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Chemical Components of Essential Oils from Basil (*Ocimum basilicum* L.) Grown at Different Nitrogen Levels

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ABSTRACT

The aim of the present study was to determined essential oil content and componets of *Ocimum basilicum* L. grown under four nitrogen applications (0, 50, 100 and 150 kg ha^{-1}). This research was carried out at the experimental fields of Field Crops Department at Agricultural Faculty of Ankara University in 2006. The essential oil content obtained using a Clevenger-type apparatus was analyzed by GC-MS, to determine individual components in essential oil. Average essential oil contents from four applications were ranged from 0.46% in the 100 kg N ha^{-1} application to 0.48% in the 50 kg N ha^{-1} and 150 kg N ha^{-1} applications. The highest essential oil ratio was recorded in the second cutting of control (0.53 %), followed by 50 kg N ha^{-1} (0.50%) and 100 kg N ha^{-1} (0.50%) applications. Major components of basil investigated were linalool and naphthalene. The essential oil components of plants exhibited different profiles with respect to nitrogen applications. Average linalool content ranged from 57.93% in the 100 kg N ha^{-1} application to 61.10% in the control and 150 kg N ha^{-1} application. The highest and lowest average naphthalene content were 13.87% in the 50 kg N ha^{-1} application and 11.58% 150 kg N ha^{-1} application, respectively.

Key Words: *Ocimum basilicum* L., essential oil, nitrogen fertilization, linalool, naphthalene.

INTRODUCTION

Basil or sweet basil (*Ocimum basilicum* L.) belonging to the plant family Lamiacea, comprising many different species, is an annual, 20-60 cm long, white-purple flowering plant. Basil with high economic value is grown and utilized throughout the world [1]. *O.basilicum* is the commonly used for cookery, pharmaceutical and cosmetic purposes. Some of its oil components, such as 1,8-cineole, linalool and and campor, are known to be biologically active [1,2]. In addition, traditionally the plant has been employed in folk medicine for its carminative, stimulant and antispasmodic properties.

The essential oil content and composition in medicinal and aromatic plants is affected primarily by plants genotypes and other conditions such as soil and climatic conditions, growing techniques, harvest time, irrigation, as well as fertilization [3-5]. There are some supporting studies that nitrogen fertilization affects content and composition of essential oil from plants.

It was recorded that N fertilization up to 160 kg ha^{-1} and 240 kg ha^{-1} increased essential oil yield of geranium (*Pelargonium graveolens*) and *Mentha citrata* and *M. arvensis,* respectively [6-8]. It was reported that the combination of inorganic and organic N resulted in significantly greater oil yield and also affected the chemical composition of essential oil since it decreased linalool and increased methyl chavical concentrations in sweet basil [9]. Similar findings were obtained by some authors [10]. Nitrogen applications generally increase oil yield in aromatic plants by enhancing the amount of biomass yield per unit land area, leaf area development and photosynthetic rate [6,11-13].

The aim of the present study was to investigate the effects of different nitrogen doses (0,50,100 and 150 kg N ha^{-1}) on content and composition of essential oil from basil.

MATERIALS AND METHODS

Experimental design and agronomic practices

This research was carried out at the experimental area of Field Crops Department at Agricultural Faculty of Ankara University (32° 51' E; 39° 57' N; 860 m above sea level) in 2006. The characteristics of experimental area were as follows: clay and loam, pH 8.06, lime 9.33%, clay 39.36%, sand 29.56%, silt 31.08%, organic matter 1.07%, total nitrogen 0.132%, phosphor 9.84 ppm and, potassium 0.024%. Total rainfall, mean relative humidity and temperature in 2007 were recorded as 305.2 mm, 52.5%, and 13.3 °C, respectively. The seeds of basil which were population grown at the experimental field of this department were sown at a dept of 18 cm in plastic cases containing a commercial peat substrate (KLASMANN-DEILMANN, Potgrond H .) on April 2-3, 2006. On reaching an adequate height of average 10-15 cm average 2 months (on 24 May) after sowing in the greenhouse, seedlings were transplanted to the experimental area. The trial experiment was established a random block design with four replications. In sowing, row width and intra row spacing were 30 cm and 20 cm, respectively, and plot size was 4.2 m^2. When required, irrigation and weed control was made. Three nitrogen doses (50, 100 and 150 kg ha^{-1}) with a control (0 kg ha^{-1}) were used in this research. Two fertilization and two cuttings were carried out:

The Fertilization Application

The first and second fertilization was applied 27 days (on 20 June) and 43 days (on 7 August) after transplanting, respectively by hand in the rows, and then plants were irrigated.

The Cutting Times

The first and second cuttings were made 59 days (on 23 July) and 99 days (on 1 September) after transplanting, respectively in the beginning of flowering. After each cutting, the plants were dried in the shade at room temperature.

Determination of essential oil content and composition

The essential oil content was determined separately in 50 g of ground aerial parts (leaves and stems) and using a Clevenger-type apparatus. The samples were distilled for 3 h in 500 ml water.

The essential oil was analyzed by GC-MS. The analysis was performed using a Hewlett Packard 6890 N GC, equipped with HP-5 MS capillary column (30 m x 0.25 µm) and HP 5973 mass selective detector. For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

Column temperature was initially kept at 50 °C for 3 min, then gradually increased to 150 °C at a 3 °C/min rate, held for 10 min and finally raised to 250 °C/min. Diluted samples (1/100 in acetone, v/v) of 1.0 µl were injected automatically and in the splitless mode [14]. Individual components were identified by spectrometric analyses using computer library.

RESULTS

The essential oil contents and components identified in the herbage of the basil plants grown at varying nitrogen doses are listed in Table 1, together with their relative percentages, in order of their retention indices.

Table 1. Essential oil content and components with respect to applied nitrogen doses and cutting time in *O.basilicum* L.

Compounds	R.T.	Applied Nitrogen Doses (kg ha^{-1})							
		0		50		100		150	
		Cutting		Cutting		Cutting		Cutting	
		1	2	1	2	1	2	1	2
α- pinen	8.56	-	0.27	0.15	0.23	-	-	-	-
Sabinen	10.19	-	0.24	0.13	0.19	-	-	-	-
β- pinen	10.29	-	0.49	0.30	0.41	0.38	0.23	-	0.20
Myrcene	11.01	-	0.57	0.30	0.47	0.39	0.37	-	0.29
p-cymene	12.43	-	0.13	-	-	-	-	-	-
Limonen	12.61	-	0.40	0.30	0.36	0.41	0.24	-	0.28
Eucalyptol (1,8 cineole)	**12.71**	**2.92**	**6.29**	**5.29**	**5.60**	**4.32**	**4.22**	**5.50**	**3.93**
Ocimeine	13.57	-	0.55	0.33	0.75	0.47	0.48	-	0.39
γ- terpinen	14.38	-	1.62	1.88	1.58	-	-	-	-
Terpinolene	15.37	-	0.13	-	-	-	-	-	-
Linalool	**16.11**	**64.5**	**57.72**	**59.68**	**57.08**	**57.78**	**58.08**	**64.05**	**58.1**
Camphor	17.97	0.70	0.84	0.76	0.83	0.84	1.00	0.84	0.81
γ- terpinen	19.51	1.30	-	-	-	1.85	0.91	1.06	1.97
Terpinyl acetate	20.15	-	0.87	-	-	0.79	0.73	0.83	0.76
Octyl acetate	21.24	-	0.26	-	0.31	0.37	0.38	-	0.36
Borneol	24.51	1.86	1.57	1.43	1.66	2.06	1.86	1.49	2.03
Eugenol	27.65	0.99	0.97	0.43	1.88	-	0.97	0.65	0.89
Linalyl formate	28.85	0.93	-	-	-	-	-	-	-
β- elemene	29.11	1.11	1.04	0.87	0.99	1.57	1.26	1.02	1.33
Eugenol methylether	29.70	-	0.20	-	0.28	-	-	-	-
Trans-caryophyllene	30.21	-	0.18	0.23	-	-	-	-	-
α- bergamotene	30.95	2.27	3.70	-	3.10	2.92	3.42	2.68	3.47
Azulene(α-guiene)	31.05	-	0.71	0.91	0.71	1.08	0.79	0.81	0.81
α-humulene	31.62	1.15	0.82	0.92	0.86	1.28	0.92	1.03	0.90
Trans- β-franesene	31.85	-	0.30	0.23	0.41	-	-	-	-
Farnesol	31.86	-	-	-	-	-	-	-	0.24
Epi-bicyclosesquiphellandrene	32.02	-	0.39	0.36	0.35	0.34	0.40	-	0.37
Germacrene-D	**32.77**	**3.89**	**3.59**	**4.04**	**3.67**	**4.40**	**4.14**	**3.96**	**4.13**
Caryophyllene	32.95	-	-	0.22	-	-	0.29	-	-
Germacrene-B	33.39	0.96	0.78	1.04	0.98	0.99	0.90	1.02	0.87
Germacerene-A	33.74	-	-	-	-	2.30	-	1.64	-
Guaiyl acetate	33.78	1.55	-	-	1.29	1.82	3.01	-	3.03
Naphthalene	**34.10**	**13.69**	**10.24**	**14.6**	**13.13**	**12.23**	**12.86**	**11.04**	**12.11**
α-ylangene	34.49	-	0.21	-	-	-	-	-	-
İsolongifolene	36.56	-	0.31	0.26	0.37	-	0.43	0.37	-
Cadına -1.4-Diene	38.05	0.75	0.77	0.82	0.89	0.89	1.01	0.73	0.96
Cyclohexene	39.27	-	-	0.43	-	-	-	-	0.50
Valenece	39.13	-	0.16	-	0.19	0.46	0.20	-	0.20
Cyclohexanol	39.25	-	0.36	-	-	-	-	-	-
6.10.11.11-tetramethyl-trycyclo..	39.26	-	-	-	0.42	-	-	-	-
Epizonarene	39.27	-	-	-	-	-	0.50	-	-
β-bisabolene	40.06	-	0.12	-	-	-	-	-	-
Neophytadiene	46.14	-	0.15	-	-	-	-	-	0.19
Total		98.57	96.95	95.91	98.99	99.94	99.60	98.72	99.12
Essential oil content (%)		**0.41**	**0.53**	**0.46**	**0.50**	**0.42**	**0.50**	**0.50**	**0.45**

The control application

The contents of essential oil were obtained 0.41 % in the first cutting and 0.53 % in the second cutting. In the first cutting, 15 compounds representing 98.57% of the oil were identified, with major compounds being: linalool (64.50%) and naphthalene (13.69%). Naphthalene was followed by relative small amounts of germacrene-D (3.89%), eucalyptol (2.92%), α- bergamotene (2.27%), borneol (1.86%), guaiyl acetate (1.55%), γ- terpinen (1.30%), α-humulene (1.15%) and β- elemene (1.11%). Also, the other five components were found below 1%.

In the second cutting, 34 compounds representing 96.95% of the oil were identified, among which linalool (57.72%), naphthalene (10.24%) and eucalyptol (6.29%) were the major ones. In addition, the ratio of α- bergamotene and germacrene-D were recorded 3.70% and 3.59%, respectively. The other compounds accounted for 0.12% to 1.62% of the total essential oil.

The 50 kg N ha^{-1} application

The total essential oil content from first and second cuttings were obtained 0.46% and 0.50%, respectively. In the first cutting, the twenty five components of which the first three components (linalool, naphthalene and eucalyptol) representing 79.57% of the oil (59.68%, 14.6% and 5.29%, respectively) were identified. The percentage amounts of *γ*- terpinen, borneol and germacrene-D were recorded 1.88, 1.43 and 1.04%, respectively. On the other hand, the percentage amounts of the other 18 compounds accounting for 7.95% of the total essential oil did not exceed 1%. The twenty eight compounds comprising 98.99 % of the essential oil were characterized in the second cutting. Linalool (57.08%), naphthalene (13.13%) and eucalyptol (5.60%) were predominant components in this oil. The percentage amounts of germacrene-D, α- bergamotene, eugenol, borneol, *γ*- terpinen and guaiyl acetate were 3.67, 3.10, 1.88, 1.66, 1.58 and 1.29%, respectively. Also, the others components identified were found below 1%.

The 100 kg N ha^{-1} application

The ratio of essential oil was recorded 0.42% in the first cutting and 0.50% in the second cutting. Twenty three compounds representing 99.94% of the oil were characterized, among which linalool (57.78%) and naphthalene (12.23%) were the major compounds in the first cutting. The amounts of eucalyptol and germacrene-D were found 4.32 and 4.40%, respectively. Eucalyptol was followed by relative small amounts of α- bergamotene (2.92%), germacrene-A (2.30%), borneol (2.06%), *γ*-terpinen (1.85%), guaiyl acetate (1.82%), *β*-elemene (1.57%), α-humulene (1.28%) and azulene (1.08%). Eleven compounds found below 1% formed 6.33% of essential oil. In the second cutting of this application, linalool (58.08%) and naphthalene (12.86%) were recorded as main components among 26 compounds comprising 99.60%of the oil. The percentage amounts of eucalyptol, germacrene-D, α- bergamotene and guaiyl acetate were found 4.22, 4.14, 3.42 and 3.01%, respectively. The ratio of the others ranged from 0.20 % (valenece) to 1.86 % (borneol).

The 150 kg N ha^{-1} application

The essential oil ratio from the first and second cuttings oh this application was 0.50% and 0.45%, respectively. The major components of the first cutting were linalool (64.05%), naphthalene (11.04%) and eucalyptol (5.50%), and 17 compounds were identified, accounting for 98.72% of total oil. In this application, the percentage amounts of α- bergamotene and germacrene-D were 2.68% and 3.96%, rerpectively. In the second cutting, 26 compounds representing 99.12% of the oil were detected, with major copmounds being: linalool (58.1%) and naphthalene (12.11%). Naphthalene was followed by relative small amounts of germacrene-D (4.13%), eucalyptol (3.93%), α- bergamotene (3.47%), borneol (2.03%), *γ*- terpinen (1.97%) and β- elemene (1.33%). The other components were obtained below 1%.

Except for the 150 kg N ha^{-1} application, the essential oil contents from the others was higher in the second cuttings than the first cuttings. As for the number of compounds identified in the essential oils, the least compounds number was obtained from the control and the 150 kg N ha^{-1} applications. The number of compounds from all applications was higher in the second cutting. The compounds of essential oils investigated changed according to both cutting times and N applications. But, first two components with the highest value were linalool and naphthalene in all applications. The highest value for linalool (64.50%) was obtained from the first cutting of the control application, followed by the first cuttings of the 150 kg N ha^{-1} (64.05%) and the 50 kg N ha^{-1} (59.68%) applications. The findings obtained from the other cuttings and applications were similar (mean 58%). In addition, except for the 100 kg N ha^{-1} application, the content of linalool was higher in the first cuttings of the others.

The highest ratio of naphthalene was recorded in the first cutting of the 50 kg N ha^{-1} application (14.60%), followed by the first cutting of the control application (13.69%), and the lowest ratio was found in the second cutting of the control application (10.24%). While the percentage of naphthalene recorded in the first cuttings of the control and the 50 kg N ha^{-1} applications was higher, this value was higher in the second cutting of the 150 kg N ha^{-1} application and similar (mean 12.6%) in both two cuttings of the 100 kg N ha^{-1} application. The lowest and highest value of eucalyptol was recorded in the control application, 2.92% in the first cutting and 6.29% in the second cutting, respectively. The values from the first and second cuttings of the 50 kg N ha^{-1} and the 100 kg N ha^{-1} applications were similar. As for the 150 kg N ha^{-1} application, the percentage of eucalyptol was lower of 1.57% in the second cutting than the first cutting. The ratio of germacrene-D ranged from 3.59% in the second cutting of the control application to 4.40% the first cutting of the 100 kg N ha^{-1} application, and great differences among the applications were not observed. The highest and lowest values for α- bergamotene were found in the control application, 3.70% in the second cutting and 2.27% in the first cutting, respectively. The value of this component was higher in the second cuttings of the control, 100 kg N ha^{-1} and 150 kg N ha^{-1} applications, and this component was not observed in the first cutting of the 50 kg N ha^{-1} application.

DISCUSSION

Generally, nitrogen applications increase oil content in medical and aromatic plants by enhancing the amount of biomass yield per unit land area, leaf area development and photosynthetic rate [6,11-13,15]. Similar findings were obtained from studies carried out by some authors [9,16,17].

In our study, average essential oil contents from four applications were ranged from 0.46% in the 100 kg N ha^{-1} application to 0.48% in the 50kg N ha^{-1} and 150 kg N ha^{-1} applications. In other words, obtained essential oil contents in plants were similar. Except for 150 kg N ha^{-1} application, the essential oil contents in the first cuttings were higher then the ones in the second cuttings in the other applications. That the essential oil content was lower in first harvest and increased gradually in subsequent harvests was stated [18,19]. It was reported that essential oil yield of the air-dried overground parts of *Ocimum basilicum* from Turkey as obtained by hydrodistillation was 1.25% (*20*). It was reported the average essential oil content in the landraces was 0.8%, ranging from 0.4 to 1.5% [21]. Oil content of the tested accessions grown in Mississippi varied from 0.07% to 1.92% in dry herbage in the field experiment [22].The essential oil content of basil herba determined in the our study was within the range reported for basil in different studies mentioned above. Also, the highest essential oil ratio was recorded in the second cutting of control, followed by 50 kg N ha^{-1} and 100 kg N ha^{-1} applications. Likewise, other study was recorded that the essential oil ratio of *O. basilicum* grown under ecological conditions of Aydin, Turkey was higher under no N application (0.837%) than with N application (5 kg/ha) (0.751%) [23].

As seen Table 1, although the first main components of basil investigated were linalool and naphthalene, the essential oil components of plants exhibited different profiles with respect to nitrogen applications. Average linalool content ranged from 57.93% in the 100 kg N ha^{-1} application to 61.10% in the control and 150 kg N ha^{-1} application. The highest and lowest average naphthalene content were 13.87% in the 50 kg N ha^{-1} application and 11.58% 150 kg N ha^{-1} application, respectively. Linalool and naphthalene were the other component followed by eucalyptol (average 4.27% in the 100 kg N ha^{-1} application 5.45% in the 50 kg N ha^{-1} application) and germacrene-D (average 3.74% in the control-4.27% in the 100 kg N ha^{-1} application). Acccroding to findings from previous studies, nitrogen fertilization has affected composition of essential oil in medicinal plants [24-27]. For example; the experiment was carried out at Dokki, Egypt, to study different rates of N fertilizer (0, 2, 4 or 8 g/pot) on essential oil and its chemical composition of basil plants. Plants which received 4 g N/pot gave the highest essential oil contents in the herbage. GC analysis of volatile oils indicated that linalool and methyl chavicol were the major compounds, followed by farnesene, in all treatments [16].

The essential oil content and composition in medicinal and aromatic plants is affected primarily by their genetics and other conditions such as harvest time, climate and the use of fertilizer [3-5].

O.basilicum L. is very popular and widely grown herbs in the worldwide. Nowadays, several studies have been caried out about cultivation, breeding, high herbage yield and high essential oil content etc. But, few studies have been conducted with the nitrogen fertilization effect on essential oil content and quality of basil. That nitrogen fertilization influences both the amount and components of essential oil were stated by some researchers. However, essential oil contents obtained in plants were similar in our study (average 0.47%). Major components in essential oil of basil investigated were linalool and naphthalene. The essential oil components of plants exhibited different profiles with respect to nitrogen applications.

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