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HERB YIELD AND CHEMICAL COMPOSITION OF BASIL (*OCIMUM BASILICUM* L) ESSENTIAL OIL IN RELATION TO THE DIFFERENT HARVEST PERIOD AND CULTIVATION CONDITIONS

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Abstract

Background: *Ocimum basilicum* L., commonly known as sweet basil, is an important aromatic plant cultivated in many parts of the world for its essential oil. Basil does not show natural distribution in Turkey but they are cultivated as medicinal, seasoning or oil plants especially in the western and southern Anatolia. In this study, introduction of new production patterns of green and purple basil into Rize province in the northern parts of Turkey is the main objective of the present study. In this context, herb yield and chemical composition (essential oil and its components) of green and purple basil plants grown under open field and underneath or between kiwi plantations were determined and compared.
Materials and Methods: The experiments were arranged as a split plot design with three replications in May in 2010 and 2011 in open-field conditions and between kiwi plantations. Three harvests were done for each year. After each harvest, above ground parts were dried at 35 ºC and essential oils from aerial parts after each harvest were extracted using 30 g of dried material by hydro distillation (4 h) with Neo-Clevenger apparatus. The essential oil compositions were identified by GC-MS.
Results: There were decline trends with respect to the plant height, fresh and dry herb yield in the second year. For both experimental years, the highest fresh and dry herb-yields were obtained in open-field conditions in green basil. There were no statistically significant differences in relation to essential oil yield in open-field conditions and kiwi plantations but the highest essential content was ascertained in open-field conditions for green basil. The major aroma constituents of basil were methyl cinnamate and linalool and the highest percentage was determined in open-field conditions for green basil.
Conclusion: The lowest essential content obtained in open-field and between kiwi plantations for green and purple basil plants was even higher than the limit (%0.3) established by Turkish Standards Institution and total yield concerned with fresh and dry herb yield complied with the previously proposed literatures. Hence, basil cultivation underneath kiwi plantations may be implemented.

Introduction

Ocimum basilicum L., commonly known as sweet basil, is an important aromatic plant cultivated in many parts of the world for its essential oil. *Ocimum* genus is represented with more than 150 species in many countries. Within the *Ocimum* genus, there are many chemical and morphological variations, which result from the crossing-easiness and genetic polymorphism (Nurzyńska-Wierdak 2011) in addition to the climatic conditions associated influences on growth, flowering and consequently chemical components of plants (Nurzyńska-Wierdak 2007a, b). Basil does not show natural distribution in Turkey but they are cultivated as medicinal, seasoning or oil plants especially in the western and southern Anatolia. Its leaves contain 0.5-2 % essential oil and methyl chavicol (estragol), eugenol, linalool, methyl cinnamate and camphor are the important components of essential oil of basil leaves. Due to the chemicals contained in essential oils, essential oils of sweet basil are used for treatment of dry mouth and dental complaints, diarrhea and chronic dysentery, respiratory disorders, and effective in the treatment of fungal diseases and stomach discomfort in addition, the influential antitussive, diuretic, anthelminthic, tranquilizer and expectorant roles in medicinal approach. Moreover, ceasing nasal-bleeding and preventing constipation, good for fatigue and insomnia, and uses for healing migraine headaches and incomplete paraplegia were reported (Telci et al. 2006). However, recently the potential uses of *O. basilicum* L. essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Lee et al. 2005, Politeo et al. 2007, Sartoratotto et al. 2004, Suppakul et al. 2003, Wannissorn et al. 2005).

Rize Province is a province of north-east Turkey, on the eastern Black Sea coast and has a rough topography and dispersed settlement. It is between 40º 52' 44"- 41º 11' 21" N latitudes and 40º 45' 26"- 41º 02' 04 " E longitudes. The mean altitude of study area is 856.55 m and it spreads out 0-2321 m altitudes. 75% of the city land cover consists of forests and tea plantations. Tea plantation is the only way of production and income for the inhabitants in the city and that causes monoculture farming. For this reason, researches in relation to determine supportive crop or products and improve production systems, which may bring about economic benefit from small area in addition to tea plantations have been conducted in recent years. In this context, kiwi cultivation, in addition to the tea, has been implemented as a result of studies conducted in recent years in the province. The minimum distance between rows and row cultivation of kiwi is 4m x 4m, and this field is not used for any agricultural activities. In this study, introduction of new production patterns of green and purple basil, which are used for medicinal and seasoning purposes, into Rize province, where there is very little arable fields, is the main objective of the present study. In this context, herb yield and chemical composition (essential oil and its components) of green and purple basil plants grown under open field and underneath or between kiwi plantations were determined and compared.

Material and Methods

Plant Material

This research was conducted in field trial of Pazar Vocational High School, Rize University in 2010 and 2011. Green basil seeds as a research material were provided from the Faculty of Agriculture, Gaziosmanpasa University and commercial purple basil seeds were used for the study.

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Morphological characteristics of research materials are represented in Table 1. Seeds were sown in viols in March in both years in the greenhouse conditions. The experiments were arranged as a split plot design with three replications in May in 2010 and 2011 in open-field conditions and between kiwi plantations. *Ocimum basilicum* L. was regularly irrigated to demonstrate good progress in its period vegetation since irrigation is a very important factor for cultivation of basil.

Table 1: Morphological characteristics of research materials

Research material	Morphological characteristics
Green basil	Green branches and leaves, white flowers, calyx glabrous, equal-length calyx teeth
Purple basil	Purple branches and leaves, pink flowers, calyx sparsely hairy, equal-length calyx teeth

Properties of Experimental Soil

The soils of the field area were sandy clay loam with medium acidity (pH=5.2), low lime content (2.02%), medium nitrogen (0.14%), salt (0.56%), and phosphorus (1.86 ppm), rich in potassium (250 ppm), and with little organic material (1.25%). During the vegetation period (May–Oct.), the experimental area had an average temperature of 18.1 °C and17.9 °C in 2010 and 2011, respectively; total precipitation was 793.6 mm and 757.2 mm; and average humidity was 61.3% and 72.88% respectively. Testing was conducted in 3 repetitions such that row spacing was 30 cm and intra-row spacing was 20 cm according to the testing design of the split plot. The experiment used 6 kg/da di-ammonium phosphate as base fertilizer and 12 kg/da calcium ammonium nitrate (26% N) as top-dressing. Three harvests were done for each year: 26th June, 18th July, 20th August, 2010 and 9th July, 29th July, 21th August, 2011.

Essential Oil Isolation

After each harvest, above ground parts were dried at 35 ºC and essential oils from aerial parts after each harvest were extracted using 30 g of dried material, a 1000 ml round-bottomed flask by hydro-distillation (4 h) with Neo-Clevenger apparatus.

Chemical Composition of Essential Oils Identification

Essential oil content was volumetrically determined. The essential oil compositions of green and purple basils grown in open-field conditions and between kiwi plantations were identified by GC-MS. GC–MS analysis was conducted on an Agilent 7890A gas chromatography and 5975C inert MSD, with Triple-Axis detector (Agilent Technology). A fused silica capillary Agilent Technology DB-WAX (Polyethylene glycol) column (60.0 m, in length, 0.32 mm inner diameter and 0.25 mm film thickness) was used for the separation. The initial temperature of column was 60 °C and temperature was gradually increased to 130°C at a rate of 5 ºC/min for 3 min, then 5 °C/min to 200 °C for 3 min, then 15 °C/min to 250°C for 10 min. The flow rate of helium carrier gas was 1 mL/min at a split ratio of 2:1 .EI was used as the ion source, and the ion source temperature was 230°C. The sector mass analyzer was set to scan from 30 to 550 amp, scan time, 1 s. the identification of the components was performed by matching their recorded mass spectra with the Standard mass spectra from the NIST05, Wiley and flavor2 libraries data provided by the software of the GC–MS system.

Statistical Analysis

The experiments were arranged as a split plot design with three replications. MSTAT-C statistical program was used to determine statistical significance levels and the differences between individual averages were considered to be statistically important at $p<0.05$.

Results and Discussion

Results obtained from three harvests were separately discussed in order to determine the influences of different growth conditions - ***open-field conditions*** and ***between kiwi plantations***. The yield of fresh and dry herb yield and then the essential oil yield was determined using Neo-Clevenger apparatus. The essential oil components of green and purple basils were chemically characterized with the use of GC/MS method.

Plant Height (cm)

There were no statistical significant differences for neither green nor purple basil populations grown in open-field and between kiwi plantations with respect to the plant height in 2010 but there was a statistical significant difference for the second harvest in 2011 (Table 2). Plant heights changed from 38.9 to 98.7 cm, 37.9 to 63.5 cm, and 36.0 to 80.7 cm for three harvests, respectively in 2010 and they ranged from 59.3 to 66.9 cm, 38.9 to 58.8 cm, and 57.3 to 64.4 cm for three harvests, respectively in 2011 (Table 2).

The different basil genotypes and populations have been studied by many researchers to determine the plant height under different ecological conditions. 22.9-57.0 (Telci et al. 2006), and 40.0-76.9 cm (Erşahin 2006). The results of the present study with respect to the plant height are in agreement with the above-mentioned findings of some researchers.

Fresh Herb Yield (kg / da)

Growing green and purple basil plants in open-field and between kiwi plantations did not elicit any statistical significant differences concerned with fresh herb yield (kg/da) for both years (Table 3). Fresh herb yield (kg/da) varied between 516.3-2988.0 kg/da from different harvests in the first year. Highest fresh herb yields (4370.9- 6184.2 kg/da) for both green and purple basils were ascertained in open-field conditions. Once compared to different harvest averages, the highest fresh herb yield was recorded in the first harvest whereas the lowest yield was determined in third harvest. Accordingly, green basil plants had higher fresh herb yield than purple basils. Furthermore, basil plant growing in open-field conditions had higher fresh yield than the plant growing between kiwi plantations.

Table 2: Changes in plant height in relation to the different harvest and years in different growing conditions

Population	Trial area	2010				2011			
		I. Harvest	II. Harvest	III. Harvest	Average	I. Harvest	II. Harvest	III. Harvest	Average
Green Basil	Open-field	98.7	55.53	88.0	80.73 a	60.87	56.77 a	57.8	58.47
	Kiwi Plant.	47.2	40.13	45.43	44.27 b	64.6	38.87 b	60.6	54.7
Purple Basil	Open-field	85.4	63.47	78.63	75.90 a	66.90	58.80 a	64.4	63.40
	Kiwi Plant.	38.97	37.93	36.0	37.70 b	59.3	53.80	57.3	56.80
LSD (%5)		NS	NS	NS	NS	NS	13.89	NS	NS

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)
NS: Non Significant

Table 3: Changes in fresh herb yield (kg/da) in relation to the different harvest and years in different growing conditions

Population	Trial area	2010				2011			
		I. Harvest	II. Harvest	III. Harvest	Total	I. Harvest	II. Harvest	III. Harvest	Total
Purple Basil	Open-field	2165.8	1257.1	977.6	4371.0	2029.8	1114.4	871.2	4015.4
	Kiwi Plant.	856.3	798.4	516.3	2180.0	744.2	693.4	431.6	1869.3
Green Basil	Open-field	2988.0	1839.4	1356.8	6184.2	2521.3	1705.8	1190.1	5417.2
	Kiwi Plant.	1415.9	1235.1	828.2	3479.1	1182.2	1034.4	876.2	3092.8
LSD (%5)		NS	NS	NS	NS	NS	13.9	NS	NS

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

The highest (2521.3 kg/da) and lowest (431.63 kg/da) fresh herb yield were determined in the first and third harvest, respectively, second year. Fresh herb yield changed between 1869.3-5417.2 kg/da in the second year. Highest total yields were found to be 6184.2 kg/ha and 5417.2 kg/da for green basil for both years, respectively, in open-field conditions. Total yield was higher in the first experimental year (4053.56 kg/da) than the second experimental year (3598.66 kg/da). As a result, highest total yield was ascertained in open-field conditions for both years and the better agronomic performance was determined for green basil than purple one for both years (Table 4). Total fresh herb yields were proposed to be 1609.1-5345.8 kg/da in different basil genotypes (Telci et al. 2006), 1719.2-4450.0 kg/da under Diyarbakır (Turkey) ecological conditions (Erşahin 2006) and 3740.2-11643.1 kg/da under Aegean ecological conditions (Ekren et al. 2009). The results in the presents study comply with the previously proposed data except Ekren et al. (2009).

Dry Herb Yield (kg/da)

Dry herb yield for the years 2010 and 2011 are collectively represented in Table 4. There were no statistically significant differences among all harvests for both experimental years with respect to the dry herb yield (kg/da). Dry herb yield for harvests were 156.0-427.6, 106.8-238.4 and 82.2- kg/da for the first year, respectively whereas they ranged from 113.5-397.2, 102.5-239.4 and 68.9-195.1 kg/da in the second year, respectively. Similar to the fresh herb yield, the highest yield was obtained from first harvest and the lowest yield was determined from the third harvest in relation to the dry herb yield for both experimental years. Green basil populations had higher dry herb yield than purple ones in both experimental years. Moreover, both green and purple basil plants demonstrated higher dry herb yield in open-field conditions than between kiwi plantations for both years. The basil plants have been studied by many researchers to examine the effects of different ecological conditions on yield parameters using various basil genotypes. Total dry herb yields were proposed to be 255.7-712.7 kg/da in different basil genotypes cultivated in Turkey (Telci et al. 2006), and 683.6-1566.2 kg/da under Aegean ecological conditions (Ekren et al. 2009). The present results that we determined comply with the mentioned results except Ekren et al. (2009) with respect to the total dry herb yield for both experimental years.

Essential Oil Content (%)

No statistically significant differences were determined with respect to the influences of growing conditions and harvests on essential oil production in green and purple basil plants in the first experimental years but there were statistically significant differences between the averages obtained from the third harvests in the second year (Table 5). Essential oil content ranged from 0.69-1.83% in the first year of the study. The highest essential content was determined by 1.66 % in green basil grown in open-field conditions even there were no significant differences green and purple basil plants in different ecological conditions. Essential oil content ranged between 0.47 and 1.63% in the second year. The highest essential oil yield was 1.45 % in relation to the average obtained from three harvests in green basil grown in open-field conditions. The essential oil yield was higher in first year (1.23%) than the second experimental year, which may be attributed to differences in climatic changes. The highest essential oil content for both green and purple basil plants grown in open-field and underneath kiwi plantations were obtained from second harvests. Even the highest essential oil yield was obtained in basils in open-field conditions for both experimental years, the lowest

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content that we determined in the present study was 0.80 %, which was over the limit (% 0.3) established by Turkish Standards Institution. Consequently, the present results demonstrate that basils growing between kiwi plantations meet the quality standards. Essential oil biosynthesis, storage and release in plants depend on environmental conditions, genetic structure or phenology of the plant in addition to the growth conditions and agricultural techniques. The essential oil content in *Ocimum basilicum* L. in the previous reports were 0.73-0.83 % (Sarıhan et al. 2004), 0.35-1.28 % (Telci et al. 2006), 0.49-1.25 % (Erşahin 2006), 0.62-0.74 % (Gürbüz et al. 2006), 0.62 % (Politeo et al. 2007) and 0.5-0.8 % (Hussaina et al. 2007). The present results were in agreement with the previously proposed reports.

Table 4: Changes in dry herb yield (kg/da) in relation to the different harvest and years in different growing conditions

Population	Trial area	2010				2011			
		I. Harvest	II. Harvest	III. Harvest	Total	I. Harvest	II. Harvest	III. Harvest	Total
Purple Basil	Open-field	339.2	209.2	142.2	609.6	317.2	182.1	113.9	613.2
	Kiwi Plant.	156.0	106.8	82.2	323.5	113.5	102.5	68.8	284.8
Green Basil	Open-field	427.5	238.4	199.8	865.8	397.2	239.4	195.1	835.7
	Kiwi Plant.	239.2	189.4	117.8	546.6	195.2	157.7	127.7	480.6
LSD (%5)		NS	NS	NS	NS	NS	13.9	46.4	139.3

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 5: Changes in essential oil yield (%) in relation to the different harvest and years in different growing conditions

Population	Trial area	2010				2011			
		I. Harvest	II. Harvest	III. Harvest	Average	I. Harvest	II. Harvest	III. Harvest	Average
Green Basil	Open-field	1.80	1.83	1.35	1.66	0.57	1.63	1.23	1.45 a
	Kiwi Plant.	1.43	1.73	0.62	1.32	0.57	1.04	0.47	0.80 bc
Purple Basil	Open-field	0.69	1.35	0.80	0.88	0.94	1.39	0.80	0.94 b
	Kiwi Plant.	0.95	1.54	0.78	1.09	0.61	1.01	0.65	0.76 c
LSD (%5)		NS	NS	NS	NS	NS	13.89	NS	0.16

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

Essential Oil Component (%)

The essential oil components of green and purple basils were chemically analyzed with the use of GC/MS method. Essential components in samples in second experimental years were identified (Table 8). Methyl cinnamate and linalool were major components in basil. Eguneol, arnesene % 1.8 cineole, citral azulene and limonen are the other components. Methyl cinnamate (8.2-19.5 %) and linalool (6.9-42.7 %) were in all harvests with respect to open-field conditions and kiwi plantations, respectively. The highest linalool percentage was determined in green basil plants grown in open-field conditions in second harvest and was followed by first and third harvest, respectively. The highest methyl cinnamate was ascertained in second and third harvest in purple basil plants growing under open-field conditions. Furthermore, eugenol (2.3-13.0 %), germacrene-D (1.5-15.9 %) and farnesene (0.2-21.1 %) were found to be high in green basil and the highest values were obtained from second harvest in open-field conditions. The percentage of 1.8 cineole in green basil plants growing under open-field conditions was 10.9 %. According to Gurbuz et al. (2006), linalool (41.2%) was the main compound, identified in the hydro-distilled *O. basilicum* essential oil from Turkey. Hussaina et al. (2007), the essential oils consisted of linalool as the most abundant component (56.7–60.6%), followed by epi-α-cadinol (8.6–11.4%), α-bergamotene (7.4–9.2%) and γ-cadinene (3.2–5.4%). Taghi Khani et al. (2013) identified 20 essential oil component and the most abundant one was linalool (% 34.95-42.58). Grayer et al., (1996) The major aroma constituents of basil were 3.7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-methoxy-4-(2-propenyl) benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g). Purkayastha and Nath (2006) reported the camphor, limonene and β-selinene were the major components in *O. basilicum* essential oils from northeast India. Methyl chavicol and citral percentage were 16.49-28.59 % and 23.30-40.69 %, respectively in the report proposed by Ekren et al. (2009). There were similar ranges with respect to the major compound and their percentage with the studies by Gurbuz et al. (2006) and (Hussaina et al. (2007). The observed differences in the constituents of basil essential oils across countries may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

A field experiment was conducted on purple and green basil (*Ocimum basilicum* L.) in Pazar/Rize in the years of 2010 and 2011 to evaluate the effects of growing conditions (in open-field conditions and between kiwi plantations on yield parameters and essential oils). The following results were obtained:

1. There were decline trends with respect to the plant height, fresh and dry herb yield in the second year.
2. For both experimental years, the highest fresh and dry herb yields were obtained in open-field conditions in green basil.
3. There were no statistically significant differences in relation to essential oil yield in open-field conditions and kiwi plantations but the highest essential content was ascertained in open-field conditions for green basil.
4. The major aroma constituents of basil were methyl cinnamate and linalool and the highest percentage was determined in open-field conditions for green basil.

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Table 6: Changes in essential oil components (%) in relation to the different harvest in 2011 in different growing conditions

Essential oil components	I. Harvest								II. Harvest								III. Harvest							
	Green Basil				Purple Basil				Green Basil				Purple Basil				Green Basil				Purple Basil			
	Trial Area				Trial Area				Trial Area				Trial Area				Trial Area				Trial Area			
	Open-field		Kiwi Plant.		Open-field		Kiwi Plant.		Open-field		Kiwi- Plant.		Open-field		Kiwi Plant.		Open-field		Kiwi Plant.		Open-field		Kiwi Plant.	
	*RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%	RT	%
Linalool	27.7	6.9	27.7	23.1	27.5	39.7	27.5	14.6	27.5	42.7	27.6	12.8	27.6	22.8	27.6	21.8	27.5	9.1	27.5	26.6	27.7	26.0	27.6	35.5
Methyl cinnamate	46.3	19.1	46.3	8.2	46.3	9.1	46.3	16.0	46.2	16.2	44.6	9.3	46.2	19.2	46.3	16.3	46.3	10.4	46.4	17.0	46.3	19.0	46.3	11.7
Eugenol	48.3	8.9	44.1	7.8	48.3	4.6	48.3	2.3	48.3	13.0	48.3	5.4	48.2	4.2	48.3	2.8	48.3	10.3	48.2	7.8	48.3	3.0	48.3	2.5
1.8-Cineole	15.1	0.2	15.0	1.2	15.2	3.6	15.1	2.1	15.1	1.9	15.0	0.7	15.2	10.9	15.1	7.1	15.1	1.1	15.2	3.1	15.1	5.4	15.1	10.5
Azulene	34.2	5.1	34.6	2.9	34.6	2.9	29.6	3.5	34.5	0.3	34.2	6.7	34.5	2.5	34.5	3.5	34.7	6.4	34.6	4.5	29.9	3.8	34.1	1.7
Limonene	14.6	1.1							14.7	0.4			14.7	0.7							14.7	0.4	14.7	0.7
β-Cubenene	32.9	1.7	29.3	1.1	34.4	3.3			32.8	1.0	32.8	1.7					32.3	1.7			29.6	0.4		
Trans-caryophyllene	29.9	2.7	29.7	1.4	29.8	2.8	29.3	2.3	29.9	1.2	29.8	1.4	29.9	2.2	29.8	3.0	29.8	1.8	29.3	2.3	29.9	2.6	29.8	2.6
Farnesene	29.2	16.1	29.3	11.7	32.2	3.92	29.1	2.2	29.2	21.1	29.3	14.7	29.1	2.5	32.0	1.0	29.2	10.2	29.3	8.3	31.0	0.8	29.1	0.2
Germacrene-D	32.8	1.6	34.4	1.5	38.4	13.5	34.4	6.5	32.8	1.6	34.4	1.5	34.4	15.9	34.5	5.8	34.4	1.3	34.0	3.8	34.5	4.5	34.1	3.7
ɣ-Cadinene	36.4	7.0	36.3	9.7	36.3	4.8	35.9	3.5	36.4	8.9	36.3	8.8	36.1	2.8	36.2	2.7	36.4	9.2	36.2	0.8	36.3	2.9	36.1	1.8
Camfor			26.9	3.5					26.7	1.0			26.9	1.2	26.7	0.9			26.9	0.9			26.9	2.0

*RT= Retention Time

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5. The lowest essential content obtained in open-field and between kiwi plantations for green and purple basil plants was even higher than the limit (%0.3) established by Turkish Standards Institution and total yield concerned with fresh and dry herb yield complied with the previously proposed literatures. Hence, basil cultivation underneath kiwi plantations may be implemented.

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