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ORIGINAL ARTICLE

Differences in Essential oil Composition of *Ocimum basilicum* L. Related to Different Levels of Nitrogen

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ABSTRACT
Ocimum basilicum L. belonging to Lamiaceae family and widely planted in Mediterranean countries for commercial use. In traditional medical Ocimum basilicum has various benefits such as anti-spasm, carminative effects, and sanitization. Since in cultivated condition use of fertilizers especially nitrogen is essential and common in this research we study the effects of various nitrogen levels on chemical composition of essential oil of ocimum basilicum L. in 4 levels (0, 80, 110, and 140 kg/ha). The major same components of 4 treatments were E-citral (20.73-28.74%), Z-citral (17.81-22.78%), Caryophyllene oxide (9.81-15.23%), Caryophyllene (1.47-5.46%) and Vinylcyclooctane (4.66-8.75%). Also results show that with increasing nitrogen levels up to 110 kg increase the amount of E-citral and Z-citral then be subtracted, But in the case of Caryophyllene oxide and Vinylcyclooctane with increasing nitrogen levels without reducing their value increases.

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INTRODUCTION

Ocimum basilicum L. belonging to Lamiaceae family, and it is utilized as an medical herb, spice and in form a fresh vegetable. The genus of *Ocimum* consist of 30 species which *Ocimum basilicum* L. can be considered as the most important and economical species this genus[1]. Although this plant originates in India, it is widely planted in Mediterranean countries such as Bulgaria, Italy and Hungary for commercial use[2]. In traditional medical *Ocimum basilicum* L. has various benefits such as anti-spasm, carminative effects, and sanitization[2]. The essence of *Ocimum basilicum* L. has various benefits involving anti-bacterial or anti-fungus effects which is widely employed in food and cosmetic industries[1]. So far the majority of researches have not considered the potencies of this plant in respect of fertilization and enhancement soil elements.

Raiesh [3] show the major components of essential oil of *Ocimum basilicum* L. are methyl eugennol and methyl chavicol as well as its anti-bacterial effects in India. Maskri *et al.,* (2011) [4] determined that the major compositions of basil comprise Cineol, Geraniol, and L-linalool during an investigation in Oman.The traces of Methyl Eugenol, Tetramethyl Ticyclo, and Ethyldecaborane have also been verified alongside with other characteristics of basil such as its anti-fungus effects by Nourbakhsh *et al*., in 2010 [5]. Earlier investigation by Ozan *et al*., (2002) [6] in respect of *Ocimum basilicum* L. had also suggested traces of Methyl Eugenol, α-Cubebene, Negrol, and E- muurolene in this plant.

Since in cultivated condition use of fertilizers especially nitrogen is essential and common in this research we study the effects of various nitrogen levels on chemical composition of essential oil of ocimum basilicum.

MATERIAL AND METHODS

This experiment was done in Shahre-Rey area in Iran(latitude: 35° 42´ , longitude : 51° 25´ , altitude:1060 m) with 216mm of mean annual precipitation. The chemical characteristics of field soil were shown in table 1.

Table 1. The chemical characteristics of field soil

Ec (Ds/m)	Ph	N (%)	P (%)	K (mg/l)
3.43	7.95	0.1	12.46	240

Treatments including 4 levels of fertilizer nitrogen (0, 80, 110, and 140 kg/ha). The seeds of *Ocimium basilicum* were sown in May and then harvested in August in each treatment of nitrogen. Plant materials were dried at ambient temperature and shade condition. Voucher specimen is identified and deposited at the herbarium of Islamic Azad University, Shahrerey branch. The essential oil of air-dried samples (100g) of each species was isolated by hydro distillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4ºc until analyzing time. GC analysis was performed by using a thermoquest gas chromatography Shimadzu 9A, with a Flame Ionization Detector (FID) and carried out using fused silica capillary DB-5 column (60m*0.25mm i.d., film thickness 0.25 *m).The operating conditions were as follows: Injector and detector temperatures were 250ºc and 300ºc, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min^{-1} , oven temperature programmed 60ºc -250ºc at the rate of 5ºc min^{-1} and finally held isothermally for 10 min. GC-MS analysis was performed by using a thermoquest-finigan gas chromatograph Varian 3400, equipped with above mentioned column and coupled to trace Mass quadrupled detector. Helium was used as carrier gas with ionization voltage of 70 ev. Ion source and interface temperature were 200 ºc and 250ºc, respectively. Mass range was form m/z 43-456. Gas chromatographic conditions were as given for GC. The chemical compounds of essential oil were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes and the oil on DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their Mass spectra with those of the internal reference Mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds in literature Adams [7]. For quantitative purpose, relative area percentages obtained by GC/FID were used without the use of correction factors.

RESULTS AND DISCUSSION

Table 2. Chemical composition and percent of the essential oil of *Ocimum basilicum* L.in different treatments

	RT	Levels of Nitrogen (Kg/ha)			
		0	80	110	140
Compounds		GC%	GC%	GC%	GC%
Benzaldehyde	12.54	0.364	-	0.306	-
Sabinene	13.29	0.960	0.658	0.997	0.615
1-Octen-3-ol	13.58	0.419	-	-	-
Benzene	15.91	0.533	-	-	-
3-cyclohepten-1-one	17.87	0.459	0.30	-	-
ALPHA.-FENCHONE	19.13	0.405	-	-	-
4-Acetyl-1-methylcyclohexene	21.32	0.379	-	-	-
epi-Photocitral	21.74	0.267	0.265	-	-
Vinylcyclooctane	24.02	4.667	6.60	8.18	8.78
Acetic acid	25.41	-	0.119	-	-
TRANPHOTS-ONEROL	25.74	-	-	-	8.31
Oxiranecarboxaldehyde	25.92	-	3.634	8.189	8.89
Z-Citral	26.94	24.37	18.14	24.46	18.51
E-Citral	28.53	33.76	19.97	36.18	30.7
Epoxy-linalooloxide	29.18	1.164	1.438	1.01	0.936
Phenol	32.16	-	-	-	2.146
Propanoic acid	32.45	0.739	1.158	1.505	-
alpha.-Copaene	32.95	2.098	0.917	1.039	1.218
2,6-Octadien-1-ol, 3,7-dimethy	33.28	0.869	0.958	1.012	1.535
beta.-Cubebene	33.56	0.872	0.856	0.961	1.041
Caryophyllene	34.94	5.460	4.376	4.703	1.471
Bicyclo[3.1.1]hept-2-ene	35.53	2.656	2.249	2.854	2.403
trans-.beta.-Farnesene	35.78	-	-	-	0.552
4,7,10-Cycloundecatriene	36.29	-	-	1.201	0.520
Naphthalene	36.58	1.266	1.266	-	-
trans-.beta.-Farnesene	37.49	0.801	0.801	-	-

Naphthalene	38.67	-	-	0.257	0.225
beta.-cadinene	39.03	0.356	0.356	0.423	0.577
(E,Z)-.ALPHA.-FARNESENE	39.37	0.316	0.316	0.343	-
Caryophyllene oxide	40.22	2.94	2.940	2.635	1.208
Benzene	40.44	0.593	0.593	-	-
Caryophyllene oxide	41.72	9.810	9.810	13.40	15.23
alpha.-Caryophyllene	42.08	2.815	2.815	-	-
Diepicedrene-1-oxide	43.21	1.396	1.396	1.484	-
cis-Z-.alpha.-Bisabolene epoxide	43.87	0.412	0.412	-	-
Caryophyllenol-II	44.86	0.276	0.407	-	0.698
ACORENONE B	45.44	0.496	0.315	-	-
Caryophyllenol-II	44.86	0.376	0.407	-	0.698
ACORENONE B	45.44	0.496	0.315	-	-
2-Pentadecanone	50.79	-	0.235	-	0.510
Total		96.18	96.392	96.089	96.103

The average yield of essential oil in 4 treatment were 0.4% to 0.45% (without nitrogen =0.4%, 80 kg/ha nitrogen =0.4%, 110 kg/ha nitrogen =0.42% and 140 kg/ha nitrogen=0.45%). The identified compounds of essential oil were different in these 4 treatment (without nitrogen= 32 , 80 kg/ha nitrogen=30, 110 kg/ha nitrogen= 18 and 140 kg/ha nitrogen= 21 compounds) and are shown in table 2. Based on identified compounds we recognized over 96% of total oil of *Ocimium basilicum* L. in 4 treatment (without nitrogen= 96.18% , 80 kg/ha nitrogen=96.39%, 110 kg/ha nitrogen= 96.08% and 140 kg/ha nitrogen= 96.10%).

Overall 40 components were identified over 96% of total essential oil of *Ocimium basilicum* L. (table 2)based on GC/MS data in all of treatments. The major same components of 4 treatments were E-citral (20.73-28.74%), Z-citral (17.81-22.78%), Caryophyllene oxide (9.81-15.23%), Caryophyllene (1.47-5.46%), Vinylcyclooctane (4.66-8.75%) (table 2).

Also results show that with increasing nitrogen levels up to 110 kg increase the amount of E-citral and Z-citral then be subtracted, But in the case of Caryophyllene oxide and Vinylcyclooctane with increasing nitrogen levels without reducing their value increases(table 3).

Anyway the existence of compositions such as E-citral, Z-citral and Caryophyllene and antimicrobial, anti-inflammatory and antinociceptive effects of these compositions show this plant could be used as a medicine plant, and if necessary use of chemical fertilizers of nitrogen application up to 110 kg in order to exploit more of the active ingredients plant in the study area is recommended.

Table 3.Effects of levels of nitrogen on major compositions of *Ocimum basilicum* L.

Compounds	Levels of Nitrogen (Kg/ha)			
	0	80	110	140
E-citral	33.76	19.97	36.18	30.7
Z-citral	24.37	18.14	24.46	18.51
Caryophyllene oxide	9.80	9.80	13.40	15.23
Vinylcyclooctane	4.667	6.60	8.18	8.78

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