Identification of the Seed Essential Oil Composition of Four Apiaceae Species and Comparison of their Biological Effects on *Sitophilus oryzae* L. and *Tribolium castaneum* (Herbst.)

Amini Sh (M.Sc.)[1], Tajabadi F (Ph.D.)[1], Khani M (Ph.D.)[1*], Labbafi MR (Ph.D.)[1], Tavakoli M (M.Sc.)[1]

1- Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

* Corresponding author: Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, 55th Kilometer of Tehran-Qazvin Freeway, Karaj, Iran
Tel: +98-26-34764010-19, Fax: +98-26-34764021
E-mail: khani@imp.ac.ir

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Abstract

Background: The Apiaceae family is defined with the diversity of essential oil. Some of composition could be used to control of storage pests.

Objective: This study was conducted to estimate and identification of the seed essential oil composition of four Apiaceae species.

Methods: The essential oil composition of the seeds of the four Apiaceae's species including (*Pimpinella anisum* L., *Foeniculum vulgar* Mill, *Coriandrum sativum* L. and *Petroselinum hortense*) were identified by Gas chromatography- mass spectrometry (GC-MS) and compared with each other. Finally, biological effects of the four different species of Apiaceae family were evaluated against adult insects of two important storage pests.

Results: The results of analysis showed Anethol with 76.56% and 76.22% is the original component of *Pimpinella anisum* L., *Foeniculum vulgar* Mill, respectively. Also, Linalool with 58.80% and *α*-Pinene with 42.15% are the original components of *Coriandrum sativum* L. and *Petroselinum hortense*, respectively.

Discussion: Regarding to the identified components of the essential oils and obtained results, *Pimpinella anisum* L. and *Coriandrum sativum* L. showed the most fumigant toxicity on the storage pests.

Keywords: Apiaceae, Essential oil, Gas chromatography-Mass spectrometry, Storage pests

Introduction

Essential oil (EO) is one of the most active and effective components of medicinal plants which can be extracted from different parts. These compounds are derived from main parts of plants such as leaves, seeds and bark. The extraction methods of essential oils are usually solvent extraction, hydro-distillation, and steam distillation [1].

The Apiaceae (Umbelliferae) is a large family of flowering and usually aromatic plants mostly growing in temperate areas. There are about 300 genera and 3000 species worldwide [2]. The plants of Apiaceae family possess a characteristic spicy or aromatic smell which is due to the occurrence of essential oil in their different parts of the plants. Some of the plant families known as excellent sources of EOs with insecticidal properties that Apiaceae family is one of them. The family Apiaceae is known as a family with the diversity of essential oils [3].

Pimpinella anisum L., *Foeniculum vulgar* Mill, *Coriandrum sativum* L. and *Petroselinum hortense* are the most important species of Apiaceae. *Pimpinella anisum* L. also called aniseed is one of the oldest medicinal plants. It is an annual grassy herb with 30–50 cm high, white flowers, and small green to yellow seeds, which grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain [4]. *Foeniculum vulgar* Mill is a tall perennial herb native to the Mediterranean region, now widely cultivated as an annual or perennial in Bulgaria, Romania, Hungary, Greece, Turkey, Italy, France, Germany, Egypt, India, and China [5]. It is a traditional and popular herb with a long history of use as a medicine. Fennel is one of Germany's more important medicinal plant crops [5]. *Coriandrum sativum* L. (Coriander) is a glabrous aromatic, herbaceous annual plant, which has a long history as a culinary herb being the source of aroma compounds and EOs with biologically active components possessing antibacterial, antifungal and antioxidant activities, and thus *C. sativum* is useful in food preparation (as a flavouring agent and adjuvant) and preservation as well in preventing food borne diseases and food spoilage [6]. *Petroselinum hortense*, commonly called as Parsley (English) and Jafari (Persian), is a herbaceous biannual species of Umbelliferae distributed throughout the world and known for its aromatic fruits, leaves and seeds [7]. The plant has long history of use as food and medicine in many countries especially in Iran. Therefore, it is extensively cultivated in various regions of Iran. The total amount of essential oils obtained from Apiaceae family have been reported differently. Studies have shown the percentage of volatile oil from 0.5% (v/w) up to 9% (v/w) [3, 8, 9]. Besides the amount, the total constituents of the plants are also reported different. On the other hand the major constituents and their percentage in evaluated samples are reported different.

In this regard, present study was done to evaluate the major components of four different species of Apiaceae family using GC/MS analysis. Finally, biological effects of the four different species of Apiaceae family were evaluated against adult insects of two important storage pests *S. oryzae* and *T. castaneum*.

Material and Methods

The seeds of *P. anisum* L., *F. vulgar* Mill, *C. sativum* L. and *P. hortense* were purchased from Giah Gostar Company (Isfahan, Iran). *T. castaneum* and *S. oryzae* were reared as target insects on 300 gr rice and 200 gr flour in the bottles in the germinator GC 400 (Grouc Co., Tehran, Iran) under defined conditions (27 ±1 °C,70 ± 5% R.H. with a 12:12 h light: dark cycle), respectively. After 7-14 days from the first appearance of the insects on the rice and flour, the produced insects were used in the subsequent experiments. All essential oil solutions were prepared in acetone (Merck, Darmstadt, Germany).

Extraction of Essential Oils

We used an all-glass Clevenger-type apparatus to conduct 2.5 h of hydro-distillation on the powdered seeds (100 gr) of each target plants and the pale yellow essential oil produced. This method for the extraction of oils is recommended by the European Pharmacopoeia [10]. The oils were dried over anhydrous sodium sulphate (Merck, Darmstat, Germany) and stored in sealed vials at 2 °C before analysis.

Gas Chromatography–Mass Spectroscopy (GC–MS)

Gas Chromatography–Mass Spectroscopy (GC–MS) analysis of the essential oils was performed on Agilent 6890 system (Agilent, Littleton, Colorado, USA) coupled with Agilent 5973 N mass selective detector equipped with a BPX5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Following injection, 5 min after injection, oven temperature was increased from 50 to 240 °C at a rate of 3 °C min^{-1} and then reached to 300 °C at rate of 15 °C min^{-1} and hold 3 min in this temperature. Other operating conditions were as follows: carrier gas, He (99.999%), with a flow rate of 0.5 mL min^{-1}; injector temperature, 250 °C; and split ratio, 1:35. Mass spectra were taken at 70 eV a scan time of 1 s and a mass range of 40–500 amu.

Identification of Components of Essential Oil Extracts

The components of the oils were identified by comparing their obtained mass spectra with both of those held in a computer library and obtained using authentic compounds. For confirmation of the results, identities of the components were confirmed by comparing their retention indices, either with those of authentic compounds or with data published in the literature [11]. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

Toxicity bioassay

Toxicity tests of the plant essential oils against *S. oryzae* and *T. castaneum* were carried out in the laboratory according to the methods described by Negahban *et al* [12] and Allotey and Azalekor [13]. To evaluate the fumigant toxicity effects of essential oils from *P. anisum* L., *F. vulgar* Mill, *C. sativum* L. and *P. hortense* against adults of *S. oryzae* and *T. castaneum* based on LC_{50} values. To calculate dosages, essential oils at 71.5 to 430 μL/L air for *S. oryzae* and 30 – 170 μL/L air for *T. castaneum* were dissolved in1 mL acetone, respectively. The prepared treatments were

applied on filter papers (Whatman No. 1, cut in to 2 cm diameter pieces. After evaporating the solvent the impregnated filter papers were attached inside screw caps of 70 ml glass vials [14]. Rice was added into the vials and 15 *S. oryzae* and *T. castaneum* were released into the vials. The caps were tightly screwed on and the vials were sealed with parafilm. The experiments were conducted in five replicates for each treatment. The mortality were recorded after 24, 48 and 72 hours to determine LC_{50} values. In fumigant experiments, new groups of both insects were prepared each time and mortality for each exposure time was determined independently.

Results

The water distilled essential oils of the seeds were analyzed by GC-MS system. The essential oil yields were in the range of 0.2-0.5% (v/w), from the seeds of the plants. In the result of analysis of *F. vulgare,* sixteen compounds were identified representing 100% of the oil. The main compounds of *F. vulgare* were *E*-Anethol (76.22%), Fenchon (10.91%) and Estragole (9.54%) (Table 1). The Obtained GC-MS results for *P. anisum* represented, twenty four compounds that contained 99.44% of the essential oil. *E*-Anethol (76.56%), Estragole (13.01%) and Linalool (7.42%) were the major components of *P. anisum* essential oil (Table 2). The major component of *F. vulgare* and *P. anisum* (*E*-Anethol) were the same and their percentages were approximately equal. The identified components of *C. sativum* were thirty four and contained 97.97% of the essential oil. Linalool (58.80%), Menthol (12.89%), α-Pinene (5.29%) and γ-Terpinene (4.76%) were the major components of *C. sativum* (Table 3). Also, in total twenty eight components of *P. hortense* and 99.45% of essential oil were identified. α-Pinene (42.15%), β-Pinene (30.21%) and Myristicine (4.37%) were the major components of *P. hortense* (Table 4).

Based on the results of bioassay experiments, the fumigant toxic effects of *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum* on *S. oryzae* and *T. castaneum* showed the medium lethal concentration (LC_{50}) of the essential oils after 24-72 hours from commencement of exposure are presented in Table 2 and 3, respectively. Toxicity effects of the essential oils showed considerable differences between the different concentrations against *S. oryzae* and *T. castaneum* (Table 4 and 5). Highest toxicities were observed against *T. castaneum* populations treated with *P. anisum* and *F. vulgare* essential oils with LC_{50} values of 43.75 and 91.28 µL/L air after 24 hours of commencement, respectively (Table 5).

Discussion

As can be found from the results, the profile of identified components from essential oils of different species are different. Qualitative and quantitative differences were reported in these species essential oils may be due to the genetic, differing chemotypes, drying conditions, mode of distillation and/or extraction and geographic or climatic factors [15]. *E*-Anethol and α-Pinene are two components that exist in all essential oils of the four plants.

Among components of EOs, terpenes especially monoterpenoids and sesquiterpenes

have been shown to be toxic to a variety of insects [16]. Previous studies have also shown that the toxicity of EOs obtained from aromatic plants against insect pests is related to the oil's main components such as 1.8-cineole, carvacrol, eugenol, limonene, linalool, α-pinene and thymol [17]. In this study, EOs from seeds of *C. sativum*, was tested in the laboratory contained linalool (58.80%) as the main product active against the two pests and show higher toxicity than other seeds.

In addition, these two insects were exhibited different responses to increasing exposure time and concentrations of essential oils.

Table 1- Constituents of the essential oil from *P. anisum* L., *F. vulgar* Mill, *C. sativum* L. and *P. hortense*

No.	KI	Components	*F. vulgare*	*P. anisum*	*C. sativum*	*P. hortense*
1	802	Hexanal	-	0.04	-	-
2	902	Heptanal	-	0.02	-	-
3	930	*α*-Thujene	-	-	-	0.65
4	939	*α*-Pinene	0.70	0.22	**5.29**	**42.15**
5	954	Camphene	0.06	-	-	0.44
6	690	Benzaldehyde	-	0.05	-	-
7	960	thuja-2,4(10)-diene	-	-	-	0.06
8	975	Sabinene	0.05	-	0.21	**2.26**
9	979	*β*-Pinene	0.05	0.05	0.45	**30.21**
10	991	Myrcene	0.29	0.04	0.21	1.67
11	1003	*α*-Phellandrene	0.17	0.04	-	0.30
12	1017	*α*-Terpinene	-	-	-	0.13
13	1025	*p*-Cymene	0.05	0.22	-	1.36
14	1026	*ortho*-Cymene	-	-	1.51	-
15	1029	Limonene	1.20	0.31	0.25	**2.02**
16	1030	*β*-Phellandrene	0.17	-	-	**6.03**
17	1031	1,8-Cineole	-	0.07	0.35	-
18	1037	*β-(Z)*-Ocimene	-	-	-	0.13
19	1060	*γ*-Terpinene	0.27	0.16	**4.76**	1.28
20	1068	1-Octanol	-	0.03	0.19	-
21	1073	*trans*-Linalol oxide	-	-	0.11	-
22	1087	Fenchone	**10.91**	-	-	-
23	1089	Terpinolene	0.04	-	-	0.45
24	1091	*p*-Cymenene	-	-	-	0.66
25	1097	Linalool	-	**7.42**	**58.80**	-
26	1098	*trans*-Sabinene hydrate	-	-	0.21	-
27	1101	Nonanal	-	0.03	-	-
28	1110	1,3,8-*p*-Menthatriene	-	-	-	0.55
29	1126	*α*-Champholenal	-	-	-	0.15
30	1143	Geijerene	-	0.12	-	-
31	1146	Camphor	0.27	0.11	0.31	0.22

Table 1- Continue

No.	KI	Components	*F. vulgare*	*P. anisum*	*C. sativum*	*P. hortense*
32	1149	(*2Z*)-Nonen-1-al	-	0.04	-	-
33	1153	Menthone	-	-	1.93	-
34	1163	*iso*-Menthone	-	-	0.53	-
35	1164	Menthofuran	-	-	0.9	-
36	1166	*neo*-Menthol	-	-	1.01	-
37	1172	Menthol	-	-	**12.89**	-
38	1177	Terpinene-4-ol	-	-	-	0.36
39	1181	Naphthalene	-	-	-	0.65
40	1183	*iso*-Menthol	-	-	0.27	-
41	1187	Dill ether	-	0.02	-	-
42	1189	*α*-Terpineol	-	-	0.29	-
43	1196	Myrtenal	-	-	-	**2.14**
44	1196	Estragole	**9.54**	**13.01**	-	-
45	1201	*trans*-Dihydrocarvone	-	0.04	-	-
46	1202	Decanal	-	-	0.12	-
47	1226	Citronellol	-	-	0.15	-
48	1235	Thymol, methyl ether	-	0.03	-	-
49	1237	Pulegone	-	-	1.02	-
50	1247	Carvotanacetone	-	-	0.73	0.13
51	1253	*Z*-Anethol	0.11	0.69	-	-
52	1274	*neo*-Menthyl acetate	-	-	0.15	-
53	1285	*E*-Anethol	**76.22**	**76.56**	0.21	0.31
54	1290	Thymol	-	-	0.22	-
55	1381	Geranyl acetate	-	-	1.83	-
56	1388	*β*-Bourbonene	-	-	0.1	-
57	1419	*E*-Caryophyllene	-	-	0.98	-
58	1443	*(Z)-β*-Farnesene	-	-	0.17	-
59	1466	*2E*-Dodecenal	-	-	0.79	-
60	1483	*γ*-Himachalene	-	0.13	-	-
61	1485	Germacrene D	-	-	0.76	-
62	1519	Myristicine	-	-	-	**4.37**
63	1557	Elemicin	-	-	-	0.2
64	1593	Viridiflorol	-	-	0.29	-
65	1595	Carotol	-	-	-	0.13
66	1678	Apiol	-	-	-	0.47
		Total	**100.00**	**99.44**	**97.97**	**99.45**

Percentage>2% are shown in bold

Table 2- Mean mortality (%) of *Sitophilus oryzae* at 24, 48 and 72 hours after commencement of exposure to essential oils of *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum*

Essential oils	Dose (µl/ L air)	Time (h)		
		24	48	72
P. hortense	71.5	0	4	7
	143	0	3	7
	214	0	4	12
	286	3	7	15
	357	3	19	50
	430	3	27	76
P. anisum	71.5	0	5	12
	143	0	7	16
	214	0	13	39
	286	0	17	41
	357	0	19	53
	430	1	31	82.5
F. vulgare	71.5	0	0	1
	143	0	0	3
	214	0	4	15
	286	1	7	20
	357	3	20	71
	430	7	35	91
C. sativum	71.5	0	1	4
	143	0	4	7
	214	0	11	24
	286	0	43	85
	357	0	84	100
	430	8	100	-

Table 3- Mean mortality (%) of *Tribolium castaneum* at 24 hours after commencement of exposure to essential oils of *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum*

	Dose (µl/ L air)					
Essential oils	30	57	85	114	142	170
F. vulgare	1	19	32	61	92	100
P. anisum	10	78	100	-	-	-

Table 4- LC_{50} of *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum* essential oils against *Sitophilus oryzae* at 72 hours after commencement of exposure.

Essential oils	degrees of freedom	LC_{50} (µl/ L air)	LC_{95} (µl/ L air)	Chi-Square	Heterogeneity
C. sativum	4	161.29 (140.81-184.57)	263.11 (220.62-377.52)	16.175	4.04
F. vulgar	4	254.71 (226.42-287.95)	500.94 (408.08-748.37)	9.41	2.35
P. anisum	4	292.04 (232.97-390.02)	1281.12 (734.68-6554.51)	13.340	3.33
P. hortense	4	306.43 (257.91-391.32)	845.7 (573.51-2278.21)	6.622	1.65

Table 5- LC50 of *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum* essential oils against *T. castaneum* at 24 hours after commencement of exposure

Essential oils	degrees of freedom	LC_{50} (µl/ L air)	LC_{95} (µl/ L air)	Chi-Square	Heterogeneity
P. anisum	4	43.75 (39.74-47.34)	72.98 (65.74-84.2)	2.22	0.556
F. vulgar	4	91.28 (76.87-104.87)	180.95 (146.7-282.58)	16.67	4.16

Conclusion

Due to the growing use of chemicals in pest control and side effects of these compounds on environment, researchers are focused on the production and use of safe and low-risk compounds for pest control**.** Using of herbal compounds among which essential oils as an insecticide and repellent have a particular importance. Regarding to the identified components of the essential oils and obtained results, *P. anisum* L. and *C. sativum* L. had the most fumigant toxicity on the storage room pests. Fumigant bioassays with essential oils from *F. vulgar*, *P. hortense*, *C. sativum* and *P. anisum* showed different toxicity effects. The essential oils from *P. anisum* were more toxic against *T. castaneum* than *S. oryzae*. Thus, the toxicity effects of tested plant essential oils were attributed to their major chemical components. Due to the toxicity and repellency effects of essential oils of Apiaceae family on *S. oryzae* and *T. castaneum* and low-risk of secondary metabolites of these plants on the human body and other non-target organisms, it is recommended the essential oils be applied as an alternative, to control pests.

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