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Insecticidal effect of two plant extract seeds, on *Agonoscena pistaciae* (Hemiptera: Aphalaridae) under laboratory conditions

Mansore Hassanshahi, Mohammad Reza Hassani and Zahra Sheibani

Mansore Hassanshahi
Department of Entomology, Rafsanjan Branch, Islamic Azad University, Rafsanjan, Iran

Mohammad Reza Hassani
Department of Entomology, Rafsanjan Branch, Islamic Azad University, Rafsanjan, Iran

Zahra Sheibani
Department of Entomology, Rafsanjan Branch, Islamic Azad University, Rafsanjan, Iran

Correspondence
Mohammad Reza Hassani
Department of Entomology, Rafsanjan Branch, Islamic Azad University, Rafsanjan, Iran

Abstract

The pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae), is a severe pest of pistachio trees in all pistachio planted area. The toxicity of seed extracts of two plants *Amygdalus scoparia* and *Prunus dulcis* var *amara* were tested against fifth instar nymphs of *A. pistaciae* under laboratory conditions. Bioassay was carried out using a dipping technique. The results showed that *A. scoparia* and *P. dulcis* var *amara* seed extracts were effective against *A. pistaciae.* Mortality of fifth instar nymphs were significantly increased with increasing concentrations of both plant extracts. Probit analysis of concentration-mortality data were conducted to estimate the LC_{50} value. The results showed that *A. scoparia* with LC_{50} value of 1494.00 ml/L was more toxic than *P. dulcis* var *amara* (1968.78 ml/L) on fifth instar nymphs of *A. pistaciae*. According to LC_{50} values, no significant differences were observed between *A. scoparia* and *P. dulcis* var *amara* 24 h post- treatment. Our results suggest that seed extracts of *A. scoparia* and *P. dulcis* var *amara* are suitable potentially compounds to control of *A. pistaciae*.

Keywords: *Agonoscena pistaciae*, *Amygdalus scoparia*, *Prunus amygdalus* var. *amara*, botanical insecticide, mortality

1. Introduction

The pistachio, *Pistacia vera* L., is one of the most important horticultural products in Iran. The pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae), is an economic pest of pistachio trees in all pistachio planted area. This pest sucks sap from leaves and caused damage and loss product [22, 11]. This pest has six complete generations per year in Rafsanjan, Iran. Therefore, chemical insecticides apply several times each year for control this pest [12]. Pest management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of the synthetic pesticide chemicals [6]. Botanical pesticides have long been touted as one of the important means to be used in protection of crop produce for pest management because botanicals reputedly pose little threat to the environment or to human health [18, 6]. The main advantage of botanicals is that they are easily produced by farmers, small-scale industries and are potentially less expensive [17]. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products [6]. Direct applications of plant extracts from various parts like leaves, stem, roots and whole plants are effective biologically against insect pests especially for the control of soft bodied insect pests, which feed on the leaves and tender plant parts as flowers and developing grains etc. Aphids, jassids and even caterpillars can be managed by this approach [18].

The effect of botanical extracts has been investigated against many pests. So that, Salehi *et al*. [23] assayed the effect of ethanolic extracts of *Thymus vulgaris* L., *Rosmarinus officinalis* L., *Ricinus communis* L., and *Sophopra alopecuriodes* L. on the fifth instar nymph of *A. pistaciae*. The effect of *Viola odorata* L. extract on *A. pistaciae* nymph mortality percentage was assayed by Razavi and Mahdian [18]. Sheibani and Hassani [24] investigated the effect of three botanical insecticides, Sirinol (garlic extract), Tondexir (pepper extract) and Palizin (eucalyptus extract) on *A. pistaciae* in field conditions. Botanicals extracted from fruit peels of *Citrus reticulate* Blanco (Ponkan), seeds of *Rubia tinctorum* L. and leaves of *Lawsonia inermis* L. were tested for their insecticidal activity against *A. pistaciae* [21].

Toxicity effect of aqueous extract of *Melia azedarach* L. was stadied by Abedi *et al*. [3]. Flores-Davila *et al*. [9] Presented the effect of extracts of *Carica papaya* L. seeds, *Sapindus saponaria* L., leaves, and *Annona muricata* L. leaves on nymph of *Bactericera cockerelli* (Sulcen). Ethanolic crude extracts of *Azadirachta indica* A. Juss, *Lippia graveolens* Kunth, *Psidium guajava* L., *Citrus reshni* Hort ex Tan) and *Allium sativum* L., were evaluated in doses of 50 and 500 ppm the *Diaphorina citri* Kuwayama, by Cázares Alonso *et al*. [5]. Results by Erler [8] suggest that botanical natural product (AkseBio2) can be used in *Cacopsylla pyri* (L.) control instead of synthetic insecticides and may serve as an integrated pest management (IPM) component in pear orchards.

Different phenolic compounds were characterized and identified in almond seed extract and its skin, shell and hull as almond by-products [13]. The almond genotypes that have high quality unsaturated fatty acids such as high oleic acids and low linoleic acids content were determined [15]. The bitter almond (*Prunus dulcis* var *amara* (Duhameh) H. L. Moore), reported as an undesirable botanical substances in animal feed lists, because of low to moderate toxicity [26].

The objective of this study to determine the effects of seed extracts of mountain almond (*Amygdalus scoparia* Spach) and bitter almond (*P. dulcis* var *amara*) on the fifth instar nymphs of *A. pistaciae* in laboratory conditions. These compounds can be used as botanical insecticides to control of this pest and other pests.

2. Material and Methods

2.1 Rearing of the test insects: Adults of pistachio psyllid were collected from untreated pistachio orchard in Rafsanjan, Iran. The laboratory rearing of egg and nymph of *A. pistaciae* was carried out in pistachio leaf disk cages under controlled conditions. The rearing of insects and bioassay experiments were performed under controlled laboratory conditions (25±1 °C, 55 ± 5% RH and a photoperiod of 16:8 [L:D] h). *A. pistaciae* nymphs reared on pistachio leaf disk, until the nymphs reached the fifth instar. The fifth instar nymphs were used for bioassay experiments. The experiments was done from May to October, 2015.

2.2 Plant Extract: The seeds of *A. scoparia* Spach and *P. dulcis* var *amara* were harvested from untreated trees in Raviz, Rafsanjan, Iran. Freshly collected seeds, air dried, and ground in a laboratory mill to a moderately fine powder. The extraction method was made following the methods described by Rehman *et al*. [20]. Powdered material (1 kg) was extracted with 95% ethanol (2.5 L). The solution was held at 25 ± 1 °C for 8 d, with the glass jar shaken twice daily to help dissolve the powdered seeds. The solution was filtered through Whatman No.1 filter paper. The extracts were evaporated under reduced pressure in a rotary evaporator, bulked, and stored in a freezer at -20 °C.

2.3 Bioassay Technique: Responses of fifth instar nymphs of *A. pistaciae* to various concentrations of plant extracts were determined using a dipping bioassay technique that was used from previous studies [4]. One-day-old fifth instar nymphs of pistachio psyllid were dipped in each concentration of each plant extract for 3 s. Treated nymphs were allowed to air dry and then placed on the pistachio leaf disk and assayed. Agar beds were layered in the base of each petri dish for maintenance of humidity of pistachio leaves. Freshly cut leaf discs of pistachio sized to fit in the base of each petri and were then placed on the agar beds. Each bioassay included three replications of each concentration for each insecticide. Each replication consisting of 20 fifth instar nymphs per petri dish). Distilled water was used as the control. Mortality of nymph was recorded after 24 h. Leaf disks were maintained in a growth chamber at temperature 25 ± 1 °C. Based on preliminary tests, graded concentrations of pesticides (597, 1145, 2195, 4209, and 8072 ml/L for *A. scoparia*; and 451, 919, 1871, 3808, and 7753 ml/L for *P. dulcis* var *amara*) were prepared with distilled water.

2.4 Statistical Analysis: The LC_{50}, 95% Confidence Interval (CI), the slope and intercept of the probit mortality regression and the relevant statistical tests were estimated by probit analysis using POLO – PC Software. Differences in LC_{50} values were considered significant between pesticides and insect species if there was no overlap of 95% fiducial limits. Failure of 95% to overlap was used as criterion for significant difference at LC_{50}. Efficacy data were analyzed by analysis of variance (ANOVA) for completely randomized design, with mean separation by Tukey (P = 0.05) (SPSS, 16)

3. Results and Discussion

The toxicity of botanical seed extract from *A. scoparia* and *P. dulcis var amara* against fifth instar nymph of *A. pistaciae* was evaluated using dipping mortality bioassay methods. The results indicated that seed extracts of *A. scoparia*, and *P. dulcis* var *amara* are effective on fifth instar nymphs of *A. pistaciae*. The LC_{50} of *A. scoparia* and *P. dulcis* var *amara* seed extracts was obtained 1494.00 and 1968.78 ml/l, respectively. According to LC_{50} values, no significant differences were observed between *A. scoparia* and *P. dulcis* var *amara* after 24 h of treatment, based on the overlap of 95% CL (Tables 1).

Table 1: Toxicity of seed extracts of *A. scoparia* and *P. dulcis amara* on fifth instar nymph of *A. pistaciae* by using a dipping bioassay method after 24 hours

	n	Slope ± SE	LC_{50} ML/L	95% Confidence Interval	t	χ^2
A. scoparia	360	1.86 ± 0.26	1494.00	1054.32-1950.00	7.05	4.63(13)
P. dulcis var *amara*	360	1.648 ± 0.31	1968.78	1197.33-2756.06	5.38	8.17(13)

During the experiment, it was observed a difference between the levels of mortality percentage caused by different concentrations of these extracts. Naturally, with increasing the concentrations, the effect of extracts was increased. The mortality percentage of *A. pistaciae* nymph enhanced with increasing insecticide concentration (Table 2). There were significant difference between insecticide concentrations for *A. scoparia* (F = 89.60; df = 5, 12; $P<0.0001$) and *P. dulcis* var *amara* (F = 95.52; df = 5, 12; $P<0.0001$). Morality percentages were significant among different treatments and classified in different groups (Table 3).

Table 2: The mortality percentage of fifth instar nymphs of *A. pistaciae* treated with *A. scoparia* and *P. dulcis* var *amara* after 24 hours

A. scoparia		***P. dulcis var amara***	
Concentration (ml/L)	**Mean ± Std. Error**	**Concentration (ml/L)**	**Mean ± Std. Error**
control	11.67 ± 1.67^{a}	control	11.67 ± 1.67^{a}
597	33.33 ± 3.33^{b}	451	35.00 ± 2.89^{b}
1145	45.00 ± 2.89^{b}	919	35.00 ± 2.89^{b}
2195	68.33 ± 4.41^{c}	1871	48.33 ± 1.67^{b}
4209	83.33 ± 4.41^{c,d}	3808	71.67 ± 4.41^{c}
8072	91.67 ± 1.67^{d}	7753	90.00 ± 2.89^{d}

** Different letters indicate significant differences (*P*<5%)

Table 3: Analysis of variance of the percentage mortality of different concentrations of *A. scoparia* and *P. dulcis* var *amara* on fifth instar nymph of *A. pistaciae* after 24 hours

	Resources changes	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
A. scoparia	Between Groups	11940.28	5	2388.06	95.52	.000
	Within Groups	300.00	12	25.00		
	Total	12240.28	17			
P. dulcis var *amara*	Between Groups	14311.11	5	2862.22	89.60	.000
	Within Groups	383.33	12	31.94		
	Total	14694.44	17			

4. Discussion

In the context of agricultural pest management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post-harvest protection of food in developing countries [16]. In general, they act and degrade rapidly and have low mammalian toxicity with a few exceptions [6]. In this bioassay, seed extracts of *A. scoparia* and *P. dulcis* var *amara* were effective on *A. pistaciae* nymphs after 24 hours under laboratory conditions. Probit analysis revealed that *A. scoparia* (LC_{50} = 1494.00 ml/L) was more toxic to *A. pistaciae* than *P. dulcis* var *amara* (LC_{50} = 1968.78 ml/L)) (Table 1). This toxic effect can be explained by existence cyanogenic glycosides compounds. The HPLC analysis of metabolites synthesized, showed that the amount of cyanogenic content in bitter genotypes was much more than the sweet ones. This means bitter genotypes could accumulate amygdalin in their seeds [27]. Emam *et al.* [7] reported that quantity of amygdaline in sweet sample of *A. scoparia* is less from bitter ones [7]. Cyanogenic glycosides are common secondary compounds in ripe fruits. These substances are toxic to some mammals. Effects of cyanogenic glycosides on insects vary dramatically among species [25]. Morever, Glendinning and Slansky [10] noted amygdalin compound reduced food intake in two noctuid caterpillars.

Razavi, and Mahdian [19], showed that *V. odorata* extract and spirotetramat insecticide have effective on *A. pistaciae* mortality percentage. Sheibani and Hassani [24] investigated the effect of three botanical insecticides, Sirinol (garlic extract), Tondexir (pepper extract) and Palizin (eucalyptus extract). The results showed that the highest mortality in Palizin treatment occurred after 2 and 7 days. The sampling of 14, 21 and 28 days post-treatment were showed the highest and lowest mortality in Sirinol and Tondexir treatments, respectively. Generally, there were no significant differences between Sirinol and Palizin 28 days post-treatment, but these compounds showed significant differences with Tondexir. Abbasipour *et al.* [2] reported that the seed extract of *Peganum harmala* L. had a significant effect on larval and pupal weight of the diamondback moth. These researchers found that seed extract of this plant caused a mortality of 66 and 100% in the third instar larvae that had fed for two days on the cabbage leaves treated with the ethanol extract at concentrations of 30 and 40 mg/ml, respectively and Significant differences were also observed on oviposition. Percentage of egg hatching was reduced significantly in 30 and 40 mg/ml but not in 10 and 20 mg/ml concentrations. Morever, Jbilou *et al.* [14] showed that methanol extracts *P. harmala* L. seeds had a significant decreasing on larval weight of stored grain pest, *Tribolium castaneum* (Herbst) but adult emergence of pupae were similar to control. Flores-Dávila *et al.* [9] presented that At 72 hours, most potato psyllid nymphs died (98 and 100% mortalities) from *A. muricata* extract from seeds, at concentrations of 2,500 and 5,000 ppm, respectively, followed by *A. indica* oil that caused 91 and 100% mortality at concentrations of 2,000 and 2,500 ppm, respectively. Botanicals extracted from fruit peels of *C. reticulata*, seeds of *R. tinctorum* and leaves of *L. inermis* were tested for their insecticidal activity against *A. pistaciae*. These results showed that *L. inermis* (LC_{50} = 33.99 µL/mL) had highest insecticidal effect and *C. reticulata* (LC_{50} = 38.84 µL/mL) and *R. tinctorum* (LC_{50} = 59.01 µL/mL) had the least, respectively [21]. Toxicity effect of Confidor® (400 ppm), Consult® (1500 ppm), Amitraz® (700 ppm), Actara® (300 ppm) and aqueous extract of *M. azedarach* (25% and 50%) were surveyed by Abedi, *et al.* [3]. Among the treatments bitter olive aqueous extract (%50) and Actara had highest toxicity effect on *A. pistaciae*. Salehi *et al.* [23] showed that the LC_{50} value for *T. vulgaris, R. officinalis, R. communis* and *S. alopecuriodes* on the fifth instar nymph of *A. pistaciae* were 92002, 569592, 321218 and 132393 ppm respectively, after 24 h. Abbassi *et al.* [2] reported that the alkaloids extracted from *P.* harmala L. seeds caused significant mortality, reduction in fecundity of female and egg hatching in desert locust, *Schistocerca gregaria* Forskal.

In conclusion, the finding of present study suggest that the extracts from *A. scoparia* and *P. dulcis* var *amara* have a good effect on fifth instar nymphs of *A. pistaciae* after 24 hours. It was found out that the extract of *A. scoparia* caused higher mortalities than *P. dulcis* var *amara* on *A. pistaciae*. Therefore, the extracts of *A. scoparia* and *P. dulcis* var *amara* can be useful to control *A. pistaciae* in pistachio orchard and other insect pests through Integrated Pest Management program and organic systems of agriculture.

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