

Research Article

Effect of some botanical compounds on pistachio psylla *Agonoscena pistaciae* (Hemiptera: Psyllidae) under laboratory and field conditions

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Abstract: Pistachio psylla, *Agonoscena pistaciae* Burkhardt & Lauterer (Hem.: Psyllidae) is one of the most important pests of pistachio orchards in Iran. The application of chemical pesticides early in the growing season until harvest has affected farmers' and consumers' health. In this research, the efficacy of arugula *Eruca sativa* cold press seed oil, methanolic spinach *Spinacia oleracea* seed extract, and dayabon® (SL 10%) was studied against nymphs and adults of pistachio psylla in a laboratory at 26 ± 2 °C, $65 \pm 5\%$ RH, and photoperiod of 16: 8 (L: D) h. LC₅₀ values of nanoemulsion formulation (NEF) of spinach seed extract and its nonformulated extract on the early nymphal stage were 468 and 4381 mg/l, respectively. In contrast, the LC₅₀ value of arugula seed oil and dayabon was 2281 and 4380 mg/l, respectively. NEF of spinach seed extract (LC₅₀ = 124 mg/l) was significantly more toxic than other compounds against the 5th nymphal stage. There was no significant difference in nonformulated spinach seed extract (LC₅₀ = 3946 mg/l), arugula seed oil (LC₅₀ = 3189 mg/l) and dayabon (LC₅₀ = 3930 mg/l) against 5th instars. The efficiency of dayabon and arugula seed oil and a chemical pesticide fenitrothion was studied in the field located in Dehmolla County, Shahrood, Iran. Sampling was carried out before and after treatment. There were no significant differences between days after treatments. It means that the botanicals were effective until 21 days after treatment. Findings indicated that botanical pesticides used in this study could be effective against pistachio psylla.

Keywords: *Agonoscena pistaciae*, nanoemulsion, spinach extract, dayabon, arugula oil

Introduction

Pistachio is considered one of the top agricultural export products of Iran (Razavi, 2005). Pistachio is one of the most cultivated

crops in most dryland areas of Iran (Talaie and Panahi, 2001). Due to the importance of this crop, pest control is very vital. One of the most critical pistachio pests is *Agonoscena pistaciae* Burkhardt & Lauterer (Hem.: Psyllidae), which has caused lots of extensive damage to pistachio orchards in recent years (Mehrnejad, 2001a, b; Samih *et al.*, 2005). Both the nymphs and adults suck the sap of the leaves (Mehrnejad and Copland, 2005). Every year

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farmers apply chemical pesticides from the outset until harvest, which causes economic concerns and endangers farmers' and consumers' health (Hassani *et al.*, 2009). Over the years, the prolonged use of chemical pesticides has made pests resistant to many pesticides (Basirat, 2003; Alizadeh *et al.*, 2014; Mostafavi *et al.*, 2017). The efficacy of many plant extracts has been studied on *A. pisaciae*. For instance, the ethanol extract of *Thymus vulgaris* L., *Rosmarinus officinalis* L., *Ricinus communis* L., and *Sophora alopecuriodes* L. have been tested on *A. pisaciae* (Salehi *et al.*, 2015; Salehi and Samih, 2016; Azimi Zadeh and Ahmadi, 2018). In other studies, the insecticidal properties of the extracts from *Lawsonia inermis* L., *Citrus sinensis* L. Osbeck, *Rubia tinctorum* L. (Madder), and *Ferula assa-foetida* L. have been investigated under laboratory conditions (Izadi *et al.*, 2012; Rouhani *et al.*, 2019). Also, the extract of *Azadirachta indica* A. Juss, *Melia azadirach* L. (Homayonfar and Zohdi, 2012; Abedi *et al.*, 2012), *Amygdalus scoparia* Spach, and *Prunus dulcis* var Amara (Hassanshahi *et al.*, 2016) have been tested on *A. pisaciae*. Some pesticides such as acetamiprid (Izadi *et al.*, 2012, Amirzade *et al.*, 2014), actara, hexaflumuron, amitraz, and confidor have been studied (Abedi *et al.*, 2012). Also, fenitrothion has been used on some psyllids (Monobrullah and Singh, 1997; Kobori *et al.*, 2011). In this study, the effect of three botanical pesticides, including dayabon®, *Eruca sativa* oil, spinach seed extract and, spinach nanoemulsion formulation, has been tested on *A. pisaciae* under laboratory conditions. Moreover, the effect of dayabon and *Eruca sativa* oil compared with fenitrothion (EC 50%) was tested in a pistachio orchard in the Dehmolla region, Shahrood Iran.

Materials and Methods

Spinach seed extract

Spinach seeds were purchased from Karaj, Iran, and powdered by a blade grinder. 100 g of the seed powder was mixed in 500 ml methanol,

and the mixture was shaken for 48 h. The mixture was filtered, and a rotary evaporator concentrated the solution at 35 °C and 100 rpm. The residue was 5.5 g at the bottom of the flask. It was extracted by 43.7 ml ethanol 80% to provide an aliquot of 125.86 g/l.

Spinach and arugula oil emulsion formulation

The extract was mixed with polyethylene glycol at 2000 rpm for 15 min, then polysorbate 80 was added to make the nanoemulsion formulation, and the whole mixture was mixed at 3000 rpm for 5 min. The final product contained 20% pure spinach extract or arugula oil. The particle size was determined by Malvern Zetasizer (Nano ZS, UK). Dayabon (castor oil, SL 10%), a botanical pesticide, was provided by Daya Nanotechnology Company, Iran.

Bioassays

Preliminary tests were conducted to determine the effective concentrations to cause from 20% to 80% mortality in nymphs. Then, three concentrations were selected between the minimum and maximum lethal concentrations based on the logarithmic intervals. Therefore, concentrations of 1500, 2410, 3872, 6223, and 10000 mg/l for dayabon, 1000, 1778, 3162, 5624, and 10000 mg/l, for *Eruca sativa* oil were made to measure LC₅₀ values. Hence, the mortality tests were done at 500, 1000, 2000, 4000, and 8000 mg/l for formulated *E. sativa* oil and concentrations of 1000, 1732, 3000, 5196, and 9000 mg/l for nonformulated spinach seed extract and five logarithmic concentrations of 50, 100, 200, 400, and 800 mg/l for nanoemulsion of the spinach seed extract. Distilled water was used as the solvent and in control. The infected leaves were collected from the pistachio orchards of Shahrood, Iran. The psyllid nymphal stages were identified with their winged appearance, body color, and movements (Dezianian, and Sahragard, 2004; Mehrnejad, 2014). Nymphs of the same stage on leaves were soaked into the solutions for 5 s and then were left to dry. Each treated leaf was placed on a wet tissue and filter paper in Petri dishes (8 cm diameter)

and kept in a standard condition set at 26 ± 2 °C, $65 \pm 5\%$ RH, and LD 16:8 h. Then, the number of dead nymphs was counted 24 h after treatment. Nymphs that did not move their antennae and legs in response to fine brush stimulation were considered dead. Each test was repeated three times.

The field experiments

For field experiments, the efficacy of dayabon, *Eruca sativa* oil, and a chemical pesticide, fenitrothion (EC 50%), was investigated. Fenitrothion was tested because farmers use it. This experiment was performed in a randomized complete block design in 2016. A pistachio orchard located in Dehmolla, 10 km far from Shahrood, was selected to test pesticides. The tested area was divided into three blocks, and each block was subdivided into six plots. Six trees in each plot were separated from the others by placing a tree between them to prevent the spray from drifting to the adjacent plot. Dayabon (4, 6, and 8 g/l), *Eruca sativa* oil (4 g/l), fenitrothion (2 g/l), and control (water spray) were implemented as treatments. A motor hydraulic sprayer with a conical nozzle was used. The sprayer was washed each time a different treatment was to be used. Sampling was carried out a day before treatment and one, three, seven, 14, and 21 days after treatment. The leaves were selected randomly from five points on each tree. The leaves were taken to the laboratory in a box containing ice. The number of nymphs on both sides was counted in the laboratory, and then the efficacy was calculated by the Henderson Tilton formula (Henderson and Tilton, 1955). Henderson Tilton formula:

$$Efficiency(\%) = (1 - \frac{T_a \times C_b}{T_b \times C_a}) \times 100$$

In this formula, C_b and C_a are the number of nymphs in control before and after treatment. T_b and T_a are the number of nymphs in treatment before and after spray, respectively. The data was normalized by the arcsine square root transformation before analysis of variance (ANOVA).

Statistical analysis

The median lethal concentration (LC_{50}) of the tested pesticides was calculated based on the data obtained in contact toxicity bioassays following the probit analysis method described by Finney (1971). Significant differences between LC_{50} s were compared based on the 95% confidence limits of relative median potency estimates. A heterogeneous factor was used to calculate the confidence intervals, provided that the significant probability was more than 0.15. Relative median potency was calculated to test significant differences in treatments between early and late nymphs. Log transformation was used to normalize the nymphs' density before ANOVA. One-way ANOVA was used to test differences among treatments. Groups were separated by post hoc Tukey's test at $p < 0.05$ if ANOVA was significant. All statistical analyses were performed by SPSS version 20.

Results

The laboratory tests

According to the data, nanoemulsion of spinach seed extract had the lowest LC_{50} values on early nymphs (468 mg/l) (Table 1) and for the 5th instar nymphs (124 mg/l) (Table 2). However, dayabon and nonformulated spinach seed extract had the highest LC_{50} (4380 mg/l) for early-stage nymphs. Also, dayabon (3930 mg/l) and nonformulated spinach seed extract (3946 mg/l) had a high LC_{50} on the fifth instars. Comparison of the LC_{50} s in early nymphs showed a significant difference between dayabon and spinach nanoemulsion. There was also a significant difference between nonformulated and nanoemulsions of spinach seed extract, but no significant difference was observed between formulated and nonformulated arugula oil emulsion. For 5th instar nymphs, there was no significant difference between dayabon, arugula oil, and spinach seed extract, but other compounds significantly different from each other. As a result, for nymphs of early instar and 5th instar nymphs, nanoemulsion of spinach seed extract caused the highest mortality. In general, the

range of confidence limits was relatively small, indicating the accuracy of the bioassay. As shown in tables 1 and 2, LC₅₀ values were low, but the probability value is higher than 0.15. This data indicates that the heterogeneity factor did not affect the calculation of the 95% confidence limits. Comparing the LC₅₀s of

treatments between early and late nymphs indicated no significant differences in susceptibility of early and late nymphs to the tested materials, except for formulated spinach seed extract, which indicated that late nymphs were more susceptible than early nymphs (Table 3).

Table 1 Values of LC₅₀ for dayabon, arugula seed oil, and spinach seed extracts on pistachio psylla *Agonoscaena pistaciae* early nymphs.

Treatments	N	P-Value	χ^2	Slope $\pm$ SE	LC ₅₀ (mg/l) (95% CL) ¹
Dayabon	214	0.811	0.961	2.325 $\pm$ 0.341	4380.4a (3640.1 – 5306.5)
Arugula seed oil (ASO)	278	0.190	3.317	1.307 $\pm$ 0.258	2280.987bc (1123.9 – 3633.2)
Formulated ASO (20%)	296	0.311	2.337	1.335 $\pm$ 0.458	2860.677ab (2213.2 – 6096.7)
Spinach seed extract (SSE)	145	0.129	5.676	1.531 $\pm$ 0.319	4380.8a (1576.8 – 121806.7)
Formulated SSE (20%)	148	0.264	3.974	1.400 $\pm$ 0.304	468.4c (3.21.1 – 898.1)

N: Number of nymphs, CL: Confidence limits. ¹ Values followed by the same letters are not significantly different based on the 95% confidence limits of relative median potency estimates.

Table 2 Values of LC₅₀ for dayabon, arugula seed oil, and spinach seed extracts on pistachio psylla *Agonoscaena pistaciae* 5th instar nymphs.

Treatments	N	P-Value	χ^2	Slope $\pm$ SE	LC ₅₀ (mg/l) (95% CL) ¹
Dayabon	860	0.990	4.310	3.247 $\pm$ 0.291	3930.1a (2463.5 – 4997.2)
Arugula seed oil (ASO)	982	0.164	5.110	1.370 $\pm$ 0.111	3189.4a (2765.2–3665.3)
Formulated ASO (20%)	758	0.087	6.527	1.491 $\pm$ 0.111	2017.9b (1341.2–2924.2)
Spinach seed extract (SSE)	327	0.587	1.930	1.058 $\pm$ 0.218	3945.7a (2835.1 – 5488.2)
Formulated SSE (20%)	579	0.175	4.953	1.487 $\pm$ 0.144	123.9c (102.8–146.9)

N: Number of nymphs, CL: Confidence limits. ¹ Values followed by the same letters are not significantly different based on the 95% confidence limits of relative median potency estimates.

Table 3 Comparison of LC₅₀s of treatments between early and late nymphs of *Agonoscaena pistaciae*.

Treatments	LC ₅₀ (mg/l)		RMP	95% confidence limits		Sig.
	Early nymph	Late nymph		lower	upper	
Dayabon	4380.4	3930.1	1.115	0.887	1.704	ns
Arugula seed oil (ASO)	2281.0	3189.4	0.715	0.201	1.045	ns
Formulated ASO (20%)	2860.7	2017.9	1.418	0.692	2.514	ns
Spinach seed extract (SSE)	4380.8	3945.7	1.110	0.714	1.964	ns
Formulated SSE (20%)	468.4	123.9	3.780	2.496	7.199	*

RMP: Relative median potency, ns: non significant, *: significant at $p < 0.05$.

Field experiments

The efficiency of plant extracts on nymphs

The efficiency of plant extracts on early nymph instars is shown in table 4. The efficiency of dayabon did not increase significantly among days after treatments. The highest efficiency of 95% was observed at 4000 and 8000 mg/l dayabon, 21 days after treatment. Therefore, it seems that a concentration of 4000 mg/l dayabon

can protect the plant from insect damage for three weeks. Also, *E. sativa* oil emulsion at 4000 mg/l 81.31% control two weeks after treatment. The efficacy of fenitrothion was 49.13% one day after treatment, and then it dropped to 24.99% 21 days after treatment, but this change was not significant.

The population density of early nymph instars in the field experiment is shown in Table 5.

Table 4 Efficiency of dayabon, arugula seed oil, and fenitrothion on nymphs of pistachio psylla *Agonoscapa pistaciae* under field conditions.

Time (DAT)	Efficiency (Mean $\pm$ SE) (%)				
	Dayabon (4000 mg/l)	Dayabon (6000 mg/l)	Dayabon (8000 mg/l)	Arugula seed oil (4000 mg/l)	Fenitrothion (2000 mg/l)
1	59.50 $\pm$ 13.52	62.45 $\pm$ 18.11	57.31 $\pm$ 16.62	57.46 $\pm$ 6.15	49.13 $\pm$ 19.89
3	59.18 $\pm$ 9.51	69.58 $\pm$ 8.01	65.90 $\pm$ 17.23	65.23 $\pm$ 11.14	45.33 $\pm$ 22.71
7	69.47 $\pm$ 5.88	76.99 $\pm$ 6.46	80.86 $\pm$ 5.24	64.87 $\pm$ 14.01	38.74 $\pm$ 27.60
14	86.73 $\pm$ 5.69	94.91 $\pm$ 2.82	80.22 $\pm$ 7.16	81.31 $\pm$ 11.58	33.85 $\pm$ 18.33
21	91.68 $\pm$ 4.77	94.70 $\pm$ 2.64	95.43 $\pm$ 1.87	79.37 $\pm$ 17.30	24.99 $\pm$ 15.17
F	4.480	2.884	3.193	4.253	0.464
df(t, e)	4, 8	4, 8	4, 8	4, 8	4, 8
p-value	0.034	0.095	0.076	0.039	0.761
Tukey HSD ¹	0.066	0.152	0.054	0.067	0.772

DAT: Days after treatment. ¹Tukey's test was not significant for treatments.

Table 5 Mean population density of early nymphs (1st and 2nd instar nymphs) of pistachio psylla *Agonoscapa pistaciae* before and after treatments under field conditions.

Days after treatment	Number of nymphs per leaf (Mean $\pm$ SE)					
	Control	Dayabon (4000 mg/l)	Dayabon (6000 mg/l)	Dayabon (8000 mg/l)	Arugula seed oil (4000 mg/l)	Fenitrothion (2000 mg/l)
0	5.33 $\pm$ 2.60 b	6.00 $\pm$ 2.08	6.33 $\pm$ 2.96	4.33 $\pm$ 2.96	3.33 $\pm$ 2.40	2.67 $\pm$ 2.19
1	3.67 $\pm$ 0.33 b	5.67 $\pm$ 3.28	3.00 $\pm$ 1.00	7.00 $\pm$ 4.04	5.67 $\pm$ 1.45	1.67 $\pm$ 1.20
3	3.67 $\pm$ 0.67 b	2.33 $\pm$ 0.67	3.00 $\pm$ 1.00	2.33 $\pm$ 0.67	2.67 $\pm$ 0.88	3.00 $\pm$ 1.73
7	6.33 $\pm$ 0.88 ab	1.33 $\pm$ 0.33	1.33 $\pm$ 0.88	2.00 $\pm$ 0.00	3.33 $\pm$ 1.45	2.00 $\pm$ 0.58
14	14.33 $\pm$ 3.18 a	2.67 $\pm$ 0.88	1.33 $\pm$ 0.88	2.33 $\pm$ 0.88	3.00 $\pm$ 1.53	7.33 $\pm$ 2.33
21	14.33 $\pm$ 3.48 a	2.00 $\pm$ 0.58	1.67 $\pm$ 0.67	0.67 $\pm$ 0.33	3.67 $\pm$ 2.67	8.00 $\pm$ 3.61
F	3.962	1.385	1.769	1.220	2.342	0.367
df (t, e)	5, 10	5, 10	5, 10	5, 10	5, 10	5, 10
P-value	0.031	0.308	0.207	0.368	0.118	0.860

Means followed by the same letters in each column are not significantly different (Tukey's test, $P < 0.05$).

Treatments of dayabon and arugula oil did not show a significant difference in the population density of nymphs in the days after treatment. Moreover, fenitrothion did not show a significant population decrease before and after treatment. In general, the population density of young nymphs was low and ranged from 3.67 to 14.33 nymphs per leaf in control and from 0.67 to 8.00 nymphs per leaf in treatments at the time of field experiment.

The population density of middle-aged nymphs (3rd and 4th instars) is shown on the

day before treatment, and the density up to 21 days after treatment (Table 6). The population density of nymphs in dayabon treatment was significantly lower after treatment than before treatment. However, it was not significant at 8000 mg/l. No significant difference was observed between the day before and after treatment for the arugula oil and fenitrothion.

The population density of fifth instar nymphs in field experiments is shown in Table 7.

Table 6 Mean population density of mid nymphs (3rd and 4th instar nymphs) of pistachio psylla *Agonoscena pistaciae* before and after treatments under field condition.

Days after treatment	Number of nymphs per leaf (Mean $\pm$ SE)					
	Control	Dayabon (4000 mg/l)	Dayabon (6000 mg/l)	Dayabon (8000 mg/l)	Arugula seed oil (4000 mg/l)	Fenitrothion (2000 mg/l)
0	26.33 $\pm$ 12.25	18.33 $\pm$ 4.41 a	12.67 $\pm$ 2.67 a	8.00 $\pm$ 5.13	4.67 $\pm$ 1.76	8.67 $\pm$ 5.67
1	9.67 $\pm$ 6.69	5.67 $\pm$ 1.45 ab	9.00 $\pm$ 6.03 ab	2.67 $\pm$ 1.20	7.67 $\pm$ 1.76	3.67 $\pm$ 0.88
3	7.33 $\pm$ 1.76	6.67 $\pm$ 0.33 ab	3.67 $\pm$ 0.67 abc	2.67 $\pm$ 1.20	3.33 $\pm$ 0.88	1.67 $\pm$ 0.88
7	8.00 $\pm$ 0.00	2.00 $\pm$ 0.58 bcd	2.00 $\pm$ 0.58 abc	1.33 $\pm$ 0.33	1.67 $\pm$ 0.88	2.67 $\pm$ 1.45
14	8.00 $\pm$ 1.15	1.33 $\pm$ 0.88 cd	0.67 $\pm$ 0.33 bc	2.00 $\pm$ 0.58	1.00 $\pm$ 1.00	5.00 $\pm$ 2.31
21	10.67 $\pm$ 0.67	0.67 $\pm$ 0.67 d	0.33 $\pm$ 0.33 c	0.33 $\pm$ 0.33	2.67 $\pm$ 2.19	8.00 $\pm$ 3.06
F	1.982	12.854	7.652	1.834	1.061	2.538
df (t, e)	5, 10	5, 10	5, 10	5, 10	5, 10	5, 10
P-value	0.167	< 0.0001	0.003	0.194	0.436	0.099

Means followed by the same letters in each column are not significantly different (Tukey's test, $P < 0.05$).

Table 7 Mean population density of 5th instar nymphs of pistachio psylla *Agonoscena pistaciae* before and after treatments under field condition.

Days after treatment	Number of nymphs per leaf (Mean $\pm$ SE)					
	Control	Dayabon (4000 mg/l)	Dayabon (6000 mg/l)	Dayabon (8000 mg/l)	Arugula seed oil (4000 mg/l)	Fenitrothion (2000 mg/l)
0	38.33 $\pm$ 13.02	46.33 $\pm$ 8.21 a	45.33 $\pm$ 2.60 a	41.67 $\pm$ 4.41 a	57.33 $\pm$ 8.69 a	21.00 $\pm$ 4.58 a
1	4.33 $\pm$ 1.45	6.33 $\pm$ 1.67 b	3.67 $\pm$ 0.88 b	4.00 $\pm$ 1.15 b	4.33 $\pm$ 0.88 b	2.33 $\pm$ 0.33 b
3	11.33 $\pm$ 4.91	6.33 $\pm$ 2.85 b	4.00 $\pm$ 1.00 b	1.67 $\pm$ 1.20 bc	5.67 $\pm$ 1.20 b	2.67 $\pm$ 0.67 b
7	16.00 $\pm$ 4.58	6.00 $\pm$ 2.08 b	2.67 $\pm$ 0.33 b	0.67 $\pm$ 0.33 bc	3.67 $\pm$ 0.88 bc	0.67 $\pm$ 0.33 b
14	17.00 $\pm$ 8.62	0.67 $\pm$ 0.33 c	0.00 $\pm$ 0.00 c	1.00 $\pm$ 0.00 bc	1.67 $\pm$ 0.88 bc	2.00 $\pm$ 1.00 b
21	18.67 $\pm$ 6.84	0.33 $\pm$ 0.33 c	0.33 $\pm$ 0.33 c	0.33 $\pm$ 0.33 c	0.33 $\pm$ 0.33 c	12.00 $\pm$ 2.08 a
F	1.308	21.376	74.320	24.455	15.195	23.931
df (t, e)	5, 10	5, 10	5, 10	5, 10	5, 10	5, 10
P-value	0.335	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means followed by the same letters in each column are not significantly different (Tukey's test, $P < 0.05$).

The population density of nymphs in the treatment of dayabon, arugula oil, and fenitrothion on the days after treatment was significantly lower than on the day before treatment. However, the pest density did not change significantly in control. In general, the population density of fifth instar nymphs is

much higher than young nymphs. It means that in field experiments, the last instar nymphs were the dominant population. Population density of total mixed stages is shown in table 8. Again, the data showed a significant decrease in the density of treatments the days after treatments.

Table 8 Mean population density of total instar nymphs of pistachio psylla *Agonoscena pistaciae* before and after treatments under field conditions.

Days after treatment	Number of nymphs per leaf (Mean $\pm$ SE)					
	Control	Dayabon (4000 mg/l)	Dayabon (6000 mg/l)	Dayabon (8000 mg/l)	Arugula seed oil (4000 mg/l)	Fenitrothion (2000 mg/l)
0	70.00 $\pm$ 11.55a	70.67 $\pm$ 13.86a	64.33 $\pm$ 3.84a	54.00 $\pm$ 12.06a	65.33 $\pm$ 10.68a	32.33 $\pm$ 12.39
1	17.67 $\pm$ 7.06c	17.67 $\pm$ 6.06ab	15.67 $\pm$ 7.22ab	13.67 $\pm$ 4.26b	17.67 $\pm$ 2.33ab	7.67 $\pm$ 0.88
3	22.33 $\pm$ 6.44bc	15.33 $\pm$ 3.53ab	10.67 $\pm$ 2.33bc	6.67 $\pm$ 2.33bc	11.67 $\pm$ 2.67ab	7.00 $\pm$ 2.08
7	30.33 $\pm$ 5.17abc	9.33 $\pm$ 2.60c	6.00 $\pm$ 1.15bc	4.00 $\pm$ 0.58bc	8.67 $\pm$ 1.86c	5.33 $\pm$ 1.67
14	39.33 $\pm$ 5.21abc	4.67 $\pm$ 2.03c	2.00 $\pm$ 1.15c	5.33 $\pm$ 1.33bc	5.67 $\pm$ 2.73c	14.33 $\pm$ 5.17
21	43.67 $\pm$ 4.48ab	3.00 $\pm$ 1.15c	2.33 $\pm$ 1.33bc	1.33 $\pm$ 0.33c	6.67 $\pm$ 5.17c	23.33 $\pm$ 8.84
F	6.454	10.041	13.840	19.107	6.926	3.474
df (t, e)	5, 10	5, 10	5, 10	5, 10	5, 10	5, 10
P-value	0.006	0.001	< 0.0001	< 0.0001	0.005	0.051

Means followed by the same letters in each column are not significantly different (Tukey's test, $P < 0.05$).

Discussion

The results showed that the tested plant compounds were effective on nymphs of pistachio psyllids. The effect of NEF on spinach seed extract was significantly higher than the non-formulated extract and the other compounds. It has been shown that the use of NFEs causes higher mortality in insects because NFEs increase good wetting and dispersing ability and cover the body of the insect (Lai *et al.*, 2006). For instance, the formulation of *Artemisia arborescens* L. extract with solid lipid nanoparticles improved the type, quality, and quantity of the oil during storage and caused higher larval mortality when nanoparticles were attached to the larvae *Bmisia tabaci* (Gennadius) (Moretti *et al.*, 2002). Also, studies have shown that insect mortality significantly increases when larvae are exposed to nanoformulations (Passino *et al.*, 2004). Moreover, by controlling the release of active ingredients, nanoformulations improve the

persistence of efficacy (Passino *et al.*, 2004; Mahdavi *et al.*, 2018).

The more significant effect of nanoformulation of spinach seed extract in the laboratory can be increased permeability, dispersibility, and reducing the surface tension of droplets. Studies have shown that spinach seed and leaf extracts contain the 20-hydroxyecdysone and polypodine B (Greibenok *et al.*, 1991). Also, insecticidal properties of phytoecdysteroids extracted from spinach and other plants such as *Silene aucheriana* Boiss and Ostrich fern *Matteuccia struthiopteris* (L.) on several insects such as diamondback moth *Plutella xylostella* (L.) (Kouhjeni Gorji *et al.*, 2014; Tabebordbar and Moharramipour, 2014, 2015), termite *Reticulitermes* sp (Shahini *et al.*, 2015), and Mediterranean flour moth *Ephestia kuehniella* Zeller (Sahaf and Moharramipour, 2013, 2014) has been proven. These compounds can cause death and deformity in the pupae of these insects.

Arugula oil has been shown to contain significant amounts of glucosinolates (Vahabi

Mashhoor *et al.*, 2021) and has significant insecticidal properties. In the studies of Tsao *et al.* (1996), glucosinolates extracted from the Brassicaceae family were effective on several insects. Li *et al.* (2000) proved that *Brassica juncea* (L.) Czern leaf extract contains high levels of myrosinase that convert glucosinolates to an isothiocyanate, reducing the feeding and leaf damage caused by the larvae of *P. xylostella*. Also, a high level of isothiocyanate erucin has been reported from arugula oil highly toxic to *Tetranychus urticae* (Seifi *et al.*, 2018). Therefore, the pesticide activity of arugula oil affects mites also. In this regard, the insecticidal activity of arugula oil containing erucin has been reported in *Blatella germanica* (Rezaei *et al.*, 2019) and *Xanthogaleruca luteola* (Muller) (Vahabi Mashhoor *et al.*, 2021a, b).

In this study, dayabon had a significant effect on the mortality of *A. pistaciae* in laboratory and field conditions. In addition to the acaricidal activity, the noticeable insecticidal activity of dayabon has been reported on several insect pests. For instance, the contact toxicity of dayabon on *Aphis nerii* Boyer and *Aphis fabae* Scopoli with the LC₅₀ of 4590 and 4130 mg/l has been reported, respectively (Vahabi *et al.*, 2016a). In contrast, it was safe for adults and larvae of *Coccinella septempunctata* L. at LC₈₀ level (Vahabi *et al.*, 2016a). The assessment of dayabon on larvae and adults of *X. luteola* at 4000 to 6000 mg/l causes 50% mortality and reduces leaf damage (Vahabi *et al.*, 2016b). Dayabon (SL 10%) contains castor oil as an active ingredient. Castor oil is obtained by pressing the castor bean *Ricinus communis* L., which consists of ricinoleic acid and other 16 and 18 carbon fatty acids (Majnon Hoseini and Davazdah Emami, 2007). Although the insecticidal activity of the studied plants on pistachio psyllids is reported for the first time, previously, other plant extracts have been tested on pistachio psyllids, some of which are significant. For example, the LC₅₀ value of ethanolic extract of *Thymus vulgaris*, *Rosmarinus officinalis*, *Ricinus communis*, and *Sophora alopecuriodes* L. on the first instar nymphs of *A. pistaciae* has been reported from

131 to 921 g/l (Salehi *et al.*, 2015). The extracts of henna *Lawsonia inermis* leaves and orange peels *Citrus sinensis* at 80 µl/ml on *A. pistaciae* nymphs showed significant control (Rouhani *et al.*, 2019). The use of neem extract *Azadirachta indica* in the control of pistachio psyllids in the laboratory at 75 and 100 mg/l was effective on nymphs and adult insects, respectively (Homayonfar and Zohdi, 2012). The study on the insecticidal activity of Acetamiprid and alcoholic extract of *Ferula assafoetida* on the 5th instar nymphs showed that *F. assafoetida* extract was more effective (Izadi *et al.*, 2012). Abedi *et al.* (2012) have tested several chemical pesticides and plant extracts such as *Melia azadirach* L. against pistachio psyllid nymphs and concluded that Actara (WG 25%) was the most effective at 300 mg/l. In the field experiments, the number of higher instars, especially the fifth instar nymphs of pistachio psyllids, was approximately 8 to 10 times higher than the early instars on the day before treatment. Like other phosphorus pesticides, it seems that fenitrothion appears to be more effective in the early nymphal stage (Reyes *et al.*, 2007). Therefore, in terms of the population's age structure, the low toxicity of fenitrothion compared to dayabon can be reasonable. Research has shown that oil-based plant pesticides significantly affect older insects because larger insects receive more oil coverage of the body surface (Rezaei, and Moharramipour, 2019). However, our studies show that the effect of dayabon on young and old nymphs was not significantly different. It was also observed that dayabon maintained its relatively good activity for up to three weeks after treatment. This activity may be due to the high effect in the early days after treatment.

However, the colony will take time to increase again. Therefore, the higher impact for two weeks can be explained by the time-consuming formation of new colonies. This impact should not be attributed to the persistence of toxic compounds on the plant, as most plant-based compounds become ineffective after foliar application. However, sublethal doses can also reduce the population (Havasi *et al.*, 2020).

Therefore, if they can have a decisive effect in the first days after treatment, they can delay the colony's establishment and pest population growth. Since the field experiment was conducted in the summer in July, due to high temperatures, the population growth rate is high, and the age structure of the population changes more rapidly so that the emergence of adult insects increases the number of eggs laid. It could be concluded that it is better to wait until the oviposition period of adults be completed as much as possible and the maximum number of eggs is hatched. Since, unlike most pesticides, dayabon is effective in old nymphal stages. Therefore, determining the exact time of treatment can have a significant impact on the effectiveness of pesticides.

Because dayabon is effective at 4 to 8 g/l, farmers can decide on its economic cost compared to other chemical pesticides. Since dayabon is a plant-based pesticide that is low-risk to humans and has been reported to be safe for some natural enemies, including parasitoids, more studies are needed. Although spinach seed extract had high efficacy in the laboratory, it was ineffective in field experiments. A review of pistachio psyllids studies shows that plant extracts are more or less effective on psyllid nymphs. However, consideration should be paid to the cost of production, storage ability, persistence on the pest, and its side effects on natural enemies for the practical use of plant extracts. Despite laboratory studies, few studies have been performed on the effectiveness of plant compounds in the field, and more field studies are necessary to find plant compounds as an alternative to pesticides.

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تأثیر برخی ترکیبات گیاهی بر پسیل پسته (*Agonosca pistaciae* (Hemiptera: Psyllidae) شرایط آزمایشگاهی و صحرایی

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چکیده: پسیل پسته، *Agonosca pistaciae* Burkhardt & Lauterer (Hem.: Psyllidae) یکی از مهم‌ترین آفات باغ‌های پسته در ایران است. استفاده از سموم شیمیایی در اوایل فصل رشد تا زمان برداشت بر سلامت کشاورزان و مصرف‌کنندگان تأثیر گذاشته است. در این پژوهش، اثر روغن دانه گیاه منداب *Eruca sativa*، عصاره دانه اسفناج *Spinacia oleracea* و Dayabon® (SL 10%) روی پوره پسیل پسته در آزمایشگاه در دمای 26 ± 2 درجه سلسیوس، رطوبت نسبی 5 ± 65 درصد و دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی مورد بررسی قرار گرفت. مقادیر LC_{50} نانوامولسیون (NEF) عصاره دانه اسفناج و عصاره فرموله نشده آن روی پوره‌های سنین اولیه به ترتیب ۴۶۸ و ۴۳۸۱ میلی‌گرم در لیتر بود. در حالی که مقدار LC_{50} روغن دانه منداب و دایابون به ترتیب ۲۲۸۱ و ۴۳۸۰ میلی‌گرم در لیتر بود. NEF عصاره دانه اسفناج ($LC_{50} = 124$ میلی‌گرم در لیتر) نسبت به سایر ترکیب‌ها روی پوره سن پنجم به طور قابل توجهی مؤثرتر بود. تفاوت معنی‌داری در عصاره دانه اسفناج فرموله نشده ($LC_{50} = 3946$ میلی‌گرم در لیتر)، روغن بذر منداب ($LC_{50} = 3189$ میلی‌گرم در لیتر) و دایابون ($LC_{50} = 3930$ میلی‌گرم در لیتر) روی پوره سن پنجم وجود نداشت. علاوه بر این، کارایی دایابون، روغن دانه منداب و سم فنیتروتیون، در باغ پسته واقع در شهرستان دهملا، شاهرود مورد مطالعه قرار گرفت. نمونه‌گیری قبل و بعد از تیمار انجام شد. در روزهای پس از تیمار، تفاوت معنی‌داری بین تیمارها مشاهده نشد. بدین معنی که تا ۲۱ روز پس از تیمارها از اثر ترکیبات گیاهی کاسته نشده است. یافته‌ها نشان داد که آفت‌کش‌های گیاهی مطالعه شده در این پژوهش می‌توانند روی پسیل پسته مؤثر باشند.

واژگان کلیدی: پسیل پسته، نانوامولسیون، عصاره اسفناج، دایابون، روغن منداب