ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Short communication / JAPR
Mina et al., 2020, 4 (1), 1-6

Journal of Advanced Pharmacy Research

Impact of Foliar Spray of Ethephon, Water Stress, Organic and Inorganic Fertilizers on The Essential Oil Composition of *Foeniculum vulgare* Mill, var. Dulce Roots

Suzan Adib Mina[1]*, Mokhtar M. Bishr[2], Hoda Mazyoun Hassan[1], Soad M. Abdel Khalik[1]

[1]*Pharmacognosy Department, Faculty of Pharmacy, Helwan University, POB 1179, Cairo, Egypt.*
[2]*Pharmaceuticals and Medicinal Plants (Mepaco). Enshas El Raml, Sharkyah, Egypt.*

***Corresponding author:** *Suzan Adib Mina, Pharmacognosy Department, Faculty of Pharmacy, Helwan University, POB 1179, Cairo, Egypt. Tel. +2001147311226*
Email address: suzanmina@yahoo.com

Submitted on: 18-11-2019; Revised on: 7-12-2019; Accepted on: 14-12-2019

To cite this article: Mina, S. A.; Bishr M. M.; Hassan, H. M.; Abdel Khalik, S. M. Impact of Foliar Spray of Ethephon, Water Stress, Organic and Inorganic Fertilizers on The Essential Oil Composition of *Foeniculum vulgare* Mill, var. Dulce Roots. *J. Adv. Pharm. Res.* **2020**, *4* (1), 1-6. DOI: 10.21608/aprh.2019.19770.1093

ABSTRACT

Objectives: Essential oils (EOs) are valuable natural products whose composition pattern, concentration of individual components and yield depend on many intrinsic and extrinsic factors. This study aimed to evaluate the impact of foliar spray of ethephon, water stress, organic and inorganic fertilizers on the EOs composition of *Foeniculum vulgare*, Miller, var. Dulce roots. **Methods:** Automated rapid headspace solid phase micro extraction (SPME) was applied for the extraction of volatile components combined with gas chromatography coupled to mass spectrometry GC/MS for identification of the volatile compounds. **Results:** forty two volatile components were identified in *Foeniculum vulgare*, Miller, var. Dulce roots which were dominated by hydrocarbons (55.36%-87.2%) with monoterpenes being the major class of components (53.15%-87.08%) in the six various applied conditions**.** Dehydro-p-cymene represented the major constituent (55.8%) in the oil of the non-treated control plant while limonene was the major component in both condition 4 and 6 upon applying drought stress, while alpha terpinolene is the major component in conditions 1, 2, 3 and 5 (30.59%, 39.93%, 50.37% and 35.41% respectively) using different organic and inorganic fertilizers. **Conclusion:** It is concluded that manipulation of agricultural conditions strongly affects biosynthesis and accumulation of essential oils obtained from *Foeniculum vulgare* Miller var. dulce roots.

Keywords: *Essential oils; Ethephon; Foeniculum vulgare; GC/MS; Headspace analysis*

INTRODUCTION

Fennel is a member of the family Apiaceae. It is classified into two sub species *vulgare* and *piperitum*. The most important cultivated fennel varieties belong to subspecies *vulgare*. Sweet fennel (*Foeniculum vulgare* Mill, var. Dulce), is a biennial medicinal plant and it is native to the Mediterranean area[1], considered a good source of essential oil possessing various pharmacological activities[2].

Essential oils (EOs) are a valuable class of natural products used as raw materials in many fields, such as pharmaceuticals, food, cosmetic, and perfume industries. The composition pattern of the EOs, concentration of individual components, and its yield depend on many intrinsic factors such as genetics, plant population, plant origin, plant part, stage of development; and extrinsic factors including environmental factors e.g. climate[3], cultivation conditions e.g. irrigation dose, fertilization, and mineral nutrition[4], postharvest techniques[5] e.g. drying methods and extraction. Soil mineral fertilization has been reported as one of the agricultural practices that can most strongly influence EOs biosynthesis, yield and constituents[6]. Nitrogen (N) and phosphorous (P) are two of the major nutrients in fertilizers and key factors controlling production of primary and secondary metabolites[7]. In addition, the interaction of minerals is often stronger than their individual actions. The production of EOs in aromatic plants may be affected positively or negatively by the type and amount of fertilizer used[8]. Previous reports were concerned with the study of the effect of the addition of both N and P during aromatic plants cultivation[4], as well as analyzing the effect of different climatic factors and different geographical position on the variability of essential oil content[9]. A negative correlation was detected between the increase in temperature and the total oil yield although it had a positive correlation with the content of trans-anethole specifically.

It has been reported that drought has an influence on the content of secondary metabolites in aerial parts of *Salvia officinalis* L. and can be used as a cultivation practice to enhance the health-promoting phytochemicals[10].

The use of foliar fertilizer technique, where the fertilizer is directly sprayed on the plant's leaves, has been proven to raise the amount of oil due to a more rapid absorption of nutrients directly to the location of demand in the leaves[11]. Growth regulators applied through foliar spraying have been previously proven to increase fruit production and quality[12]. Also ethephon foliar spraying in addition to water stress application on *Thymus vulgaris* herb caused a 57% increase in oil yield against normal cultivation condition[13].

The objective of this study is to determine the impact of application of different organic and inorganic fertilization with different irrigation doses and foliar spray on the accumulation of the essential oils of *Foeniculum vulgare* Miller var. Dulce roots.

MATERIAL AND METHODS

Plant material

Foeniculum vulgare, Miller, var. Dulce, family Apiaceae, seeds were obtained from Mepaco Company, Egypt. The plant was botanically identified and authenticated by Dr. Abdul Halim Abdul Majali Mohammed, Head of Flora Research and Plant Classification Department, agricultural research Centre. A voucher specimen number (2Fvu2/2017) was deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University.

Materials for cultivation

Ethephon 48% (Ripex 48%) StarChem Company, Egypt, Super Phosphate (12.5% P_2O_5).Abo Zabal Company for fertilizers. Ammonium sulfate (20.6 % N) Kian for international commerce, Egypt. Urea (46% N) Mopoco, Egypt. Potassium sulfate, Al-Nassr for chemicals, Al-Fayoum, Egypt. Chicken Fertilizer, Chicken Farm, Sharkyah, Egypt.

EXPERIMENTAL

The experimental design and treatment before and after sowing

Experiments and cultivation were carried out in Faculty of Pharmacy farm, Helwan University Cairo/Egypt during November 13th, 2014. For six different conditions of soil treatment, irrigation schedule and addition of growth factor as described in **Table 1**, the land to be cultivated was divided into 7 plots and each plot area was 15 m^2. Each plot was divided into 7 rows. Each row had 5 holes. The fennel seeds were planted at the rate of 5 seeds per hole at distance 40 cm. Germination was practically completed after 12 days from sowing for all conditions except control in which germination was completed after one month.

Headspace GC sampling

Ten grams of fresh roots (in fruiting stage) of each condition were separated from the rest of the plant and subjected to headspace extraction followed by GC-MS analysis. The extraction procedures performed at incubation temperature (130 °C), incubation time (7200 s), syringe temperature (140 °C) and agitator speed (250 rpm).

Gas chromatography/mass spectrometry

GC/MS analysis was performed on a GC/MS system (Shimadzu-2010 Ultra GC/MS) with software (Class 5000). Gas chromatograph equipped with a column Tr-5MS (5% Phenyl polysil Phenylene Siloxane), column (DB-5, 30 m × 0.25 mm i.d × 0.25 um film thickness). The analyses were carried out under the following conditions: Carrier gas: He with flow rate 1 ml/min; Detector temp. FID: 230°C; Injector temp.: 210°C; split ratio; 1: 10; Oven temp. Program: initial temp.; 40°C (2 min) increasing to 210°C at 5°C/min - 210°C (5 min). The capillary column was directly coupled with mass spectrometer HP 5973 (Agilent). EI-MS were recorded at 70 ev. The analysis has been done at the Quality Control Department, Arab CO. for Pharmaceutical and Medicinal Plants (MEPACO), Cairo, Egypt.

Table 1. Summary of the six applied cultivation conditions before and after sowing fennel seeds

Treatments / Condition	Before sowing	After 45 days	After 75 days	Irrigation schedule
Control (1st plot)	-------	--------	--------	Every 15 days
Condition 1 (2nd plot)	Chicken manure (rate of 0.1 m^3)	Ethephon (10 ppm)	Ethephon (10 ppm)	Every 15 days
Condition 2 (3rd plot)	P_2O_5 (250 g)	$(NH_4)_2SO_4$ (720 g) + Ethephon (10 ppm)	$(NH_4)_2SO_4$ (720 g) + Ethephon (10 ppm)	Every 15 days
Condition 3 (4th plot)	P_2O_5 (250 g) + K_2SO_4 (110 g)	K_2SO_4 (110 g)	---	Every 15 days
Condition 4 (5th plot)	Chicken manure (rate of 0.1 m^3)	Ethephon (10 ppm)	Ethephon (10 ppm)	first 4 months every 15 days remaining irrigation every 45 days
Condition 5 (6th plot)	P_2O_5 (250 g)	Urea (200 g)	Urea (200 g)	Every 15 days
Condition 6 (7th plot)	P_2O_5 (250 g)	$(NH_4)_2SO_4$ (720 g) + Ethephon (10 ppm)	$(NH_4)_2SO_4$ (720 g) + Ethephon (10 ppm)	First 4 months every 15 days Remaining irrigation every 45 days

Identification of essential oil constituents

Essential oil components peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its kovat retention index (KI) relative to n-alkanes (C6-C20), mass spectrum matching to NIST, WILEY library database and with authentic standards when available.

RESULTS AND DISCUSSION

Fennel root essential oils are a complex mixture of chemical compounds which are represented by two main classes: hydrocarbons (terpenes) and oxygenates. The relative concentration of these compounds varies considerably depending on the origin of fennel[14]. *Trans*-anethole, fenchone, estragol (methyl chavicol) and *α*-phellandrene are known as marker compounds in fennel oil[15].

The present study reveals the differences in volatile oil composition of root of *Foeniculum vulgare* Miller var. Dulce. arising from altering the agricultural techniques. Components ranging from 14-26 were identified in all the root oils obtained under different cultivation conditions with a percent identification from 98.53 to 99.89%.

Previous studies showed that essential oils of fennel root composed of dillapiole (> 90%) [16]. Others reported phenylpropanes (dillapiol and myristicin), monoterpenes hydrocarbone (*α* and *β*-pinene, *α*- and γ-terpinene and camphene) and organic acids (cinnamic, ferulic and caffeic acids) [17]. In the present study, fennel root oils composition was dominated by hydrocarbons with the major class being monoterpenes in all applied conditions, which contribute to the flavor and aroma of the plant. In the control plot, without any alteration in cultivation conditions, Dehydro-p-cymene represents more than half the constituent of the oil (55.8%) although it was completely absent from the oil composition in all the applied cultivation conditions versus the appearance of *α*-terpinolene, limonene, and γ-terpinene as the major three constituents in the six different tested samples conditions.

Urea addition exclusively to plot number 6 (5th condition) affected oil composition by the increased production of certain components (e.g. myricitin and apiol) and lead to the formation of the highest percentage of oxygenated compounds in all tested oil samples.

It is worth mentioning that applying drought stress in both condition 4 and 6 led to a significant increase in the concentration of limonene which possess a strong orange-like odor and strong antiviral activity, it is a common ingredient in countless cleaning and cosmetic products, and it is commonly used in food manufacturing.

Several class of oxygenated compounds were detected in all tested samples, where ethers constituted the major constituent in all applied conditions including control plot with the highest percentage in condition 5 (38.59%), the only plot subjected to urea fertilizer with normal irrigation schedule, followed by esters as the second major class of oxygenated compounds.

Table 2. Effect of different cultivation conditions on oil composition of *Foeniculum vulgare* Miller var. dulce roots (in fruiting stage)

Peak No	Rt	KI	Oil Composition	Control	C1	C2	C3	C4	C5	C6
1	3.024	600	Acetic acid	-	-	-	-	-	-	0.15
2	4.627	799	Hexanal	0.95	-	-	0.26	-	-	-
3	5.178	806	2,3-Butanediol	-	-	-	-	-	-	0.19
4	5.396	830	Furfural	3.41	0.39	0.21	-	0.11	0.47	-
5	7.275	902	N Heptanal	-	0.14	0.12	0.22	-	-	0.1
6	8.027	923	α-Thujene	-	-	0.13	-	-	-	0.15
7	8.322	933	α-Pinene	-	3.76	4.55	2.78	7.89	2.97	2.62
8	8.67	952	Camphene	-	0.74	0.77	-	0.63	0.4	0.35
9	9.531	980	β-Pinene	-	-	-	-	0.38	-	-
10	10.054	991	β-Myrcene	-	3.63	3.97	2.05	3.53	1.99	2.74
11	10.39	995	2-Pentylfuran	1.02	-	-	-	-	-	-
12	10.412	1004	Octanal	0.47	-	-	-	-	-	-
13	10.417	1007	α-Phellandrene	-	1.44	2.24	2.19	1.2	1.39	0.81
14	10.797	1012	α-Terpinene	-	0.44	0.49	-	0.26	0.19	0.56
15	11.03	1024	p-Cymene	-	0.76	1.89	0.4	0.18	0.44	-
16	11.425	1031	limonene	1.74	19.73	14.62	8.92	35.38	6.78	47.97
17	11.58	1040	Cis-Ocimene	-	4.1	2.67	7.2	2.52	2.06	3.35
18	12.151	1059	γ-Terpinene	-	10.41	9.66	0.8	1.95	1.52	12.04
19	13.079	1084	α-Terpinolene	-	30.59	39.93	50.37	24.53	35.41	16.26
20	13.3	1099	Undecane	-	0.13	-	-	-	-	-
21	13.442	1105	Dehydro-p-cymene	55.8	-	-	-	-	-	-
22	13.65	1108	Nonanal	0.63	-	-	-	-	-	-
23	13.845	1115	D-Fenchyl alcohol	-	-	0.21	-	0.17	-	-
24	14.266	1132	neoallocimene	-	0.26	0.15	0.54	0.14	-	0.23
25	14.6	1144	Mentha-1,4,8-triene	-	0.12	0.12	-	-	-	-
26	15.138	1160	6-butyl-1,4-cycloheptadiene	-	-	-	0.23	0.1	-	-
27	15.985	1183	p-Cymen-8-ol	1.4	-	-	0.31	-	-	-
28	16.176	1185	α-Terpineol		-	-	-	0.1	-	-
29	16.474	1200	Estragol	-	0.65	0.12	2.38	1.15	0.41	3.04
30	17.049	1231	β-Fenchyl acetate	1.01	7.44	4.89	2.54	5.86	4.95	2.5
31	17.45	1236	α-Fenchyl acetate	1.42	1.17	1.69	0.49	1.23	0.32	0.43
32	19.333	1283	trans-Anethole	1.16	0.74	1.22	3.54	2.84	3.84	2.34
33	19.716	1313	2-Methoxy-4-vinylphenol	-	-	-	-	-	0.24	-
34	22.603	1406	cis-Caryophyllene	-	0.52	0.43	1.22	-	-	-
35	22.61	1420	trans-Caryophyllene	5.11	-	-	-	0.34	1.71	0.12
36	24.807	1508	β-Bisabolene	-	-	-	0.22	-	0.29	-

Continue Table 2.

37	25.197	1523	β-Sesquiphellandrene	0.49	0.6	-	-	-	0.21	-
38	25.256	1532	Myristicin	-	-	5.48	3.61	3.34	19.88	0.33
39	25.377	1576	Globulol	-	-	-	0.4	0.84	-	0.29
40	25.969	1622	cis-Asarone	-	-	-	-	0.1	0.36	-
41	27.6	1644	Dillapiol	23.92	11.83	0.19	7.92	0.2	0.62	0.8
42	28.968	1685	Apiol	-	-	4.14	0.77	4.31	13.24	2.21
			T. Hydrocarbons%	63.14	77.23	81.62	76.92	79.03	55.36	87.2
			T. Monoterpene%	57.54	75.98	81.19	75.25	78.59	53.15	87.08
			T. Sesquiterpene %	5.6	1.12	0.43	1.44	0.34	2.21	0.12
			Oxygenated compounds%	35.39	22.36	18.27	22.44	20.25	44.33	12.38
			Acids	-	-	-	-	-	-	0.15
			Alcohol	1.4	-	0.21	0.71	1.11	-	0.48
			Ethers	25.08	13.22	11.15	18.22	11.94	38.59	8.72
			Esters	2.43	8.61	6.58	3.03	7.09	5.27	2.93
			Aldehydes	5.46	0.53	0.33	0.48	0.11	0.47	0.1
			Total identified compounds	14	22	24	23	26	23	23
			% identification	98.53	99.59	99.89	99.36	99.28	99.69	99.58

Rt= retention time, T= total, KI= kovats index, C1=condition 1, C2= condition 2, C3=condition 3, C4= condition 4, C5= condition 5, C6= condition 6

CONCLUSION

The cultivation conditions including foliar spray, water stress, organic and inorganic fertilizers strongly affect biosynthesis, accumulation, and distribution of essential oil and they can be applied to improve the quantity and the quality of the yielded oil. Drought effect is a factor leading to high limonene content in fennel oil and the undesirable component estragol is found to be minimal in oil obtained under cultivation condition 2. Finally to obtain fennel root oil with the highest hydrocarbon content application of cultivation condition number two is recommended.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Muckensturm, B.; Foechterlen, D.; Reduron, J.P.; Danton, P.; Hildenbrand, M. Phytochemical and chemotaxonomic studies of *Foeniculum vulgare*. *Biochemical Systematics and Ecology*. **1997**, *25* (4), 353–358.
2. Oktay, M.; Gülçin, İ.; Küfrevioğlu, Ö. I. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts LWT—*Food Sci. Technol.* **2003**, *36*, 263-271.
3. Deepshekha, P.; Geeta, T.; Chitra, P.; Sunita, B.; Effect of Climatic Conditions on the Volatile Compounds of the Aerial Parts of *Foeniculum vulgare* Mill. *J. Essent. Oil Bear Plants* **2019**, *22* (4), 1093-1103.
4. Rioba, N. B.; Itulya, F. M.; Saidi, M.; Dudai, N.; Bernstein, N. Effects of nitrogen, phosphorus and irrigation frequency on essential oil content and composition of sage (*Salvia officinalis* L.). *J. Appl. Res. Med. Arom. Plant*. **2015**, *2* (1), 21–29.
5. Formisano, C.; Delfine, S.; Oliviero, F.; Tenore, G.C.; Rigano, D.;Senatore, F. Correlation among environmental factors, chemical composition and antioxidative properties of essential oil and extracts of chamomile (*Matricaria chamomilla* L.) collected in Molise (South-central Italy). *Ind. Crop. Prod.* **2015**, *63*, 256–263.
6. Karamanos, A.J.; Sotiropoulou, D.E. Field studies of nitrogen application on Greek oregano (*Origanum vulgare* ssp. hirtum (Link) letswaart) essential oil during two cultivation seasons. *Ind. Crop. Prod.* **2013**, *46*, 246–252.
7. Omer, E.; Hussein, E.A.; Hendawy, S.; Azza, E.; Gendy, A. Effect of nitrogen and potassium fertilizers on growth, yield, essential oil and

artemisinin of *Artemisia annua* L. plant. *Int. Res. J. Hort.* **2014**, *2*, 11–20.

8. Ramezani, S.; Rezaei, M.R.; Sotoudehnia, P. Improved growth, yield and essential oil content of basil grown under different levels of phosphorus sprays in the field. *J. Appl. Biol. Sci.* **2009**, *3* (2), 96–101.
9. Rahimmalek, M.; Maghsoudi, H.; Sabzalian, M. R.; Pirbalouti. A. G. Variability of Essential Oil Content and Composition of Different Iranian Fennel (*Foeniculum vulgare* Mill.) Accessions in Relation to Some Morphological and Climatic Factors, *J. Agric. Sci. Tech*. **2014**, *16*, 1365-1374.
10. Battaieb, I.; Sellami-Hamrouni, I.; Bourgou, S.; Limam, F.; Marzouk, B. Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta. Physiol. Plants* **2011**, *33*, 1103-1111.
11. Mondal, A. B.; Al Mamun, A. Effect of foliar application of urea on the growth and yield of tomato. *Front. Agric. China*. **2011**, *5* (3), 372–374.
12. Dutta, P.; Banik, A.K. Effect of foliar feeding of nutrients and plant growth regulators on physicochemical quality of Sardar guava grown in West Bengal. *Acta Hort.* **2007**, *335*, 407-11.
13. Hassan, H. M.; Mina, S. A.; Bishr, M.M.; Khalik, S.M.A. Influence of foliar spray of ethephon and water stress on the essential oil composition and impact on the cytotoxic activity of *Thymus vulgaris* aerial parts. *Nat. Prod. Res.* **2018**, *33* (18), 2714-2717
14. M.C. Diaaz-Maroto, M.S. Pearez-Coello, J. Esteban, J. Sanz. Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. *J. Agric. Food Chem*, **2006**, *54*, 6814-6818
15. M. Mogh, Comparative survey on the essential oil composition from the seeds and flowers of *Foeniculum vulgare* Mill. from Kerman province, *J. Hortic. Forest.* **2013**, *5* (3), pp. 37-40.
16. Toth, L. Studies on the essential oil from *Foeniculum vulgare*. I. Composition of fruit-and root oil. *Planta Med.* **1967**, *15*, 157-72.
17. Trenkle, K. Fennel (*Foeniculum vulgare*). 2. Essential oils of the fruit, herb, and roots of the plant. *Pharmazie* **1972**, *27* (5), 314-324.