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Composition of *Pistacia khinjuk* (Anacardiaceae) Leaf Essential Oil and its Insecticidal Activity on Common Pistachio Psyllid, *Agonoscena pistaciae* (Hem., Psylloidea)

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Abstract: Wild pistachio species, *Pistacia khinjuk* Stocks, is the non-preferred host plant of common psyllid, *Aganoscena pistaciae*, the key pest of cultivated pistachios, *P. vera* L. in many Asian countries. *P. khinjuk* essential oil (EO) was obtained from leaf samples via hydro-distillation. EO chemical composition was identified by GC and GC-MASS. Forty compounds out of 48 total compounds were identified in oil. The major constituents of EO were myrcene (18.7 %), α-eudesmol (12.3 %), β-eudesmol (9.3 %), 1,7-di-epi-β-cedrene (7.3 %), bicyclogermacrene (5.6 %) and δ-eudesmol (4.9 %). Insecticidal activity of EO and pure myrcene, as the major EO constituent, were separately determined through contact bioassay tests against the psyllid eggs and nymphs. Both EO and myrcene caused approximately equal mortalities of 30-45 % on eggs and 65-90 % on nymphs. This is the first report about the insecticidal activity of *P. khinjuk* essential oil against *A. pistaciae*.

Key words: *Agonoscena pistaciae,* insecticidal activity, essential oil composition, myrcene, *Pistacia khinjuk*.

Introduction

Pistacia genus belongs to the Anacardiaceae family and consists of at least 11 species of dioecious trees and shrubs, among which pistachio, *Pistacia vera* L., is the most important species due to its commercially valuable edible nuts [1,2]. *P. khinjuk* could be a descendent of *P. vera* [3] that is primarily used as rootstocks for pistachio [4]. It has common names of Gulungoor, Gwun, Khinjuk, Shurumma (Afghanistan), Ushgai, Buzgai (Baluchistan) [3], Kakarsinghee in India [5], Bittim in Turkey [6] and Kasour in Iran. Geographical distribution of *P. khinjuk* extends from southeastern Turkey to northern Syria, northern Iraq, the mountains of western and southern Iran, and from there through Pakistan, Baluchistan, and into eastern Afghanistan. Towards the west, this species penetrated into southern Jordan, Hijaz, south Sinai and the eastern desert of Egypt [7].

Literatures are available about EO properties of some *Pistacia* species like *P. lentiscus* [8] and that of *P. atlantica* [9]. Limited studies are available on some antibacterial activities of *P. khinjuk* leaf EO [10] and its composition of EO which was derived from gum and fruits [11,12]. There is still no information about *P. khinjuk* possible detrimental effects on *A. pistaciae* that feeds on leaf phloem sap of pistachios. The insect has dominant activity on pistachios while it acquires rarely on its non-preferred hosts including wild-growing

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P. khinjuk trees [13].

The objectives of the present study were to determine the chemical composition of *P. khinjuk* essential oil and also to evaluate the insecticidal activity of EO on egg and nymphal stage of the common pistachio psyillid.

Materials and methods

Plant material

Leaf samples were collected from 20 years old *Pistacia khinjuk* trees adapted in a research orchard, located at pistachio research station (Rafsanjan, Iran). Fresh leaves were collected in the middle of spring (2012). The voucher specimen has been deposited in the national herbarium of Iran (TARI).

EO isolation procedure

Sampled leaves were air-dried and then subjected to hydro-distillation using a Clevenger-type apparatus for 2.5 h. The oils separated from water and dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis [14].

GC and GC/MS analysis

The oil was analyzed according to the method described by Sefidkon, *et al.*[14] using capillary gas chromatography. The oils obtained from three distillations were mixed and then injected to a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed to be held at 40°C for 5 minutes and then increased to 280°C at a rate of 4°C/min. Injector and detector (FID) temperatures were 290°C, and helium was used as carrier gas with a linear velocity of 32 cm/s, and split ratio 1/60.

GC-MS analysis was accomplished according to the method described by Sefidkon, *et al*.[14] using a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.). Oven temperature was 40°C increasing to 250°C at a rate of 4°C, transfer line temperature 260°C. The carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV, scan time 1 s and mass range of 40-300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors.

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds, and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature [15,16]. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Insecticidal activity assays

Insects

Adult psyllids were collected by shaking infected pistachio shoots and leaves into hyaline plastic containers [17] and then immediately covered with a fine mesh. Adults were transferred to the laboratory. Eggs and nymphs were obtained by introducing adults (100-120 unsexed adults per container) on fresh pistachio leaves from Ahmadaghaei cultivar. After 24 h, the adults were removed, and a part of the leaves, containing eggs, were used in egg tests. Other leaves were allowed until their eggs developed to nymphs for nymph tests. 24-36 hours-old eggs, 1-4th instar nymphs and 5th (the last) nymphs were separately used in bioassays. Insects were reared in laboratory condition with of 25±3°C, 65±5 % R.H. and light regime 16:8 hours (L:D).

EO and myrcene preparations

EO was diluted in distilled water plus Tween-20 (Amersco) (0.5 %) to 0 % (control), 0.125, 0.25 and 0.50 % (v/v) concentrations. These three concentrations of EO were applied in contact toxicity experiments. In separate experiments, Myrcene (95 % Sigma-Aldrich) was diluted in distilled water plus Tween-20 (0.5 %) to 0.5 % concentration.

Contact toxicity bioassays

Bioassays were conducted according to the method by Yang *et al*.[18] with some modifications. The number of insect or nymphs was counted on each test leaf before treatment. The leaflets (each pistachio leaf include three to five leaflets) with eggs were dipped in essential oils (0.125 %, 0.25

% and 0.5 %) for 5 s [19,20]. For the controls, leaves with eggs were dipped in distilled water plus tween-20 (0.5 %). The number of hatched nymphs was counted after 24 and 48 h after treatment. Died eggs were recognized from the live ones with their different color and shrunk surface. The leaves with either the 1-4th instar nymphs or 5th instar ones were sprayed with EO preparations. Number of survived nymphs was counted after 24 and 48 h. The experiments were conducted in laboratory condition at 25±3°C, 65±5 % R.H. and light regime 16:8 hours (L:D).

Experimental design and data analysis

Each toxicity tests was made in layout of completely randomized design with five treatments including 0 % EO or myrcene (control), 0.125 %, 0.25 % and 0.50 % *P. khinjuk* EO and 0.50 % myrcene. Each test consisted of 6-8 replications, with 100 insects per replication and the total number of 600-800 eggs or nymphs per treatment. Comparisons were made between mortalities recorded after 8 and 24 h. In order to normalization of percent data, mortality data were transformed using Arcsine method prior to analysis. Toxicity data were analyzed using one-way ANOVA in SPSS 10.0.

Results

Essential oil composition

The oil isolated from *P. khinjuk* leaves by hydro-distillation was as a pale yellow liquid. The mean oil yield was 1.82 ± 0.12 mg Kg^{-1} dry mass leaves. The gas chromatogram of the essential oil is shown in Fig. 1. Forty compounds were identified in oil, accounted 58.3 % of the total (Table 1). The major constituents of three EO samples were: myrcene (18.7 %), α-eudesmol (12.3 %), β-eudesmol (9.3 %), 1,7-di-epi-β-cedrene (7.3 %), bicyclogermacrene (5.6 %) and δ-eudesmol (4.9 %).

Effect of *P. khinjuk* EO and myrcene on eggs

After 8 h of spraying, no significant difference was observed in eggs hatchability between control and different concentrations of *P. khinjuk* EO and even myrcene (F=2.827, df=4, 27, p=0.07) (Fig. 2 and 4) while these effects were significant after 24 h (F=4.07, df=4,27, p<0.001). Treatments of 0.25 % and 0.50 % EO and 0.50 %

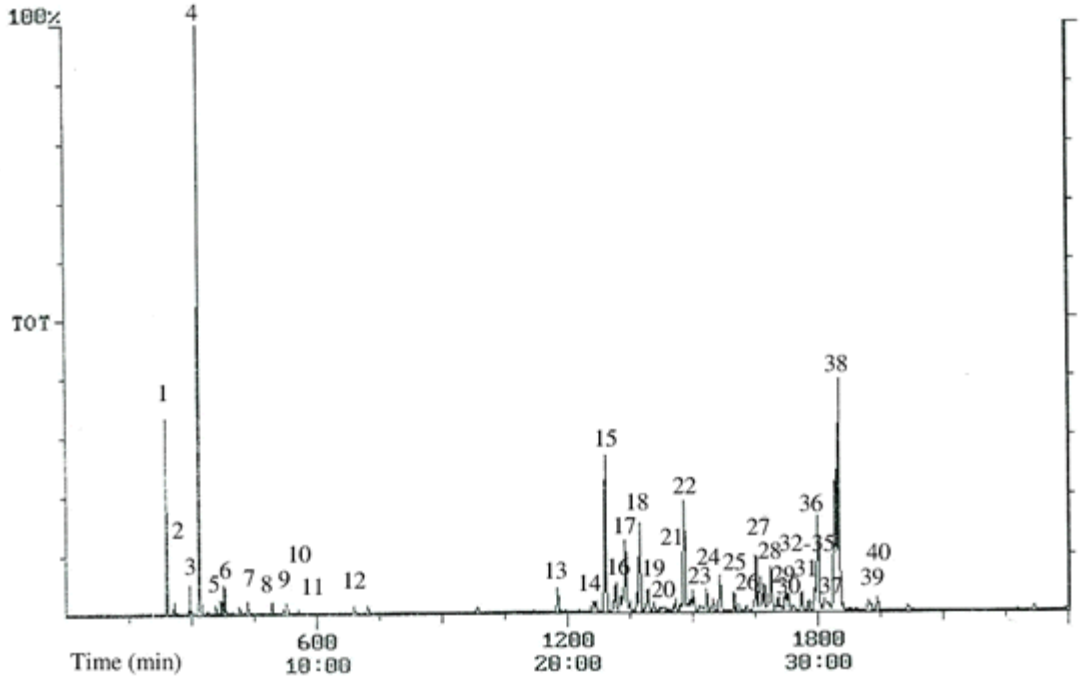

Figure 1. Typical gas chromatogram of the essential oil of *Pistacia khinjuk* adapted in a research orchard located at pistachio research station (Rafsanjan, Iran). The different compounds are shown by Arabic numerals according to Table 1.

Table 1. Chemical composition of the leaf essential oil of *Pistacia khinjuk* Stocks

No.	Component	RI	% composition
1	α-Pinene	936	3.8
2	Camphene	950	0.3
3	β-Pinene	976	0.6
4	Myrcene	985	18.7
5	α-Terpinene	1016	0.3
6	*p*-Cymene	1022	0.4
7	Limonene	1026	0.7
8	e-β-Ocimene	1041	0.2
9	γ-Terpinene	1051	0.4
10	Terpinolene	1081	0.4
11	n-Nonanal	1172	0.4
12	Terpinene-4-ol	1177	0.2
13	Cyclosativene	1362	0.9
14	α-Gurjunene	1396	0.6
15	1,7-di-epi-β-Cedrene	1399	7.3
16	*cis*-Thujopsene	1431	1.2
17	γ-Elemene	1436	0.7
18	Aromadendrene	1437	3.8
19	α-neo-Clovene	1454	0.8
20	α-Humulene	1455	3.9
21	*allo*-Aromadendrene	1463	1.2
22	β-Acoradiene	1476	0.3
23	*cis*-β-Guaiene	1483	0.4
24	Bicyclogermacrene	1498	5.6
25	γ-Patchoulene	1499	0.6
26	Cuparene	1505	1.1
27	β-Vetivene	1508	0.8
28	δ-Cadinene	1518	0.5
29	10-epi-Cubebol	1534	1.5
30	Germacrene B	1552	0.7
31	(E)-Nerolidol	1558	2.6
32	Z-3-Hexenyl benzoate	1574	1.6
33	Spathulenol	1578	1.3
34	Caryophyllene oxide	1582	2.7
35	Longiborneol	1597	0.9
36	Eremoligenol	1614	0.9
37	γ-Eudesmol	1624	4.9
38	Hinesol	1638	0.3
39	β-Eudesmol	1649	9.3
40	α-Eudesmol	1654	12.3

The components are listed in the order of their elution on the DB-5 column.

myrcene had shown higher egg mortalities of 17.99±3.48 %, 46.15±3.74 % and 31.94±6.18 %, respectively (Fig. 3 and 4).

Effect of *P. khinjuk* EO and myrcene on nymphs

After both 8 and 24 h, there was a significant difference between mortalities of 1-4th and 5 instar nymphs in control and different treatments (8 h: F=2.47, df=4, 55, p<0.05; 24 h; F=4.03, df=4, 55, p<0.001) while no significant different were observed between different treatments. The lowest mortality rate was observed in control (4.50 ± 1.87 % after 8 h and 6.97 ± 1.47 % after 24 h). The highest ones were in EO and myrcene treatments with 45-58 % mortalities after 8 h (Fig. 2

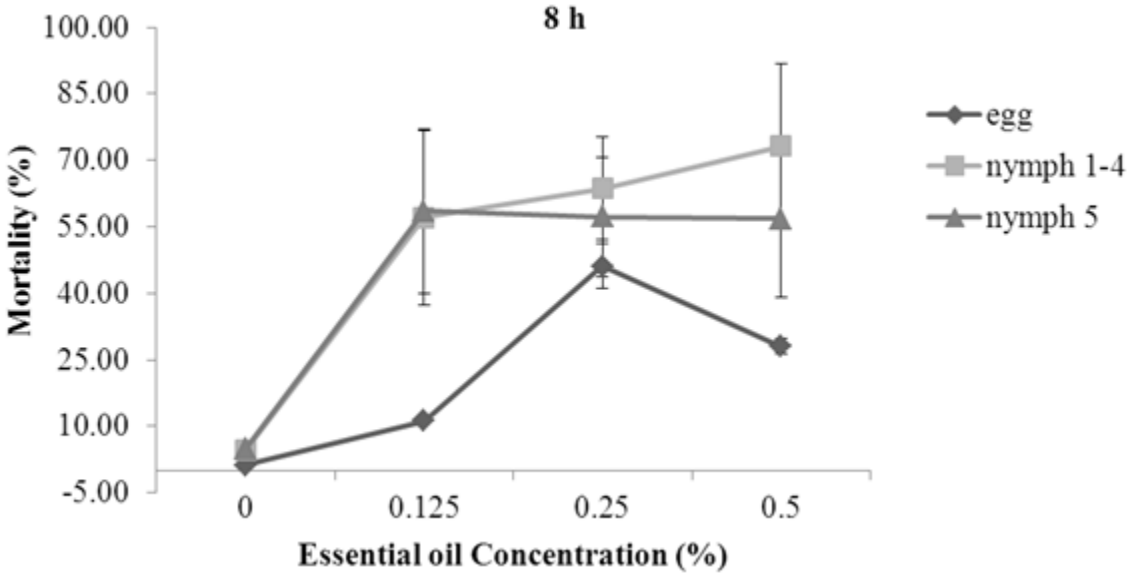

Figure 2. Mean percent mortality (±SE) of *Agonoscena pistaciae* eggs and nymphs (1-4th instar and 5th instar) after 8 h of spraying different concentrations of *Pistacia khinjuk* essential oil preparations and myrncene

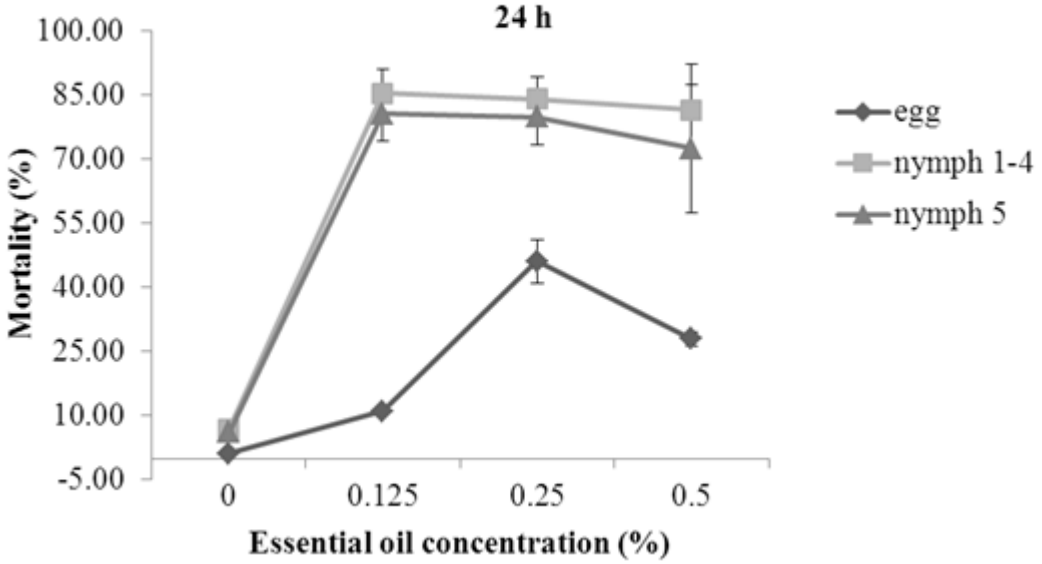

Figure 3. Mean percent mortality (±SE) of *Agonoscena pistaciae* eggs and nymphs (1-4th instar and 5th instar) after 24 h of spraying different concentrations of *Pistacia khinjuk* essential oil preparations and myrncene

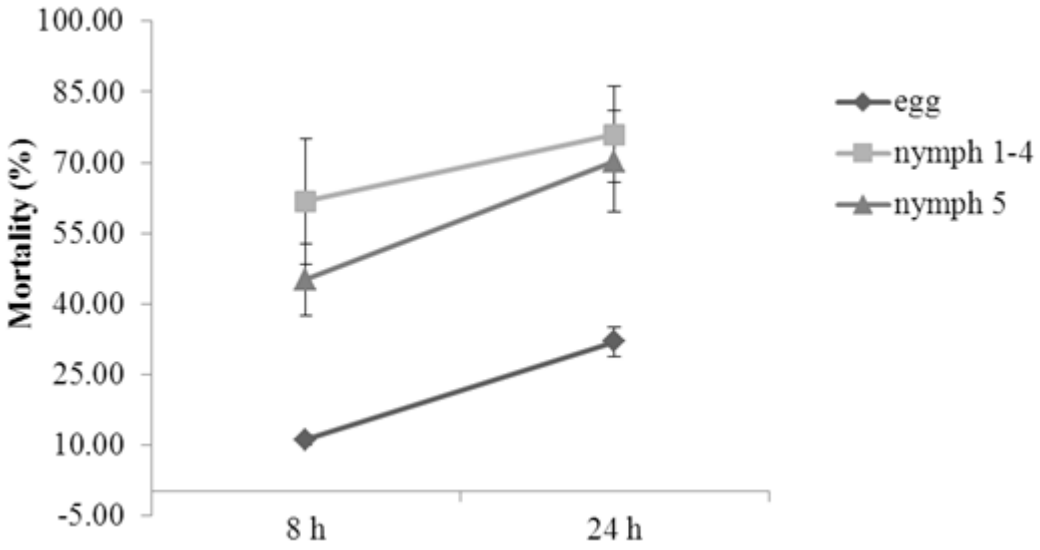

Figure 4. Mean percent mortality (±SE) of *Agonoscena pistaciae* eggs and nymphs (1-4th instar and 5th instar) after 8 and 24 h of spraying 0.50 % myrncene preparation

and 4) and 65-90 % after 24 h (Fig. 3 and 4).

Discussion

EO composition was qualitatively different from the previously determined compositions of previously determined *Pistacia* species leaf-EO including *P. lentiscus* with fifty seven constituents and the major ones of α-pinene (24.9 %), limonene (17.8 %), germacrene D (13.5 %), terpinen-4-ol (10.6 %), *p*-cymene (7.5 %), β-pinene (6.9 %), sabinene (6.7 %), α-terpineol (4.0 %) and γ-terpinene (3.6 %) [7]. It was also varied with that of *P. atlantica* which were α-pinene + α-thujene (66.6 %), camphene (20.8 %), β-pinene (13.1 %), *p*-cymene (10.2 %), terpinen-4-ol (15.9 %) and spathulenol (32.6 %) [8]. Due to the lack of published data on *P. khinjuk* leaf EO, it is impossible to compare the current results with EO composition of other *P. khinjuk* trees from other Iran regions or countries.

The great promise is that EO from leaves of *P. khinjuk* had certain insect effects on both the eggs and the nymphs of *A. pistaciae* although its efficacy on nymph was approximately two-folds higher then the latter. However, these effects have shown to be increased with the time. Different susceptibilities of eggs and nymphs to the EO might be attributed to differences in one or more physical or physiological characteristics like relative permeability of the nymph cuticle and egg shell and also their respiration activities. Myrcene as a knwon monoterpene and the major component of EO had also shown a comparable insecticidal effects. It is more efficient on the nymphs than the eggs while different nymphal stages have shown equal susceptibility to myrcene (Fig. 4). The high toxicity of myrcene preparations may also justify its role in high toxicity of *P. khinjuk* EO to *A. pistaciae*. In conclusion, the EO from *P. khinjuk* had strong contact toxicity to *A. pistaciae*. As a natural EO it is environmentally non-persistent and safe for humans as well as other mammals and it may be used as an active ingredient in a botanical insecticide. However, further researches need to be conducted on its mode of insecticidal action, formulation for improving the potency and stability and also on its incorporating into an integrated pest management program for pistachio.

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