

Essential oil composition of some spices treated with nitrogen in arid regions

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Introduction

Anise (Pimpinella anisum L., Apiaceae) has been used as an aromatic herb. In folk medicine, anise is used as an appetizer, tranquillizer and diuretic drug (Tyler et al., 1988; Lawless, 1999). The traditional use of Pernod, Ouzo, Anisette, Raki, and many other anise-flavored drinks after a heavy meal is a familiar example of its antispasmodic effect, especially in the digestive tract (Hänsel et al., 1999). Dried ripe fruits of anise, commercially called aniseeds (Anisi fructus), contain the whole dry cremocarp of anise (P. anisum L.). For medical purposes, they are used to treat dyspeptic complaints and catarrh of the respiratory tract, and as mild expectorants. It was also reported that extracts from anise fruits have therapeutic effects on several conditions, such as gyne - ecological and neurological disorders (Czygan and Anis, 1992; Lawless, 1999). Coriander (Coriandrum sativum L.) is a culinary and medicinal plant and belongs to the Apiaceae family. This plant has economic importance since it has been used as flavoring agent in food products, perfumes and cosmetics. The essential oils and various extracts from coriander have been shown to possess antibacterial (Burt, 2004), antioxidant (Wangensteen et al., 2004), anti-cancerous and antimutagenic (Chithra and Leelamma, 2004) activities. Due to their unique and preferred flavor and aroma, the swollen bases of sweet fennel (Foeniculum vulgare var. dulce, Apiaceae) are freshly consumed in salads or cooked as a kitchen vegetable. The major constituents of sweet fennel essential oil such as anethole and limonene are also used as essence

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This study was designed to find correlations between the rate of nitrogen and essential oil extracted from anise, coriander and sweet fennel plants grow in arid regions. The highest essential oil contents were recorded under the level of 200 kg N ha⁻¹ with the values of 3.2% and 0.3 ml plant⁻¹ (for anise); 0.3% and 0.1 ml plant⁻¹ (for coriander); 3% and 0.5 ml plant⁻¹ (for sweet fennel). An increase for nitrogen levels of the plants resulted in the enhanced accumulation of essential oil, as well as in a rise in the major constituent's concentration. Groups of essential oils (monoterpenes and sesquiterpenes) were changed with various nitrogen levels.

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in cosmetics and perfumes and for some medicinal purposes (Marotti et al., 1993; Stuart, 1982).

Plant nutrition is one of the most important factors that increase plant productivity. Nitrogen (N) is the most recognized in plants. N fertilization has been reported to reduce essential oil content in creeping juniper (Juniperus horizontalis) (Robert, 1986), although it has been reported to increase total essential oil yield in thyme (Thymus vulgaris L.) (Baranauskienne et al., 2003). Munsi (1992) indicated that for improvement in production of essential oil from a crop like Japanese mint (Mentha arvensis L), a judicious application of nitrogen is required. N fertilization increased the essential oil, Apiaceae and plants (Khalid, 1996). Zheljazkov and Margina (1996) established that essential oil content of mint (Mentha piperita and Mentha arvensis) was increased with increasing N fertilizer rates. N fertilization has been shown improve the essential oil yield and composition of Japanese mint (Mentha arvensis) (Saxena and Singh, 1998). Baranauskiene et al. (2003) investigated the influence of N fertilizers on the essential oil of thyme. It was found that N fertilizer increase the yield of essential oil. N fertilization increased the amount of essential oil concentration and essential oil yield of Ocimum basilicum L. (Arabaci and Bayram, 2004). Akbarinia et al. (2007) indicated that the highest essential oil was obtained with 90 kg N ha⁻¹. Senthil Kumar et al. (2009) revealed that application of N at 93.8 kg ha⁻¹ gave the highest essential oil yield of Davana (Artemisia pallens Wall.). Hellal et al. (2011) indicated that N fertilizer increased the essential oil yield of dill (Anethum graveolens L.) plant compared with the untreated control.

Arid regions in Egypt are characterized with poor nutrients (especially N), lacking organic and inorganic colloids, low available water and unfavorable environmental conditions which negatively affect growth and productivity of medicinal and aromatic plants including anise, coriander and sweet fennel (Abd-Allah *et al.*, 2001). The main objective of the present investigation was to study the effect of different levels of N fertilizers on the essential oil of anise, coriander and sweet fennel plants under arid regions conditions.

Materials and Methods

Experimental

Experiments were carried out in arid region at the Experimental Farm of Desert Development Center (DDC) in Sadat City, American University, Egypt, during two successive seasons, 2005/2006 and 2006/2007. The area of DDC had been recently reclaimed and had not cultivated before. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) presented in Table 1. Seeds of coriander and anise, which were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt; whereas sweet fennel seeds were imported from France. Sweet fennel seeds were sown in the third week of October during both seasons. The seedlings of sweet fennel were transplanted into the open field 45 days after sowing. At the same time, the seeds of coriander and anise were sown directly in the open field. The experimental design was a complete randomized block with four replicates. The experimental area (plot) was 30 m² (4 m x 7.5m) containing 15 rows; the distance between hills was 25 cm and 50 cm apart. Thinning for two plants per hill was made 45 days after cultivating the plants in the open field. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into four main groups subjected to four levels of nitrogen i.e. $N0 = 0 \text{ kg N ha}^{-1}$, $N1=100 \text{ kg N ha}^{-1}$, N2 $= 150 \text{ kg N ha}^{-1}$, N3 $= 200 \text{ kg N ha}^{-1}$. N source was ammonium sulphate $[(NH_4)_2SO_4] (20\% N)]$.

Harvesting

At fruiting stage, the plants were harvested at the end of two seasons and fruit yield (g plant⁻¹) were recorded.

Essential oil isolation

Ripening fruits were collected from each

treatment during the first and second season, and then 100 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage and ml plant⁻¹.

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 μ m coating thickness, fused silica capillary column (DB5), 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.

Qualitative and quantitative analyses

Identifications were made by library searches (Adams, 2007) combining MS and retention data of authentic compounds by comparison of their GC retention Indices (RI) with those of the literature or with those of standards available in our laboratories. The retention Indices were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature (Adams, 2007). Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis

The averages of data from both seasons were statistically analyzed using analysis of variance (ANOVA) and the values of least significant difference (LSD) at 5% according to Snedecor and Cochran (1990).

Results

Essential oil content

Anise, coriander and sweet fennel essential oil contents (% and ml plant⁻¹) were changed with various nitrogen levels (Table 2). The highest essential oil contents were recorded under the level of 200 kg N ha⁻¹ with the values of 3.2% and 0.3 ml plant⁻¹ (for anise); 0.3% and 0.1 ml plant⁻¹ (for coriander); 3% and 0.5 ml plant⁻¹ (for sweet fennel) compared with control and other treatments. ANOVA indicated that the changes in anise essential oil (%), sweet fennel

Table 1. Mechanical and chemical analysis of the soil

Sa	nd	Silt		Clay Gravel		pН	EC (d	S m ⁻¹)		
		9	%							
7	9.7	13.0	7.3	18.7		18.7		8.7	2.0	
Ca ⁺⁺	Mg ⁺⁺	Na ⁺⁺	K^+	CO3		HCO3-	C1-	SO4-		
				mgg -1						
4.9	5.6	11.9	0.6	1.8		1.9	18.6	1.2		
	K		Fe	Cu		Zn	N	ĺn		
	mgg -1					mg g -1				
	0.5		5.4	0.4		0.3	1	.6		

Table2. Effect of N fertilization on essential oil content

Nitrasturanta	Essential oil							
IN treatments	Anise		C	oriander	Sweet fennel			
(kg na ·)	%	ml plant ⁻¹	%	ml plant ⁻¹	%	Ml plant ⁻¹		
0	2.6	0.1	0.2	tr.	1.3	0.1		
100	2.7	0.1	0.2	tr.	1.9	0.4		
150	2.8	0.2	0.2	tr.	2.0	0.4		
200	3.2	0.3	0.3	0.1	3.0	0.5		
LSD								
0.05	0.1	NS	NS	NS	NS	0.1		
$tr_{1} = < 0.1$								

 Table 3. Effect of N fertilization on essential oil constituents of anise.

NI-	C = max = m tr $(0/)$	1/1*		Nitro	gen na ·		
NO	Components (%)	KI*	0	100	150	200	
1	α–Pinene	939	1.1	1.0	1.1	1.2	
2	Camphene	953	1.2	1.3	1.3	1.2	
3	Sabinene	976	0.9	1.1	1.2	1.3	
4	β-Pinene	980	0.2	0.1	0.4	0.3	
5	Myrcene	991	0.5	0.6	0.4	0.5	
6	α-Phellandrene	1005	1.4	1.2	1.4	1.6	
7	Limonene	1031	1.9	2.3	2.4	2.2	
8	γ-Terpinene	1062	0.6	0.6	0.4	0.5	
9	Fenchone	1073	5.2	5.3	5.3	5.4	
10	Linalool	1098	1.8	2.1	1.2	1.1	
11	Camphor	1143	2.4	2.5	2.6	2.3	
12	Menthol	1173	1.9	1.8	1.4	1.3	
13	Dihydrocarvone	1193	0.8	0.9	1.1	1.2	
14	Estragole	1195	4.9	5.1	5.2	5.5	
15	2-Hydroxy-1,8-cineole	1219	1.4	1.5	1.4	1.3	
16	Fenchyl acetate	1224	0.6	0.6	0.5	0.5	
17	Carvone	1242	0.7	0.6	0.8	0.5	
18	Cis-anethole	1265	0.8	1.1	1.2	1.1	
19	Trans-anethole	1283	64.7	64.8	65.4	65.0	
20	Methyl eugenol	1401	1.1	1.3	1.2	1.5	
21	E-B-Farnesene	1458	1.5	1.2	1.4	1.1	
22	Germacrene D	1480	1.7	1.6	1.4	1.2	
23	E,Z-Farnesol	1742	1.6	1.3	1.2	1.5	
Monot	erpene hydrocarbons		10.2	10.7	11.2	11.1	
Oxyge	nated monoterpenes		83.9	85.0	84.9	84.0	
Sesqui	terpene hydrocarbons		3.2	2.9	2.6	2.7	
Oxygenated sesquiterpene 1.6 1.3 1.2 1.5							
Total identified 98.9 99.9 99.9 99.3							

KI^{*} = Confirmed by comparison with Kovats indices on DB5 column (Adams, 2007)

Table 4. Effect of N fertilization on essential oilconstituents of coriander.

No	Components (%)	KI*	Nitrogen ha-1				
INO.			0	100	150	200	
1	α-pinene	939	1.3	1.1	1.5	1.4	
2	Camphene	953	0.1	0.2	0.3	0.3	
3	Sabinene	976	0.5	0.2	0.4	0.1	
4	ß -pinene	980	0.2	0.1	0.3	0.5	
5	Myrcene	991	0.9	1.1	1.2	0.7	
6	P-Cymene	1026	0.5	0.6	0.4	0.5	
7	Limonene	1031	6.8	7.1	7.3	7.4	
8	γ-Terpinene	1062	1.9	1.6	1.3	1.1	
9	Linalool	1098	75.5	75.6	76.5	76.9	
10	Camphor	1143	3.7	3.5	3.6	3.9	
11	Borneol	1165	0.9	0.5	0.6	0.4	
12	Terpine-4-ol	1177	2.9	2.8	1.9	1.9	
13	Geraniol	1255	1.9	1.5	0.9	1.1	
14	Geranylacetate	1383	1.7	1.3	1.4	1.9	
15	Tetradecane	1399	0.7	0.6	0.9	1.1	
Monoterpene hydrocarbons			12.2	12.0	12.7	12.0	
Oxygenated monoterpenes			86.6	85.2	84.9	86.1	
Sesqu	iterpene hydrocarbons		0.7	0.6	0.9	1.1	
Totali	dentified	99.5	97.8	98.5	99.2		

KI * = Confirmed by comparison with Kovats indices on DB5 column (Adams, 2007)

(ml plant⁻¹) were significant. On the other hand the changes of coriander essential oil contents (% and ml plant⁻¹), anise essential oil (ml plant⁻¹) and sweet fennel essential oil (%) were insignificant.

Chemical constituents of anise essential oil

Twenty three constituents amounting 98.9 -99.9% of the oil were found in the anise essential oil extracted by the hydro - distillation method. Different nitrogen levels had no effect on the number of chemical components of anise essential oil (Table 3). The main components were trans-anethole (64.7% - 65.4%), estragole (4.9% - 5.5) and fenchone (5.2% - 5.4%). The values of main constituents were increased by nitrogen levels compared with control treatments. It is clear that the highest amounts of major constituents were produced under the level of 200 kg N ha-1. Also Table 3 represents the compounds obtained from anise essential oil grouped into four classes i.e monoterpene hydrocarbons (10.2% - 11.2%), oxygenated monoterpene (83.9% - 85%), sesquiterpene hydrocarbons (2.6% - 3.2%) and oxygenated sesquiterpene (1.2% - 1.6%%). It is evident that the concentration of monoterpene hydrocarbons as highest in the essential oil of plants grown under 150 kg N ha-1. The highest values of oxygenated monoterpene resulted from the treatment of 100 kg N ha⁻¹. Sesquiterpene hydrocarbons and oxygenated monoterpene were the highest in essential oil of plants grown under control conditions.

Chemical constituents of coriander essential oil

Fifteen compounds amounting 97.8 - 99.5% of the oil were found in the coriander essential oil extracted by the hydro - distillation method. Different nitrogen levels had no effect on the number of chemical components of anise essential oil (Table 4). The major components were linalool (75.5% - 76.9%), limonene (6.8% - 7.4%) and camphor (3.7% - 3.9%). The values of major constituents were increased by nitrogen levels compared with control treatments. It is clear that the highest amounts of major constituents were produced under the level of 200 kg N ha⁻¹. Also Table 4 represents the compounds obtained from coriander essential oil grouped into three classes i.e monoterpene hydrocarbons (12% -12.7%), oxygenated monoterpene (84.9% - 86.6%) and sesquiterpene hydrocarbons (0.6% - 1.1%). It is evident that the concentration of monoterpene hydrocarbons was the highest in the essential oil of plants grown under 150 kg N ha⁻¹. The highest values of oxygenated monoterpene resulted from the control treatment. Sesquiterpene hydrocarbons were the highest in essential oil of plants grown under 200 kg N ha⁻¹.

Chemical constituents of sweet fennel essential oil

Twenty three constituents amounting 98.2 - 100% of the oil were found in the sweet fennel essential

No	Components (%)	KI*	Nitrogen ha				
			0	100	150	200	
1	Camphene	953	tr.	tr.	tr.	tr.	
2	Sabinene	976	tr.	0.1	0.1	0.2	
3	B-Pinene	980	tr.	tr.	tr.	tr.	
4	Myrcene	991	tr.	tr.	tr.	tr.	
5	α-Phellandrene	1005	0.2	0.1	0.1	0.2	
6	p-Cymene	1026	0.1	0.3	0.2	0.1	
7	Limonene	1030	0.7	0.5	0.6	0.5	
8	β-Phellandrene	1031	0.1	0.2	0.3	0.3	
9	2-Hydroxy-1,8-cineole	1033	0.1	0.1	0.3	0.2	
10	(E) - b-Ocimene	1049	0.1	0.2	0.2	0.1	
11	γ-Terpinene	1062	0.1	0.1	0.2	0.3	
12	Fenchone	1075	17.0	17.2	17.2	17.5	
13	Linalool	1098	tr.	tr.	tr.	tr.	
14	Fenchol	1123	0.1	0.2	0.3	0.2	
15	Limonene oxide	1133	tr.	tr.	tr.	tr.	
16	Camphor	1143	0.4	0.3	0.3	0.2	
18	Menthone	1154	tr.	tr.	tr.	tr.	
19	α- Terpineol	1185	tr.	tr.	tr.	tr.	
20	Estragole	1195	21.0	21.2	21.3	21.4	
21	Carveol	1229	0.1	0.1	0.3	0.2	
22	Trans-anethole	1283	58.0	58.1	58.2	58.2	
23	E-β-Farnesene	1458	0.1	0.1	0.2	0.2	
Monoterpene hydrocarbons			1.4	0.3	0.4	0.4	
Oxygenated monoterpenes			96.8	98.5	99.4	99.3	
Sesquiterpene hydrocarbons			0.1	0.1	0.2	0.2	
Total identified			98.2	98.9	100.0	99.9	

 Table 5. Effect of N fertilization on essential oil constituents of sweet fennel

KI* = Confirmed by comparison with Kovats indices on DB5 column (Adams, 2007). tr. = < 0.1%.

oil extracted by the hydro - distillation method. Different nitrogen levels had no effect on the number of chemical components of anise essential oil (Table 5). The main components were trans-anethole (58% - 58.2%), estragole (21% - 21.4%) and fenchone (17% - 17.5%). The values of main constituents were increased by nitrogen levels compared with control treatments. It is clear that the highest amounts of major constituents were produced under the level of 200 kg N ha⁻¹. Also Table 5 represents the compounds obtained from sweet fennel essential oil grouped into three classes i.e monoterpene hydrocarbons (0.3% - 1.4%), oxygenated monoterpene (96.8% - 99.4%) and sesquiterpene hydrocarbons (0.1% - 0.2%). It is evident that the concentration of monoterpene hydrocarbons was the highest in the essential oil of plants grown under control treatment. The highest values of oxygenated monoterpene resulted from the treatment 150 kg N ha⁻¹. Sesquiterpene hydrocarbons were the highest in essential oil of plants grown under 150 and 200 kg N ha-1.

Discussion

The variations in essential oil content and composition could be due to its effect of different N levels on enzymes activity and metabolism improvements (Burbott and Loomis, 1969). These results are in accordance with those obtained by previous studies such as Khalid (1996) who reported that N fertilization increased the essential oil of *Apiaceae* plants. N fertilization increased the amount of essential oil concentration and essential oil yield of *Ocimum basilicum* L. (Arabaci and Bayram, 2004). Hellal *et al.* (2011) indicated that N fertilizer

increased the essential oil yield of dill (Anethum graveolens L.) plant. Sarab et al. (2008) obtained the essential oil concentration in the herb in the case of the application of the highest rate of nitrogen. Kandil et al. (2009) obtained that the highest basil essential oil yield when the highest N rates were applied. The enhanced accumulation of essential oil under the conditions when plants are well supplied with nitrogen results from the increased production of biomass as well as from the direct impact on the biosynthesis of this substance (Sangwan et al., 2001). The above cited studies and the present study prove the significant effect of an increased amount of nitrogen on the concentration of linalool and chemical composition of the essential oil obtained from the basil herb (Özcan and Chalchat, 2002; NurzyńskaWierdak, 2007; Chang et al., 2008). An increase for nitrogen in the nutritional environment of the plants resulted in the enhanced accumulation of essential oil, as well as in a rise in linalool and germacrene D concentrations (Nurzynska-Wierdak, 2013).

Conclusion

It may be concluded that Anise, coriander and sweet fennel essential oils content (% and ml plant⁻¹) were changed with various nitrogen levels. The highest essential oil contents were recorded under the level of 200 kg N ha⁻¹). An increase for nitrogen levels of the plants resulted in the enhanced accumulation of the major constituent's concentration. Groups of essential oils were changed with various nitrogen levels.

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