koenigii+C. reticulata, M. koenigii+C. longa, C. reticulata+C. longa at 0.1% each and M. koenigii+C. reticulata+C. longa at 0.07% each were found highly effective against R. dominica fumigated after 5, 10, 15 and 20 days. Only M. koenigii at 0.2% was found highly effective against T. castaneum fumigated after 5, 10, 15 and 20 day. The fumigation of grain with M. koenigii at 0.2% completely suppress the infestation and weight loss when it was fumigated after 5, 10, 15 and 20 days while very low infestation and weight loss was observed in grain treated with M. koenigii +C. reticulata at 0.1% each. Essential oils did not affect the organoleptic properties and germination of wheat.

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Fumigant toxicity of *Haplophyllum tuberculatum* (Rutaceae) and *Nepeta crispa* (Lamiaceae) on the Indian meal moth

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Abstract

The insecticidal activity of essential oil vapors of *Haplophyllum tuberculatum* (Sapindales: Rutaceae) and *Nepeta crispa* (Lamiales: Lamiaceae) were evaluated on third instar larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae) as one of the major insect pests of stored products. Essential oils of the plants were obtained using Clevenger-type water distillation. GC-MS analyses of the oils demonstrated that the main compounds of *H. tuberculatum* were p-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %). It is an important plant that has many medicinal properties. 1,8-Cineole (32.98 %), β-Pinene (8.70 %), 4aa,7a,7aa-Nepetalactone (8.08 %) and 4aβ,7a,7aβ-Nepetalactone (6.1 %) were detected as the predominant component in *N. crispa*. The aerial parts of that are used in traditional medicine. The LC₅₀ values were estimated after 24 hours for *H. tuberculatum* and *N. crispa* as 4.301 and 5.579 µl L⁻¹ air, respectively. LC₅₀ values were projected using probit analysis. Results on *H. tuberculatum* showed more toxicity against the Indian meal moth compared to the *N. crispa*. In conclusion, the essential oil of the two plants could have potential for application to stored grain and agricultural commodities to control of stored crop pests in IPM programs.

Key Words: Essential oils, insecticidal toxicity, Plodia interpunctella, Haplophyllum tuberculatum, Nepeta crispa.

Introduction

Plodia interpunctella Hubner (Lepidoptera: Pyralidae), the Indian meal moth, is a common domestic pest, feeding on stored food products including nuts, beans, processed foods and dried fruits (Simmons and Nelson 1975). The larvae spoil stored food by spinning a silky web inside and on top of the food surface and feed in it (Phillips et al., 2000). According to earlier experiences, fumigants are commonly utilized for control of stored-products pests (Cox et al., 1984) Chemical insecticides led to various problems to our environment and health (Taylor, 1989). Plant-derived products have proved to be excellent source of a variety of volatiles with the potential for development as alternatives to conventional insecticides (Atta-ur-Rahman et al., 1999; Tripathi et al., 2000; Lee et al. 2004; Ebadollahi et al., 2010; Wu et al., 2015; Plata-Rueda et al., 2017).

The genus *Haplophyllum*, belongs to the Rutacea family, comprises about 70 species represented in Mediterranean, Saharo-Arabian, Irano-Turanian, and Sudano-Zambezian regions (Willis, 1980; Takhtajan, 1986). *Haplophyllum* species are generally used in traditional medicine as a remedy for headaches and arthritis, skin discoloration, wart removal, and against parasitic diseases and other infections. *Haplophyllum tuberculatum* Forssk is considered to protect livestock from biting insects and flies (Miller et al., 1988). Insecticidal activity of the aerial parts of *H. tuberculatum* against *Culex quinquefasciatus* has been proved (Zohair et al., 1989).

The genus *Nepeta* (Lamiaceae) contains almost 280 species endemic to Europe, Asia and a few parts of Africa (Rechinger, 1982). Some of the species of *Nepeta* are grown as a garden herb and some of the species used as medicinal herb for cancer, toothache, colds, anemia, headache, diarrhea, indigestion, tuberculosis, and other various ailments (Duke and Ayensu, 1985; Duke, 2002). This genus, with 67 species in Iran, also is reported to be used as phytotherapy in Iranian traditional medicine (Amin, 1991). Essential oils of some species of *Nepeta* have been described to possess insecticidal activity (Zhu et al., 2006; Ali et al., 2016).

The review of the literature revealed the scarcity of information over the toxicity of *H. tuberculatum* and *N. crispa* Willd essential oils. Hence at the present study, the potential of fumigant toxicity of the essential oils from the two aromatic plant species examined against the third instar larvae of the Indian meal moth, *P. interpunctella*, in order to contribute for the development of new approaches for controlling this insect pest.

Materials and methods

Rearing of insects

P. interpunctella was collected from the Department of Plant Protection, Faculty of Agriculture of Urmia University, Iran. It was reared using the method of Adler (2010), with some modifications, on a diet containing 400 g of wheat bran, 15 g of broken almonds, 48 g of glucose, 80 g of dried yeast,

80 ml of glycerin and 20 ml of water. Stock cultures were maintained in a temperature-controlled chamber with 25±5 °C and 65±5 % R.H. and photoperiod of 14:8 (L:D) h.

Plant material

Aerial parts of *Nepeta crispa* and *Haplophyllum tuberculatum* were collected at the flowering stages in the middle of August (from the mountain areas of West Azerbaijan province (Northwestern Iran)) and November (from the fields of Kerman province (Southern Iran)) 2016, respectively. Samples were dried at room temperature and chopped into small pieces. The essential oils were extracted from the samples using a Clevenger-type apparatus for 4 h. The obtained essential oils were kept in the refrigerator at 4 °C for subsequent experiments.

Analysis of the essential oil

Essential oils were analyzed using GC–MS. For GC–MS analysis an Agilent 7890. A gas chromatograph coupled to a 5975A mass spectrometer using a HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used. The oven temperature was programmed as follows: 3 min at 80 °C, subsequently 8 °C min⁻¹ to 180 °C, held for 10 min at 180°C. Helium was used as carrier gas at a flow rate of 1 mL min⁻¹ and Electronimpact (EI) was 70 eV. The injector was set in a split mode (split ratio of 1:500) and mass range acquisition was from 40 to 500 m/z. Essential oil constituents were identified by using the calculated linear retention indices (Wiley 2007; NIST 2005) and mass spectra with those reported in the NIST 05 and Wily 07.

Bioassays

Fumigant toxicity of essential oils from *H. tuberculatum* and *N. crispa* investigated against *P. interpunctella*. Fumigation experiments carried out using the method of Ziaee et al. (2013) with some modifications. Ten third instar larvae of the insect were presented to the 100 ml glass jars as experiment units. The jars were covered with muslin cloth for inhibition of larvae contact to filter paper. Different concentrations of the essential oils (2, 2.91, 4.24, 6.18 and 9 µl L⁻¹ air for *H. tuberculatum*, 15, and 2, 3.31, 5.48, 9.06 and 15 µl L⁻¹ air for *N. crispa*) were pipetted onto a filter paper (Whatman No.1) and appended to the under surface of the jar's lid. After 24 h, the data were recorded in terms of the number of dead larvae and percentage mortality determined for each insect. Each treatment was replicated six times. The bioassay was conducted at 25 ± 5 °C and 65 ± 5 % R.H. and photoperiod of 14:8 (L:D) h. The control groups were treated except that no essential oils were employed.

Data analysis

Analysis of variances, using SPSS 21.0 (followed by Tukey's test to compare differences among various treatments at α = 0.05 level), were carried out to determine the significance of differences between mortality of larvae of each species at different concentrations of essential oils. Probit analysis was used to estimate LC₅₀ and LC₉₅ values (Abbott, 1925).

Results

Chemical composition of essential oil

Thirty-one and 27 chemical constituents were identified which represented 93.64 and 91.62 % of the total oils of *H. tuberculatum and N. crispa*. The major components of *H. tuberculatum* oil were p-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %). Chemical components analysis of the essential oils revealed that the predominant composition of *N. crispa* oil was 1,8-Cineole (32.98 %) followed by β-Pinene (8.70 %), 4aα,7a,7aa-Nepetalactone (8.08 %) and 4aβ,7a,7aβ-Nepetalactone (6.1 %) (Tables 1 and 2).

No.	Component	RI	%
1	Alpha-Pinene	931	0.45
2	Sabinene	960	0.53
3	α-phellandrene	1001	0.68
4	3-Carene	1011	0.90
5	<i>p</i> -Cymene	1026	1.16
6	β-phellandrene	1030	2.78
7	Linalool	1099	1.14
8	p-menth-2-en-1-ol-cis	1125	20.15
9	p-menth-2-en-1-ol-trans	1142	16.92
10	Isoborneol	1164	0.55
11	Terpinine-4-ol	1180	1.92
12	p-Cymen-8-ol	1186	0.60
13	Alpha-Terpineol	1193	0.80
14	cis-Piperitol	1198	6.72
15	trans-Piperitol	1210	13.23
16	Carvone	1246	1.22
17	Piperitone	1258	7.34
18	Bornyl acetate	1288	0.49
19	Thymol	1290	0.68
20	Carvacrol	1300	1.38
21	Piperitenone	1344	0.56
22	Alpha-Copaene	1380	0.43
23	Beta. Bourbonene	1389	0.75
24	trans-Caryophyllene	1425	2.39
25	Alphacurcumen	1484	1.13
26	Germacrene D	1486	4.70
27	7-epialphaselinene	1524	0.47
28	Germacrene B	1563	1.07
29	Caryophyllene oxide	1590	0.99
30	betaGurjunene	1593	1.02
31	(-)-Spathulenol	1633	0.49
	total		93.64

Table 1. Chemical constituents of Haplophyllum tuberculatum essential oil.

* RI= Retention indices

Insecticidal activity of essential oils against P. interpunctella larvae

The essential oils of *H. tuberculatum* and *N. crispa* possessed fumigant toxicity on third instar larvae of *P. interpunctella* with the LC₅₀ and LC₉₅ values (P < 0.0001) of 4.301, 13.538 and 5.579, 24.808 µl L⁻¹ air, respectively (Table 3).

The results of one-way analysis of variances represented that effect of concentrations of the essential oils of *H. tuberculatum* (F= 76.054; df= 4, 25; *P* < 0.001) and *N. crispa* (F= 81.709; df= 4, 25; *P* < 0.001) on the mortality of third instar larvae of *P. interpunctella* were significant. The mortalities of the larvae of *P. interpunctella* were 86.7 and 88.3 % after 24 h exposure to the highest concentrations of *H. tuberculatum* and *N. crispa* essential oils, respectively. The increasing concentration of essential oils created significant increase in mortality. (Table 4).

No.	Component	RI	%
1	a-Thujene	928	0.78
2	a-Pinene	936	2.89
3	Sabinene	971	2.66
4	β-Pinene	978	8.70
5	Myrcene	983	0.80
6	Dehydro-1,8-cineol	989	0.51
7	a-Terpinene	1014	1.86
8	ρ-Cymene	1017	0.18
9	1,8-Cineole	1033	32.98
10	γ-Terpinene	1054	1.18
11	trans-Sabinene hydrate	1061	1.92
12	a-Terpinolene	1086	0.34
13	cis-Sabinene hydrate	1089	2.52
14	Linalool	1094	1.94
15	α-Campholenal	1113	0.23
16	trans-Pinocarveol	1124	0.39
17	trans-Verbenol	1132	0.04
18	Sabinol	1137	0.68
19	δ-Terpineol	1154	2.69
20	4-Terpineol	1167	3.38
21	a-Terpineol	1179	4.26
22	4aα,7α,7aα-Nepetalactone	1337	8.08
23	4aβ,7α,7aβ-Nepetalactone	1348	6.10
24	$4a\beta$, 7α , $7a\beta$ -Nepetalactone	1368	5.78
25	trans-β-Farnesene	1444	0.46
26	α-Farnesene	1492	0.17
27	Germacrene-B	1510	0.10
	total		91.62

Table 2. Chemical constituents of Nepeta crispa essential oil.

* RI= Retention indices

Table 3. Probit analysis data for *H. tuberculatum* and *N. crispa* essential oils against third instar larvae of *P. interpunctella*

Essential oil							**X2
	n	LC₅₀ (µl L⁻¹)	*CI (μl L ⁻¹)	LC ₉₅ (µl L ⁻¹)	*CI (μl L ⁻¹)	Slope ± SE	
H. tuberculatum	10	4.301	3.847–4.814	13.538	10.725–19.278	3.029±0.374	0.641
N. crispa	10	5.579	4.822-6.464	24.808	18.369–39.007	3.53± 0.284	0.637

^{*}CI: confidential interval; ^{**} χ^2 : chi-squared value

Table 4. Fumigant activity of *N. crispa, S. hortensis* and *A. graveolens* essential oils against third instar larvae of *P. interpunctella*

Pest species	Concentration (µl L ⁻¹ air)	Mortality (%) mean±SE
H. tuberculatum	2	$15.0 \pm 3.34 a^*$
	2.91	26.7 ± 3.33 a
	4.24	50.0 ± 3.65 b
	6.19	68.3 ± 3.07 c
	9	86.7 ± 3.33 d
N. crispa	2	13.3 ± 3.33 a
	3.31	28.3 ± 3.07 b
	5.48	50.0 ± 3.65 c
	9.06	66.7 ± 3.2 d
	15	88.3 ± 3.07 e

* Means within column with the same letter(s) are not significantly different (P > 0.05) according to Tukey's test.

Discussion

Fumigation is one of the most effective methods of rapidly controlling insects infesting stored foods. A new approach for the control strategies that are environmentally sustainable and avoids the use of conventional pesticides is of paramount important. The essential oils of plants are assumed as viable alternative of controlling many insect pests (Mauchline et al., 2005; Lopez et al., 2008; Razavi 2012). Some reports have been stated that the most promising botanical insect-control agents belong to the families of Annonaceae, Asteraceae, Canellaceae, Apiaceae, Lamiaceae, Meliaceae and Rutaceae (Jacobson, 1989; Kim and Ahn, 2001; Chaubey, 2006, 2007; Taghizadeh-Saroukolai et al., 2010).

Chemical composition of essential oil

The insecticidal compositions and essential oils obtained of many plants are monoterpenoids (Ahn et al. 1998; Ayvaz et al., 2008). Monoterpenoids may inhibit the nervous system of insects (Tong, 2010).

In the current study, the principal constituents of *H. tuberculatum* essential oil were p-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %) that the toxic impacts of the oil could be attributed to the major constituents. Previous studies on the essential oil of this species demonstrated variable chemical components (Raissi et al., 2016). The main composition of the essential oil of *H. tuberculatum* were characterized as β -phellandrene (23.3%), limonene (12.6%), (Z)- β -ocimene (12.3%), β -caryophyllene (11.6%), myrcene (11.3%), and α -phellandrene (10.9%) (Al-Burtamani et al., 2005). Giles and Bisits (2007) isolated the chemical compounds of *H. tuberculatum* essential oil and found cis-p-menth-2-en-1-ol and trans-p-menth-2-en-1-ol (22.9 and 16.1%, respectively) as the main constituents in the oil. *H. tuberculatum* essentials oils were constituted by oxygenated monoterpenes (71.0% of the whole oil). In another investigation in Larestan, Iran, main constitue of *H. tuberculatum* was borneol (25.73%) followed by α -Pinene (14%), Bornyl acetate (18.07%) and β -caryophyllene (7.43%) (Vahdania et al., 2011). According the current review we revealed that all samples comprise mainly of monoterpenes, and the differences could be due to differences in time of harvest, plant parts applied, agroclimatic and geographic conditions.

The dominant compounds obtained from *N. crispa* essential oil contained 1,8-Cineole (32.98 %) followed by β -Pinene (8.70 %), 4aa,7a,7aa-Nepetalactone (8.08 %) and 4a β ,7a,7a β -Nepetalactone (6.1 %). The major composition of *N. crispa* (71 %) and *N. menthoides* (41.1 %) oils were identified 1,8-cineole (Mojab et al., 2009). Ali et al (2016) stated 1,8-cineole was the main component of *Nepeta racemosa* and *N. faassenii* essential oils. While *N. sibirica* and *N. subsessilis* essential oils mainly possessed of sesquiterpenes: (Z)- β -farnesene, β -bisabolene, δ -cadinene or β -caryophyllene, and caryophyllene oxide. Previous investigations represented that the essential oils of *Nepeta* species consist mainly of 1,8-cineole (Sonboli et al., 2004; Sefidkon et al., 2006). It has been found that the main constitutes of *N. racemosa* essential oil, collected from a wild source in western Iran, were 1,8-cineole (37%) and nepetalactone (2.3%) (Daryasari et al., 2012). 1,8-cineole has been indicated as a toxic volatile against insect pests (Obeng-Ofori et al., 1997; Lee et al., 2004; Kordali et al., 2006; Stamopoulos et al., 2007; Rozman et al., 2007).

Insecticidal activity of essential oils against P. interpunctella larvae

Some previous studies have described that many of the plant's essential oils possess an insecticidal and antifeedant activity on stored-product pests (Negahban et al. 2007; Rajendran and Sriranjini 2008; Karabörklü et al. 2010, 2011; Saeidi and Yousefi, 2013; Wu et al., 2015; Plata-Rueda et al., 2017).

Current study is the first investigation on the insecticidal activity of the essential oils of *H. tuberculatum* and *N. crispa* against stored product pests. Based on the LC₅₀ values (Table 3) the examined essential oils were strongly toxic against *P. interpunctella*. Mortality rate of 86.7 and 88.3 % observed in third instar larvae of *P. interpunctella* exposed to different concentrations of *H.*

tuberculatum and N. crispa oils, respectively. In both oils, mortality of larvae increased as concentration of the essential oils increased. Similar findings were obtained by El-Khyat et al. (2017) who reported insecticidal activity of Matricaria chamomilla L., Origanum majorana L. and Citrus aurantium L. on Ephestia Cautella and stated that the insecticidal activity increased with the increase of concentration. Rafiei-Karahroodi et al. (2011) investigated characterization of essential oil of different essential plant oils on Plodia interpunctella and detected that the oils have toxicant effect on first instar larvae of Indian meal moth. These reports are confirmed by Ebadollahi et al. (2010), Moazeni et al. (2013), and Pandir and Bas (2016) who indicated that essential plant oils were very toxic on insects due to their active volatiles. Ahmad et al. (2016) stated that crude extracts and fractions from N. leavigata and N. kurramensis indicated insecticidal activity against Tribolium castaneum which supports the traditional anti insect value (mosquito replant) of the Nepeta species (Baser et al., 2000). Insecticidal properties of N. recomena on Sitophilus aranaries, E. kuehniella and Lasioderma serricorne has been reported (Aslan et al., 2005). The essential oil of S. hortensis has been reported to possess high toxic effect against E. kuehniella and P. interpunctella (Mollaei et al., 2011). Insecticidal activity of the essential oils varies depending on the experimental method, exposure time, origin of the essential oil, concentration of the oil, insect stage and species (Chiasson et al., 2001; Choi et al., 2003; Sedy and Koschier, 2003; Negahban et al. 2007; Ayvaz et al., 2010; Mollaei et al., 2011).

In conclusion, findings of this investigation revealed the essential oils from *H. tuberculatum* and *N. crispa* showed potent fumigant activities on *P. interpunctella*. Nevertheless further studies, including evaluation of the residues of the oils in food, flavor quality of food and persistence experiments, are required to clarify the competency of *H. tuberculatum* and *N. crispa* to reduce stored-products insect populations in IPM programs.

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Efficiency of ozone gas treatment against *Plodia interpunctella* (Hübner) (Lepidoptera:Pyralidae) (Indianmeal Moth) in hazelnut

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