International Food Research Journal 22(6): 2396-2402 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Quality and quantity of *Pimpinella anisum* L. essential oil treated with macro and micronutrients under desert conditions

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Article history

Received: 26 January 2015
Received in revised form: 24 April 2015
Accepted: 27 April 2015

Keywords

Pimpinella anisum
Anise
Essential oil
Macronutrients
Micronutrients
NP fertilization

Abstract

The essential oil of anise (*Pimpinella anisum* L.) is largely used as a substrate for synthesis of various food and pharmaceutical substances. Desert regions in Egypt are characterized by poor nutrients (macro and micro) and unfavorable environmental conditions which negatively affect aromatic plants including anise plants. Plant nutrition (macro and micro nutrients) one of the most important factors that affected the essential oil production. Fertilization with N_3 (200 kg N ha^{-1}), P_3 (75 kg P_2O_5 ha^{-1}) x micronutrients resulted the maximum mean values of essential oil content (3.3%) and yields (0.3 ml $plant^{-1}$). The lowest essential oil content (2.4%) and yield (t ml $plant^{-1}$) were recorded at control treatments. The highest amount of major compounds [trans-anethole (65.6%), estragole (5.6%), fenchone (5.6%) and camphor (3.1%)] resulted from the N_3P_3 x micronutrients treatment. The chemical classes of essential oil such as monoterpene hydrocarbons decreased in all treatments compared with untreated control, however oxygenated monoterpenes increased. On the other hand sesquiterpene hydrocarbons and oxygenated sesquiterpenes were changed in all treatments compared with untreated control.

Introduction

Anise (*Pimpinella anisum* L.), a grassy annual plant with white flower and small green to yellow seed grows in Iran, Turkey, India, Egypt, and other worm regions throughout the world (Besharati-seidani *et al*., 2005). According to the study by Askari *et al*. (1998) anise seed contains 1.5 - 3.5% mass of volatile oil consisting primarily of trans-anethole and cis-anethole. The major component of anise, trans-anethole, is largely used as a substrate for synthesis of various pharmaceutical substances (Kosalec *et al.*, 2005). Anise and specifically its essential oil have been used in Egyptian folklore medicine for many years (Besharati-seidani *et al*., 2005). Volatile compounds of the essential oil obtained from seeds have exhibited invitro activity against Saccharomyces cerevisiae and some clinical yeast isolates (Fujita and Kubo, 2004; Kosalec *et al*., 2005).

Plant nutrition (macro and micro nutrients) one of the most important factors that affected the essential oil production. Nitrogen (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, 2003). Increase N caused a significant increase in coriander (*Coriandrum sativum* L.) and Davana (*Artemisia pallens* Wall.) essential oil (Akbarinia 2007, Senthil Kumar *et al*., 2009). Three different concentrations of phosphorous (P) (5, 30, and 60 mg L^{-1}) in the nutrient solution were used for the cultivation of *Origanum dictamnus*, significant differences (qualitative and quantitative) were observed between the essential oil samples (Economakis *et al.*, 2002). P fertilization increased essential oil content of some *Apiaceae* and *Origanum* sp. plants (Khalid, 1996). The P had a stimulating effect on the essential oil production of chamomile flowers compared with the control (Nassar *et al*., 2004). Five levels of P (0, 30, 60, 90 and 120 kg P_2O_5 ha^{-1}) were evaluated, results showed that 90 kg P_2O_5 ha^{-1} resulted a significant increase in essential oil of *Trichosanthes cucumerina* L. compared with other levels (Adebooye and Oloyede, 2009). Saharkhiz and Omidbaigi (2008) indicated that feverfew essential oil concentration increased with increasing the P levels. Phosphorus treatments had marked effects on the essential oil content of basil (Ramezani *et al*., 2009). Hornok (1980) indicated that NP fertilization effective on the essential oil content of dill (*Anethum graveolens* L.). The application of 100 kg N and 26 kg P per hectare produced the highest essential oil yields of davana (*Artemisia pallens* Wall.) (Rao, 1989).

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The highest essential oil of chamomile (*Chamomilla recutita* (L.) were achieved when the ratio between the major nutrients N: P was 1:1 (Nikolova *et al*., 1999). Micronutrients are involved in all metabolic and cellular functions (Hänsch and Mendel, 2009). Plants differ in their need for micronutrients (Hänsch and Mendel, 2009). Several of these elements are redox-active that makes them essential as catalytically active cofactors in enzymes, others have enzyme-activating functions, and yet others fulfill a structural role in stabilizing proteins (Hänsch and Mendel, 2009). Kandeel (1991) reported that using micro elements as foliar application at 2000 mg L^{-1} + NP had a significant effect on essential oil content of parsley (*Petroselinum crispum* Mill).

Desert regions in Egypt are characterized by poor nutrients (macro and micro) and unfavorable environmental conditions which negatively affect aromatic plants including anise plants. In this study we represented results obtained from experiments dealing with essential oil extracted from Egyptian anise fruits submitted to some macro (N and P) and micro nutrients (Mn, Zn, Fe, Mg, Zn, Cu, Mo, Ni and Co) treatments.

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) and are presented in Table 1. Seeds of anise were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Seeds were sown directly in the open field in the third week of October during both seasons. The experimental area (plot) was 30m² (4m 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 two main groups. The first group was subjected to different levels of NP combinations: N_0P_0, N_1P_1, N_2P_2 and N_3P_3. N_0 = 0 kg N ha^{-1}, N_1=100 kg N ha^{-1}, N_2 = 150 kg N ha^{-1}, N_3 = 200 kg N ha^{-1}; P_0 = 0 kg P_2O_5 ha^{-1}, P_1 = 37.5 kg P_2O_5 ha^{-1}, P_2 = 56.3 kg P_2O_5 ha^{-1}, P_3 = 75 kg P_2O_5 ha^{-1}. The second group was subjected to the same NP treatments but foliar spray (micronutrients) was added at 1g L^{-1}. N source was ammonium sulphate [$(NH_4)_2SO_4$] (20% N). P_2O_5 source was calcium superphosphate (15% P_2O_5). Foliar spray source was commercial solution (Greenzite) which contains EDTA Na_2 Mn (40%), EDTA Na_2 Zn (48%), Fe (5.4 mg L^{-1}), Mg (0.54mg L^{-1}), Mn (50.54 mg L^{-1}), Zn (570.27 mg L^{-1}), Cu (0.054 mg L^{-1}), Mo (0.027 mg L^{-1}), Ni (0.005 mg L^{-1}) and Co (0.005 mg L^{-1}). Greenzite was added as foliar spray during 2 times, the first one after 2 weeks from thinning while the seconded one after 21 days from the first one.

Table 1. Mechanical and chemical analysis of the soil

Property	Value
Sand (%)	79.7
Silt (%)	13.0
Clay (%)	7.3
Gravel (%)	18.7
Texture	Sand
pH	8.7
EC (dS m^{-1})	2.0
Soluble anions (mg 100 g^{-1} soil)	
SO4⁻	1.2
Cl^-	18.6
HCO_3^-	1.9
CO_3	1.8
Soluble cations (mg 100 g^{-1} soil)	
Ca^{++}	4.9
Mg^{++}	5.6
Na^{++}	11.9
K^+	0.6
Saturation (%)	10.1
K (mg g^{-1})	0.5
Fe (mg g^{-1})	5.4
Cu (mg g^{-1})	0.4
Zn (mg g^{-1})	0.3
Mn (mg g^{-1})	1.6

Harvesting

At fruiting stage, the plants were harvested during both seasons and fruit yield (g $plant^{-1}$)] were recorded.

Essential oil isolation

Ripe fruits were collected from each treatment during the first and second season; air dried and weighed to extract the essential oil, then 100 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oils (ml $plant^{-1}$) were calculated by using the dry fruits. The essential oils extracted from anise were collected during the first and second seasons from each treatment, and then 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 gas chromatography mass spectrometer (GC/MS), 70 eV, using a DB5, 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 split less 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 (Adams, 2007) 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. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis

In this experiment, two factors were considered: NP treatments (N_0 P_0, N_1 P_1, N_2 P_2, and N_3 P_3) and trace elements. For each treatment there were 4 replicates, each of which had 3 plots. The number of experimental pots was 96 plots. The experimental design followed a complete random block design. According to Snedecor and Cochran (1990) the averages of data were statistically analyzed using 2-way analysis of variance (ANOVA-2). Significant values determined according to P values (P " 0.05 = significant, P < 0.01 = more significant and P < 0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

Results

Data presented in Table 2 indicated that NP, micronutrients and their interactions had a positive effect on essential oil content (%) and yield (ml plant^{-1}). Application of NP x micronutrients caused a pronounced increment in both essential oil content and yield compared with the treatments of NP without micronutrients. Fertilization with N_3P_3 x micronutrients resulted the maximum mean values of essential oil content (3.3%) and yields (0.3 ml plant^{-1}). The lowest essential oil content (2.4%) and yield (t ml plant^{-1}) were recorded at control treatments.

Table 2. Effect of NP, trace elements and their interactions on essential oil content

Treatments		Essential oil content %	Essential oil content ml plant $^{-1}$
No trace elements	N_0P_0	2.4	t.
	N_1P_1	2.5	0.1
	N_2P_2	2.8	0.2
	N_3P_3	2.9	0.2
Overall with No trace elements		2.7	0.1
With trace elements	N_0P_0	2.5	0.1
	N_1P_1	2.7	0.1
	N_2P_2	2.9	0.2
	N_3P_3	3.3	0.3
Overall with trace elements		2.9	0.2
Overall NP	N_0P_0	2.5	0.1
	N_1P_1	2.6	0.1
	N_2P_2	2.9	0.2
	N_3P_3	3.1	0.2
F value			
NP		12.7***	5.8**
Trace elements		5.5*	0.4
NP x trace elements		0.0	0.4

ANOVA indicated that the increases in essential oil contents and yield were highly significant for NP treatments. The increase in essential oil (%) was significant for micronutrients while it was insignificant for NP x micronutrients. the increases in essential oil contents and yield were insignificant for micronutrients and NP x micronutrients treatments.

Twenty - three constituents were identified in essential oil extracted from anise fruits, accounting for 98.1 – 99.8% of total constituents, and belong to four chemical main classes. Oxygenated monoterpene class was the major one, the remaining fractions as monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes formed the minor classes (Table 3).

The main constituents of anise fruits essential oil as detected by GC/MS were trans-anethole, estragole, fenchone and camphor which increased as NP level increase. NP x micronutrients treatments resulted in higher values of main components compared with the treatments of NP alone. The highest amount of major compounds [trans-anethole (65.6%), estragole (5.6%), fenchone (5.6%) and camphor (3.1%)] resulted from the N_3P_3 x micronutrients treatment compared with other treatments and control. The chemical classes of essential oil such as monoterpene hydrocarbons decreased in all treatments compared with control, however oxygenated monoterpenes increased. On the other hand sesquiterpene hydrocarbons and oxygenated sesquiterpenes were

Table 3. Effect of the interactions between NP and trace elements on essential oil constituents of anise

No	Components (%)	RI*	RT (min.)	Class	IM	Treatments NP				NP x trace elements				F value
						N_0P_0	N_1P_1	N_2P_2	N_3P_3	N_0P_0	N_1P_1	N_2P_2	N_3P_3	
1	α-Pinene	939	4.35	MH	RI & MS	1.1	0.8	0.9	1.3	1.2	1.3	1.2	1.2	0.6
2	Camphene	953	4.50	MH	RI & MS	1.2	0.7	1.2	1.2	0.9	1.1	0.3	1.0	7.1***
3	Sabinene	976	5.33	MH	RI & MS	0.9	1.3	1.1	0.8	0.6	1.0	0.4	0.6	1.1
4	ß-Pinene	980	5.56	MH	RI & MS	0.2	0.3	0.2	0.4	0.5	0.7	1.1	0.3	3.5**
5	Myrcene	991	6.11	MH	RI & MS	0.5	0.4	0.3	0.4	0.6	0.6	0.8	0.5	1.4
6	α -Phellandrene	1005	6.37	MH	RI & MS	1.4	1.5	1.3	1.3	1.3	1.1	1.1	0.9	0.5
7	Limonene	1031	7.31	MH	RI & MS	1.9	2.1	1.3	1.1	1.2	1.3	1.1	1.7	8.1***
8	γ –Terpinene	1062	7.59	MH	RI & MS	1.4	1.7	1.5	1.6	1.3	1.5	1.6	0.8	3.7**
9	Fenchone	1073	8.47	OM	RI & MS	0.6	0.3	0.5	0.7	0.6	0.5	0.7	0.9	0.3
10	Linalool	1098	8.59	OM	RI & MS	1.8	1.9	1.6	1.4	1.5	1.5	1.1	1.3	0.5
11	Camphor	1143	9.56	OM	RI & MS	5.2	5.3	5.4	5.5	5.5	5.4	5.6	5.6	0.0
12	Menthol	1173	10.34	OM	RI & MS	2.4	2.3	2.4	2.6	2.7	2.8	2.9	3.1	0.1
13	Dihydrocarvone	1193	11.56	OM	RI & MS	1.9	2.1	1.8	1.6	1.5	1.1	1.8	1.4	2.3
14	Estragole	1195	12.45	OM	RI & MS	0.8	0.7	1.1	0.5	1.4	0.7	0.5	0.9	9.0***
15	2-Hydroxy-1,8-cineole	1219	16.54	OM	RI & MS	0.7	0.6	0.9	0.8	0.6	1.5	0.2	0.3	7.9***
16	Fenchyl acetate	1224	17.21	OM	RI & MS	4.9	5.1	5.2	5.2	5.4	5.5	5.5	5.6	0.2
17	Carvone	1242	17.57	OM	RI & MS	0.6	0.7	0.8	0.7	0.5	0.6	0.8	0.4	0.5
18	Cis-Anethole	1265	18.56	OM	RI & MS	0.8	0.9	1.1	1.1	0.8	0.3	1.2	0.4	2.7*
19	trans-Anethole	1283	19.22	OM	RI & MS	64.7	64.8	64.8	64.9	65.0	65.2	65.3	65.6	1.7
20	Methyl Eugenol	1401	19.59	OM	RI & MS	1.1	1.2	1.3	1.2	1.4	0.7	0.9	1.2	3.1*
21	Ê-β-Farnesene	1458	21.45	SH	RI & MS	1.5	1.4	1.2	1.6	1.3	1.6	1.7	1.3	3.8*
22	Germacrene D	1480	22.11	SH	RI & MS	1.7	1.8	1.4	1.9	1.8	1.6	1.9	1.6	5.7***
23	Ê-β-Farnesol	1742	23.87	OS	RI & MS	1.6	1.7	2.0	1.9	1.9	1.8	2.1	1.5	1.6
MH = Monoterpene hydrocarbons						7.8	7.4	6.8	7.2	6.9	7.6	6.7	7.1	2.3*
OM = Oxygenated monoterpenes						86.3	87.3	87.9	87.1	87.6	86.8	87.4	86.6	3.7**
SH = Sesquiterpene hydrocarbons						3.2	3.2	2.6	3.5	3.1	3.2	3.6	2.9	13.3***
OS = Oxygenated sesquiterpene						1.6	1.7	2.0	1.9	1.9	1.8	2.1	1.5	1.4
Total identified						98.9	99.6	99.3	99.7	99.5	99.4	99.8	98.1	

*RI = Confirmed by comparison with Kovats indices on DB5 column (Adams, 2007).
IM = Identification method.
RT = Retention time.
$^{*}P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

changed in all treatments compared with control.

ANOVA indicated that the changes in the most constituents were insignificant for NP X micronutrients treatments while the constituents of camphene, limonene, Dihydrocarvone, carvone and germacrene D were highly significant. On the other hand, the changes in ß-Pinene and 2- hydroxyl-1, 8-cineole were more significant. The changes in cis-anethole, methyl eugenoland Ê-β-farnesene were insignificant. On the other hand, the changes in the essential oil classes were significant for monoterpene hydrocarbons, more significant for oxygenated monoterpene, highly significant for sesquiterpene hydrocarbons and insignificant for oxygenated sesquiterpene (Table 3).

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). Phosphorous was significantly affected essential oil of coriander fruits, fennel, mint and linalool constituent (Ram *et al*., 2003; Moslemi *et al*., 2013). The specific effect of the mode of action of the P on the production of these compounds could be explained on the basis of different primary metabolic pathways of carbon. α-pinene, d-fenchone, and camphene are monoterpenes that are biosynthesized from acetyl-CoA via the mevalonate pathway. α-Pinene and d-fenchone are derived from the pinyl branch of this pathway, while camphene is derived from the closely related bornyl branch of the pathway. This explains why changes in their content in the essential oil correlate positively. In contrast, anethole and methylchavical are phenylpropanoids biosynthesised from phosphoenolpyruvate (PEP) and d-erythrose-4-phosphate via the shikimic acid pathway. They differ chemically only in the position of their instauration in the side chain. Thus, we can anticipate that changes in their concentrations might also be parallel. The common biosynthetic point of the two pathways is that PEP is a precursor via pyruvate for the synthesis of acetyl-CoA. This explains why increased production of the phenylpropanoids is at the expense of biosynthesis of the monoterpenes, since the precursor required for monoterpenes is being diverted into the shikimate pathway (Buntain and Chung, 1994; Robbers *et al*., 1996; Dewick, 1997). Although there are a few reports on fruit yield, essential oil content and yield, harvesting time, etc.

(Hussain and Abou-el-Magd, 1993; Morra *et al*., 1993; Damato *et al*., 1994), information on the effect of P on their constituents is meager. However, László (1979) worked out that increasing doses of P increased carvone content (a monocyclic monoterpene) in *Anethum graveolens*. It is further concluded that basal as well as foliar fertilizer application of P could be employed to obtain the desired quality of essential oil extracted from anise, coriander and sweet fennel fruits. These results are in accordance with those obtained by previous studies such as Khalid (1996) he reported that N fertilization increased the essential oil, Apiaceae and plants. 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 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 (Özcan and Chalchat, 2002). 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 (Sangwan *et al*., 2001; Nurzynska-Wierdak *et al*., 2013). An increase in the amount of 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 concentration. (Chang *et al*., 2008).

The data of this investigation essential oil were positively affected by phosphorus application. Phosphorus significantly increased essential oil concentration. The most effective P rate was 75 kg P ha^{-1}. These results are in agreement with the data of Nikolova *et al*. (1999) who showed P fertilization increased the essential oil concentration of chamomile. It is well documented that phosphorus is an essential element in reproductive of plants (Nikolova *et al*., 1999) and thus, the essential oil increased by applied P was expected in our study. Phosphorus is also known to have multifarious cellular functions in plants, including: Signal-ling and trans membrane metabolic flux and therefore, the secondary metabolism (essential oil) are modulated by these mechanisms (Marschner, 1986). Our results agree with those obtained by Kandeel (1991) who reported that using micro elements as foliar application at 2000 mg L^{-1} + NP had a significant effect on essential oil content of parsley (*Petroselinum crispum* Mill). Previous results of Misra *et al*. (2010) on geranium (*Pelargonium graveolens* L. Her.' ex. Ait.) plants indicated that micronutrients effect on the CO_2 assimilation rate, photosynthetic pigments content and ultimately the accumulation of geranium essential monoterpenes oil (s) and the production of biomolecule geraniol (main component of geranium essential oil).

Conclusion

In conclusion, it appears that application of NP X micronutrients caused a pronounced increment in both essential oil content (%) and yield (ml plant $^{-1}$) of anise. Therefore, It is strongly recommended that on arid region (characterized by low in available nutrients), the crop be supplied with adequate NP and macronutrients. Furthermore, the influence of NP and macronutrients addition on the essential oil of anise is thoroughly studied on locations with wide range of climatology, physical and chemical properties and mineralogical characteristics.

Acknowledgment

The author would to thank the National Research Centre (NRC) and Desert Development Center (DDC) in Sadat City, American University, Egypt for their financial support of this work.

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