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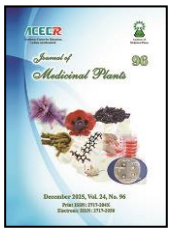

Research Article

Chemical components and fumigant toxicity of *Satureja hortensis* L., *Nepeta crispa* Willd, and *Anethum graveolens* L. essential oils against *Plodia interpunctella* (Hübner)

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ABSTRACT

Background: Botanically derived compounds are increasingly recognized as potential alternatives to synthetic insecticides due to their bioactive properties against insect pests. **Objective:** This study investigated the chemical composition and insecticidal activity of essential oil vapors obtained from *Satureja hortensis* L. (Lamiales: Lamiaceae), *Nepeta crispa* Willd (Lamiales: Lamiaceae), and *Anethum graveolens* L. (Apiales: Apiaceae) against larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae). **Methods:** Essential oils were extracted from the aerial parts of the selected medicinal plants using Clevenger-type hydrodistillation. The chemical constituents were identified by gas chromatography–mass spectrometry (GC–MS). **Results:** GC-MS analysis revealed that Carvacrol (34.74), γ-terpinene (34.27) and ρ-cymene (16.96 %) were the predominant compounds in *S. hortensis* oil. The essential oil of *N. crispa* was mainly composed of 1, 8-cineole (57.69), *β*-pinene (6.53) and *α*-terpineol (4.44 %). In *A. graveolens*, the major constituents were L-phellandrene (34.19), Carvone (23.67), Limonene (21.47), *α*-terpineol (5.58), and *ρ*-cymene (5.50 %). These plants are important for their medicinal properties. Fumigant toxicity assays revealed LC_{50} values after 24 hours of exposure as 26.974, 5.579 and 16.34 µl L^{-1} air for *S. hortensis*, *N. crispa* and *A. graveolens*, respectively. Results indicate the strong insecticidal activity of *N. crispa* against the *P. interpunctella* compared to other oils. **Conclusion:** Essential oils of these plants show capability for use in IPM programs to control stored pest populations.

1. Introduction

The Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), is one of the most destructive insects affecting stored agricultural and food products such as cereals, nuts, legumes, dried fruits, and processed foods

Abbreviations: GC/MS, Gas chromatography-mass spectrometry; CI, confidential interval; χ^2, chi-squared value; LC_{50}, Lethal concentration 50 %; LC95, Lethal concentration 95%

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[1, 2]. It causes significant economic losses due to direct crop damage, treatment costs, and consumer complaints resulting from product contamination [3]. Although chemical insecticides effectively reduce pest damage in stored products, their use has led to various environmental concerns during production, sale, using in farms and ultimately as residues in food [4]. In recent years, attention has shifted toward plant-based compounds as safer alternatives to conventional insecticides. Many plant species produce secondary metabolites with biological activity that can be exploited for pest management [5-6].

Satureja hortensis (Savory) (Lamiaceae) has been reported to possess antimicrobial [7] and insecticidal properties [8, 9], suggesting its potential use in insect control programs. Species of the genus *Nepeta* are widely cultivated and traditionally used in herbal medicine for the treatment of various ailments [10, 11]. Iran hosts a high diversity of *Nepeta* species, which are commonly used in traditional phytotherapy [12]. Although insecticidal activity has been demonstrated for several *Nepeta* species [13], information regarding the toxicity of *Nepeta crispa* essential oil against insect pests remains limited. *Anethum graveolens* is another aromatic plant recognized for its bioactive properties [14]. Its essential oil contains compounds such as carvone, phellandrene, limonene, and dill ether, which have been associated with insect deterrent and toxic effects [15]. Previous studies have demonstrated its antifeedant activity against *Cochlochila bullita* (Stål) (Hemiptera: Tingidae) [14], and also the insecticidal potential against aphids [16], and beetles [17]. Additionally studies show that the essential oil from *Satureja* genus on *Acrobasis advenella* (Zinck.) (Lepidoptera: Pyralidae) [9], *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [18], several stored-product pests [19] and some different insect pests had an insecticidal effect. Despite these findings, comparative information on the chemical composition and fumigant toxicity of essential oils from *S. hortensis*, *N. crispa*, and *A. graveolens* against *P. interpunctella* larvae is still scarce. Therefore, the present study aimed to analyze the chemical constituents of these essential oils and to evaluate their fumigant insecticidal effects on third-instar larvae of the Indian meal moth, with the goal of identifying potential botanical alternatives for stored-product pest management.

2. Materials and methods

2.1. Insect culture

A laboratory population of the Indian meal moth, *Plodia interpunctella*, was initiated using individuals supplied by the Department of Plant Protection, Faculty of Agriculture, Urmia University (Iran). The colony was maintained according to the Adler rearing protocol [20], incorporating several adjustments. Rearing was carried out on a formulated artificial medium consisting of wheat bran (400 g), ground almonds (15 g), glucose (48 g), dried yeast (80 g), glycerol (80 mL) and distilled water (20 mL). Stock populations were maintained in a growth chamber regulated at 25 ± 5 ˚C and 65 ± 5 % relative humidity, with a 14:8 (L:D) h photoperiod.

2.2. Plants

During the flowering stage, aerial parts of *S. hortensis*, *N. crispa* and *A. graveolens* were collected from the mountainous regions of West Azerbaijan province in Northwestern Iran. The collected plant materials were dried under ambient laboratory conditions (23-24 °C) and subsequently chopped into small fragments.

Plant identification was verified by Ghahreman [21], in Flora of Iran, with voucher specimens (SH-101, NC-111, AG-212) were deposited in the Herbarium of Shahid Bakeri High Education Center, Miandoab. Essential oils were collected through hydrodistillation with a Clevenger-type apparatus for four hours. The extracted oils were stored at 4 ˚C until further use [22].

2.3. Essential oil analysis

Essential oils constituents were characterized using gas chromatography coupled with mass spectrometry (GC-MS). The analyses were conducted on an Agilent 7890A gas chromatographic system linked to a 5975A mass detector and fitted with an HP-5 MS fused-silica capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness; stationary phase: 5 % Phenyl Methylpolysiloxane). The column oven was initially set to 80 °C and maintained for 3 min, then heated to 180 °C at a rate of 8 °C min^{-1} and held at this temperature for an additional 10 min. Helium was employed as the carrier gas with a constant flow of 1 mL min^{-1}. Ionization was achieved by electron impact at 70 eV. Samples were introduced in split injection mode using a 1:50 split ratio, and mass spectra were collected within an m/z range of 40 – 500. Compound identification was accomplished by comparing calculated linear retention indices with published data and by matching mass spectral fragmentation patterns against the Wiley 2007 and NIST 2005 reference libraries.

2.4. Bioassay procedures

The fumigant activity of essential oils obtained from three selected medicinal plants was evaluated on third-instar larvae of *P. interpunctella*. Preliminary range-finding tests were conducted to determine suitable concentration levels of each essential oil [23]. Bioassays were performed in 100 ml glass jars, each containing ten larvae as a single experimental unit. To avoid physical contact between the insects and treated substrates, the jars were sealed with muslin cloth. Measured quantities of essential oils were dispensed onto Whatman No.1 filter paper discs, which were then attached to the inner side of the jar lids. The concentrations evaluated were 15.00, 20.75, 28.70, 39.70 and 55.00 µL/L air for *S. hortensis*, 2.00, 3.31, 5.48, 9.06 and 15.00 µL/L air for *N. crispa* and 8, 11.57, 16.73, 24.20 and 35.00 µl/l air for *A. graveolens*. Larval mortality was assessed after 24 h, and individuals showing no movement when gently prodded or exposed briefly to mild heat were classified as dead. Each concentration was tested with six independent replicates. Assays were conducted under standardized laboratory status (25 ± 5 °C, 65 ± 5 % relative humidity, and a 14:8 h light:dark photoperiod). Untreated groups were maintained under identical conditions without exposure to essential oils. Treatments were randomized to avoid any positional bias.

2.5. Data analysis

The experiment followed a randomized complete block design. Mortality percentages were subjected to analysis of variance (ANOVA), and differences among treatment means were evaluated using Tukey's HSD test at a 5 % probability level (α = 0.05). Lethal concentration values (LC_{50} and LC_{95}), along with their corresponding 95 % confidence limits, were calculated through probit analysis using SPSS software (version 21.0) [24, 25]. Treatment effects were regarded as statistically significant when their confidence intervals showed no overlap.

3. Results

3.1. Essential oils components

Analysis of the volatile fractions revealed the presence of 20 components in S. hortensis and 14 components in N. crispa essential oils, which together comprised 99.16 % and 84.22 % of the respective oil compositions. The essential oil of A. graveolens was composed of 12 detectable components, which together made up 97.69 % of the total oil. The predominant constituents of *S. hortensis* essential oil were carvacrol (34.74), *γ*-terpinene (34.27), and *ρ*-cymene (16.96 %) (Table 1).

GC–MS analysis showed that the essential oil of *N. crispa* was dominated by 1,8-cineole (57.69 %), with *β*-pinene (6.53 %) and *α*-terpineol (4.44 %) present as the next most abundant components (Table 2).

The results of the GC–MS analysis indicated that the volatile oil extracted from *A. graveolens* was dominated by L-phellandrene (34.19 %), carvone (23.67 %), and limonene (21.47 %). Lesser yet notable amounts of *α*-terpineol (5.58 %) and *p*-cymene (5.50 %) were also detected (Table 3).

3.2. Fumigant toxicity of essential oils against P. interpunctella larvae

Probit analysis data ($P < 0.0001$) of fumigant activity indicated that impact of *N. crispa* essential oil (LC_{50} = 5.579 (4.822–6.464) µl/l air) on third-instar larvae of *P. interpunctella* was more potent followed by the essential oils of *A. graveolens* (LC_{50} = 16.34 (14.621–18.238) µl/l air) and *S. hortensis* (LC_{50} = 26.974 (24.376–29.735) µl/l air) (Table 4).

Table 1. Essential oils components of *Satureja hortensis*

No.	Constituent	Retention Index, RI	Retention Time, RT (min)	Composition (%)
1	*α*-Thujene	929	5.12	1.53
2	*α*-Pinene	934	5.28	1.97
3	*β*-Pinene	978	6.14	0.86
4	*β*-Myrcene	990	6.38	1.86
5	*α*-Phellandrene	1005	6.69	0.30
6	*α*-Terpinene	1017	6.96	2.74
7	*ρ*-Cymene	1025	7.14	16.96
8	Limonene	1030	7.24	0.72
9	1,8-Cineole	1032	7.30	0.29
10	*γ*-Terpinene	1060	7.91	34.27
11	Menthol	1174	10.53	0.50
12	Terpinene-4-ol	1180	10.67	0.20
13	Carvacrol methyl ether	1245	12.16	0.16
14	*trans*-Anethole	1287	13.12	0.48
15	Thymol	1296	13.33	0.27
16	Carvacrol	1305	13.54	34.74
17	Carvacryl acetate	1373	15.03	0.16
18	*trans*-Caryophyllene	1425	16.15	0.28
19	*β*-Bisabolene	1509	17.89	0.67
20	Caryophyllene oxide	1589	19.48	0.20
	Total			**99.16**

Table 2. Essential oils components of *Nepeta crispa*

No.	Constituent	Retention Index, RI	Retention Time, RT (min)	Composition (%)
1	α-Thujene	929	5.12	1.01
2	α-Pinene	934	5.28	2.51
3	Sabinene	971	6.04	2.09
4	β-Pinene	978	6.14	6.53
5	β-Myrcene	990	6.38	0.41
6	ρ-Cymene	1025	7.14	1.40
7	Limonene	1030	7.24	1.39
8	1,8-cineole	1032	7.30	57.69
9	γ-Terpinene	1060	7.91	1.18
10	Linalool	1099	8.79	1.24
11	Terpinene-4-ol	1180	10.67	1.75
12	α-Terpineol	1192	10.95	4.44
13	*trans*-Anethole	1287	13.12	0.85
14	*cis*-α-Bisabolene	1502	17.76	1.73
	Total			**84.22**

Table 3. Essential oils components of *Anethum graveolens*

No.	Constituent	Retention Index, RI	Retention Time, RT (min)	Composition (%)
1	α-Thujene	929	5.12	0.26
2	α-Pinene	934	5.28	1.41
3	β-Myrcene	990	6.38	0.73
4	L-Phellandrene	1005	6.70	34.19
5	ρ-Cymene	1025	7.14	5.50
6	Limonene	1030	7.24	21.47
7	γ-Terpinene	1059	7.89	0.74
8	α-Terpineol	1188	10.86	5.58
9	*trans*-Dihydrocarvone	1207	11.30	3.21
10	Carvone	1249	12.27	23.67
11	*trans*-Anethole	1287	13.12	0.16
12	Carvacrol	1300	13.42	0.77
	Total			**97.69**

Table 4. Insecticidal effects of essential oil derived from *Nepeta crispa*, *Satureja hortensis* and *Anethum graveolens* on *Plodia interpunctella*

Essential oil	LC_{50} (µL/L)	CI (µL/L)	LC_{95} (µL/L)	CI (µL/L)	Slope ± SE	χ^2
S. hortensis	26.974	24.376 – 29.735	74.451	60.833 – 101.463	3.73 ± 0.434	0.74
N. crispa	5.579	4.822 – 6.464	24.808	18.369 – 39.007	2.53 ± 0.284	0.99
A. graveolens	16.34	14.621 – 18.238	50.636	40.340 – 71.583	3.34 ± 0.384	0.79

Each treatment contained 10 larvae per replicate (N = 10)

A clear dose–response relationship was observed, with larval mortality increasing significantly as the concentration of each essential oil increased. One-way ANOVA revealed a highly significant influence of oil dosage on the mortality of third-instar *P. interpunctella* larvae for *S. hortensis* ($F = 72.574$; $df = 4, 25$; $P < 0.001$), *N. crispa* ($F = 81.709$; $df = 4, 25$; $P < 0.001$), and *A. graveolens* ($F = 74.102$; $df = 4, 25$; $P < 0.001$). Following 24 h of exposure to the maximum concentrations tested, mortality levels reached 91.7 % for *S. hortensis*, 88.3 % for *N. crispa*, and 90.0 % for *A. graveolens* essential oils (Table 5).

Table 5. Mortality (%) of *Plodia interpunctella* larvae exposed to *Nepeta crispa*, *Satureja hortensis* and *Anethum graveolens* essential oils

Plants	Concentration (µl/l air)	Mortality (%) mean ± SE
S. hortensis	15.00	$18.3 \pm 3.07_{e}$
	20.75	35.0 ± 2.23^{d}
	28.70	51.7 ± 4.01^{c}
	39.70	68.3 ± 1.66^{b}
	55.00	91.7 ± 4.77^{a}
N. crispa	2.00	13.3 ± 3.33^{e}
	3.31	28.3 ± 3.07^{d}
	5.48	50.0 ± 3.65^{c}
	9.06	66.7 ± 3.2^{b}
	15.00	88.3 ± 3.07^{a}
A. graveolens	8.00	15.0 ± 3.42^{d}
	11.57	31.7 ± 3.11^{c}
	16.73	53.3 ± 3.31^{b}
	24.20	65.0 ± 4.28^{b}
	35.00	90.0 ± 2.58^{a}

Means with different letters in a column indicate significant differences (Tukey's HSD test, $P < 0.05$)

4. Discussion

Fumigant treatments are widely recognized as an efficient approach for the quick suppression of insects infesting stored commodities. Growing concern over environmental safety has intensified the search for alternative pest control methods that minimize reliance on synthetic insecticides. Previous research indicates that essential oils are generally safe for non-target vertebrate organisms [27], and plants with fragrant properties have long been employed in traditional pest control practices. The volatile oils derived from these plants demonstrate a wide range of biological effects on insects, including toxicity, behavioral deterrence, and various sublethal impacts [26].

In the present investigation, GC–MS analysis showed that the essential oil obtained from *N. crispa* was characterized by a high proportion of 1,8-cineole (57.69 %), with *β*-pinene (6.53 %) and *α*-terpineol (4.44 %)

occurring at lower levels. This compositional pattern is consistent with earlier findings, in which 1,8-cineole was reported as the dominant compound in the essential oils of *N. crispa* (71 %) and *N. menthoides* (41.1 %) [28]. Comparable results were also documented by Ali et al. [13], who identified 1,8-cineole as the principal constituent in oils extracted from *N. racemosa* and *N. faassenii*. In contrast, oils derived from *N. subsessilis* and *N. sibirica* have been reported to contain mainly sesquiterpene hydrocarbons, including *δ*-cadinene, *β*-bisabolene, *β*-caryophyllene, (Z)-*β*-farnesene and caryophyllene oxide. Moreover, analysis of the flowering aerial parts of *N. grandiflora* revealed a different volatile profile, dominated by p-cymene (43.46 %), *γ*-terpinene (18.58 %), carvacrol (12.95 %), and o-cymene (10.99 %) [29]. Taken together, these findings indicate that essential oils from *Nepeta* species frequently exhibit high concentrations of 1,8-cineole [30, 31]. For example, essential oil obtained from wild populations of *N. racemosa* in west of Iran was rich in 1,8-cineole (37 %), along with nepetalactone (2.3 %) [32]. The pronounced insecticidal effectiveness observed for *N. crispa* essential oil in this study is therefore likely linked to the presence of these major volatile constituents, particularly 1,8-cineole.

In the current study, the dominant components of *S. hortensis* oil were characterized as summarized in Table 1. Previous literatures have been reported that the oil contains a comparable profile of constituents to those identified in the current work, with thymol also described among its components [8, 9]. Among phytochemicals, carvacrol and thymol have been recognized as highly effective and toxic fumigants against pests of stored products [33, 34, 35]. Differences in the major constituents of essential oils reported across studies, including those observed in the present work, may result from factors such as geographic origin, genetic diversity, cultivation practices, seasonal effects, and the methods used for extraction.

The major components of *A. graveolens* essential oil were identified, as presented in Table 3. These results are consistent with previous studies [17, 36, 37, 38]. The insecticidal activity of *A. graveolens* oil is largely attributed to the presence of carvone, limonene, and trans-dihydrocarvone [17]. Variations in the qualitative and quantitative composition of the dominant volatile compounds may be influenced by the plant's geographic origin as well as the specific plant part used for oil extraction [38].

Fumigation assays revealed that essential oils derived from members of the Lamiaceae and Apiaceae families were effective in controlling *P. interpunctella*. Mortalities of the insects were proportional to essential oils concentration. Our results confirm previous reports and indicate that essential oils from some species of *Nepeta* [13], *S. hortensis* [9], and *A. graveolens* [17] have shown insecticidal effect against several stored pests. According to our results, *N. crispa* essential oil exhibited the strongest activity against third-instar *P. interpunctella* larvae, with lower effects recorded for *A. graveolens* and *S. hortensis*, (which were estimated LC_{50} = 5.579, 16.34 and 26.974 µl/L air of the respective oil). The greatest level of mortality (88.3 %) in *P. interpunctella* occurred under the highest dose of *N. crispa* oil. Previous work [39] demonstrated that extracts of *N. leavigata* and *N. kurramensis* were toxic to *T. castaneum*, further confirming the insecticidal and biting-deterrent potential of plants in the genus *Nepeta* [13]. As well as an investigation that has reported extracts of *N. italica* L. exhibited

insecticidal activity against wheat weevil and confused flour beetle [40].

At the highest concentration tested, *S. hortensis* essential oil caused 91.7 % mortality in third-instar larvae of *P. interpunctella*. Studies conducted earlier have identified *S. hortensis* oil as toxic to *P. interpunctella*, *E. kuehniella*, and *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) [8]. In addition, its insecticidal efficacy has been documented against *Lipaphis pseudobrassicae* [41], the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) [42], and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) [9]. Exposure of *P. interpunctella* larvae to *A. graveolens* essential oil resulted in 90 % mortality. It should be noted that the oil extracted from *A. graveolens* exhibits insecticidal effects on *Sitophilus zeamais* Motschulsky [43].

5. Conclusion

The results found in this investigation demonstrate that the oils extracted from *S. hortensis*, *N. crispa*, and *A. graveolens* exhibit strong fumigant activity on *P. interpunctella*, a major pest of stored products, suggesting their potential use as alternative agents for protecting stored foods. Moreover, *N. crispa* essential oil had greater insecticidal effectiveness on *P. interpunctella* larvae than the other medicinal plants evaluated in this research.

Author contributions

RN: Project management, Investigation, Validation, Writing and Editing, RT: Project management, Investigation, Conceptualization, Writing and Editing, SGH: Project management, Investigation, Methodology, Validation, Data analysis, Writing, Review and Editing, SHM: Investigation, Validation, Resources and Editing. All authors read the manuscript and approved it.

Conflicts of interest

The authors declared no conflict of interest.

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