.4 ± 0.25	3.7 (3)	21.3 (10.12–44.70)
Mianeh2	174	2831.5 [1880.10–5259.80]	1.1 ± 0.23	2.7 (3)	23.3 (9.74–55.50)
Urmia2	91	3707.9 [2227.90–6971.10]	1.1 ± 0.28	3.0 (3)	30.4 (12.35–75.00)
Padena	121	5713.9 [4650.50–6827.53]	2.9 ± 0.68	0.2 (3)	46.9 (23.52–93.50)

RR resistance ratio = LC_{50} of target population/LC_{50} of Urmia population

[a]Total number of mites used for all concentrations, including controls

Table 2 Synergistic effect of PBO (1000 mg L^{-1}), TPP (1500 mg L^{-1}), and DEM (500 mg L^{-1}) on fenpyroximate resistance in *Panonychus ulmi* populations, compared to the most susceptible population of Urmia

Population	Treatment	n[a]	LC_{50} (mg a.i. L^{-1}) [95% CI][b]	Slope ± SE	χ^2 (df)	RR (95% CI)	SR (95% CI)
Urmia	Fenpyroximate	230	121.8 [61.61–193.66]	1.0 ± 0.16	0.6 (4)		
	+ PBO	231	112.3 [51.24–216.24]	1.3 ± 0.18	7.1 (3)		1.1 (0.52–2.26)
	+ TPP	134	112.3 [51.24–216.24]	1.3 ± 0.18	7.1 (3)		0.7 (0.24–1.86)
	+ DEM	202	170.4 [87.98–293.44]	1.3 ± 0.16	3.8 (3)		0.4 (0.17–0.82)
Salmas	Fenpyroximate	217	154.6 [101.60–233.53]	0.9 ± 0.14	2.8 (3)	1.3 (0.56–2.88)	
	+ PBO	167	189.9 [149.00–243.68]	1.9 ± 0.32	2.5 (4)	1.7 (1.08–2.63)	0.8 (0.46–1.44)
	+ TPP	207	157.8 [51.51–324.97]	1.2 ± 0.15	6.3 (3)	0.9 (0.36–2.08)	1.0 (0.52–1.83)
	+ DEM	174	105.6 [48.48–190.97]	1.2 ± 0.20	3.4 (3)	0.03 (0.010–0.060)	1.5 (0.73–2.93)
PSR-TK	Fenpyroximate	195	1748.2 [958–3465.45]	1.0 ± 0.17	3.2 (3)	14.3 (6.32–36.36)	
	+ PBO	107	260.0 [117.23–438.43]*	1.3 ± 0.28	0.2 (2)	2.1 (1.03–5.19)	6.7 (2.90-16.16)
	+ TPP	216	652.9 [379.30–438.43]*	1.3 ± 0.28	0.2 (2)	3.6 (1.53–8.39)	2.7 (1.50–4.79)
	+ DEM	229	791.4 [418.72–1938]*	1.1 ± 0.17	4.6 (3)	4.6 (2.48–8.69)	2.2 (1.14–4.29)
Shahin Dej	Fenpyroximate	132	2539.8 [1645.70–4296.48]	1.9 ± 0.30	1.2 (3)	20.5 (8.95–48.50)	
	+ PBO	138	1541.0 [1044.00–2311.71]	1.4 ± 0.33	0.2 (2)	13.7 (7.87–23.92)	1.7 (0.95–3.29)
	+ TPP	168	418.2 [325.58–551.38]*	1.9 ± 0.30	1.3 (2)	2.3 (0.98–5.33)	6.1 (3.28–11.24)
	+ DEM	190	1426.4 [1083.97–1873.48]	1.7 ± 0.31	0.2 (2)	8.4 (4.92–14.24)	1.8 (0.96–3.31)
Semirom3	Fenpyroximate	198	2591.3 [1493.20–4161.43]	1.4 ± 0.25	3.7 (3)	21.3 (10.12–44.70)	
	+ PBO	137	1560.1 [1092.83–2117.51]*	1.6 ± 0.30	0.8 (2)	13.9 (8.35–23.10)	1.7 (1.36–1.96)
	+ TPP	204	1112.9 [743.10–1517.01]*	1.3 ± 0.23	0.9 (2)	6.1 (2.51–14.78)	2.3 (1.36–3.97)
	+ DEM	178	720.7 [459.74–1119.54]*	0.9 ± 0.17	1.6 (3)	4.3 (2.17–8.24)	3.6 (1.93–6.68)

RR resistance ratio = LC_{50} of target population/LC_{50} of susceptible (Urmia) population, *SR* synergistic ratio = LC_{50} of fenpyroximate/LC_{50} of [synergist + fenpyroximate]

[a]Total number of mites used for all concentrations, including controls

[b]An asterisk indicates whether there is a significant difference between [synergist + fenpyroximate] treatment and treatment with fenpyroximate alone (based on 95% CI of SR)

Table 3 Mean (±SEM) detoxification enzyme activities in various populations of *Panonychus ulmi*

Population	Esterases		GSTs		P450s	
	α-NA (nmol min^{-1} mg $protein^{-1}$)	Ratio	CDNB (nmol min^{-1} mg^{-1} protein)	Ratio	TMBZ (U mg^{-1} protein)	Ratio
Urmia	5362.2 ± 395.72a		233.7 ± 35.30ab		15.4 ± 1.30a	
Marand	3539.3 ± 121.90a	0.66	126.7 ± 57.78b	0.54	4.8 ± 1.38b	0.31
PSR-TK	5466.7 ± 58.91a	1.01	373.9 ± 23.84a	1.59	6.7 ± 0.71b	0.43
Shahin Dej	3714.5 ± 98.15a	0.69	404.1 ± 49.61a	1.72	7.6 ± 0.55b	0.49
Semirom3	4631.3 ± 916.54a	0.86	227.5 ± 38.45ab	0.97	14.8 ± 0.68a	0.96

Means within a column followed by the same letter are not significantly different (LSD test: $P > 0.05$)

Enzyme activity assays

The in vitro activities of CarEs, GSTs and P450s in the Urmia, Marand, PSR-TK, Shahin Dej and Semirom3 populations are presented in Table 3. The PSR-TK and Shahin Dej populations showed higher GST activities (1.6- and 1.7-fold, respectively), whereas CarEs and P450 activities were not significantly higher in the resistant population compared to the fenpyroximate susceptible population Urmia.

Target site mutations

A few *P. ulmi* populations, exhibiting different resistance levels to fenpyroximate, were screened for the presence of PSST mutations. None of the screened populations—Padena, Semirom3, PSR-TK and Marand—carried the H92R or A94V mutation.

Discussion

The European red mite is an economic pest of apple orchards and its control is strongly dependent on the application of acaricides. Resistance to acaricides belonging to different mode of action groups has been previously reported for Iranian *P. ulmi* populations (Badieinia et al. 2020; Rameshgar et al. 2019a, b). However, the resistance status of Iranian *P. ulmi* populations to fenpyroximate, an acaricide that has been registered in Iran since 1996 to control *P. ulmi* in pome and stone fruit orchards (Nourbakhsh 2019), has not yet been monitored. In this study, *P. ulmi* populations from Iran exhibited low to high resistance levels to fenpyroximate. The lowest LC_{50} was detected in the Urmia population and the highest RR was found for the Padena population (46.9-fold). For *T. urticae* and *P. citri*, higher resistance ratios against fenpyroximate have been reported (i.e., >500- and >76-fold, respectively) (Nauen et al. 2001; Stumpf and Nauen 2001; Van Pottelberge et al. 2009; Alavijeh et al. 2020). However, in these studies, fenpyroximate toxicity towards the most susceptible population was much higher (LC_{50} of 6.7 mg a.i. L^{-1} for *P. citri* Alavijeh et al. 2020 and LC_{50} of 6.8 mg a.i. L^{-1} for *T. urticae* Van Pottelberge et al. 2009). Considering that Kumral and Kovanci (2007) observed a fenpyroximate LC_{50} of 3.0 mg a.i. L^{-1} against *P. ulmi*, it is reasonable to speculate that the

susceptible Urmia population included in this study is in fact moderately resistant to fenpyroximate.

The role of P450s in METI-I resistance has previously been documented using either PBO synergism tests or by measuring P450 activity (Stumpf and Nauen 2001; Kim et al. 2004; Van Pottelberge et al. 2009). It was also shown that an in vitro expressed P450, CYP392A11, was able to hydroxylate fenpyroximate and its ectopic expression in *Drosophila* flies conferred resistance to fenpyroximate (Riga et al. 2015), providing functional evidence for its role in fenpyroximate resistance. In this study, PBO could only strongly synergize fenpyroximate toxicity in the PSR-TK strain and minor differences were found between P450 activity of the resistant Iranian populations and the susceptible Urmia population, suggesting either a higher basal expression level per se or that the role of P450s in fenpyroximate resistance in *P. ulmi* populations from Iran is minimal. Of peculiar note, Kramer and Nauen (2011) reported that spirodiclofen resistance in PSR-TK, mediated by P450 monooxygenase detoxification, also provided relatively high cross-resistance to the METI-I compound tebufenpyrad (RR 30-fold). On the other hand, Alavijeh et al. (2020) found that P450s did not seem to have an important role in fenpyroximate resistance in Iranian *P. citri* populations. Alternatively, it is also possible that some P450 enzymes may be involved in metabolizing fenpyroximate but are not inhibited by the synergist PBO.

Esterases have been suggested to play a role in a Korean *T. urticae* strain selected for fenpyroximate resistance (Kim et al. 2004) and in vertebrates ester hydrolysis has been shown to be the key step in fenpyroximate detoxification (Motoba et al. 2000). In line with these studies, TPP pre-treatment, resulted in about 6-fold enhanced fenpyroximate toxicity towards the Shahin Dej population, whereas a 2.7 × and 2.3 × higher fenpyroximate toxicity was observed for the PSR-TK and Semirom3 populations, respectively. However, none of these enhanced toxicities were associated with a higher in vitro esterase activity (Tables 2, 3), suggesting that compositional changes in esterases or qualitative differences are involved in esterase mediated fenpyroximate resistance of Shahin Dej, Semirom3 and PSR-TK. Last, similar to other METI-Is, exposure to fenpyroximate is known to induce oxidative stress (Sherer et al. 2007; Na et al. 2009) and GSTs are known to play a critical role in cellular detoxification against this stress (Vontas et al. 2001; Allocati et al. 2018). The GST inhibitor DEM, significantly synergized fenpyroximate toxicity in the Semirom3 and PSR-TK populations, suggesting that GST overexpression might have a role in fenpyroximate resistance of PSR-TK.

Overall, none of the synergists were able to decrease fenpyroximate resistance to full susceptibility. These results are in line with previous METI-I resistance studies and might suggest that synergists are not able to fully suppress enzymatic detoxification or the synergist concentrations were lower than those needed to completely block detoxification enzymes (Van Pottelberge et al. 2009; Alavijeh et al. 2020). Alternatively, a target-site resistance mechanism might be at play. A mutation, H92R, in the gene encoding the PSST subunit of Complex I has been linked to METI-I resistance (Bajda et al. 2017; Alavijeh et al. 2020). The introgression of H92R mutation into a susceptible background confirmed its role in resistance to fenpyroximate and other METI-I compounds such as pyridaben and tebufenpyrad (Bajda et al. 2017). In addition, another PSST mutation, A94V, was found to be associated with resistance to fenpyroximate in *P. citri* (Alavijeh et al. 2020). PCR sequencing revealed, however, that none of these mutations were present in the *P. ulmi* populations of this study. However, we cannot completely exclude the presence of other mutations in PSST.

Considering the results of this study and our previous studies on the *P. ulmi* resistance to pyrethroids (Rameshgar et al. 2019b), abamectin (Rameshgar et al. 2019a), spirodiclofen

and spiromesifen (Badieinia et al. 2020), most Iranian *P. ulmi* populations have been shown to be multi-acaricide resistant. In general, metabolic resistance mechanisms seemed to be at play, with the exception of pyrethroid resistance, which was linked to an altered target site (Rameshgar et al. 2019b).

To conclude, the fenpyroximate resistance status of *P. ulmi* populations from Iran was monitored and possible resistance mechanisms were investigated for a number of resistant populations. A high synergism ratio was observed for only a few populations but none of the synergists caused fenpyroximate resistance to drop to full susceptibility. Also, enzyme activities of resistant populations showed only minor differences compared to the most susceptible population. Sequencing of the partial PSST subunit did not reveal target-site mutations (H92R and A94V) in the tested populations. Overall, this might suggest that previously undocumented resistance mechanisms are at play. Of particular note, in Alavijeh et al. (2020) an iron-cluster scaffold protein, known to be crucial in Complex I stability and for protection against oxidative stress, was highly overexpressed (more than 60-fold) in fenpyroximate-resistant *P. citri* strains and it might be worth to explore whether this is also the case for the resistant *P. ulmi* populations investigated in this study.

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