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Insecticidal Activity of Essential Oil from Juniperus communis L. subsp. hemisphaerica (Presl) Nyman against Two Stored Product Beetles

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Abstract. In the current study, insecticidal activity of essential oil from fruits of *Juniperus communis* L. subsp. hemisphaerica (Presl) Nyman was evaluated against *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) by fumigation at 24, 48, and 72 h exposure times. Dry fruits were subjected to hydrodistillation using a Clevenger-type apparatus and the chemical composition of the volatile oil studied by gas chromatography-mass spectrometry (GC-MS). The major components were identified α−pinene (59.70%), and limonene (9.66%). Insecticidal activity was varying with essential oil concentration and exposure time. Results showed that *R. dominica* is more susceptible than *T. castaneum* for all exposure times. LC_{50} values at 24 h were estimated 36.96 µl/l air for *R. dominica*, and 107.96 µl/l air for *T. castaneum*. These results suggested that *J. communis* subsp. hemisphaerica fruit oil may have potential as a control agent against *R. dominica*, and *T. castaneum*.

Keywords: *Juniperus communis* subsp. hemisphaerica, essential oil, fumigation, stored product beetle.

Introduction

Juniperus L. (Cupressaceae) is a genus of evergreen shrubs or trees and the second most diverse of the conifers, with some 67 species in the world (ADAMS, 2004). In Iran, Cupressaceae family consists of one species of *Platycladus*, one species of *Cupressus*, and five species of *Juniperus*. *Juniperus communis* L., *Juniperus excelsa* M. Bieb., *Juniperus foetidissima* Willd., *Juniperus oblonga* M. Bieb and *Juniperus sabina* L. are represented species (ASSADI, 1998).

Amongst the *Juniperus* L. genus, the most renowned species used in traditional medicine is *J. communis* (GAUTAM *et al.*, 2007; GONZALEZ-TEJERO *et al.*, 2008). Its dried bluish-black cones, known as "juniper berries", are said to stimulate the appetite and are used as a flavoring agent for culinary purposes and in the preparation of gin spirits (FOSTER, 1999; DARWIN, 2000). They have also been used for various medicinal purposes, including as an antiseptic, contraceptive, diuretic, and as a remedy for urinary tract infections, scrofula, chest complaints, diabetes, rheumatism and backache. The smoke from burnt juniper branches has been used as a fumigant to prevent the spread of infections (TILFORD, 1997; DARWIN, 2000; NEWTON *et al.*, 2002; ALLEN & HATFIELD, 2004). In addition, there are numerous reports on the biological activity of the essential oil of *J. communis* (EMAMI *et al*., 2007a, b; GORDIEN *et al*., 2009; MICELI *et al*., 2009; REZVANI *et al*., 2009).

The present work was carried out to identification of chemical compounds as well as determines the possible fumigant toxicity of the essential oil of the fruits of *Juniperus communis* L. subsp. hemisphaerica (Presl) Nyman against *Rhyzopertha dominica*, and *Tribolium castaneum*.

Material and methods

Insect culture

Rhyzopertha dominica and *T. castaneum* were reared on whole wheat and wheat flour mixed with yeast (10:1, w/w), respectively. Adult insects, 1–7 days old, were used for fumigant toxicity tests. The cultures were maintained in the dark in a growth chamber set at 27±2°C and 60±5% r.h. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experiments were carried out under the same environmental conditions.

Plant material

The fruits (berries that formed in the current year) of *J. communis* subsp. hemisphaerica were collected from plants growing natural in Mazandaran Province, region of Khashvash (36, 14', 992'' N; 52, 13', 407'' E; 2300 m above sea level), 75 km of Amol, North of Iran. The fruits were collected during the June 2011. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. The voucher specimens with number JCH 740 have been deposited at the herbarium of the Department of Horticulture at Urmia University.

Extraction of essential oil

Dry fruits of the plant were hydro distilled in a Clevenger type apparatus where the plant materials subjected to hydro distillation. Conditions of extraction were: 25 g of dry sample; 550 ml water, 4 h distillation. Anhydrous sodium sulphate used to remove water after extraction. Extracted oil transferred to glass flasks that were filled to the top and kept at the temperature of 4 °C in a refrigerator.

Fumigant toxicity

In order to test the toxicity, same concentrations of essential oil including 20, 27, 36, 48 and 65 µl/l air were tested on *T. castaneum* and *R. dominica*. They were applied on a filter−paper (Whatman No.1) strip measuring 4 × 5 cm that attached to the lower side of the jars lid. Twenty adults (1–7 days old) of insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical center of 1 L glass jars, which then sealed with air−tight lids (HASHEMI & SAFAVI, 2012). Thus, there was no direct contact between the oil and the insects. In the control jars, oil was not applied on the filter papers. Mortality determined after 24, 48 and 72 h from commencement of the exposure. Each experiment was replicated four times for each concentration. When no leg or antennal movements observed, insect considered dead.

Data analysis

The mortality data were corrected using Abbott's formula (ABBOT, 1925) for the mortalities in the controls, and then subjected to probate analyses to estimate LC_{50} and LC_{95} values. The percentage of mortality was determined for analysis of variance (ANOVA) according to the general linear model (GLM). Significant differences identified by honest significant difference (HSD) tests at the 5% level and entered in the fig. Data processing conducted by the SPSS software version 16.0 for Windows.

Results

Yield and chemical constituent

In this research the essential oil of the fruits from *J. communis* subsp. hemisphaerica collected from region of Khashvash, gave yellowish oil with a yield of 2.31% (w/w) based on dry weights. GC-MS analyses of the fruit oil identified 14 compounds (90.58%). The main components of the oil were α−pinene (59.70%), limonene (9.66%), myrcene (6.03%), and germacrene D (5.06%) (Table 1).

Table 1. Chemical composition of *Juniperus communis* subsp. hemisphaerica fruit oil.

No	Compound	Retention Index	Percentage
1	α-thujene	923	0.76
2	α-pinene	935	59.70
3	Sabinene	974	4.69
4	β-pinene	980	1.32
5	Myrcene	986	6.03
6	δ-2-carene	1002	-
7	α-terpinene	1017	0.27
8	Limonene	1034	9.66
9	γ-terpinene	1061	0.53
10	Terpinolene	1090	0.86
11	Terpinen-4-ol	1179	0.53
12	Z-caryophyllene	1410	–
13	E-caryophyllene	1414	1.17
14	Germacrene D	1482	5.06
	Monoterpenes		84.36
	Sesquiterpenes		6.23
	Total		90.58

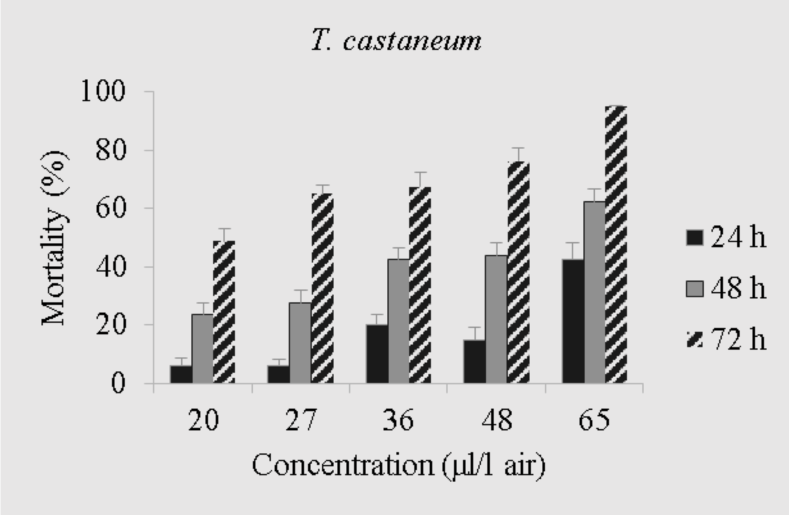
Fumigant toxicity

Toxicity data indicate a remarkable difference in susceptibility between the insects (Table 2, and Fig. 1). *Tribolium castaneum* were the most resistant species to the essential oil with LC_{50} value of 107.96 µl/l air, whereas the *R. dominica* was more susceptible with LC_{50} value of 36.96 µl/l air, at a 24 h exposure time. Furthermore, with the increase of exposure time to 72 h, mortality increased and LC_{50} values decreased to 34.48 µl/l air for *T. castaneum*; and 7.94 µl/l air for *R. dominica* (Table 2). Based on the results from fumigant bioassays, the essential oil testing showed high toxicity when that was applied against insects with insecticidal activity dependent on oil concentration and exposure time. When experimental insects were fumigated for 24 h, for *R. dominica* a concentration of 48 µl/l air oil was necessary to cause mortality higher than 50%, while for *T. castaneum* concentration 65 µl/l air and 48 h exposure time was enough to cause equal mortality when were used (Fig. 1). Moreover, slopes of probit lines estimated that any increase in essential oil concentration, was imposed the high mortality to *R. dominica* (3.47 at 72 h) when compared to *T. castaneum* (3.20 at 72 h) (Table 2). Furthermore, intercept of probit line in all exposure times for *R. dominica* was higher than *T. castaneum*, showing the higher response threshold (Table 2).

Table 2. Result of probit analysis to calculate LC_{50} and LC_{95} values.

Insects	Exposure time	LC_{50}	LC_{95}	$\chi 2$ [df = 3]	*p*	Intercept	Slope
T. castaneum	24	107.96	340.95	1.94[a]	0.58	-1.69	3.29
	48	68.61	306.59	0.69[a]	0.87	0.35	2.53
	72	34.48	112.28	1.54[a]	0.67	0.07	3.20
R. dominica	24	36.96	181.40	0.44[a]	0.93	1.27	2.38
	48	18.11	76.00	1.27[a]	0.73	1.68	2.64
	72	7.94	23.62	0.81[a]	0.84	1.88	3.47

[a] Since goodness−of−fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.



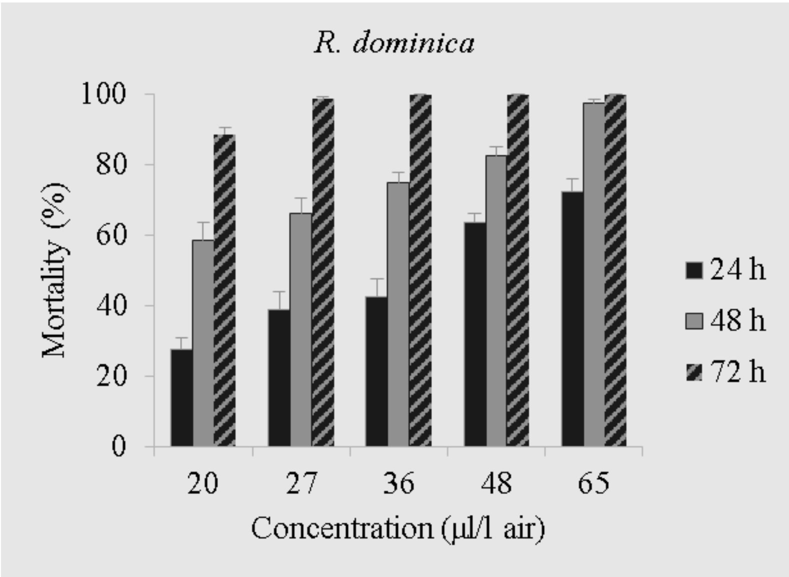


Fig. 1. Mean mortality (%) of *Rhyzopertha dominica* and *Tribolium castaneum* exposed to different concentrations of *Juniperus communis* subsp. hemisphaerica fruit oil. Different letters over columns indicate significant differences according to Tukey test at α= 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

Discussion

The yield of essential oil from the fruits is relatively higher than other studies on *J. communis* subsp. hemisphaerica in Iran (EMAMI *et al.*, 2007a, b). The fruit oil had compositions similar to those of other *J. communis* essential oils analyzed in Iran. REZVANI (2010) reported the main components of the oil that were α-pinene (46.63%), α-cedrol (12.36%), and β-pinene (4.64%). EMAMI *et al.* (2007a) also studied the composition of the *J. communis* subsp. *hemisphaerica* fruit oil. The oil contained sabinene (25.10%), α-pinene (13.60%), and limonene (9.10%) as main components. In comparison with published data, it could be clearly shown that ingredients of the essential oil of the fruits of *J. communis* subsp. hemisphaerica are similar, but with differences in their percentage depending distinctly on the region in which they are grown. Most notable differences observed in the composition of *J. communis* subsp. hemisphaerica grown in Amol (Khashvash) included the absence of α–cedrol, Δ3-carene, β-caryophyllene, and caryophyllene oxide, and the high percentage of myrcene (EMAMI *et al.*, 2007a; REZVANI, 2010).

The insecticidal activity of essential oil from *J. communis* has been evaluated against a number of insects. LANS *et al*. (2008) used the essential oil of *J. communis* L. var. depressa Pursh. to treat fleas and flies on cats and dogs in British Columbia, Canada. In another study, CHOI *et al*. (2003) tested insecticidal activity of *J. communis* oil against eggs, nymphs, and adults of *Trialeurodes vaporariorum* Westwood. In addition, essential oil of *J. communis* was evaluated for repellency against adult *Aedes aegypti* (L.), *Amblyomma americanum* (L.), *Ixodes scapularis* Say, and for toxicity against *Ae. aegypti* larvae and adults (CARROLL *et al*., 2011).

The toxicity of different essential oils used to protect against *T. castaneum* and *R. dominica* infestation has been previously studied, and these beetles have shown susceptibility to some plant−derived chemicals. Experiment has shown that, *R. dominica* is more susceptible than *T. castaneum* (Table 2 and Fig. 1). ROZMAN *et al*. (2007) studied toxicity of naturally occurring compounds of Lamiaceae and Lauraceae against *Sitophilus oryzae* (L.), *R. dominica* and *T. castaneum*. They observed that *R. dominica* was more susceptible than *T. castaneum.* EBADOLLAHI (2011) evaluated toxicity of essential oil of *Agastache foeniculum* [Pursh] Kuntze against *R. dominica* and *T. castaneum.* Results showed that, *R. dominica* (LC_{50}= 14.17 µl/l) was more susceptible than *T. castaneum* (LC_{50}= 22.24 µl/l), at 24 h exposure time. LEE *et al*. (2004) tested the fumigant toxicity of six essential oils and 1, 8−cineole against *S. oryzae*, *T. castaneum*, and *R. dominica*. In that experiment, *R. dominica* was found more susceptible than the other species. HOSSEINI *et al*. (2013) reported fumigant toxicity of essential oil from *Salvia leriifolia* (Benth.) against *Sitophilus granarius* (L.) and *R. dominica*. LC_{50} values at 24 h were estimated 79.17 µl/l air for *S. granarius*, and 25.87 µl/l air for *R. dominica*.

GC−MS analyses of the oil revealed that the percentage of monoterpenoids was higher than the other compounds (Table 1). The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility, they have fumigant action that might be of great importance for stored product insects (LEE *et al*., 2002, 2004; HASHEMI & SAFAVI, 2012; HOSSEINI *et al*., 2013). The α−pinene is one of these monoterpenoids. It is characterized as the main component (59.70%) of the fruits of *J. communis* subsp. hemisphaerica essential oil. There are numerous reports on toxicity of the α-pinene to our experimental insects. LEE *et al*. (2002) reported toxicity of α−pinene to *T. castaneum*. The oils extracted from leaves and the fruits of *Platycladus orientalis* (L.) Franco containing *a*−pinene as a major component (35.2%, 50.7%), respectively, was found to be the most effective against *T. castaneum* (HASHEMI & SAFAVI, 2012). α−pinene as a major compound (15.89%) of *S. leriifolia* was toxic on *R. dominica* (HOSSEINI *et al*., 2013).

This study indicates that essential oil of *J. communis* subsp. hemisphaerica is a source of biologically active vapor which may potentially prove to be efficient insecticide. Toxicity screening of essential oil showed significant activity against *T. castaneum* and especially *R. dominica*. This study will provide a basis in the future work with *J. communis* subsp. hemisphaerica particularly from Iran.

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