J. Agr. Sci. Tech. (2021) Vol. 23(3): 575-588

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Detoxifying Enzyme Activities in the Common Pistachio Psylla and the Coccinellid Predator

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ABSTRACT

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hem: Aphalaridae) is one of the main and most destructive pests of pistachio orchards in Iran. Chemical control is a widely applied method to manage this pest problem. The intensive use of insecticides has led to the development of resistant populations of the common pistachio psylla. In this research, the activities of detoxifying enzymes (general esterase, glutathione S-transferase and cytochrome P_{450}) were assessed against two populations of the common pistachio psylla, and the coccinellid predator, *Oenopia conglobata* L. (Col: Coccinellidae) in Kerman Province, under treatment of three rational insecticides, namely, acetamiprid, spirotetramat, and hexaflumuron in four concentrations (control, LC_{25}, LC_{50} and LC_{75}). The results indicated that the activities of detoxifying enzymes were higher in the resistant population of psylla compared to the susceptible one. Esterase was the predominant detoxifying enzyme in the pest and its predator. Based on the results, the activity of detoxifying enzymes were higher at the higher concentrations of the pesticides. Esterase activity was greater in the psyllid populations than the coccinellid predator; which may indicate a higher sensitivity of the lady beetle to insecticides than its prey.

Keywords: Agonoscena pistaciae, Esterase, Lady beetle, Oenopia conglobata.

INTRODUCTION

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae) is a major pest of pistachio orchards distributed in all pistachio-producing areas of Iran (Burckardt and Lauterer, 1989; Samih *et al*., 2005). *A. pistaciae* has 6–7 generations per year. (Mehrnejad and Copland, 2005). The high rate of reproduction and the easy adaptations to harsh environmental conditions has led to well-adapted exploitation of *A. pistaciae* on the pistachio plantations, particularly that of the winter forms, which allow the establishment of very large colonies in early spring or even late winter (Mehrnejad, 1998).

Among coccinellid beetles, *Oenopia conglobata* L. (Coleoptera: Coccinellidae) is considered as the most abundant predatory beetles in the pistachio orchards in Kerman Province (Mehrnejad, 2007). This coccinellid predator is the prevalent natural enemy of the common pistachio psylla, feeding on both eggs and nymphs (Hodek, 1973; Kabiri Raeis Abbad and Amiri Besheli, 2012).

Chemical control is commonly used for the population management of this pest. However, pesticide application has an adverse effect on natural enemies and the environment and is considered as one of the possible factors influencing the insecticidal susceptibility of the pest (Amirzade *et al*., 2014; Kabiri Raeis Abbad and Amiri

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Besheli, 2012). *A. pistaciae* has a high potential to develop insecticidal resistance due to its short life cycle and high reproductive capacity. Intensive use of insecticides has led to excessive selection pressure for resistance to synthetic insecticides in some populations of the common pistachio psylla (Talebi *et al*., 2001). So far, a large number of chemicals have been developed to protect the crop against insect pests. But, insecticides are toxic to many non-target organisms (Nath *et al*., 1997; Suh *et al*., 2000) and their use can disrupt the balance between a host and its natural enemy, resulting in an increase in pest population density (Tomberlin *et al*., 2002; Van Driesche and Bellowa, 1996). Studies have reported that insecticides cause numerous sublethal effects on the insect pests, including changes in fecundity (Takada *et al*., 2001), developmental rate (Willrich and Boethel, 2001), sex ratio, diapause and morphology (Croft, 1990); and even change in insect detoxifying enzyme activity and insect resistance (Franca *et al*., 2017).

Some insecticides such as spirotetramat, acetamiprid, and hexaflumuron are widely used in the pistachio orchards of Kerman Province, Iran (Noorbakhsh *et al*., 2001). Spirotetramat (Movento®) is a tetramic acid derivative and a new cyclic ketoenol compound introduced against sucking insect pests of agricultural crops. The mode of action of this insecticide is the inhibition of lipogenesis in the treated insects, resulting in lipid content depletion, growth inhibition of younger insects, and reduction in the ability of adults to reproduce (Bruck *et al*., 2009). Acetamiprid belongs to the neonicotinoid group with systemic, translaminar and contact activities. This group acts rapidly as an agonist of the nicotinic acetylcholine receptor in the postsynaptic membrane and results in paralysis and death of the treated insects (Elbert *et al*., 1991; Schroeder and Flattum, 1984). The benzoyl phenyl urea hexaflumuron is an insect growth regulator that interferes with chitin synthesis and interrupts hormonal balance with the molting process modification, inhibiting the insect's growth (Oberlander and Silhacek, 1998). This insecticide is widely used against homopteran insects, especially *A. pistaciae* (Alimohamadi *et al*., 2014).

The resistance of *A. pistaciae* populations to phosalone (Talebi *et al*. 2001, Alizadeh *et al*. 2011), and three common insecticides including spirotetramat, acetamiprid and hexaflumuron (Bemani *et al*., 2018), has been demonstrated in some pistachio producing areas of Kerman Province. The most common type of resistance mechanism in insects has been demonstrated by increasing enzymatic detoxification of insecticides (Devorshak and Roe, 1999; Li *et al*., 2007). A qualitative/quantitative change in detoxifying enzymes leads to resistance (Devonshire and Moores, 1982). The cytochrome P_{450}s, esterases, and Glutathione S-Transferases (GST) contribute to the degradation of insecticides (Ferrari and Georghiou, 1991; Mouches *et al*., 1986).

Keeping these points in mind, the present study aimed to assess the activities of detoxifying enzymes in two populations of the common pistachio psylla in Kerman Province. We also investigated the effects of different concentrations of these insecticides on detoxifying enzyme activities in the fifth instar nymphs of *A. pistaciae* in the Rafsanjan population (as a resistant population) versus a susceptible population (the Anar population) (Bemani *et al*., 2018) and the third instar of its coccinellid predator, *O. conglobata* in Rafsanjan, Iran.

MATERIALS AND METHODS

To choose the populations (resistance and susceptible) of the pest, the bioassay tests were done (Bemani *et al*., 2018). Five populations of *A. pistaciae* collected from Kerman Province, Iran, were chosen based on their LC_{50} of three insecticides including acetamiprid, spirotetramat, and hexaflumuron against the 5th instar nymphs, based on which the least values (11.92, 24.13 and 81.06 mg ai L^{-1}, respectively)

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were considered as susceptible population (from Anar) and the greatest (40.55, 43.65 and 95.10 mg ai L^{-1}, respectively) as the resistant population (from Rafsanjan). Fifth instar nymphs were used because they were recognizable from the other nymphs and their mortality was low. The experiments were performed with four replications, each comprising twelve nymphs (Bemani *et al*., 2018). Adult of the predatory beetle, *O. conglobata*, were collected from pistachio orchards of the Rafsanjan area and the LC_{50} values of the third instar larvae were 8.76, 5218.33 and 2268.81 mg ai L^{-1}, for acetamiprid, spirotetramat, and hexaflumuron, respectively. Bioassays on the lady beetle were performed with three replications, each comprising 10 to 12 larvae (Bemani *et al*., 2018).

The nymphs of the common pistachio psylla *A. pistaciae* were collected randomly from pistachio gardens of Rafsanjan and Anar, Iran (based on bioassay results). To remove the effects of previous chemical treatments, it was assured that the selected gardens had received no chemical pesticides one year before the onset of experiments. To obtain the fifth instar nymphs of similar age, pistachio leaf cuttings containing nymphs were maintained within ventilated plastic boxes (20×25×10 cm) in a growth chamber at 25±2°C, 50-60% RH and the photoperiod cycle of 16:8 L: D. Adults of the predatory beetle, *O. conglobata* were collected from pistachio gardens in Rafsanjan, Iran. Adults were reared in ventilated plastic boxes (20×25×10 cm) at 25±2°C, 65±5% RH with the photoperiod cycle of 16:8 L:D. They were provided with *A. pistaciae* as food and maintained for 3 weeks to adapt to the laboratory conditions. Leaf cutting carrying eggs were then removed and transferred to the new plastic box. After hatching, developmental stages were monitored daily and third instar larvae were chosen for enzymatic tests. In order to do enzymatic tests, one-day-old fifth instar nymphs and one-day-old third instar larvae were transferred into Petri dishes and sprayed with 1 mL of aqueous emulsions of different concentrations (LC_{25}, LC_{50}, and LC_{75}) of acetamiprid, spirotetramat, and hexaflumuron (concentrations explained in Tables 1 to 4). The spray was applied at 15 mbar using the Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK). Distilled water was used as a control treatment. The treated nymphs were transferred to fresh leaves of pistachio and the larvae were transferred to fresh pistachio leaves containing untreated psylla and maintained in a controlled climate chamber (25±2°C, 50-60% RH, 16:8 L:D). Other experiments were conducted after 24 hours for acetamiprid and 48 hours for spirotetramat and hexaflumuron due to their different modes of action. These experiments were conducted during the summer of 2016.

Esterase Activity

Fifth instar nymphs of *A. pistaciae* (N= 50) from two populations (resistant and susceptible) and third instar larvae of *O. conglobata* (N= 1) were homogenized in a 110 µL buffer of sodium phosphate containing 0.1% of triton X-100 (10 mM, pH 7). Then, the homogenates were centrifuged at 15,000×g for 10 minutes. The resulting supernatants were used as the enzyme source in all enzyme assays. The protein content of the enzyme sample was determined by a standard method (Lowry *et al*., 1951) in which Bovine Serum Albumin (BSA) was used as the standard.

Hydrolytic activities against the substrates, α-Naphthyl acetate, and β-Naphthyl acetate, were measured following a standard method (Van-Asperen, 1962) with some modifications. Enzyme assays were done with an addition of 50 µL of enzyme sample to 100 µL phosphate buffer (pH 7, 0.1M) and 10 µL of substrates (10 mM in acetone). Fast blue RR (50 µL, 0.5 mg mL^{-1} in buffer) was then added to the reaction mixture and the released naphthol was continuously measured at 450 nm every 5 minutes for 20 minutes using a microplate reader (Epoch,

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Table 1. Effect of acetamiprid on detoxifying enzyme activity of two populations of *Agonoscena pistaciae*.

Population	Insecticide concentration (mg ai L^{-1})	Enzyme activity (μmol min^{-1} mg^{-1} protein)±SE [a]			
		Esterase (α-)	Esterase (β-)	GST	Cytochrome P_{450}
Rafsanjan	0	0.24±0.08aA	3.38±0.78aB	0.20±0.08aA	0.006±0.001aA
	24	0.29±0.07a	4.13±0.83a	0.21±0.07a	0.006±0.001a
	40	0.35±0.09a	6.66±1.12ab	0.25±0.06a	0.008±0.000a
	70	0.46±0.09a	8.42±1.90b	0.27±0.06a	0.008±0.000a
Anar	0	0.19±0.00aA	2.86±0.21aB	0.19±0.04aA	0.005±0.000aA
	3	0.28±0.03b	3.65±0.28b	0.23±0.03a	0.005±0.000a
	11.9	0.31±0.04b	3.80±0.25b	0.28±0.03a	0.006±0.000a
	24	0.40±0.04c	5.16±0.23c	0.28±0.10a	0.006±0.001a

[a] Lowercase letters within columns compare concentrations on each psylla population (LSD's test), whereas capital letters between the same rows compare enzyme activities on control on each psylla population. Means±SE followed by the same lowercase or capital letter did not differ significantly ($P< 0.05$). Esterase (α-)= Esterase with α-naphthyl acetate as a substrate; Esterase (β-)= Esterase with β-naphthyl acetate as a substrate; GST= Glutathione S-Transferase, SE= Standard Error.

Table 2. Effects of spirotetramat on detoxifying enzyme activity of two populations of *Agonoscena pistaciae.*

Population	Insecticide concentration (mg ai L^{-1})	Enzyme activity (μmol min^{-1} mg^{-1} protein)±SE [a]			
		Esterase (α-)	Esterase (β-)	GST	Cytochrome P_{450}
Rafsanjan	0	0.24±0.08aA	3.38±0.78aB	0.20±0.08aA	0.006±0.001aA
	15	0.28±0.02a	4.70±0.46a	0.21±0.04a	0.006±0.000a
	43	0.39±0.03a	4.73±0.33a	0.22±0.02a	0.006±0.001a
	90	0.44±0.06a	5.32±0.45a	0.24±0.04a	0.007±0.001a
Anar	0	0.19±0.00aA	2.86±0.21aB	0.19±0.04aA	0.005±0.000aA
	10	0.27±0.08a	3.95±0.57a	0.25±0.04a	0.005±0.000a
	24	0.31±0.05a	4.19±0.36a	0.24±0.09a	0.005±0.001a
	50	0.33±0.09a	4.22±0.47a	0.27±0.06a	0.006±0.001a

[a] As explained under Table 1.

Table 3. Effect of hexaflumuron on detoxifying enzyme activity of two populations of *Agonoscena pistaciae*.

Population	Insecticide concentration (mg ai L^{-1})	Enzyme activity (μmol min^{-1} mg^{-1} protein)±SE[a]			
		Esterase (α-)	Esterase (β-)	GST	Cytochrome P_{450}
Rafsanjan	0	0.24±0.08aA	3.38±0.78aB	0.20±0.08aA	0.006±0.001aA
	60	0.29±0.03a	3.86±0.52a	0.20±0.02a	0.006±0.000a
	95	0.33±0.05a	3.90±0.49a	0.21±0.03a	0.005±0.000a
	160	0.39±0.03a	4.49±1.05a	0.23±0.06a	0.005±0.001a
Anar	0	0.19±0.00aA	2.86±0.21aB	0.19±0.04aA	0.005±0.000aA
	40	0.28±0.08a	3.96±0.93a	0.21±0.02a	0.006±0.000a
	81	0.25±0.04a	4.14±0.55a	0.22±0.07a	0.006±0.000a
	130	0.34±0.06a	4.67±0.42a	0.27±0.09a	0.006±0.001a

[a] As explained under Table 1.

Gen 5). A standard curve of absorbance versus concentrations of naphthol was constructed to enable calculation of the amount of naphthol produced during the esterase assay. The experiment was performed with four replications.

Glutathione S-Transferase (GST) Activity

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Table 4. Effects of insecticides on detoxifying enzyme activity of coccinellid predator, *Oenopia conglobata*.

Insecticide	Concentration (mg ai L^{-1})	Enzyme activity (μmol min^{-1} mg^{-1} protein)±SE [a]			
		Esterase (α-)	Esterase (β-)	GST	Cytochrome P_{450}
Acetamiprid	0	0.14±0.02aBC	2.71±0.11aD	0.24±0.02aBC	0.003±0.000aAB
	2	0.23±0.05a	3.04±0.36a	0.27±0.01a	0.004±0.001a
	8.7	0.29±0.06a	3.71±0.27a	0.32±0.07ab	0.004±0.000a
	20	0.37±0.06a	4.16±0.53a	0.46±0.06b	0.005±0.000a
Spirotetramat	0	0.14±0.02aBC	2.71±0.11aD	0.24±0.02aBC	0.003±0.000aAB
	2000	0.17±0.02a	2.98±0.27a	0.31±0.08a	0.004±0.000a
	5218	0.22±0.03a	3.80±0.28a	0.32±0.00a	0.004±0.000a
	8500	0.28±0.05a	4.06±0.58a	0.34±0.13a	0.005±0.000a
Hexaflumuron	0	0.14±0.02aBC	2.71±0.11aD	0.24±0.02aBC	0.003±0.000aAB
	500	0.19±0.03a	3.00±0.18a	0.31±0.08a	0.004±0.000ab
	2268	0.21±0.02a	3.34±0.28a	0.34±0.03a	0.005±0.000b
	6000	0.29±0.05a	3.82±0.37a	0.37±0.11a	0.005±0.000b

[a] As explained under Table 1

Fifth instar nymphs of *A. pistaciae* (N= 50) from two populations (resistant and susceptible) and third instar larvae (N= 1) of *O. conglobata* were homogenized in 200 μL of ice-cold phosphate buffer (pH 7, 10 mM). Then, centrifuged at 10,000×g for 10 minutes, GST activity was measured using 1-Chloro-2,4-DiNitroBenzene (CDNB) and reduced GSH as substrates with slight modifications of a standard method (Habig *et al*., 1976). Reaction mixtures (200 μL) containing 1 mM CDNB and 5 mM GSH in 0.1M sodium phosphate buffer, pH 7, were placed in a well containing 10 μL of the enzyme sample. The change in absorbance was measured continuously every 1 minute for 10 minutes at 340 nm. Four replicates were performed for each population. Changes in optical density due to GST activity per individual were converted to μmol CDNB conjugated/min/mg protein using the extinction coefficient of 2,4-dinitrophenyl glutatione (ε_{340nm}= 9.6 mM^{-1} cm^{-1}). The experiment was performed with four replications.

Cytochrome P_{450} (General Oxidase) Assay

Cytochrome P_{450} activity was measured and expressed as a general oxidase level, which is an indirect method of cytochrome P_{450} measurement by using heme peroxidation (Brogdon *et al*., 1997; Penilla *et al*., 2007). The heme peroxidation method was considered as a tool for comparing the differences in general oxidase levels based on the hemoprotein levels (Casimiro *et al*., 2006; Penilla *et al*., 2007). Since the heme constitutes the majority of cytochrome P_{450} in nonblood-fed insects, measurement of heme activity was used to compare the levels of cytochrome P_{450} on the basis of general oxidase levels (Brogdon *et al*., 1997). Heme peroxidase activity was measured by using 3,3′,5,5′-tetra-methyl benzidine (TMBZ) (Sigma Aldrich) as the substrate.

Cytochrome P_{450} activity assay was performed using a standard method (Martin *et al*., 2003). Cytochrome C was used to construct the standard curve. The reaction mixture consisted of 50 μL phosphate buffer (100 mM, pH 7.2), 50 μL enzyme, 150 μL TMBZ solution and 25 μL hydrogen peroxide 3%. After a 30 minutes incubation period, absorbance was read as an endpoint at 630 nm. The experiment was done with four replications.

Statistical Analysis

Data were initially tested for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene's test) before subjecting them to ANOVA. Statistical

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analyses were performed using a one-way Analysis Of Variance (ANOVA) followed by a post-hoc LSD's test (P= 0.05). A Student's t-test was applied to compare the means of the two groups when necessary. The results were expressed as mean ±SE and considered to be significantly different at P< 0.05.

RESULTS

The General Esterases

The activity of general esterases in the Rafsanjan (as resistant) and the Anar (as susceptible) populations of the common pistachio psylla and its coccinellid predator were measured. The esterase activity level in the Rafsanjan population was higher than the Anar population when α-naphthyl acetate (1.24 fold) and β-naphthyl acetate (1.18 fold) were used as substrates (Figure 1), but differences in the esterase activity levels among the psyllid populations were not significant (t= -0.590, P= 0.596 for α-naphthyl and t= 0.646, P= 0.542 for β-naphthyl acetate). Furthermore, the esterase activity level in the Rafsanjan population of the psylla was 1.64 and 1.24 fold higher than its coccinellid predator population when α-naphthyl acetate and β-naphthyl acetate were utilized as substrates, respectively (t= -1.157, P= 0.323 for α-naphthyl and t= 0.854, P= 0.426 for β-naphthyl acetate) (Figure 2). The esterase activity in the Anar population with α-naphthyl acetate as a substrate was significantly affected by four concentrations (control, LC_{25}, LC_{50} and LC_{75}) of acetamiprid (F= 8.855, P= 0.002), but not by the other two insecticides (F= 0.815, P= 0.510 for spirotetramat and F= 1.277, P= 0.327 for hexaflumuron). In the Rafsanjan population of the psyllid and its coccinellid predator, esterase activity with α-naphthyl acetate as a substrate was not affected by different concentrations of the insecticides (F= 1.275, P= 0.327 for acetamiprid, F= 3.197, P= 0.062 for spirotetramat, and F= 1.639, P= 0.233 for hexaflumuron in the Rafsanjan population) (Tables 1-3); (F= 3.457, P= 0.051 for acetamiprid, F= 3.253, P= 0.060 for spirotetramat and F= 3.414, P= 0.053 for hexaflumuron in coccinellid predator) (Table 4).

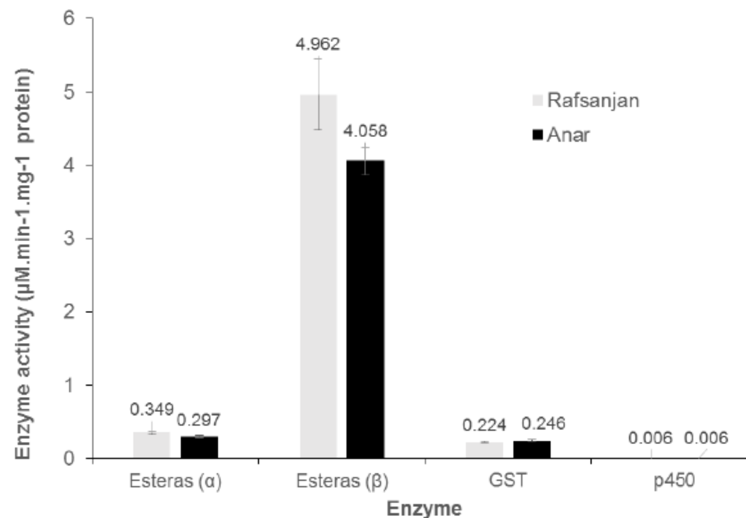

Figure 1. Difference in the activity of detoxifying enzymes in two populations (the Rafsanjan as resistant and the Anar as susceptible populations) of *Agonoscena pistaciae* that compare with a Student's t-test. Esterase (α-)= Esterase with α-naphthyl acetate as a substrate; Esterase (β-)= Esterase with β-naphthyl acetate as a substrate, GST= Glutathione S-Transferase.

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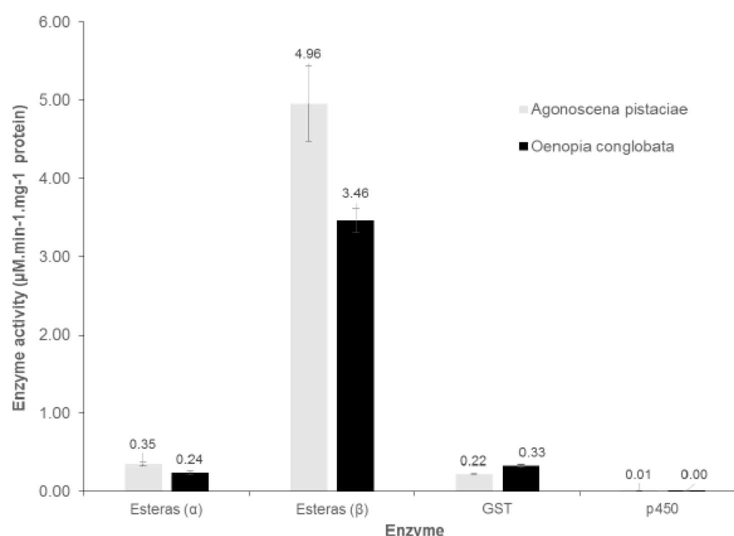

Figure 2. Difference in the activity of detoxifying enzymes in the psylla (the Rafsanjan population), *Agonoscena pistaciae* and coccinellid predator, *Oenopia conglobata* (from Rafsanjan) that compare with a Student's t-test. Esterase (α-)= Esterase with α-naphthyl acetate as a substrate; Esterase (β)= Esterase with β-naphthyl acetate as a substrate, GST=Glutathione S-Transferase.

In addition, the esterase activity in the Rafsanjan and Anar populations with β-naphthyl acetate as a substrate were influenced by different doses of acetamiprid (F= 3.480, P= 0.050 for Rafsanjan and F= 15.545, P= 0.000 for Anar population), but not by the other two insecticides (F= 2.382, P= 0.121 for spirotetramat and F= 0.373, P= 0.774 for hexaflumuron in Rafsanjan population); (F= 2.275, P= 0.133 for spirotetramat and F= 1.636, P= 0.233 for hexaflumuron in Anar population) (Table 1-3). In the coccinellid predator, however, none of the insecticides influenced the esterases activity level (F= 3.363, P= 0.055 for acetamiprid, F= 3.246, P= 0.060 for spirotetramat and F= 3.473, P= 0.051 for hexaflumuron) (Table 4).

Glutathione S-Transferase

The activities of GST in two populations of psylla and *O. conglobata* (its coccinellid predator) were measured. No significant difference in GST activity was recorded among the populations. The activity of GST in the Rafsanjan population was 1.05 fold higher than the Anar population (t= 0.138, P= 0.894) (Figure 1). GST activity in the coccinellid predator population was 1.17 fold higher than the Rafsanjan population (t= 0.419, P= 0.701) (Figure 2).

The GST activity in the Rafsanjan and Anar populations was not affected by four concentrations (control, LC_{25}, LC_{50} and LC_{75}) of insecticides (F= 0.218, P= 0.882 for acetamiprid, F= 0.108, P= 0.954 for spirotetramat and F= 0.064, P= 0.978 for hexaflumuron in the Rafsanjan) (F= 0.599, P= 0.627 for acetamiprid, F= 0.331, P= 0.803 for spirotetramat and F= 0.421, P= 0.741 for hexaflumuron in the Anar) (Table 1-3). In the coccinellid predator population, this activity was affected by acetamiprid concentrations (F= 4.090, P= 0.032), but not by the other two insecticides (F= 0.361, P= 0.782 for spirotetramat and F= 0.642, P= 0.603 for hexaflumuron) (Table 4).

Cytochrome P_{450}

The cytochrome P_{450} activity in both populations of the psylla and its predator was estimated. Results showed that there

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was no significant difference in the cytochrome P_{450} activity among the populations. The activity of cytochrome P_{450} in the Rafsanjan population was 1.16 fold higher than the Anar population (t= 0.647, P= 0.542) (Figure 1) and 1.80 fold higher than the coccinellid predator population (t= 2.285, P= 0.062) (Figure 2).

The activity of cytochrome P_{450} in both Rafsanjan and Anar populations was not affected by four concentrations (control, LC_{25}, LC_{50} and LC_{75}) of the insecticides (F= 1.867, P= 0.191 for acetamiprid, F= 0.225, P= 0.878 for spirotetramat and F= 0.109, P= 0.953 for hexaflumuron in the Rafsanjan) (F= 0.979, P= 0.435 for acetamiprid, F= 0.255, P= 0.856 for spirotetramat and F= 0.479, P= 0.703 for hexaflumuron in the Anar) (Tables 1-3). In the coccinellid predator population, however, the activity was influenced by hexaflumuron (F= 3.758, P= 0.041), but not by the other insecticides (F= 1.410, P= 0.288 for acetamiprid and F= 1.974, P= 0.172 for spirotetramat) (Table 4).

DISCUSSION

The common pistachio psylla is a serious pest of pistachio gardens. Control of this pest is highly associated with different pesticides application. Intensive field application of pesticides causes selective pressure on pest populations and, finally, results in development of resistance to most of the insecticides. Therefore, effective control of this pest is tightly related to continuous population monitoring and resistance management. General esterases, GSTs, and cytochrome P_{450}s are the most important enzymes responsible for the development of pesticide resistance (Tak *et al*., 2017). These enzymes are responsible for pesticide metabolism and gene mutations leading to metabolic resistance against chemical insecticides (Claudianos *et al*., 2006). The participation of esterase, GST and microsomal monooxygenase in insecticide resistance has been already reported in some insect species (Francis *et al*., 2017; Gong *et al*., 2013; Kristensen, 2005; Van de Baan and Croft, 1990). However, in the current study, beta esterase activity seems to be responsible for the pesticide resistance in *A. pistaciae*.

General esterases are a large group of hydrolase enzymes metabolizing exogenous and endogenous substrates with the ester bond (Devorshak and Roe, 1999). Results of the current study revealed that (when β-naphthyl acetate was used as substrate) esterase was the predominant detoxifying enzyme in both populations of the common pistachio psylla. By considering the insecticides (i.e., acetamiprid, spirotetramat, and hexaflumuron), again, esterase (when β-naphthyl acetate was used as substrate) was the predominant detoxifying enzyme. However, there were no significant differences in the activities of the esterase in the two populations and three insecticides. The most important finding is that regardless of the pesticide and population, the activity of esterase (when β-naphthyl acetate was used as substrate) was substantially more than the other enzymes. The elevation of esterase activity is one of the predominant mechanisms of an insect's resistance to most of the insecticides (Bandaraa and Karunaratne, 2017). General esterases were already determined as the main detoxifying enzymes in the common pistachio psylla, *A. pistaciae,* against phosalone (Abdallah *et al*., 2016; Alizadeh *et al*., 2011), and the cowpea aphid, *Aphis craccivora* Koch against thiamethoxam (Abdallah *et al*., 2016; Alizadeh *et al*., 2011) and the cattle tick *Rhipicephalus (Boophilus) microplus* against different acaricides (Bandaraa and Karunaratne, 2017). Mohammadzadeh *et al*. (2014) showed a significant increase in esterase activities of the treated *Xanthogaleruca luteola* (Muller) larvae with spinosad. In disagreement with our results, they found that enzyme activity with α-naphthyl acetate as a substrate was more than β-naphthyl.

GSTs are another group of detoxifying enzymes that catalyze the conjugation of the reduced form of glutathione (GSH) to

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electrophile xenobiotic substrates (Habig *et al*., 1976). In the current study, no significant differences were found in the activity of GST, α-esterase and cytochrome P_{450} in different treatments (populations and insecticides). Besides, the activity of cytochrome P_{450} in different populations and insecticides was negligible. In agreement with our results, Alizadeh *et al*. (2011) showed that GST has a minor role in the resistance of the common pistachio psylla to phosalone. Soleymanzade *et al*. (2019) demonstrated that esterase was the key detoxifying enzyme of *Plutella xylostella* L. resistant to chlorpyrifos, and GST and cytochrome P450 monooxygenase did not have any role (Soleymanzade *et al*., 2019). Also, Mohammadzadeh *et al*. (2014) showed an increase in GST activities of the *X. luteola* larvae treated with spinosad after 48 hours at LC_{50} and LC_{30} compared to the control (Mohammadzadeh *et al*., 2014).

However, in disagreement with our results, Zhang *et al*. (2017) found that enhancement of GST and cytochrome P_{450} monooxygenase activities were most probably the prevalent detoxification mechanism responsible for the diamondback moth's, *Plutella xylostella,* resistance to indoxacarb. Piri *et al*. (2014) treated *Glyphodes pyloalis* Walker larvae with spinosad and, after 48 h, their results showed that activity of GST was reduced in sublethal concentrations of LC_{20}, LC_{30}, and LC_{40} compared with control. Zhou *et al*. (2019) demonstrated that the activity of GST decreased after the treatment of *Sogatella furcifera* with LC_{10} concentration of thiamethoxam. Xu *et al*. (2015) concluded that GST is the main detoxifying enzyme in *Spodoptera litura* against some insecticides, allelochemicals, and heavy metals. The resistance of the cotton leafhopper, *Amrascabiguttula biguttula* (Ishida), to neonicotinoid insecticides was attributed to the higher activity of GST (Halappa and Patil, 2016). However, insect resistance to insecticide is an inherent trait that may differ even among individuals of the same population (Gong *et al*., 2013). The two main types of insecticide resistance mechanisms are the metabolic or enzymatic and the target site modification resistance (Tmimi *et al*., 2018). In addition, the difference in enzyme activity and, therefore, the susceptibility of the pest may be attributed to different factors e.g. insect species, developmental stage, sex, target site, and population. The insensitivity of the target site of insects against a specific pesticide is usually related to the activity of a particular detoxifying enzyme (Gong *et al*., 2013). Saha (2016) declared that variations in the activity of detoxifying enzymes in different species and populations are the main reason for the development of resistance against pesticides.

The detoxifying ability and the sensitivity of insects and their natural enemies to insecticides may be different from differences in the insects' specific characteristics and biological and ecological habitats of the species. In some cases, subtle differences in the insects' life cycles may considerably influence their susceptibility to insecticides (Cho *et al*., 2002; Mullin *et al*., 1982; Wu and Miyatab, 2005). Based on the outcomes of the current study, in coccinellid predator of the psyllid, esterase (when β-naphthyl acetate was used as substrate) was the main detoxifying enzyme and its activity was substantially more than the other enzymes in all the treatments (insecticides). Wu and Miyatab (2005) suggested that the detoxification enzyme (mixed-function oxidase, carboxylestsrase, and GST) activities may play an important role in the resistance of 18 parasitoids, predators, and herbivore pest species to methamidophos.

In general, the activity of esterase (with two substrates) in the pest (psylla) was more than in the natural enemy (coccinellid), which may indicate higher sensitivity of the lady beetle to insecticides than its prey. Therefore, from a practical viewpoint, an insecticide should be used when the predator is inactive yet.

Most insects possess the ability to detoxify pesticides; the magnitude and mechanism of this ability may vary greatly among the species, developmental stages, and

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populations. Mullin *et al* (1982) demonstrated higher activity of cytochrome P_{450} in the herbivorous mite, *Tetranichus urticae,* than in its predator mite, *Amblyseius fallacies*.

In conclusion, our results suggest that in the common pistachio psylla, the general esterase activities may play a role in conferring resistance to acetamiprid, spirotetramat, and hexaflumuron. This conclusion may help to determine the resistance mechanism of *A. pistaciae* to a different group of insecticides, and these data can be used to manage the resistance of this destructive pest and also preserve its coccinellid predator.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Ferdowsi University of Mashhad, Iran. The findings presented in this paper were a part of the PhD studies of the first author.

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فعالیت آنزیم‌های سم‌زدا در پسیل معمولی پسته و کفشدوزک شکارگر

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چکیده

پسیل معمولی پسته *Agonoscena pistaciae* Burckhardt and Lauterer (Hem: Aphalaridae) یکی از مهم‌ترین و مخرب‌ترین آفات باغ‌های پسته در ایران می‌باشد. مبارزه شیمیایی روش گسترده‌ای برای مدیریت جمعیت این آفت است. استفاده زیاد از حشره‌کش‌ها منجر به ایجاد جمعیت‌های مقاوم در پسیل معمولی پسته شده است. در این تحقیق، فعالیت آنزیم‌های سم‌زدا (استرازهای عمومی، گلوتاتیون اس-ترانسفراز و سیتوکروم P_{450}) در دو جمعیت از پسیل معمولی پسته و کفشدوزک شکارگر آن (*Oenopia conglobata* L. (Col: Coccinellidae در استان کرمان، ایران، که تحت تأثیر سه حشره‌کش رایج استامی‌پرید، اسپیروتترامات و هگزافلومورون با چهار غلظت (کنترل، LC_{25}، LC_{50} و LC_{75})، قرار گرفته بودند، مورد بررسی قرار گرفت. نتایج نشان داد که فعالیت آنزیم‌های سم‌زدا در جمعیت مقاوم پسیل پسته نسبت به جمعیت حساس بیشتر بود. استراز، آنزیم سم‌زدای غالب در آفت و کفشدوزک شکارگر بود. بر اساس نتایج، فعالیت آنزیم‌های سم‌زدا در غلظت‌های بالاتر آفت‌کش، بیشتر بود. فعالیت استراز در جمعیت پسیل پسته بیشتر از کفشدوزک شکارگر آن بود که نشان دهنده حساسیت بیشتر کفشدوزک شکارگر در مقایسه با طعمه‌ی آن نسبت به حشره‌کش‌ها است.