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Variation in Insecticidal Susceptibility of *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae), and its Coccinellid Predator, *Oenopia conglobata* L. (Coleoptera: Coccinellidae)

MARJAN BEMANI[1] , GHOLAMHOSSEIN MORAVVEJ[1*], HAMZEH IZADI[2] , AND HOSSEIN SADEGHI-NAMAGHI[1]

ABSTRACT: The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer is a key pest throughout all pistachio producing regions of Iran. Pest management depends primarily on the application of chemical insecticides. The lack of compatibility between natural enemies and pesticides is the main concern to ecologists in the integrated management of psylla. The susceptibility of the fifth instar nymphs of *A. pistaciae* from five populations of the Kerman province and third instar larvae of the lady beetle species, *Oenopia conglobata L.*, were examined against three commonly used insecticides: acetamiprid, spirotetramat and hexaflumuron, using the spraying tower method. Probit regression analysis was conducted on mortality data at various insecticide concentrations using the POLO-PLUS program to estimate LC_{50} values. Resistance ratio data revealed that usage of pesticides has led to resistance in the psylla population of Rafsanjan. The results showed that the Rafsanjan psylla population (with LC_{50} values of 40.55, 43.65 and 95.10 mg a.i. L^{-1}, for acetamiprid, spirotetramat and hexaflumuron, respectively) was the most resistant and the Anar population (with LC_{50} values of 11.92, 24.13 and 81.06 mg a.i. L^{-1} for acetamiprid, spirotetramat and hexaflumuron, respectively) was the most susceptible based on the LC_{50} ratios of insecticides. The LC_{50} values of acetamiprid, spirotetramat and hexaflumuron for the coccinellid predator *O. conglobata* in the Rafsanjan population were estimated to be 8.76, 5218.33 and 2268.81 mg a.i. L^{-1}, respectively. The predator was more susceptible to acetamiprid and more tolerant to spirotetramat and hexaflumuron than the psylla *A. pistaciae.*

KEYWORDS: Acetamiprid, hexaflumuron, spirotetramat, bioassay

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae) is a key pest in all pistachio orchards, *Pistacia vera* L. (Sapindales: Anacardiaceae) of Iran. This pest has also been reported from other countries such as Turkey, Iraq, Armenia, Turkmenistan, Syria and Greece (Anagnou-Veroniki *et al*., 2008; Burckardt and Lauterer, 1989; Mart *et al*., 1995). Both adults and nymphs feed on leaf sap and produce large amounts of honeydew. Direct feeding decreases plant growth leading to defoliation, falling of fruit buds and poor yield (Lababidi, 2002). The coccinellid, *Oenopia conglobata* L. (Coleoptera: Coccinellidae) is the most common and

[1]Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

[2]Department of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran

[*]Corresponding author: moravej@um.ac.ir, 00989150533595

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prevalent natural enemy feeding on both eggs and nymphs of the common pistachio psylla (Hodek, 1973; Kabiri Raeis Abbad and Amiri Besheli, 2012).

The common pistachio psylla, has 6-7 generations per year. Also, this species has been found to exceed 1000 eggs/female for the winter form and 900 eggs/female for the summer form, respectively (Mehrnejad and Copland, 2005). This high rate of reproduction, particularly by the winter forms, means that the pest is well adapted to the hard environment and exploitation of the pistachio plantations, as the establishment of very large colonies is achieved in early spring or even late winter (Mehrnejad, 1998). The control of this pest is heavily relies on the use of chemical insecticides. However, due to its short generation time and high reproductive rate, several insecticide applications are required during the season to mitigate the pest damage (Alizadeh *et al.*, 2014; Amirzade *et al.*, 2014). Numerous pesticide applications may adversely affect natural enemies and the environment and consequently lead to insecticidal resistance (Amirzade *et al.*, 2014; Kabiri Raeis Abbad and Amiri Besheli, 2012). Beneficial arthropods are more susceptible to insecticides compared to their prey (Croft, 1990; Ruberson *et al.*, 1998) due to their higher searching activity, lower detoxification capacity, lower genetic variation and lower food accessibility (Tabashnik and Johnson, 1999).

A number of organophosphates, insect growth regulators (IGR), neonicotinoids and pyrethroids have been widely used to control *A. pistaciae* in pistachio orchards (Samih *et al.*, 2005). The resistance of *A. pistaciae* to the organophosphate, phosalone has been demonstrated in some areas of the Kerman province of Iran (Alizadeh *et al.*, 2011; Talebi *et al.*, 2001). The recently recommended insecticides acetamiprid and hexaflumuron are frequently used against *A. pistaciae* in the Kerman province (Noorbakhsh *et al.*, 2001). The insecticide, spirotetramat has also attracted more attention of farmers due to its newly adverse mode of action to others (Bruck *et al.*, 2009).

Acetamiprid is a neonicotinoid insecticide. The neonicotinoids are a relatively new class of insecticides with systemic, translaminar and contact activity which act rapidly as an agonist of the nicotinic acetylcholine receptor in postsynaptic membrane, resulting in paralysis and death of the treated insects (Elbert *et al.*, 1991; Schroeder and Flattum, 1984). Spirotetramat belongs to the chemical class of insecticides called ketoenols in the subclass tetramic acid derivatives with a mode of action of lipogenesis disruption through inhibition of acetyl CoA carboxylase that results in decreasing lipid contents, growth inhibition of younger insects and reducing the reproduction ability of adults. This insecticide has shown an outstanding performance against sucking insect pests in laboratory and greenhouse assays as well as in semi-field and field trials (Bruck *et al.*, 2009). Hexaflumuron as an insect-growth regulator (IGR) inhibits chitin synthesis and disrupts insect cuticle formation during molting. The present research aimed to evaluate the lethal effects of the insecticides, acetamiprid, hexaflumuron and spirotetramat on *A. pistaciae* populations collected from five regions in the Kerman province, including Rafsanjan, Kerman, Zarand, Anar and Sirjan. Moreover, the insecticidal susceptibility of the common predator, *O. conglobata* was examined as part of the integrated pest management in pistachio orchards of Rafsanjan.

MATERIALS AND METHODS

Rearing of the Test Insects

This research was done in the laboratory of Entomology, Vali-e-Asr University, Rafsanjan, Iran, in 2015. The nymphs of the common pistachio psylla *A. pistaciae* were collected from pistachio gardens of Rafsanjan, Kerman, Zarand, Anar and Sirjan, Iran. To remove the effects of previous chemical treatments, it was assured that the selected gardens had received no chemical pesticides since a year before the onset of experiments. To obtain 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 bioassay experiments.

Bioassay Procedure

Based on a series of preliminary tests on psylla populations from each region, the suitable concentrations were determined for bioassay experiments of each insecticide. The commercial formulations of the following insecticides were included in this research: spirotetramat (Movento®, 10% CS); acetamiprid (Ekka®, 20% SP) and hexaflumuron (Hef®, 10% EC) (Table 1). Third instar larvae of *O. conglobata* collected from Rafsanjan were assayed using the concentrations of 2, 3.4, 6, 11 and 20 mg a.i. L^{-1} of acetamiprid, 2000, 2800, 4100, 5900 and 8500 mg a.i. L^{-1} of spirotetramat and 500, 900, 1700, 3200 and 6000 mg a.i. L^{-1} of hexaflumuron.

One-day-old fifth instar nymphs were transferred into petri dishes and sprayed with 1 ml of aqueous emulsions of different concentrations of each insecticide. The spray was applied at 15 mbar using a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK). The experiments were performed with four replications, each comprising twelve nymphs. Distilled water was used as a control treatment. Treated nymphs were transferred to fresh leaves and maintained in a controlled climate chamber (25 ± 2 °C, 50-60% RH, 16:8 L:D). Mortality was recorded after 24 h for acetamiprid and 48 h for spirotetramat and hexaflumuron due to their different modes of actions. Likewise, bioassays on the coccinellid *O. conglobata* were performed using one-day-old third instar larvae with three replications, each comprising ten to twelve larvae. This experiment was done as a completely randomized design.

Data Analysis

The data were analyzed using the probit analysis to estimate lethal concentrations

(LC_{50}) in the POLO-PLUS software. For comparison of the probit mortality lines of treatments, the program also provides the likelihood ratio tests of equality and parallelism.

Table 1. Concentrations of the insecticides against common pistachio psylla, *Agonoscena pistaciae* used for bioassay experiments.

Insecticide	Population	Concentration (mg a.i. L^{-1})				
		C1	C2	C3	C4	C5
Acetamiprid	Rafsanjan	24	30	40	52	70
	Anar	3	5	8.4	14	24
	Zarand	14	19	26.4	36.3	50
	Kerman	10	14	20	28	40
	Sirjan	10	14.4	20.8	30	44
Spirotetramat	Rafsanjan	15	23	36	57	90
	Anar	10	14	22	33	50
	Zarand	12	17	26	39	60
	Kerman	10	15	24	38	60
	Sirjan	10	15	24	38	60
Hexaflumuron	Rafsanjan	60	76	97	124	160
	Anar	40	53	71	96	130
	Zarand	50	64	83	100	140
	Kerman	50	64	83	100	140
	Sirjan	45	59	79	100	140

RESULTS AND DISCUSSION

In all the populations, mortality of the psyllid nymphs and predator larvae were proportional to the insecticide concentrations. Mortality increased as concentration of the insecticides increased. Differences between mortality rate caused by insecticides and the control were also significant.

Data presented in Table 2 indicates that acetamiprid was more toxic to common pistachio psylla nymphs than spirotetramat and hexaflumuron. The Rafsanjan population was the more resistant whereas the Anar population the most sensitive. The highest resistance ratio of 3.40, 1.80 and 1.17 for acetamiprid, spirotetramat and hexaflumuron, respectively was recorded for the Rafsanjan population. For all populations, acetamiprid showed the highest resistance ratio (Table 2).

The LC_{50} values for the predator were 8.76, 5218.33 and 2268.81 mg a.i. L^{-1} for acetamiprid, spirotetramat and hexaflumuron, respectively. Comparison between the LC_{50} values of the fifth instar *A. pistaciae* nymphs of the Rafsanjan population and the predator demonstrated that toxicity of acetamiprid to the predator is higher than the nymphs, but spirotetramat and hexaflumuron are much more toxic to fifth instar *A. pistaciae* nymphs of the Rafsanjan population than the predator. During the evaluation of *A.*

Table 2. Susceptibility of *Agonoscena pistaciae* fifth instar nymphs to insecticides from five different populations of Kerman province, Iran.

Insecticide	Population origin	Number	Probit mortality		LC_{50} (95% CL)	X2	Resistance ratio (95% CL)
			Slope (± SE)	Intercept (± SE)			
	Rafsanjan	279	2.72 (± 0.60)	-4.38 (± 0.99)	40.55 (32.27-49.17)	0.14	3.40 (2.27-5.09)
	Kerman	253	1.82 (± 0.45)	-2.38 (± 0.60)	20.32 (14.88-26.99)	0.84	1.70 (1.09-2.65)
Acetamiprid	Zarand	265	2.49 (± 0.52)	-3.64 (± 1.14)	28.79 (23.08-35.76)	1.04	2.41 (1.60-3.62)
	Sirjan	268	1.79 (± 0.47)	-2.54 (± 0.67)	26.01 (18.03-38.10)	1.97	2.18 (1.34-3.52)
	Anar	262	1.45 (± 0.32)	-1.56 (± 0.34)	11.92 (8.42-18.20)	0.43	_
	Rafsanjan	250	1.54 (± 0.46)	-2.52 (± 0.79)	43.65 (23.20-71.78)	0.92	1.80 (1.10-3.09)
	Kerman	199	1.66 (± 0.49)	-2.36 (± 0.74)	26.50 (14.28-43.08)	0.15	1.09 (0.64-1.86)
Spirotetramat	Zarand	260	2.01 (± 0.45)	-2.83 (± 0.69)	25.51 (17.18-34.34)	0.46	1.05 (0.69-1.59)
	Sirjan	273	1.50 (± 0.42)	-2.17 (± 0.64)	27.67 (15.60-43.93)	1.74	1.14 (0.68-1.91)
	Anar	265	1.99 (± 0.41)	-2.75 (± 0.59)	24.13 (17.85-31.74)	0.74	_
	Rafsanjan	232	2.67 (± 0.79)	-5.29 (± 1.63)	95.10 (63.62-22.69)	0.12	1.17 (0.84-1.63)
	Kerman	248	2.99 (± 0.81)	-5.86 (± 1.63)	91.03 (66.81-18.03)	0.45	1.12 (0.82-1.54)
Hexaflumuron	Zarand	264	3.01 (± 0.75)	-5.86 (± 1.50)	87.79 (67.21-108.96)	0.85	1.08 (0.81-1.45)
	Sirjan	264	2.58 (± 0.69)	-5.02 (± 1.36)	88.54 (64.67-16.64)	0.51	1.09 (0.79-1.50)
	Anar	262	2.66 (± 0.57)	-5.08 (± 1.11)	81.06 (65.36-100.59)	0.10	_

pistaciae against *O. conglobata* in Rafsanjan population, Chi-square values indicated that acetamiprid had the best fit to the *A. pistaciae* data, while spirotetramat and hexaflumuron had the best fits to the *O. conglobata* data (Table 3). During the evaluation of acetamiprid, spirotetramat and hexaflumuron against *A. pistaciae*, the LC_{50} values comparisons of the LC_{50} ratio and the lower and upper 95% confidence limits showed that there were significant differences between LC_{50} values of the tested populations.

For *O. conglobata* comparisons of LC_{50} ratio (2.30) and the lower and upper 95% confidence limits showed that there was a significant difference between LC_{50} values of spirotetramat (5218.33 mg a.i. L^{-1}) and hexaflumuron (2268.81 mg a.i. L^{-1}). When comparing hexaflumuron and acetamiprid, LC_{50} ratio (258.99) with the lower and upper 95% confidence limits showed that there was a significant difference between the LC_{50} values of hexaflumuron (2268.81 mg a.i. L^{-1}) and acetamiprid (8.76 mg a.i. L^{-1}). When comparing spirotetramat and acetamiprid, the LC_{50} ratio (595.69) with the lower and upper 95% confidence limits showed that there was a significant difference between LC_{50} values of spirotetramat (5218.33 mg a.i. L^{-1}) and acetamiprid (8.76 mg a.i. L^{-1}) (Table 3).

The slope of probit mortality regressions for the three insecticides were not significantly different, consequently, we did not reject the likelihood ratio test of parallelism ($\chi2=3.87$, df=2, $p>0.05$ for Anar and $\chi2=3.30$, df=2, $p>0.05$ for Rafsanjan psyllid population and $\chi2=1.99$, df=2, $p>0.05$ for the predator), this result suggest that any slopes of the probit mortality regressions would not be significantly different from the others. The interceps of probit mortality regressions for the three insecticides were significantly different, consequently, we rejected the likelihood ratio test of equality ($\chi2=50.82$, df=4, $p<0.05$ for Anar and $\chi2=20.88$, df=4, $p<0.05$ for Rafsanjan psyllid population and $\chi2=72.75$, df=4, $p<0.05$ for coccinellid predator), suggesting that at least the interceps of one of the probit mortality regressions would be significantly different from the others. Further likelihood ratio tests of equality indicated that the interceps between all possible paired combinations significantly differed from each other ($p < 0.05$), except those of acetamiprid and spirotetramat in the Rafsanjan population ($\chi2 = 2.81$, df = 2, $p > 0.05$) (Table 2).

In this study, the susceptibility of five populations of the common pistachio psylla to acetamiprid, spirotetramat and hexaflumuron via a spray tower method, was investigated. Data revealed that all populations are more sensitive to acetamiprid than spiro-

Table 3. Susceptibility of *Oenopia conglobata* third instar larvae to insecticides from Rafsanjan, Iran.

Insecticide	Number	Slope ± SE	LC_{50} (Lower CL–Upper CL)	X^2	Heterogeneity
Acetamiprid	160	1.77 ± 0.31	8.76 (6.66-12.39)	0.89	0.29
Spirotetramat	150	2.12 ± 0.49	5218.3 (4151.22-7321.46)	0.43	0.14
Hexaflumuron	154	1.37 ± 0.28	2268.81 (1592.12-3537.38)	0.26	0.08

tetramat and hexaflumuron. However, the difference between toxicity of pesticides is highly related to their mode of action. The mode of action of insecticides is responsible for their higher or lower toxicity to the treated insects (Sanchez-Bayo, 2012). Toxicity of these three insecticides to fifth instar nymphs of the common pistachio psylla in five populations of the Kerman province could be rated in the following order: acetamiprid>spirotetramat>hexaflumuron.

LC_{50} values of acetamiprid, spirotetramat and hexaflumuron for the coccinellid predator showed that spirotetramat is the least toxic insecticide, while acetamiprid is the most toxic insecticide for *O. conglobata*. The susceptibilities of the coccinellid to acetamipirid is higher than the susceptibility of the pest to the same insecticide; on the other hand, the predator showed lower susceptibility to spirotetramat and hexaflumuron than the pest. Amirzadeh et al. (2014) found that acetamiprid was most toxic to Coccinella undecimpunctata aegyptica by evaluating three neonicotinoid insecticides (acetamiprid, thiamethoxam, and imidacloprid) against the common pistachio psylla fifth instar nymphs and its predators (Adalia bipunctata L. and C. undecimpunctata aegyptiaca Reiche). Dong-Soon et al. (2006) found high toxicity of acetamiprid against Deraeocoris brevis Knight (Hemiptera: Miridae), a predatory plant bug.

Insecticide bioassay is often used to estimate the median lethal concentration (LC_{50}). The LC_{50} is the concentration of an insecticide required to kill 50% of a given population or strain under the specified conditions. The change of effect rate in relation to a unit change in concentration is expressed by the line slope which in turn shows the variability in susceptibility of the test population. A steep line means a population has small variation in susceptibility. This requires using the pesticide in the field more attentively to prevent exerting a high selection pressure that could eliminate the susceptible individuals and lead to selection of resistant individuals, like acetamiprid; whereas a flat line means a population varying widely in susceptibility, meaning that with a fairly large increase in insecticide concentration, the mortality would increase considerably, like spirotetramat and hexaflumuron (Robertson *et al.,* 2007).

LC_{50} ratio of the mentioned insecticides against fifth instar nymphs of the common pistachio psylla revealed that the Rafsanjan population is the most resistant and the Anar population is the most susceptible. Susceptibility of the populations to the evaluated pesticides was rated as: Anar>Kerman>Sirjan>Zarand>Rafsanjan.

Decrease in susceptibility of the common pistachio psylla to phosalone was first reported by Talebi et al. (2001). The level of resistance ratio in the Rafsanjan population was 1.8-fold of that of the Jabalbarez population (Talebi *et al.,* 2001). Alizadeh et al. (2011) reported a significant discrepancy in susceptibility of the common pistachio psylla to phosalone among different populations. Resistance ratio of the studied populations to the susceptible population ranged from 3.3 to 11.3 (Alizadeh *et al.,* 2011). In the current research, the LC_{50} ratios of acetamiprid, spirotetramat and hexaflumuron were 3.4, 1.8 and 1.2 folds, respectively; for Rafsanjan, suggesting the Rafsanjan population to be the most resistant and the Anar population the most sensitive to the insecticides tested in this study.

CONCLUSION

Repetitive and frequent application of hexaflumuron for controlling the common pistachio psylla in Rafsanjan rendered the pest to increase its resistance against the insecticide and caused higher levels of resistances compared to the other populations tested in this study. In comparisons between the resistance ratios of the pesticides, acetamiprid showed the highest decrease in susceptibility of the pest in all populations. Findings of the current study indicate the high sensitivity of the common pistachio psylla and its predator, *O. conglobata* to acetamiprid than spirotetramat and hexaflumuron. Because of the crucial role of predators controlling important agricultural pests, such as *O. conglobata* against the common pistachio psylla, and due to the importance of insecticide comparability between chemical and biological control, it seems reasonable to advise the application of spirotetramat in an integrated pest management program for alleviation of the common pistachio psylla problem.

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