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Evaluation of Essential Oil Constituents and Heavy Metals Accumulation of Mint, Parsley and Chamomile that are Grown at Various Planting Locations of Egypt

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Abstract

Mint, parsley and chamomile are aromatic and edible plants that are exported outside from Egypt; they contain many volatile compounds with biological properties that make them very important in food and pharmaceutical manufacturing. Egyptian exporters complain about contamination of water used to irrigate these plants. Therefore, the aim of current study was to assess the content of volatile components and heavy metals of those plants, which harvested from their planting locations (Bahariya oasis, Alfayoum and Benisuef). Obtained results reported that menthone, myristicine and bisabolol oxide A were the major components of Mint, parsley and chamomile essential oils, respectively. The maximum values of menthone (56.32%), myristicine (32.96%) and bisabolol oxide A (81.51%) were recorded with samples collected from Alfayoum, Bahariya oasis and Benisuef, respectively. Chamomile plants harvested from Bahariya oasis produced the greatest values of Cr (17.3 ppm), Pb (0.4 ppm) and Cd (0.2 ppm). Parsley samples harvested from Bahariya oasis gave the maximum value of Zn (27.6 ppm). Mint plants collected from Bahariya oasis resulted in the maximum values of Mn (202.3 ppm) and Ni (4.2 ppm). The highest value of Fe (1591.3 ppm) was recorded with mint samples collected from Benisuef. It may be concluded that planting locations resulted in variations of heavy metals and chemical constituents of mint, parsley and chamomile essential oils. To avoid the health risks resulting from contamination with heavy metals, some environmentally friendly methods should be used.

Key words: Mint, parsley, chamomile, essential oil, heavy metals.

1. Introduction

Medicinal and aromatic plants have been widely explored because of the rising demand for their uses in various objectives, like food and pharmaceutical industries. They are economically serious products and represent a major economic fountain that helps improve the national income [1]. Parsley or *Petroselinum crispum* (Mill) is one of aromatic plants of Apiaceae family; its different parts (leaves, roots and fruits) contain essential oils that have multiple biological uses, such as anti-microbial, diuretic, antioxidants and anticancer, which make them of great importance in the fields of food, pharmaceutical and medicine industries **[2-5]**. Mint (*Mentha* sp.) is aromatic herb from family Lamiaceae; its essential oil is used as anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, anticatarrhal, fungicide and antioxidant **[6]**. Flower heads of Chamomile or *Matricaria chamomilla* L (Asteraceae) contain essential oils that have a wide range of biological properties such as reduce inflammation, antibacterial, anti-inflammatory and antispasmodic **[7]**.

Cultivation sites differ in many characteristics that are in turn reflected on the growth and chemical content of medicinal and aromatic plants **[8, 9]**. These characteristics are represented in climatic conditions **[9]**, soil specifications **[10]**, and irrigation water quality **[11]**.

Mint, parsley and chamomile are medicinal, aromatic and edible crops that are exported outside from Egypt, and some producers complain about the rejection of some of them because they do not conform to international standards; they also complained about the possibility of contamination of the irrigation water.

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Therefore, the aim of this study was to collect those plants during their growing seasons from the cultivated areas (Bahariya oasis, Alfayoum and Benisuef) and evaluate their contents of essential oils and heavy metals.

2. Materials and methods

2.1. Plant material and collection sites

Aerial parts of mint (or parsley) and chamomile flowers were collected from three farms located at different planting locations in Egypt such as Bahariya oasis, Alfayoum and Benisuef during two growing seasons. Soil and irrigation water analyses are presented in Tables 1 and 2.

Table 1. Soil Characteristics

Items	Planting locations		
	Bahariya oasis	Alfayoum	Benisuef
Sand (%)	90.0	64.5	17.7
Silt (%)	3.6	16.7	27.2
Clay (%)	6.4	20.2	53.4
pH (1:25)	7.3	7.8	8.0
EC (dS/m)	1.5	3.8	1.4
Organic matter (%)	0.7	1.8	1.6
$CaCO_3$ (%)	11.3	8.2	2.7
N (%)	0.03	0.1	0.1
P (mg/kg)	28.5	9.3	15.3
K (mg/kg)	25.8	74.8	40.0
Ca (mg/kg)	122.9	81.3	30.0
Mg (mg/kg)	10.2	0.9	16.2
Cu (mg/kg)	1.1	0.6	0.8

2.2. Essential oil isolation and analysis

Essential oil isolation: 200 g of fresh aerial parts of mint (or parsley) and chamomile flowerers were subjected to hydro-distillation for 3h using a Clevenger-type apparatus **[12]**. Essential oils extracted from various plants were dried over anhydrous sodium sulphate to identify the chemical constituents. GC/MS: The essential oil was analyzed on a VG analytical 70-250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 30 m, 0.25 μm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80-270°C at 10°C/min. Diluted samples (1/100, v/v, in n-pentane) of 1 ml were injected, at 250°C, manually and in the splitless mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program. Identifications were made by library searches **[13]**.

2.3. Heavy metals determination

Plant tissue samples digestion: Classical wet decomposition technique (i.e. decomposition by mixture of inorganic acids under normal pressure and high temperature) was used for the digestion of the samples. Prior to digestion of leaves from sampled plant, all glassware were thoroughly cleaned by soaking overnight in 2M nitric acid, washed thoroughly with distilled water and dried. Prepared sample was oven dried at 105 °C for 24 hours and 0.5g of sample dissolved in 10 ml of concentrated nitric acid and digestion tablet was added **[14]**. The mixture was heated until all the fumes ceased. Hydrogen peroxide was added drop wise till a colourless solutions was obtained and 10 ml distilled water added and mixture was left to cool **[14]**. The solution was filtered through whatman No. 42 filter paper into 100 ml volumetric flask and topped with distilled water then put in clean labeled bottle for analysis using AAS (Buck Scientific 210 VGO Varian Co Ltd; Australia) with appropriate hollow cathode lamp at selected wavelength. Preparation of stock solutions and standards: All stock solutions were prepared by dissolving 0.5g to 1g of metal nitrate in 10 ml of distilled water and then made up to 100 ml of solution using distilled water. Through serial dilutions, standard working solutions of Pb, Mn, Cd, Cr, Fe, Ni and Zn were made which were used to generate calibration curves **[14]**.

2.4. Statistical analysis

In this experiment, 2 factors were considered: the three kinds of plants and three planting locations. For each treatment there were 3 replicates. The experimental design followed a complete random block design. According to Snedecor and Cochran **[15]**, the data of both seasons were statistically analyzed using 2-way analysis of variance. Significant values determined according to least significant differences (LSD) at 0.05.

3. Results

3.1. Changes in mint essential oil constituents

GC/MS analysis of mint essential oil shows twenty two constituents were detected under Bahariya oasis, Alfayoum and Benisuef locations (Table 3). The major compounds were menthone (27.69-56.32%) and menthol (26.36-29.85%). Different identified compounds belong to two chemical classes. Oxygenated components (OC, 80.1-92.41%) class was the major; while the class of hydrocarbon compounds (HC, 7.58-13.45%) formed as minor one. Different locations gave several variations in mint essential oil constituents and their classes (Table 3). Samples collected from Bahariya oasis produced the greatest amount of OC (92.41%); while those harvested from Alfayoum location resulted in the maximum value of menthone (56.32%). The highest values of menthol

(29.85%) and HC (13.45%) were obtained from samples harvested from Benisuef location. On the other hand, both menthofuran (9.29%) and menthyl acetate (21.32%) appeared as other main compounds in were harvested from Bahariya oasis and it only appeared as trace quantities of samples from both Alfayoum and Benisuef locations.

3.2. Changes in parsley essential oil constituents

Changes of parsley essential oil components (eighteen components) in response to different growth locations are presented in Table 4. Different variations were observed in parsley essential oil components due to different planting locations, and they were belonged to HC (34.79-60.16%) and OC (34.79-46.84%) classes. The main components were myristicine (22.18-32.96%), β-phellandrene (15.96-27.95%), apiol (3.19-13.88%) and β-myrcene (3.33-11.02%). Samples collected from Bahariya oasis resulted in the maximum amounts of β-phellandrene (27.95%) myristicine (32.96%), apiol (13.88%) and OC (46.84%); while those collected from Benisuef location gave the highest amounts of β-myrcene (11.02%) and HC (60.16%).

3.3. Changes in chamomile essential oil constituents

Nineteen constituents were identified in chamomile essential oil (Table 5). The OC (90.23-95.04%) was the basic class and HC (2.69-7.60%) was the minor one. Bisabolol oxide A (65.90-81.51%), α-bisabolol oxide B (7.52-19.97%) and α-bisabolol (3.07-5.87%) were detected as main components. Samples harvested from Alfayoum location gave the maximum values of α-bisabolol oxide B (19.97%) and HC (7.60%); while those collected from Benisuef site produced the greatest amounts of α-bisabolol (5.87%), bisabolol oxide A (81.51%) and OC (95.04%).

3.4. Changes in heavy metals contents

The differences in heavy metals contents (Pb, Mn, Cd, Cr, Fe, Ni and Zn) in response to various growth locations, plants and their interactions are presented in Table 6. Mint plants gave higher contents in Mn than chamomile and parsley plants. Plants collected from Bahariya oasis resulted in higher content of Mn than those from other regions. The greatest value of Mn content (202.3 ppm) was recorded in mint plants collected from Bahariya oasis. The changes in Mn contents were significant for growth locations, plants and their interactions.

Plants collected from Bahariya oasis resulted in higher Cr content than those collected from other locations. Cr Content was higher in mint plant than chamomile or parsley. The highest amount of Cr (17.3 ppm) was recorded with the chamomile samples from Bahariya oasis. The variation in Cr amounts was significant for locations, plants and their interactions (Table 6).

Plants collected from Benisuef location produced higher amount of Pb than those collected from other regions. The contents of Pb were higher in mint than chamomile or parsley. The greatest value of Pb content (0.4 ppm) was obtained from chamomile samples harvested from Bahariya oasis site (Table 6). The changes in Pb content were significant under all tested factors.

Samples obtained from Bahariya oasis produced higher Fe contents than those obtained from other sites. Mint plant resulted in higher Fe amounts than other tested plants. The maximum value of Fe content (1591.3 ppm) was observed from mint samples collected from Benisuef region (Table 6). All changes in Fe contents were significant for various examined factors.

No variations were found in Cd contents of various plants (Table 6); samples collected from Bahariya oasis contained the Cd element and did not appear in samples from Benisuef or Alfayoum. Chamomile samples obtained from Bahariya oasis gave the maximum value (0.2 ppm) of Cd element. The variation in Cd contents were non significant for plants or plants x locations, while they were significant for locations factor.

Parsley plants produced higher Zn contents than chamomile or mint; higher values of Zn were detected in the samples collected from Bahariya oasis than those obtained from Benisuef or Alfayoum (Table 6). Parsley samples harvested from Bahariya oasis gave the maximum value of Zn (27.6 ppm). The changes in Zn contents were significant for all tested factors.

Mint samples produced higher contents of Ni than those of parsley or chamomile. Bahariya oasis samples contained higher Ni than those from other locations. Mint samples harvested from Bahariya oasis gave the maximum value of Ni (4.2 ppm). The variations in Ni contents were significant for plants, locations and their interactions.

4. Discussion

This investigation reported that planting location and different plant kinds lead to several differences in the chemical contents of mint, parsley and chamomile. These diversities can be referring to modifications in the action of enzymes for formation of essential oils and heavy metals absorption **[16]**. In various planting locations, there are different factors that play an effective function in metabolisms of essential oils and heavy metals contents. These factors are soil properties, altitudes, weather and quality of irrigation water.

Different variations were observed in essential oil constituents under different rates of altitudes. The levels of altitudes resulted in highly significant variations of HC and OC from curry and thyme essential oils **[17, 18]**. Savoy and pignut planted at low altitude produced higher values of OC than those planted at high one **[19, 20]**.

Status of weather such as air temperature, soil temperature, relative humidity rates and number of hours of sunshine of different planting locations lead to many changes in essential oil composition **[9]**. The HC and OC of basil, savory and thyme essential oils were significantly changed in response to sunshine periods **[21-23].** Different relationships were observed in geranium essential oils and their components (linalool, geraniol, geranyl formate, citronellol, citronellyl formate, citronellyl tiglate and 2-phenyl tiglate) due to the rates of relative humidity **[24].** The temperature of agricultural soil deemed as a catalyst for many biological operations, soil wetness, airing and various nutrient uptakes which are needful for plant expansion and essential oil output **[25, 26]**.

Table 2: Heavy metal contents in soil and irrigation water

Locations	Sources	Heavy metals content (ppm)								
		Mn	Cr	Pb	Fe	Cd	Zn	Ni	Co	Hg
Bahariya oasis	Irrigation water	3.67	0.0036	0.0029	0.504	ND	0.123	0.096	.nd	.nd
	Total in soil	271.70	6.29	0.066	1974.23	0.058	11.64	5.96	.nd	.nd
	Available in soil	53.22	0.07	0.051	35.96	0.021	2.82	1.57	.nd	.nd
Alfayoum	Irrigation water	0.72	0.0023	0.0006	0.047	ND	0.003	0.006	.nd	.nd
	Total in soil	338.09	60.57	1.98	2344.38	0.28	55.11	38.02	.nd	.nd
	Available in soil	49.32	0.064	1.25	7.51	0.018	1.52	1.43	.nd	.nd
Benisuef	Irrigation water	0.023	0.0027	0.0028	0.109	ND	0.007	0.004	.nd	.nd
	Total in soil	476.54	66.06	1.41	13871.41	0.37	63.33	46.82	.nd	.nd
	Available in soil	45.12	0.082	1.14	24.15	0.029	1.82	1.63	.nd	.nd

Table 3: Essential oil constituents of mint under different planting locations.

No	Constituents (%)	Rt.	Class	Locations		
				Bahariyaoasis	Alfayoum	Benisuef
1.	Sabinene	6.46	HC	0.52	1.46	-
2.	α-Thujene	7.17	HC	0.77	-	-
3.	Dihydrocarvyl acetate	8.58	OC	-	5.17	-
4.	β-Pinene	10.14	HC	-	-	2.00
5.	Limonene	11.40	HC	4.00	-	2.18
6.	Eucalyptol	11.56	OC	-	-	3.76
7.	Menthone	14.07	OC	27.69	56.32	43.62
8.	Menthofuran	14.27	OC	9.29	-	-
9.	Levomenthol	14.40	OC	5.89	-	0.81
10.	Menthol	14.56	OC	26.36	28.95	29.85
11.	Pulegone	16.17	OC	1.35	-	0.59
12.	Piperitone	17.08	OC	.tr	-	1.47
13.	*p*-Menth-2-ene	17.52	HC	2.29	-	-
14.	Menthyl acetate	18.07	OC	21.32	-	-
15.	4(8)-p-Menthene	18.48	OC	0.51	-	-
16.	β-Elemene	19.35	HC	-	-	0.90
17.	Caryophyllene	20.03	HC	-	2.95	3.17
18.	.β-Cadinene	20.18	HC	-	-	0.54
19.	Germacrene D	21.19	HC	-	2.99	3.62
20.	α-Humulene	21.43	HC	-	1.50	-
21.	γ-Elemene	21.45	HC	-	-	1.04
22.	Terpinolene	24.58	HC	-	0.66	-
HC				7.58	9.56	13.45
OC				92.41	90.44	80.1

Not: Rt. = retention time; - = not detected; HC = hydrocarbon compound; OC = oxygenated compound.

Table 4: Essential oil constituents of parsley under different planting locations.

No.	Constituents (%)	Rt.	Class	Locations		
				Bahariya oasis	Alfayoum	Benisuef
1.	α-Pinene	9.05	HC	-	0.9	1.26
2.	*cis*-β-Ocimene	9.50	HC	2.01	-	-
3.	Sabinene	10.01	HC	0.88	-	-
4.	α-Thujene	10.12	HC	0.62	-	-
5.	β-Pinene	10.20	HC	-	1.2	1.55
6.	β-Myrcene	10.50	HC	3.33	7.1	11.02
7.	α-Phellandrene	10.59	HC	-	2.2	1.04
8.	D-Limonene	11.43	HC	-	-	2.27
9.	β-Phellandrene	11.56	HC	27.95	16.7	15.96
10.	α-Terpinolene	12.17	HC	-	5.9	3.49
11.	*p*-Cymene	13.17	HC	-	2.1	3.79
12.	1,3,8-p-Menthatriene	13.56	HC	-	18.5	19.78
13.	Estragole	15.57	OC	-	-	1.57
14.	Myrtenyl methyl ether	16.44	OC	-	-	1.33
15.	Caryophyllene oxide	18.07	OC	-	-	1.72
16.	Trans-*p*-Menth-2,8-dien-1-ol	18.58	OC	-	4.61	4.61
17.	Myristicine	22.36	OC	32.96	26.8	22.18
18.	Apiol	25.04	OC	13.88	10.4	3.19
HC				34.79	54.6	60.16
OC				46.84	41.81	34.6

Not: Rt. = retention time; - = not detected; HC = hydrocarbon compound; OC = oxygenated compound.

Table 5: Essential oil constituents of chamomile under different planting locations.

No	Constituents (%)	Rt.	Class	Locations		
				Bahariya oasis	Alfayoum	Benisuef
1.	D-Limonene	11.42	HC	0.21	-	-
2.	β-Phellandrene	11.52	HC	0.24	-	-
3.	*trans*-β-Ocimene	1118	HC	0.33	-	-
4.	γ-Terpinene	12.10	HC	0.18	-	-
5.	Artemisia ketone	12.26	OC	0.52	-	0.14
6.	Levomenthol	15.35	OC	0.17	-	-
7.	Borneol	15.59	OC	0.22	-	-
8.	Carvone	17.19	OC	0.16	-	-
9.	*trans*-Caryophyllene	20.33	HC	-	-	1.66
10.	*cis*-β-Farnesene	20.54	HC	3.76	3.53	-
11.	α-Bisabolene	11.42	HC	0.36	-	-
12.	Germacrene D	21.20	HC	0.75	0.85	0.25
13.	Terpinolene	21.25	HC	-	-	0.22
14.	Farnesol	21.29	OC	0.27	-	-
15.	γ-Elemene	21.47	HC	0.85	0.86	-
16.	α-Bisabolol oxide B	24.53	OC	19.97	13.26	7.52
17.	α-Bisabolol	25.13	OC	5.10	3.07	5.87
18.	Chamazulene	25.55	HC	0.92	0.74	0.56
19.	Bisabolol oxide A	26.27	OC	65.90	73.90	81.51
HC				7.60	5.98	2.69
OC				92.31	90.23	95.04

Not: Rt. = retention time; - = not detected; HC = hydrocarbon compound; OC = oxygenated compound.

Table 6: Heavy metals contents under different planting locations.

Plants	Locations	Heavy metals content (ppm)						
		Mn	Cr	Pb	Fe	Cd	Zn	Ni
Parsley	Bahariya oasis	104.7	3.4	.nd	303.6	0.1	27.6	2.4
	Alfayoum	33.3	4.2	.nd	459.1	.nd	18.5	1.9
	Benisuef	0.5	.nd	.nd	.nd	.nd	19.3	.nd
Mean Parsley		46.2	2.5	.nd	.nd	0.1	21.8	1.4
Mint	Bahariya oasis	202.3	15.4	.nd	1375.1	0.1	18.1	4.2
	Alfayoum	75.9	6.0	0.1	1118.4	.nd	15.3	2.5
	Benisuef	79.6	5.4	0.5	1591.3	0.1	25.4	4.1
Mean Mint		119.3	8.9	0.2	1361.6	0.1	19.6	3.6
Chamomile	Bahariya oasis	132.9	17.3	0.4	1133.4	0.2	23.2	3.5
	Alfayoum	39.5	1.3	.nd	200.3	0.1	16.9	1.6
	Benisuef	43.5	1.3	.nd	419.5	.nd	13.9	1.4
Mean Chamomile		72.0	6.6	0.1	584.4	0.1	18.0	2.2
Mean locations	Bahariya oasis	146.6	12.0	0.1	937.3	0.1	22.9	3.4
	Alfayoum	49.6	3.8	0.1	592.6	.nd	16.9	2.0
	Benisuef	41.2	2.2	0.2	670.3	.nd	19.5	1.8
LSD (0.05):								
Plants		5.1	0.4	0.0	26.6	ns	2.4	0.2
Locations		5.1	0.4	0.0	26.6	0.1	2.4	0.2
Species x Locations		8.8	0.8	0.1	46.1	ns	4.1	0.3

On the other hand, soil properties at various plant locations affect growth, essential oil and nutrient contents of tested plants **[27]**. Texture of soil influences growth of plant root system, which affects the transfer of elements to aerial parts. It was found that development of roots is instantaneous under sandy soils, while speed of water and nutrient absorption is fading; opposite trend was found under clay soil; this is reflected in formation and production of essential oil and nutrient accumulations in plant cells **[28, 29]**. Texture of soil also controls the transfer of hormones from the root to aerial parts, where those hormones are controlled vital plant processes and thus formation of essential oils and nutrient accumulations **[28, 29]**. Effect of soil chemical characters on essential oil and mineral contents were confirmed previously **[30]**; low soil pH facilitates plant's absorption of nutrients from soil and thus changes in the content and components of essential oil and mineral contents; and vice versa when pH is high **[31]**. Calcareous soil had a positive effect on the essential oil and nutrient accumulation in plant cells **[27]**, because it contains Ca, which acts as a catalyst works to regulate enzymes for the production of many chemical components **[28]**. Soil inorganic carbon affects the essential oil production through formation of carbohydrate derivatives during the photosynthesis process; among those derivatives is acetyl CoA is used in synthesis of terpenes compounds via mevalonic acid pathway **[28]**. Rates of soil electrical conductivity (EC) led to variations in plant mineral and essential oil constituents **[29]**. Abiotic stress conditions such as salinity (in soil or irrigation water), drought, excess of heavy metals, and lack of major nutrients affect essential oil and nutrient uptakes **[32-36]**.

This study resulted in presence of different heavy metals in irrigation water under various planting locations, which in turn moved to the soil and then to the edible parts of mint, parsley and chamomile. The permissible levels of heavy metals in medicinal plants set by the Joint Food and Agriculture Organization (FAO)-World Health Organization (WHO) committee for Mn, Fe, Cd, Pb, Cr, Co, Ni, Zn and Hg are 200 ppm, 200 ppm, 0.3 ppm, 10 ppm, 2 ppm, 0.48 ppm, 1.5 ppm, 50 ppm and 0.2-0.4ppm, respectively **[37-40]**. This means that there some heavy metals, such as Mn, Fe, Cd and Cr that exceed the permissible limit of plant contents. The heavy metals accumulations in edible plants a toxic effect on human health **[41]**. Exposure to heavy metals is risk agent for cancer, neurological, cardiovascular diseases, kidney dysfunction, hypertension, and other serious diseases of the liver, lung, nervous system, and immune system **[42, 43]** Heavy metal ions such as Cd and Pb have toxic effects on the biochemicals of human body **[42]**. To avoid the health risks resulting from contamination with heavy metals, some environmentally friendly methods must be followed to prevent the transfer of these elements to the plant edible parts such as phyto-remediation, plant growth promoting bacteria, nano tools **[44]**.

Obtained results of this investigation are confirmed by previous research papers. They reported that the same plant with the same parts gives differences in chemical constituents in response to its cultivation in different locations that differ in soil characteristics, climate and weather **[45-53]**.

5. Conclusion

This investigation indicated that mint, parsley and chamomile essential oils and heavy metals contents are related to various conditions of planting locations. Edible parts of mint, parsley and chamomile contain different rates of heavy metals; this is due to its presence in the irrigation water and therefore agricultural soil. To avoid the health risks resulting from contamination with heavy metals, some environmentally friendly methods should be used.

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