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CHANGES IN AGRICULTURAL AND TECHNOLOGIC CHARACTERISTICS OF SOME BASIL (*OCIMUM BASILICUM* L.) GENOTYPES AT DIFFERENT PLANT GROWTH STAGES AND HARVEST PERIODS

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Abstract

The purpose of this study was to assess the changes in yield, essential oil content, and composition of some basil genotypes during different growth stages. The research was conducted at Bursa Uludag University, Faculty of Agriculture, Department of Field Crops, in Turkey. Three basil genotypes (Largesweet, Midnight, and Malatya), three growth phases (the beginning of flowering, the 50% flowering, the end of flowering), and harvest numbers (1st harvest and 2nd harvest) were examined as variables in this study. A field trial was conducted using a three-level factorial randomized complete block design with three replications. For each growth stage, two harvests were made throughout the vegetation season. As a result of the study, the Malatya genotype showed to have greater values in terms of plant height, totally fresh, dry herb, and dry leaf yields (53.85 cm, 2224.03 kg da^{-1}, 334.75 kg da^{-1}, 157.01 kg da^{-1}, respectively) than other genotypes, whereas the Largesweet genotype had higher values in terms of essential oil content (0.53%). Higher values of agricultural characteristics were achieved in general at 50% flowering condition. The essential oil ratio was increased towards the end of flowering period. In all aspects, the 1st harvest outperformed the 2nd harvest. In terms of essential oil composition, Largesweet and Midnight cultivars were found to be of the linalool chemotype, while the Malatya genotype was of the linalool/methyl cinnamate Z chemotype.

Key words: Ontogenetic variability, Yield, Essential oil content, Essential oil composition.

Introduction

The Genus Ocimum, belonging to the Lamiaceae family, has 65 species, distributed throughout the world (Singh *et al*., 2004). The plant *Ocimum basilicum* L. is known as "basil." Annual and perennial species of the Ocimum genus show a natural distribution in Asia, Africa, and Central America (Simon *et al*., 1999). Basil (*Ocimum basilicum* L.) is an annual herbaceous plant that grows in hot and dry circumstances and is sensitive to cold (Ekmekci, 2013). Only cultural forms of basil are cultivated; none have spread naturally in Turkey.

Many basil varieties differ both morphologically (plant size, habitus, leaf shape, leaf, and inflorescence color) (Nurzyńska-Wierdak, 2007) and according to the chemical composition of the essential oil (Sifola & Barbieri, 2006; Dzida, 2010; Nurzyńska-Wierdak, 2013 a). Studies have shown that there are quite different chemotypes in basil in terms of essential oil composition within the *Ocimum basilicum L*., defining basil as four distinct groups, namely linalool, methyl cinnamate, methyl chavicol, and eugenol, and also numerous subtypes (Simon *et al*., 1999; Lee *et al*., 2005). The different chemotypes of *Ocimum* species, in recent years, are used in numerous fields such as food, spice, perfumery, cosmetics, aromatherapy, and medicine due to its antioxidant and antibacterial properties. Basil's aromatic components have insecticidal, antifungal, and antibacterial effects (Telci *et al*., 2006; Moghaddam, 2010). Among the *Ocimum* species, *O. basilicum L.* has the most economic importance (Dudai & Belanger, 2016) because its leaves are used to produce essential oil and are consumed as food. The essential oil content in the plant varies between 0.5-1.0% (Arabaci & Bayram, 2004; Baydar, 2016). The presence and composition of essential oils create the special aroma of plants. Although the compounds' composition changes with varieties, agricultural practices and environmental conditions affect it as well.

The purpose of producing medicinal and aromatic plants is to achieve both high yield and active substance content per unit area. To develop more suitable varieties for this purpose, it is necessary to determine appropriate climatic conditions and cultivation techniques for future breeding programs of basil. In agronomic practices, it is also important to define the appropriate harvest time concerning the development period of the plant. In this paper the changes in yield, essential oil ratio and composition of three basil genotypes, by harvest time in three plant growth stages were examined.

Material and Methods

Site description: The study was conducted in the experimental field of Bursa Uludag University, Faculty of Agriculture, Bursa (40º 11' N, 29º 04' E), in the South Marmara region of Turkey, in 2018.

Climate characteristics and soil properties: The climate of Bursa, where the field trial was conducted, shows a transitional nature between Mediterranean and Black Sea climates. Bursa's climate conditions during the long-term and experimental periods are presented in Table 1 (Anon., 2018). The soil texture of the dedicated experimental area (0-30 cm) was composed of clay and calcareous soil, unsalted (0.77 **dS/m**) and slightly alkaline (pH: 7.88), low in organic matter (1.2%), low in available phosphorus (4.98 kg da^{-1}), with exchangeable potassium (263.10 kg da^{-1}) and sufficient levels of iron, copper, and zinc.

Table 1. Climatic data recorded in Bursa Province.

Months	Long term period (1928-2008)			2018		
	Temp. (˚C)	Precipitation (mm)	Humidity (%)	Temp. (˚C)	Precipitation (mm)	Humidity (%)
May	17.4	43.4	64.2	19.9	89.8	76.5
June	22.4	33.6	58.7	23.5	59.2	70.1
July	24.6	18.9	57.5	26.1	9.6	63.5
August	24.3	13.8	60.2	26.4	1.8	59.6
Total	-	**109.7**	-	-	**160.4**	-
Mean	**22.2**	-	**60.2**	**24**	-	**67.4**

Temp: Temperature

Table 2. Morphological-characteristics of basil (*O. basilicum* L.) genotypes used in the study.

Genotype	Leaf color	Inflorescence color	Stem color	Leaf size	Leaf surface	Leaf edge
Largesweet	Green	White	Green	Med.-Large	Wavy	Few Toothed
Midnight	Dark Purple	Purplish-Pink	Purple	Medium	Slight wavy	Few Toothed
Malatya	Dark Purple	Purplish-Pink	Purple	Large	Wavy	Toothed

The plant growth stages examined in the study are defined as given below:

Table 3. Harvest dates in 2018.

Genotypes	Plant growth stages					
	I		II		III	
	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest
Largesweet	11 June	16 July	21 June	27 July	5 July	14 August
Midnight	16 June	03 August	25 June	10 August	9 July	31 August
Malatya	16 June	03 August	02 July	23 August	13 July	07 September

I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Plant material and growing process: Different Basil genotypes and plant growth phases (the beginning of flowering, the 50% flowering, the end of flowering) were evaluated as factors. Data on the characteristics of the genotypes used are given in Table 2. Commercial seeds of varieties (Largesweet and Midnight) were purchased from a private company (Vilmorin-Anadolu) in Turkey. Malatya population seeds were provided from Gazi Osmanpasa University, Agriculture Faculty, Field Crops Department.

The beginning of flowering: It is the period when plants begin to flower (10%), and budding is more intense (Fig. 1A, B, C). **The 50% Flowering:** 50% of the number of flowers on the plant is in bloom. Flowers and buds are observed together on the plant (Fig. 2A, B, C). **The End of Flowering:** Almost all the flowers on the plant are in bloom during this stage. It is the period when the flower petals on the plant begin to fall, and the lower flowers begin to set seeds (Fig. 3A, B, C).

Experimental design and treatment: The research was planned according to the Factorial Randomized Complete Block design and was conducted with three replications. Each plot (4,8 m²) consists of four rows. Basil seeds were sown in the pots with germination peat in the greenhouse on 16.03.2018 and the emergence was observed on 20.03.2018. Seedlings (approximately 3-4 cm) were transferred to viols on 11.04.2018. The seedlings were regularly watered throughout the growing process. When seedlings reached a height of about 10 cm, they were transferred to the field on 04.05.2018 at 40 cm × 30 cm distances. In the experiment, fertilizer was added in the amount of 5 kg N, and 5 kg P_2O_5 were added per decare.

Half of the nitrogen fertilizer (15-15-15) was applied during planting, and the other half (Ammonium Sulfate-21% N) was given after the 1st harvest. All the phosphorus (TSP-44% P_2O_5) was applied together with the planting seedlings. Plots were irrigated as needed during the vegetative period. Harvest was done manually by cutting the plants 10 cm above the soil surface. Basil genotypes were harvested twice during the growing season. There are generally 4-5 weeks between two harvest season and 10-15 days between plant growth periods (Table 3). Harvested plants were dried in the shade at room temperature, and leaf, inflorescence, and stem separation were done on dried plants.

Essential oil isolation and GC analysis: The content of the essential oil from 30 g of dry leaves was measured by the hydro distillation method using a modified Clevenger-type apparatus and the distillation duration was recorded as "two hours". The essential oil content was determined by the volumetric method (v w^{-1}) and expressed as a percentage (Wichtl, 1971). The essential oil was stored in dark glass bottles at 4ºC. The determination of essential oil components was identified by the Agilent 5975 C Capillary Column Gas Chromatography device in Atatürk Horticultural Central Research Ins. in Yalova. Working conditions of the device: Column used: HP-INNOWAX Capillary Column, Column length: 60 m, Oven Temperature (Programmed operation): 50°C, 50°C: 1 min., 50-175°C: 25° min^{-1}, 175°C-230°C: 4 min., 230°C: 6 min., Detector temperature: 280°C, Injector Temperature: 250°C, Carrier Gas: Helium, Flow Rate of Gas: 1,45 ml min^{-1}.

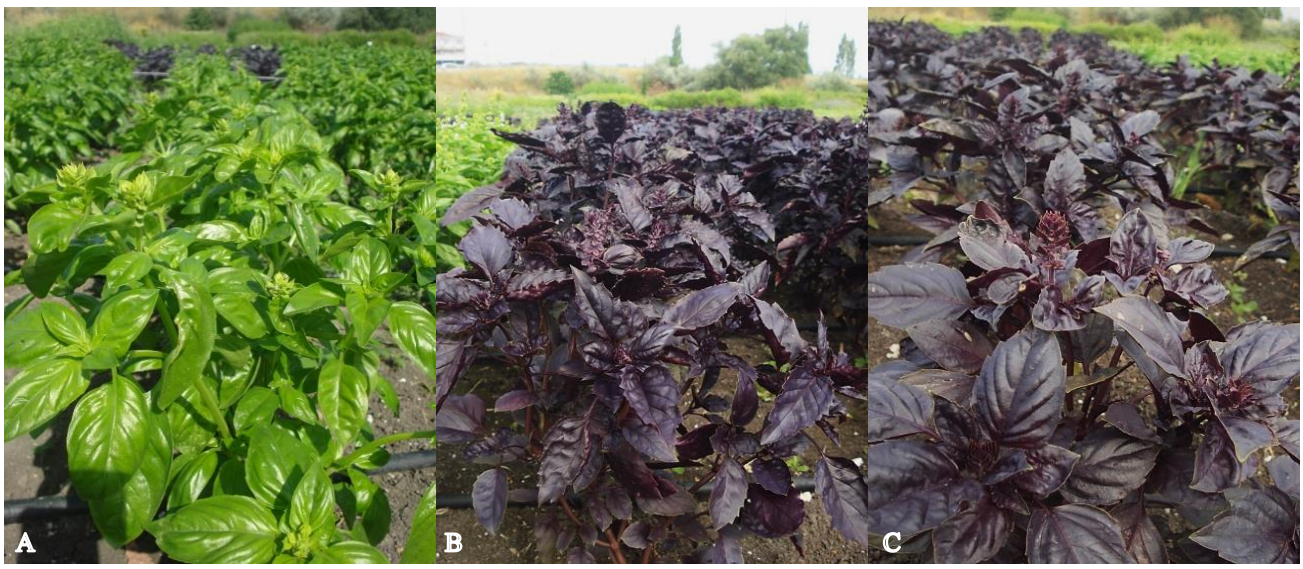


Fig. 1. The beginning of flowering (A. Largesweet, B. Midnight cultivars and C. Malatya population).

Fig. 2. The % 50 flowering (A. Largesweet, B. Midnight cultivars and C. Malatya population).

Fig. 3. The end of flowering (A. Largesweet, B. Midnight cultivars and C. Malatya population).

Statistical analyses

A field trial was arranged using a three-level factorial randomized complete block design with three replications. All data were subjected to an analysis of variance for each character using the JMP (version 7) package program. F test was applied to specify the 0.05 and 0.01 probability levels. The LSD test was used to determine the distinct groups.

Results and Discussion

Agricultural characteristics

Plant height: The differences in terms of genotype, growth stage, harvest, and genotype × growth stage × harvest interaction in basil were found to be statistically significant at the 1% probability level (Table 4). Plant height varied between 39.52 (Midnight)-53.85 cm (Malatya) according to the basil genotypes. In terms of genetic structure, Malatya stands taller than other genotypes. Considering the growth stage means, the end of flowering period had the highest plant height of 51.21 cm, and the lowest plant height value of 38.35 cm at the beginning of flowering. The plant height increased with the progression of vegetation and the elongation of flower spikes.

In studies which the field trials carried out with different basil genotypes and ecologies, plant height values were determined as 19.40-76.87 cm at the beginning of flowering and 17.16-95 cm at the flowering period (Kacar *et al*., 2009; Aslan, 2014; Karik *et al*., 2014; Ozcan, 2014; Ozkan, 2014; Naldan, 2017; Acikbas, 2018; Sonmez *et al*., 2019) and 49.29-78.64 cm at the end of flowering period (Aslan, 2014; Sonmez *et al*., 2019). In general, plant height values for each period in this study were within the range of the researchers' limit values. Plant height changes with growth, depending on genetic structure and the ecological parameters of the environment in which the plant grows. The discrepancies in the studies of the basil genotypes used are related to agronomic techniques, and ecological differences in the vegetation periods in the locations. In ecologies, day-night temperatures and day lengths affect the plant height and the development of basil (Putievsky, 1983).

When the means of harvest numbers were examined, the 1st harvest had a higher plant height of 46.65 cm than the 2nd harvest (45.34 cm). One explanation for/ Explanation of the higher plant height was that the total amount of precipitation (149 mm) received from the vegetative period until the 1st harvest (May-June) of the plants was more than 11.4 mm received during the 2nd harvest period (July-August) (Table 1).

In the 1st harvest (63.58 cm and 43.21 cm) generally taller plants heigher than the 2nd harvest (54.23 cm and 41.01 cm) were observed (Aslan 2014; Ozcan 2014). When the genotype × growth stage × harvest number interaction was examined, the highest values were recorded in Malatya (60 cm) and Largesweet (59.37 cm) genotypes at the end of flowering period, while the lowest values were recorded in the Midnight genotype (28.78 cm) at the beginning of flowering. The fact that the interaction was significant revealing that the effects of the growth stage and the number of harvests on plant height differed between genotypes. The results obtained in the interaction supported the findings of the main factors.

Fresh and dry herb yield: According to the results of fresh and dry herb yield in basil, the differences determined in the main factors (genotype, growth stage and harvest) and G x GS x H interactions at harvest numbers and G x H interactions at total values were found to be statistically significant at the 1% probability level (Tables 5, 6). The highest values in terms of fresh and dry herb yield were obtained from Malatya genotype in both harvest means (816.04-1112.02 kg da^{-1} and 89.30-167.38 kg da^{-1}, respectively) and total yields values (1406.61-2224.03 kg da^{-1} and 178.59-334.75 kg da^{-1}, respectively). This genotype was followed by Largesweet and Midnight cultivars, respectively. One of the explanations for the increased fresh herb yield is the Malatya genotype's higher plant height. In general, genotypes with high fresh herb yields have more biomass than short plant height genotypes due to their larger plant height and well-developed stem and broadleaf blades (Gunay & Telci, 2017).

Table 4. The effects of genotype, different plant growth stages and harvests on the mean of plant height (cm) of basil (*Ocimum basilicum* L.)

Genotype (G)	1st Harvest			2nd Harvest			Mean of genotype
	Plant growth stages						
	I	II	III	I	II	III	
Largesweet	34.97 ı	46.40 d	59.37 a	37.37 hı	42.80 f	46.77d	**44.61 B**
Midnight	28.78 j	43.38 ef	45.65 de	37.37 hı	42.50 f	39.43 gh	**39.52 C**
Malatya	41.61 fg	59.70 a	60.00 a	50.00 c	55.73 b	56.07 b	**53.85 A**
Mean of growth stage (GS)	**38.35 C**	**48.42 B**	**51.21 A**				
Mean of harvest (H)		**46.65 A**			**45.34 B**		
LSD (0.05)	G: 1.05. GS: 1.05. H: 0.86. G×GS×H: 2.58						
$p<0.05$	G: ** GS: ** H: ** G×GS×H: **						

There is no statistical difference between numbers containing the same letter or letters.
** Statistically significant at 1% probability level. I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Table 5. The effects of genotype, different plant growth stage and harvest on mean of fresh herb yield (kg da^{-1}) of basil (*Ocimum basilicum* L.).

Genotype (G)	1st Harvest			2nd Harvest			Mean of genotype	Total			Mean of genotype
	I	II	III	I	II	III		I	II	III	
Large sweet	486.72 h	1018.06 d	1428.32 a	435.13 h	759.13 ef	768.89 ef	**816.04 B**	921.85 e	1777.12 d	2197.22 b	**1632.06 B**
Midnight	485.12 h	1172.38 bc	576.92 gh	638.85 fg	779.89 ef	566.67 gh	**703.31 C**	1123.98 e	1952.27 cd	1143.59 e	**1406.61 C**
Malatya	801.14 e	1237.93 b	1005.34 d	1225.43 bc	1313.47 ab	1088.78 cd	**1112.02 A**	2026.58 bc	2551.40 a	2094.12 bc	**2224.03 A**
Mean of growth stage (GS)	**678.73 C**	**1046.81 A**	**905.82 B**				**Mean of GS**	**1357.47 C**	**2093.60 A**	**1811.64 B**	
Mean of Harvest (H)		**912.44 A**			**841.81 B**						
LSD (0.05)		G:60.16. GS:60.16. H:49.12. G×GS×H:147.37							G:131.63. GS:131.63. G×GS:227.99		
p<0,05		G: ** GS: ** H: ** G×GS×H: **							G: **, GS: **, G×GS: **		

There is no statistical difference between numbers containing the same letter or letters.
**Statistically significant at 1% probability level. I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering.

Table 6. The effects of genotype, different plant growth stage and harvest on mean of dry herb yield (kg da^{-1}) of basil (*Ocimum basilicum* L.).

Genotype (G)	1 stHarvest			2nd Harvest			Mean of genotype	Total			Mean of genotype
	I	II	III	I	II	III		I	II	III	
Large sweet	69.06ıjk	143.74 de	241.91 a	62.11 jk	105.07gh	114.01 fg	**122.65 B**	131.16 e	248.81 cd	355.92 ab	**245.30 B**
Midnight	56.87 k	133.88 ef	71.82 ıjk	87.50 hı	108.05 g	77.65 ıj	**89.30 C**	144.37 e	241.93 d	149.47 e	**178.59 C**
Malatya	98.51 gh	177.85 c	162.24 cd	180.16 c	207.28 b	178.22 c	**167.38 A**	278.67 c	385.13 a	340.45 b	**334.75 A**
Mean of growth stage (GS)	**92.37 B**	**145.98 A**	**140.98 A**				**Mean of GS**	**184.73 B**	**291.96 A**	**281.95 A**	
Mean of harvest (H)		**128.43**			**124.45**						
LSD (0,05)		G: 8.33. GS: 8.33. H:49.12. G×GS×H: 20.41							G:18.17. GS:18.17. G×GS:31.47		
p<0,05		G: ** GS: ** H: ** G×GS×H: **							G: **, GS: **, G×GS: **		

There is no statistical difference between numbers containing the same letter or letters. ** Statistically significant at 1% probability level. I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Table 7. The effects of genotype, different plant growth stage and harvest on mean of dry leaf yield (kg da^{-1}) of basil (*Ocimum basilicum* L.).

Genotype (G)	1 stHarvest			2 nd Harvest			Mean of genotype	Total			Mean of genotype
	I	II	III	I	II	III		I	II	III	
Large sweet	49.26 de	76.52 b	96.84 a	38.73 efg	57.50 cd	58.55 cd	**62.90 B**	80.99 e	134.02 d	155.39 bc	**125.80 B**
Midnight	41.35 ef	76.73 b	27.95 g	56.08 cd	66.52 bc	32.25 fg	**50.15 C**	97.43 e	143.27 cd	60.20 f	**100.30 C**
Malatya	65.33 bc	67.40 bc	49.80 de	104.01 a	108.34 a	76.15 b	**78.50 A**	169.34 ab	175.74 a	125.95 d	**157.01 A**
Mean of growth stage (GS)	**59.13 B**	**75.50 A**	**56.92 B**				**Mean of GS**	**118.25 B**	**151.01 A**	**113.84 B**	
Mean of harvest (H)		**61.24 B**			**66.46 A**						
LSD (0,05)		G: 4.99. GS: 4.99. H: 4.08. G×GS×B: 12.24							G:11.64. GS:11.64. G×GS:20.16		
p<0,05		G: ** GS: ** H: ** G×GS×H: *							G: **, GS: **, G×GS: **		

There is no statistical difference between numbers containing the same letter or letters.
** Statistically significant at 1% probability level. I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Total fresh herb yields were determined in the range of 921.85-2026.58 kg da^{-1} at the beginning of flowering, 1777.12-2551.40 kg da^{-1} at the 50% flowering, and 1143.59-2197.22 kg da^{-1} at the end of flowering period. While the lowest fresh herb yield values were observed at the beginning of flowering, the highest fresh herb yield was obtained at the 50% flowering. A similar situation was observed in the 1st and 2nd harvests. Fresh herb yield tended to decrease since the plant height was shorter at the beginning of flowering and the older leaves began to turn yellow and fell off at the end of flowering period. In general, increases and decreases in dry herb yield values of harvests and development periods showed parallelism with green herb yield values (Table 6). It was observed that the values of total dry herb yield varied between 184.73-281.95 kg da^{-1} according to the growth stages. The highest values were determined at the 50% flowering (241.93-385.13 kg da^{-1}) and the end of flowering (149.47-355.92 kg da^{-1}) periods, and the lowest values were determined at the beginning of flowering (131.16-278.67 kg da^{-1}).

Higher fresh herb yield was recorded in the 1st harvest at 912.44 kg da^{-1} compared to the 2nd harvest (841.81 kg da^{-1}). As a result of the onset of downy mildew damage caused by the fungus *Peronospora belbahrii* before the 2nd harvest, the lesions on the leaves negatively affected the photosynthesis mechanism and the development of the plants; thus, sufficient development could not be achieved. This situation caused a decrease in the fresh herb yields in the 2nd harvest. The susceptibility to the disease in genotypes differed. The Malatya and Largesweet genotypes, which were generally high in terms of plant height and had good mass development, were more resistant to the disease than the shorter Midnight genotype. In a study conducted by Gunay & Telci, (2017) in Isparta conditions, an analogous situation was experienced due to powdery mildew disease. When the average number of harvests was examined in terms of dry herb yield, no statistical difference was found between the 1st harvest (128.43 kg da^{-1}) and the 2nd harvest (124.45 kg da^{-1}), and similar values were obtained.

In studies conducted in different ecological conditions, similar results were obtained in terms of increases or decreases in fresh and dry herb yields determined according to harvest and development periods (Ersahin, 2006; Kacar *et al*., 2009; Aslan, 2014; Ozcan, 2014; Cabar, 2016; Naldan, 2017; Acikbas, 2018). The ecological conditions of the region including the length of the vegetation period, and the number of harvests affected the fresh herb yield. Generally, higher fresh herb yields were obtained in a warm climate and extended periods (Karik *et al*., 2014; Sonmez *et al*., 2009; Aslan, 2014). In the present study similar varieties were used in the same ecological condition, more than two harvests could not be obtained due to mildew; thus, there were differences in yield values. In general, the values in our study were found to be lower than the studies conducted in different ecologies. The factors affecting the fresh herb yield also affected the dry herb yield.

Dry leaf yield: The differences observed in terms of genotype, growth stage, and harvests in dry leaf yield in basil were found to be statistically significant at the 1% probability level while the differences determined in terms of genotype × growth stage × harvest number interaction were found to be statistically significant at the 5% probability level. The differences determined in the factors and interactions in total dry leaf yield values were found to be statistically significant at the 1% probability level (Table 7). Considering the genotypes in reliance on the main factors, it is seen that the highest dry leaf yield value was obtained in the Malatya genotype. On the other hand, the lowest dry leaf yield was obtained in the Midnight genotype in terms of both harvest periods' averages (50.15-78.50 kg da^{-1}) and total values (100.30-157.01 kg da^{-1}).

The total mass was measured as 80.99-169.34 kg da^{-1} at the beginning of flowering, 134.02-175.74 kg da^{-1} at the 50% flowering, and 60.20-155.39 kg da^{-1} at the end of flowering period. While the highest dry leaf yield values were observed at the 50% flowering period, the lowest values were determined at the beginning of flowering and the end of flowering periods. Inflorescences were not included in the dry leaf yield since our aim was to assess the changes in the leaves based on the growth stages. When the mean number of the harvests was examined, it was seen that the 2nd harvest reached a higher dry leaf yield with 66.46 kg da^{-1} compared to the 1st harvest (61.24 kg da^{-1}). Dry leaf yields are related to leaf ratios and herb yields. Hence, when these properties have a high value, dry leaf yields increased accordingly and this was caused by the difference in leaf ratios of genotypes. When the genotype × growth stage × harvest number interaction was examined, the dry leaf yield variation range was 27.95-108.34 kg da^{-1}. The highest dry leaf yield values were obtained at the 50% flowering and the beginning of flowering periods of the Malatya genotype in the 2nd harvest and at the end of flowering of the Largesweet variety in the 1st harvest. The lowest value was measured at the end of flowering period of the Midnight variety in the 1st harvest. Therefore, the investigated genotypes reacted differently to the changing climatic conditions during the vegetation periods. It is seen that the total dry leaf yield values had a varied range of 60.20-175.74 kg da^{-1} based on the genotype × growth period interaction. In parallel with the fresh and dry herb yield, the highest values were determined in the Malatya genotype at the 50% flowering period (175.74 kg da^{-1}). This value was followed by the beginning of flowering (169.34 kg da^{-1}) of the Malatya genotype, which was in the same statistical group. The lowest value was recorded at the post-flowering of the Midnight variety (60.20 kg da^{-1}). In previous works, it was shown that dry leaf yield was increased from the beginning of flowering to flowering and then tended to decrease (Ersahin, 2006; Aslan, 2014; Cabar, 2016; Naldan, 2017; Acikbas, 2018).

Technologic characteristics

Essential oil content: The differences in terms of genotype and plant growth in the essential oil content in dry leaves were found to be statistically significant at the 1% probability level, while harvest and genotype × growth stage × harvest number interaction were found to be

statistically insignificant (Table 8). When we consider the essential oil content in the leaf, it is seen that the values vary between 0.31 (Midnight)-0.53% (Largesweet) according to basil genotypes. Considering the plant growth stages, the essential oil contents were measured between 0.23-0.34% at the beginning of flowering, 0.25-0.53% at the 50% flowering, and 0.34-0.82% at the end of flowering period. At the end of flowering period highest essential oil content with a mean of 0.53% was recorded, while at the beginning of flowering period the lowest with 0.29% was found. As the growth stages progressed, an increase was observed in the essential oil content which was in parallel with the rise in the average temperature (Table 1).

Secondary metabolites and biosynthetic activities in medicinal and aromatic plants are genetically controlled (Padalia *et al*., 2017a). On the other hand, these are strongly affected by many factors such as climatic conditions, geographical location, agricultural conditions, applied agronomic processes, growth period, harvest time, methods and post-harvest processes, and chemical and biological differences arise depending on these factors (Telci, 2006; Msaada *et al*., 2007; Ebrahimi *et al*., 2008; Sellami *et al*., 2009; Nurzynska-Wierdak *et al*., 2012; Lee & Ding, 2016; Padalia *et al*., 2017a). Especially the plant growth stage is considered one of the most crucial factors affecting the amount and composition of essential oil (Sellami *et al*., 2009; Bagdonaite, *et al*., 2010; Saeb & Gholamrezaee, 2012; Verma, *et al*., 2012; Toncer *et al*., 2017).

According to the study by Toncer *et al*., (2017), it was noted that the essential oil ratio in dry herbs are increased with the progression of the growth stages, 1.2% at the pre-flowering, 1.4% at the flowering, and 1.6% at the post-flowering period. In a study, conducted in Brazil, the authors found that the highest essential oil ratio was obtained during the flowering period in the analyses made at different growth stages, including pre-flowering, flowering, and post-flowering periods. They stated that the flowering period is suitable for capturing the appropriate temperature, light intensity, and radiation rate for essential oil synthesis. They also emphasized that the presence of pollinating insects during the flowering period may have contributed to the synthesis of secondary metabolites (Paulus *et al*., 2019). The highest essential oil contents in both dry (Nurzyńska-Wierdak *et al*., 2012 Nurzyńska-Wierdak, 2013 b) and fresh herb (Verma *et al*., 2012) and dry leaves (Karik *et al*., 2014; Yasmin *et al*., 2018; Sonmez *et al*., 2019) were found during the flowering period in the research carried out in different development periods.

When the average number of harvests was examined, no statistical difference was observed between the first (0.42%) and second harvests (0.39%). In studies conducted in different ecologies, the 1st harvest had higher essential oil content than the 2nd (Aslan, 2014), and both harvests had similar rates of 0.65% and 0.69% (Ozcan, 2014). It was determined that the 2nd harvests were higher than the 1st harvests (Ozkan, 2014). In their study conducted with two green and purple genotypes, Yaldiz *et al*. (2015) reported that the essential oil ratio was increased from the 1st to the 2nd harvest in both genotypes and decreased in the third harvest. Climatic conditions such as temperature play a vital role in essential oil synthesis in the plants of Labiatae family. The fact that the average temperature was similar in both harvest periods reduced the difference between the determined essential oil content. When the genotype × growth stage × harvest number interaction was examined, the change range of the essential oil ratio was 0.23-0.82%. The identification of ontogenetic and diurnal variability in the culture of medicinal and aromatic plants and increasing the production of essential oils are important due to the fluctuations in climatic conditions, especially temperature and light, during the day (Brant *et al*., 2009).

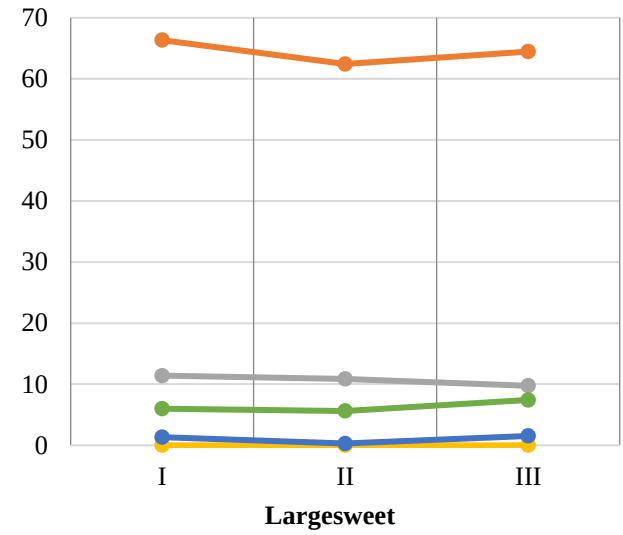
Essential oil components: Overall, more than 97% of the essential oil components were identified in all samples in both 1st and 2nd harvest results. Most of the members of the family Labiatae have a very strong aroma due to the rich essential oils of monoterpenes, sesquiterpenes and phenylproponoids. In the 1st and 2nd harvests, respectively Monoterpenoids (72.00-80.52 and 42.94-73.45 %), represented by linalool (upto 73.32 and 86.05%), sesquiterpenoids (2.82-18.48 and 2.69-21.58%), represented by *β*-Elemene (upto 13.99 and 15.32%), and phenylpropanoids (5.43-16.82 and 6.54-51.36 %), represented by (*Z*)-methyl cinnamate (upto 3.83 and 45.30%), constitute the main fraction of the composition (Tables 9, 10). The presence of essential oils with distinct compositions determines the specific aroma and flavour of the *Ocimum* taxa (Padalia *et al*., 2017b).

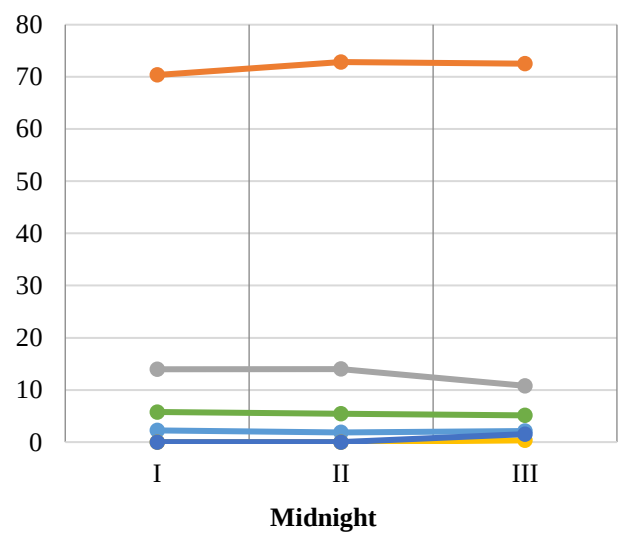
Table 8. The effects of genotype, different plant growth stage and harvests on mean of essential oil content (%) of basil (*Ocimum basilicum* L.).

Genotype (G)	1st Harvest			2nd Harvest			Mean of genotype
	Plant growth stages						
	I	II	III	I	II	III	
Largesweet	0.30	0.52	0.82	0.23	0.53	0.76	**0.53 A**
Midnight	0.29	0.25	0.43	0.28	0.29	0.34	**0.31 C**
Malatya	0.29	0.43	0.48	0.34	0.41	0.35	**0.38 B**
Mean of growth stage (GS)	**0.29 C**	**0,41 B**			**0.53 A**		
Mean of harvest (H)		**0.42**			**0.39**		
LSD (0.05)	G: 1.10. GP: 0.042						
$p<0.05$	G: ** GP: ** H: ns G×GP×H: ns						

There is no statistical difference between numbers containing the same letter or letters.

**: Statistically significant at 1% probability level. ns: non-significant, I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering





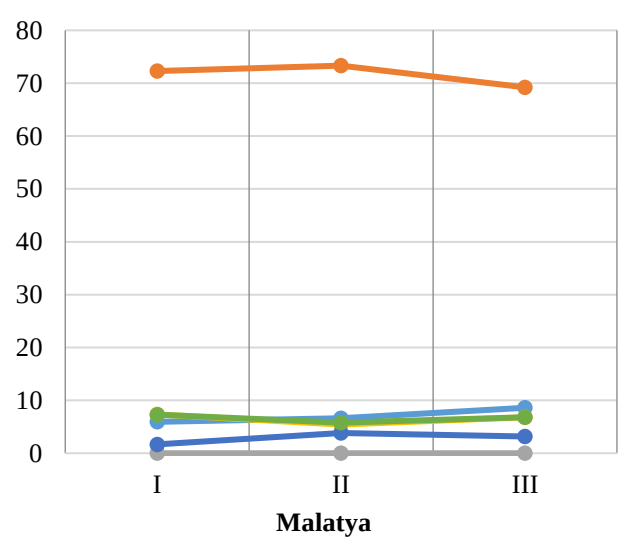
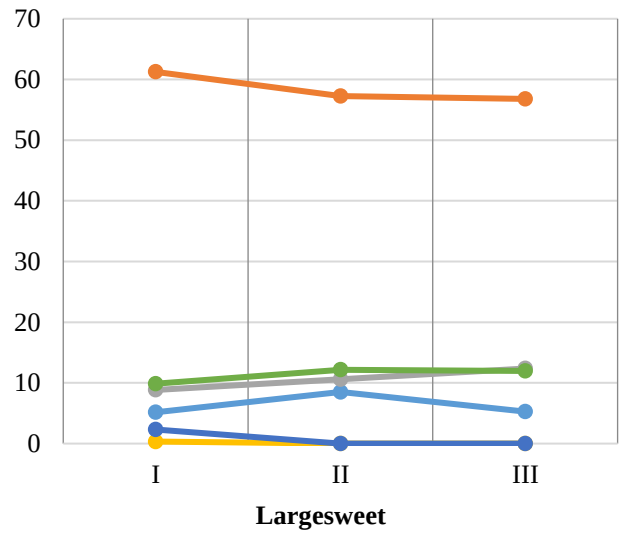
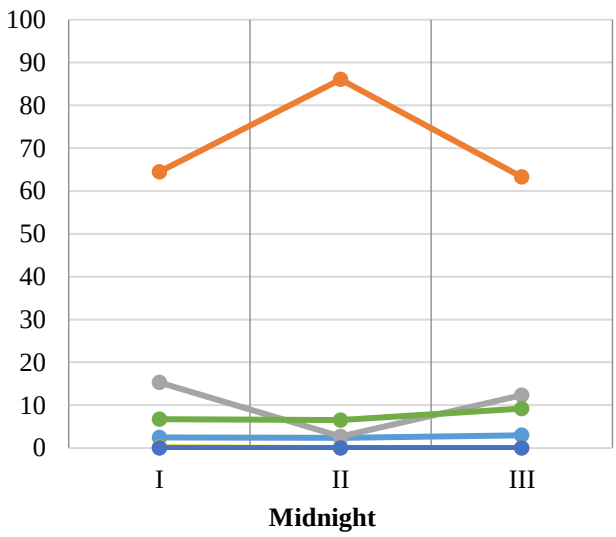


Fig. 4. Some important components in essential oils obtained from the 1st harvest of basil plant.





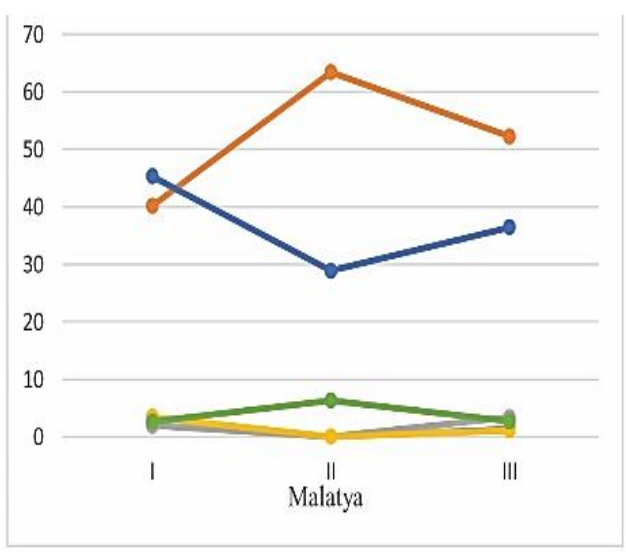


Fig. 5. Some important components in essential oils obtained from the 2nd harvest of basil plant.

I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Table 9. Essential oil components (%) in dry leaves at the 1st harvest.

No	RT	Compounds	Genotypes and plant growth stages								
			Largesweet			Midnight			Malatya		
			I	II	III	I	II	III	I	II	III
1.	14.176	1.8 Cineole	3.66	9.74	7.14	2.24	1.85	2.12	5.94	6,60	8,60
2.	16.091	*γ*-Terpinene	0.15	-	-	-	-	-	-	-	-
3.	17.310	*p*-Cymene	0.17	0.14	0.60	-	-	-	-	0,19	0,39
4.	28.050	Linalool	66.33	62.43	64.45	70.36	72.84	72.55	72.28	73,32	69,22
5.	29.278	cis-*α*-Bergamotene	0.98	0.97	1.10	-	-	-	-	-	-
6.	29.478	*β*-Elemene	11.41	10.84	9.74	13.96	13.99	10.79	-	-	-
7.	31.939	*α*-Humulene	0.53	0.55	0.43	0.36	-	0.33	-	-	-
8.	32.700	*δ*-Terpineol	1.69	1.70	1.25	2.57	1.68	1.60	0.93	-	-
9.	33.099	Germacrene D	0.50	-	-	-	-	-	-	1,74	0,97
10.	33.272	*δ*-Guaiene	1.38	1.19	1.05	0.53	-	1.01	1.11	0,41	0,53
11.	33.817	Bicyclogermacrene	-	0.69	0.60	-	-	0.46	-	0,40	0,42
12.	34.577	*γ*-Cadinene	2.45	2.35	2.15	1.80	1.71	1.43	2.45	0,83	0,90
13.	39.990	Methyl cinnamate E	-	-	-	-	-	0.34	7.35	5,42	6,83
14.	42.455	Cubenol	0.68	0.62	0.75	0.75	0.72	0.61	-	-	-
15.	43.110	Methyl cinnamate Z	1.32	0.30	1.53	-	-	1.53	1.68	3,83	3,20
16.	43.981	Spathulenol	0.23	0.21	0.25	-	-	0.35	-	-	-
17.	45.100	Eugenol	5.99	5.61	7.43	5.77	5.43	5.13	7.30	5,79	6,79
18.	46.073	Carvacrol	-	-	-	-	-	-	-	0,41	0,68
19.	46.478	*β*-Eudesmol	0.32	0.27	0.23	0.34	-	0.55	-	-	-
Class compositions											
Monoterpenoids			72.00	74.01	73.44	75.17	76.37	76.27	79.15	80.52	78.89
Sesquiterpenoids			18.48	17.69	16.30	17.74	16.42	15.53	3.56	3.38	2.82
Phenylpropanoids			7.31	5.91	8.96	5.77	5.43	7.00	16.33	15.04	16.82
Identified			**97.79**	**97.61**	**98.70**	**98.68**	**98.22**	**98.80**	**99.04**	**98.94**	**98.53**
Non-identified			**2.21**	**2.39**	**1.30**	**1.32**	**1.78**	**1.20**	**0.96**	**1.06**	**1.47**
Total			**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

Table 10. Essential oil components (%) in dry leaves at the 2nd harvest.

No	RT	Compounds	Genotypes and Plant Growth Stages								
			Largesweet			Midnight			Malatya		
			I	II	III	I	II	III	I	II	III
1.	14.176	1.8 Cineole	5.16	8.51	5.25	2.45	2.38	2.97	2.04		1.36
2.	16.091	*γ*-Terpinene	0.29	0.24	0.29	0.18	-	1.98	-	-	-
3.	17.310	*p*-Cymene	0.42	0.29	0.41	0.20	-	3.14	0.15	-	-
4.	28.050	Linalool	61.25	57.24	56.80	64.52	86.05	63.27	40.12	63.40	52.23
5.	29.278	cis-*α*-Bergamotene	1.05	0.87	0.95	0.24	-	-	-	-	-
6.	29.478	*β*-Elemene	8.84	10.62	12.37	15.32	2.69	12.33	1.91	-	3.41
7.	31.939	*α*-Humulene	0.54	0.56	0.59	0.43	-	1.48	0.16	-	-
8.	32.700	*δ*-Terpineol	1.76	1.84	1.87	1.86	2.34	2.09	0.63	1.42	0.97
9.	33.099	Germacrene D	0.46	0.55	0.51	-	-	-	-	-	-
10.	33.272	*δ*-Guaiene	1.27	1.46	1.62	1.11	-	-	1.02	-	0.79
11.	33.817	Bicyclogermacrene	0.78	0.71	0.99	-	-	-	-	-	-
12.	34.577	*γ*-Cadinene	1.92	2.31	2.89	2.22	-	2.15	1.08	-	1.05
13.	39.990	Methyl cinnamate E	0.34	-	-	0.17	-	-	3.44	-	1.14
14.	42.455	Cubenol	0.74	0.75	0.95	0.84	-	-	0.34	-	-
15.	43.110	Methyl cinnamate Z	2.32	-	-	-	-	-	45.30	28.89	36.41
16.	43.981	Spathulenol	0.25	0.18	0.26	0.40	-	-	-	-	-
17.	45.100	Eugenol	9.87	12.16	11.96	6.74	6.54	9.22	2.62	6.30	2.64
18.	46.073	Carvacrol	-	0.20	0.11	-	-	-	-	-	-
19.	46.478	*β*-Eudesmol	0.36	0.32	0.45	0.59	-	-	0.21	-	-
Class compositions											
Monoterpenoids			68.88	68.32	64.73	69.21	90.77	73.45	42.94	64.82	54.56
Sesquiterpenoids			16.21	18.33	21.58	21.15	2.69	15.96	4.72	-	5.25
Phenylpropanoids			12.53	12.16	11.96	6.91	6.54	9.22	51.36	35.18	40.19
Identified			**97.61**	**98.81**	**98.27**	**97.27**	**100**	**98.63**	**99.02**	**100**	**100**
Non-identified			**2.40**	**1.19**	**1.73**	**2.73**	**-**	**1.37**	**0.98**	**-**	**-**
Total			**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

I: The Beginning of Flowering, II: The 50% Flowering, III: The End of Flowering

More than one harvest can be made in a development period for basil and similar spices. In this case, the essential oil content and compositions may change depending on the harvesting season. In the 1st harvest, linalool stood out as the main component in all three genotypes. Linalool content varied between 62.43-64.45% in the Largesweet variety, 70.34-72.84% in the Midnight variety, and 69.22-73.32% in the Malatya variety at the growth stages. The main component, linalool, was followed by *β*-elemene, eugenol, and 1.8 cineole in cultivars. The amounts of these components were found in the ranges of 9.74-11.41%, 5.61-7.43%, and 3.66-9.74% in the Largesweet variety, and 10.79-13.99%, 5.13-5.77% and 1.85-2.24% in the Midnight variety, respectively. In the Malatya genotype, the following components were methyl cinnamate E (5.42-7.35%), eugenol (5.79-7.30%), 1.8 cineole (5.94-8.60%), and methyl cinnamate Z (1.68-3.83%). *β*-elemene, which was the highest in amount after the linalool, was not determined in the Malatya genotype in the 1st harvest. Also, methyl cinnamate E was not measured in the Largesweet and Midnight cultivars (Table 9, Fig. 4).

In the 2nd harvest, the main component was linalool, and it was in the range of 56.80-61.25% in the Largesweet variety, 63.27-86.05% in the Midnight variety, and 40.12-63.40% in the Malatya genotype according to the growth stages. In general, the amount of linalool was decreased in the 2nd harvest of all genotypes. However, only at the 50% flowering period of the Midnight genotype, the amount of linalool raised to the highest value of both harvests (86.05%) and tended to decrease again at the following period. It was determined that linalool in this cultivar was 33.37% and 36% higher, respectively, compared to other growth stages (Table 10, Fig. 5).

It has been reported in numerous studies that plants increase secondary metabolite production depending on density in stressful growth environments (Bernstein *et al*., 2010; Bekhradi *et al*., 2015; Kaya & Inan, 2017). At the 2nd harvest period, mildew damage, which increased in intensity towards the flowering period, was more prevalent than other genotypes in this cultivar, and accordingly, the reaction of the cultivar might have changed in various secondary metabolites. This situation changed the balance of linalool and *β*-elemene in particular. As the amount of linalool was increased, its ratio to *β*-elemene decreased. The amount of *β*-elemene, which was 15.32% and 12.33% at the beginning and during post-flowering, decreased to 2.69% during the 50% flowering.

The *β*-elemene component, which was not found in Malatya genotype in the 1st harvest, was detected at the pre-flowering (1.91%) and at the post-flowering periods (3.41%) in the 2nd harvest, but not found at the 50% flowering period. In the Malatya genotype, methyl cinnamate Z was the component with the highest ratio following the linalool component in the 2nd harvest and its ratio changed between 28.89-45.30%. As the linalool ratio was decreased in this genotype, the methyl cinnamate Z ratio increased. Other components following the main component in the Largesweet variety were eugenol (9.87-12.16%), *β*-elemene (8.84-12.37%), and 1.8 cineole (5.16-8.51%). Most especially, as the linalool ratio was decreased and the amounts of eugenol and *β*-elemene were increased. In terms of these values, great differences between the growth periods were not observed. Methyl cinnamate Z was the other key component following the main component linalool in the Malatya genotype. As the linalool ratio decreased, the amount of this component increased. In the Malatya genotype, methyl cinnamate Z was 45.30% higher than the linalool (40.12%) during the pre-flowering period. In other periods, this component was decreased due to the increase in linalool (Table 10, Fig. 5).

In our study, the linalool contents of the genotypes were found close to each other in the 1st harvest compared to the growth stages, while in the 2nd harvest, it was found to be 50% higher during the flowering period. The important biochemical changes that occur during plant growth stages ensures that the harvest times and post-harvest processes are adjusted to obtain quality products. The seasonal variation of linalool was not high in most of the samples except for the 2nd harvest of Midnight. This situation showed that the compound was not affected much by the climate changes during the harvest periods, and the genetic control of linalool synthesis was greater (Telci *et al*., 2015). In a study Toncer *et al*. (2017) demonstrated that the main components of the essential oil were linalool at the flowering and the post-flowering periods, and eugenol at the pre-flowering period. It was noted that the linalool was increased proportionally from the pre-flowering period to the flowering period. In a study conducted by Padalia *et al*., (2017b) it was reported that the content of linalool was higher at the 50% of the flowering stage (43.8%) compared to seed setting (42.8%), vegetative stages (42.2%), and full flowering stage (39.1%). In the same study, the content of (E)-methyl cinnamate was maximal at the full flowering stage (42.8%) followed by 40.4% at the vegetative stage, 39.8% at the 50% flowering stage, and a minimal 36.6% at the seed setting stage. On the other hand, the content of (Z)-methyl cinnamate was found to be maximal at the full flowering stage (5.9%) and minimal at the vegetative stage (5.4%) (Padalia *et al*., 2017b).

Based on essential oil composition of *O. basilicum*, four major chemotypes (methyl chavicol, linalool, methyl eugenol, and methyl cinnamate type) and numerous subtypes with mixed proportion of these constituents were identified in *O. basilicum* (Lawrence, 1988). However, a wide variety of chemotypes, the main components are camphor, citral, ocimene, methyl cinnamate, methyl eugenol, *trans-β*-ocimene, *β*-caryophyllene, and *β*-bisabolene, have been identified in germplasms around the world (Baydar, 2016). In this study conducted with domestic and foreign basil genotypes in Turkey, according to essential oil components, twelve chemotypes were identified; these ones are Linalool, Linalool/Methyl chavicol, Linalool/Methyl eugenol, Linalool/Eucalyptol, Linalool/Eugenol, Methyl chavicol, Methyl eugenol, Methyl cinnamate/Linalool, Citral, Guaiene, Bisabolene/Estragole and Caryophyllene (Telci *et al*., 2015). In the present study, it was determined that Largesweet and Midnight cultivars were of the linalool chemotype, while the Malatya genotype was of the linalool/methyl cinnamate Z chemotype. Especially in the Malatya genotype, the increase in methyl cinnamate Z ratio in the 2nd harvest was remarkable. Linalool is a more common compound as the main component in basil samples. Besides, most of the basil population and genotypes are classified as linalool-rich chemotypes (Telci *et al*., 2006). Linalool is a strong aromatic compound in the monoterpene group, while methyl cinnamate is a strong aromatic compound in the phenylpropanoid group, which is the methyl ester of cinnamic acid. Linalool, methyl cinnamate and their mixture are preferable for cultivation in pharmaceutical, cosmetic and food industries (Letizia *et al*.,

2003; Telci, *et al*., 2006; Bhatia *et al*., 2007) and some authors have reported their repellent, insecticidal and larvicidal activities (Peterson *et al*., 2000; Nour *et al*., 2009; Dekker *et al*., 2011). Linalool and Methyl cinnamate is an important ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Linalool's worldwide use is in the region of >1000 metric tons and Methyl cinnamate's worldwide use is in the region of 10–100 metric tonnes per annum (Letizia *et al*., 2003; Bhatia *et al*., 2007). Effective substances in medicinal and aromatic plants vary according to the genetic structure of the plant, the ecological conditions in which it grows, and agronomic processes (Sangwan *et al*., 2001; Telci *et al*., 2010; Telci *et al*., 2011). Differences determined in essential oil components have been shaped by different growth periods and changing climatic conditions, especially by the genotypic structure of the plants.

Conclusion

As a result of this study, it has been demonstrated that basil cultivation can be performed successfully in Bursa. When the genotypes of the factors discussed in the study were evaluated, it was found that the Malatya genotype stood out in terms of agronomic characteristics, followed by the Largesweet and Midnight varieties. It was also noted that the 50% flowering period was in the 1st place in terms of agricultural characteristics. In terms of the essential oil ratio, higher values were obtained at the end of flowering period. It was concluded that it would be appropriate to harvest at the 50% flowering period for a higher dry leaf yield, and towards the end of flowering for a higher essential oil ratio. In general, the 1st harvest achieved higher data than the 2nd harvest. In terms of essential oil composition, it was determined that Largesweet and Midnight cultivars were of the linalool chemotype, while the Malatya genotype was of the linalool/methyl cinnamate Z chemotype. In addition, knowing the important biochemical changes that occur during plant development periods ensures that the harvest times and post-harvest processes are adjusted to obtain quality products. This knowledge of genotype in terms of agricultural and technological characteristics can be exploited in the planning and execution of future breeding programs in basil.

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