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DIURNAL VARIATION OF ESSENTIAL OIL RATIO AND COMPOSITION OF SOME BASIL GENOTYPES

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Abstract

The change of essential oil ratios and components of five different basil genotypes harvested at different times (00:00, 06:00, 12:00, 18:00) in day was investigated. Plants were grown under the field conditions and essential oils of the plants were extracted by hydro-distillation method. Identification of essential oil components were performed by GC-MS. It was the G5 genotype that had the highest essential oil content in all plant parts, regardless of the time of day. The highest essential oil content (2.8%) was obtained from the dry leaves of the G5 genotype from the sampling made at 18:00. The main components of essential oils were determined as linalool, eucalyptol, trans α-bergamotene, methyl cinnamate, α-muurolol and eugenol. However, while the ratios of these components differed between genotypes, the changes in time of day were similar for some components. Linalool content tended to increase mostly in the middle of the day. This study indicated that essential oil ratio and components of different basil genotypes significantly differed according to harvesting times in day.

Introduction

Ocimum tenuiflorum L., *O. gratissimum* L., *O. canum* Sims, *O. basilicum* L., *O. kilimandscharicum* Güerke, *O. americanum* L., and *O. micranthum* Willd. are some of the important species of the *Ocimum* genus which grow in different parts of the world (Paton 1992) and is known for its various medicinal properties and source of flavouring ingredient and fragrance (Prakash and Gupta 2005). Lesser known ones may contain a wide variety of flavours such as lemon, rose, camphor, liquorice, woody and fruit (Simon *et al*. 1999).

O. basillicum belonging to Lamiaceae is generally used in dry and fresh form in the food, flavouring, perfume and medicine sectors (Telci *et al*. 2006). Its leaves and flowering tops are known as degassing and antispasmodic in folk medicine (Sajjadi 2006). It has the most common spice sources containing antimicrobial and antioxidant properties Candela *et al*. 2019, Goudjil *et al*. 2020, Tohidi *et al*. 2020). In addition, this observation has been supported by various studies (Wannissorn *et al*. 2005, Lee *et al*. 2005, Politeo *et al*. 2007).

The major common ingredients are monoterpenes and phenylpropanoids, but their essential oils may vary (Padalia *et al*. 2013). The chemical compositions of plants can be different depending on plant origin, environmental conditions (Atis *et al*. 2012a, Yilmaz *et al*. 2021, Ertekin *et al*. 2022, Ertekin, 2022), harvesting stages (Atis *et al*. 2012b) and storage methods especially for essential oils, as well as the developmental (ontogenetic) stage (Chang *et al*. 2009). The change in the content of secondary metabolites and their ratio within the plant may vary not only according to these factors but also according to the time of day (Gurbuz *et al*. 2006). In particular, this change can affect the proportion of a specific ingredient. In addition to the fact that genotypic differences are enormously effective in the ratio of active ingredients, especially the changes of

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different genotypes according to the time of the day have become a subject that needs to be examined. The present study was aimed to investigate the change of essential oil ratios and components of five different basil genotypes harvested at different times in day.

Materials and Methods

In present study, five different green basil genotypes showing morphological differences were used as plant material. Experiment was carried out according to Randomized Complete Block design with 3 replications in Hatay Mustafa Kemal University Research and Application Field in Türkiye. The plant material used for the production of essential oil was obtained by sampling from field trial parcels where 8.3 plants/ m^2 were set up. Physical weed control was applied throughout the growing period.

Fresh and dry leaves and flowers were used to obtain essential oil. A total of 50 g of each of the ground plant samples was used for the separate hydro-distillation experiment. Sample was weighed before being placed in 1 liter flask. Deionised water was added to cover the sample fully. Hydro-distillation method, which was carried out in all-glass Clevenger type distillation, was used to obtain the essential oil. The essential oils were dried over anhydrous sodium sulphate and stored in dark vial bottles at 4°C until analysis (Caliskan *et al*. 2019, Mohan *et al*. 2020).

Gas chromatographic method was used to determine the components of essential oils of plants. Determination of essential oil components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatographic device under the following conditions. The 5 % Phenyl Polysilphenylene-siloxane with TR-FAME MS model which is 0.25 mm inner diameter x 60 m length and 0.25 µm film thickness column was used. Helium (99.9 %) was used as the carrier gas at a flow rate of 1 ml/ min. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50°C and the temperature was increased to 220°C with a rate of heat increase of 3°C/min.

The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9) (Maral *et al*. 2018). The individual compositions were determined by comparing their retention index and with Wiley Library (Wiley Interscience, New York). The relative quantities of individual compounds were calculated with the Xcalibur Report program. The compounds were identified from the GC-MS spectra by comparison of their retention indices (RI) with homologous series of n-alkanes. Retention indices were determined using retention times of n-alkanes (C8-C40) injected under the same chromatographic conditions, co-injection with standards compared with those data from Wiley 9. RI's were compared with the reported values. Identification of each compound was made by comparison of their retention times with those of authentic samples and by computer searching and matching with mass spectral data held in computer libraries (Loying *et al*. 2019, Manabi *et al*. 2020).

Results and Discussion

When the parts of the plant from which essential oil was obtained were examined independently of the harvest time, it was observed that the essential oil ratios of the genotypes are different from each other (Table 1). The highest essential oil ratios for each plant part belonged to the G5 genotype (Fig. 1).

When the average of essential oil ratios of all genotypes was examined according to the harvest hours, no difference was observed between the hours (Table 2).

The diurnal variability of the essential oil ratio obtained from different plant parts belonging to five different genotypes is shown in Fig. 1. The highest essential oil content (2.8%) was obtained from the dry leaves of the G5 genotype from the sampling made at 18:00.

Table 1. Essential oil ratios of genotypes as daily average.

Genotype	Fresh leaf	Fresh flower	Dry leaf	Dry flower
G1	0.23±0.02bc	0.48±0.12bc	1.45±0.09c	1.42±0.31b
G2	0.19±0.05bc	0.35±0.03c	1.31±0.04c	0.99±0.12b
G3	0.14±0.02c	0.63±0.06ab	0.79±0.06d	1.58±0.08ab
G4	0.27±0.03b	0.51±0.05bc	1.87±0.04b	1.54±0.23b
G5	0.51±0.03a	0.80±0.09a	2.53±0.10a	2.19±0.10a

abcd Mean values with different letter in the same column indicate a significant difference ($P < 0.05$).

Table 2. Essential oil ratios at harvest time as average of genotypes.

Harvest hour	Fresh leaf	Fresh flower	Dry leaf	Dry flower
0	0.26±0.05	0.60±0.10	1.58±0.26	1.44±0.25
6	0.26±0.08	0.55±0.09	1.57±0.31	1.66±0.25
12	0.26±0.07	0.56±0.08	1.59±0.26	1.57±0.29
18	0.28±0.08	0.50±0.13	1.62±0.35	1.51±0.21

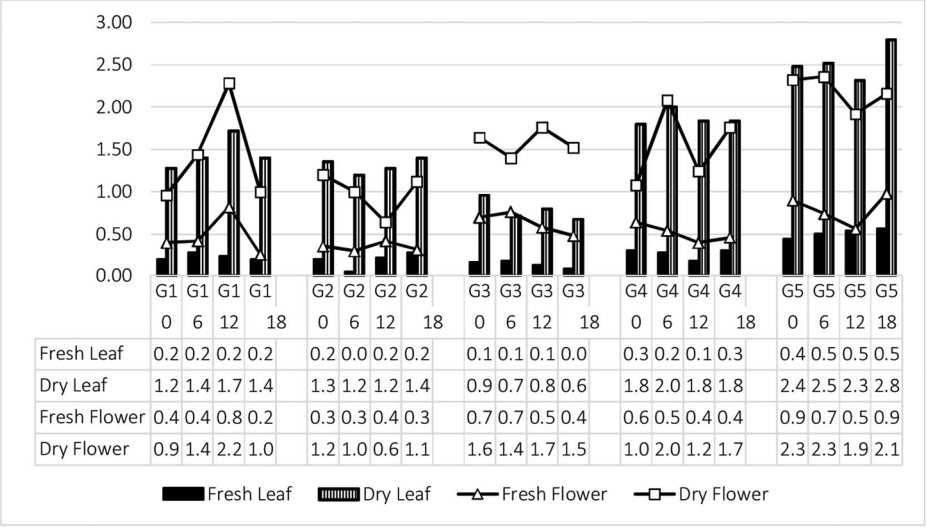

Fig. 1. Diurnal variability of essential oil ratios of different plant parts of each genotype.

In the hourly harvest of the G1 genotype, the main components of the essential oils obtained from the fresh leaves were determined as linalool and eugenol (Fig. 2). The rate of linalool at 06:00 was 45.43%, at 12:00 this rate increased and was determined as 51.73%. The lowest linalool rate was found at 18:00 with 38.56%. The highest eugenol ratio was detected at 00:00 with 40.33%, followed by 37.34% at 18:00 and 33.69% at 06:00, respectively. The lowest eugenol rate was 30.93% at 12:00.

When the efficiency of the hourly harvest on the dry leaves of this genotype was examined, it was observed that the linalool ratio increased compared to the wet leaves. The highest linalool ratio was determined at 12:00 (63.79%), 00:00 (61.27%), 06:00 (56.34%) and 18:00 (53.24%) harvest hrs, respectively. When the components in dry leaves were examined, it was determined that the eugenol ratios decreased compared to the wet leaves, while the eucalyptol ratios increased.

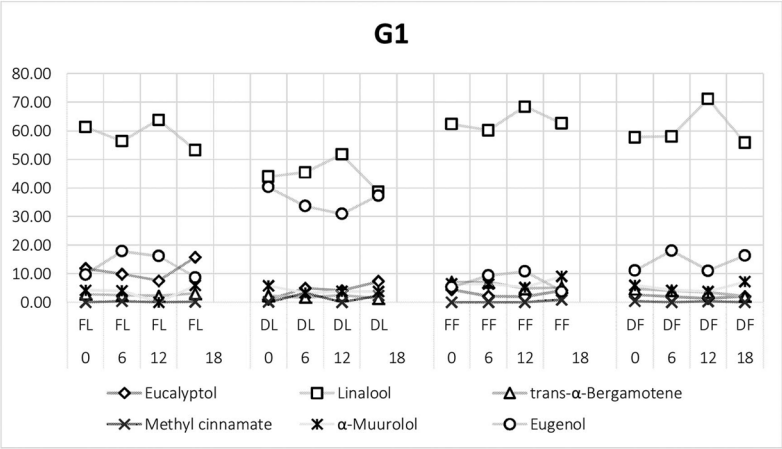

Fig. 2. Diurnal variability of essential oil components of the G1 genotype.

In the components of essential oils obtained from the flowers of the G1 genotype, the ratio of linalool was found to be higher in both fresh and dry flowers than that in the leaves. The highest linalool ratio with 71.11% was observed in the fresh flowers harvested at 12:00, and the lowest linalool ratio with 55.89% was observed in the fresh flowers harvested at 00:00. Eugenol ratios in flowers were found to be less than those in leaf essential oils. However, there was an increase in trans-α- bergamotene and α-muurolol ratios.

In the G2 genotype, the linalool ratio varied between 71.24 and 40.08%, with the effect of hourly harvests. The linalool ratio was higher in the flowers, but unlike the G1 genotype, the highest linalool ratio was detected at 12:00 harvest of the dry leaves of the G2 genotype with 71.24%. The highest rate of linalool (53.68%) in fresh leaves was at 12:00, followed by 00:00 (50.40%). The lowest linalool rate (40.08%) was at 06:00. Another major component was identified as eugenol (Fig. 3). The recorded eugenol rates were 26.58% at 06:00, 27.15% at 12:00, 33.75% at 16:00 and 27.34% at 00:00, respectively. The rate of α- muurolol was determined at the highest level with 10.25% at 06:00 and the lowest ratio with 4.53% at 18:00.

The highest linalool rate (71.24%) in dry leaves was at 12:00, while the lowest linalool rate (55.67%) was recorded at 06:00. Eugenol ratio was determined as 15.13% at 06:00, 4.94% at 12:00, 8.74% at 16:00 and 12.16% at 00:00, respectively. α muurolol ratios varied between 4.15% and 7.5%. Eucalyptol ratios increased in dry leaves compared to fresh leaves.

When the linalool ratios of the fresh flowers of the G2 genotypes were examined, the highest linalool ratio was 68.85% at 12:00, while the lowest linalool ratio was 59.01% at 06:00. Eugenol was 12.19% at 06:00, 11.92% at 12:00, 11.84% at 18:00 and 13.35% at 00:00. α- muurolol ratios varied between 5.58% and 5.89%.

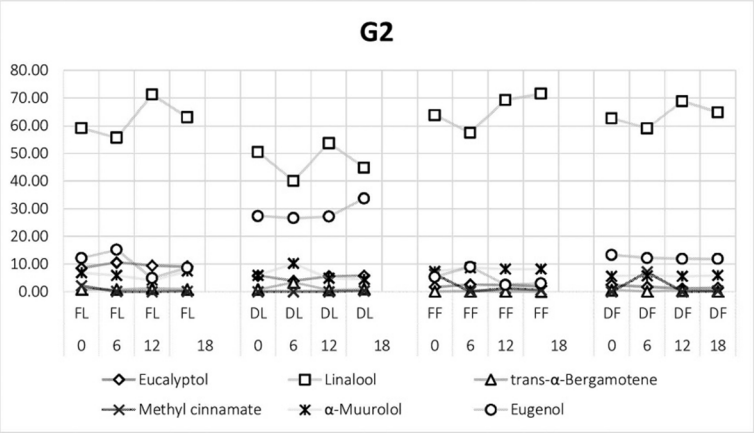

Fig. 3. Diurnal variability of essential oil components of the G2 genotype.

While the highest linalool was 71.58% at 18:00 in essential oil components obtained from dry flowers, the lowest linalool ratio was determined at 06:00 with 57.45%. The highest eugenol ratio was determined at 06:00 with 8.98%. It was determined that this was followed by 00:00 with 5.39%, 18:00 with 3.04% and 12:00 with 2.50%, respectively. α- muurolol ratios increased in dried flowers, while Eucalyptol ratios decreased in essential oils of flowers compared to leaves.

While the rate of linalool in fresh leaves of the G3 genotype was 20.80% at 06:00, this rate was determined as 39.67% at 12:00. In the harvesting time at the 18:00 and 00:00 hrs, linalool ratios were recorded as 29.64 and 22.49%, respectively. In the G3 genotype, the main component was anethole at 06:00 (30.99%) and 00:00 (49.54%) harvests of fresh leaves (Fig. 4). However, while no anethole was found in the harvest of this genotype at 12:00, a very low rate (0.04%) of anethole was found at 18:00. The highest eugenol ratio was 38.12% at 18:00, and the lowest ratio was at 00:00 with 6.84% in fresh leaves. The linalool ratio in dry leaves was the highest at 61.32% at 12:00 and the lowest at 06:00 with 36.43%. Anethole rates were again high at 00:00 (31.46%) and 06:00 (30.47%). Anethole rates were low (0.62% vs. 0.68%) at 12:00 and 18:00. Eugenol ratio varied between 4.37% and 7.11%.

The linalool content of essential oils obtained from the flowers of the G3 genotype according to hourly harvests was higher than that of the leaves. The linalool ratios at 12:00 were higher in each part of the plants compared to those atother harvest hours. The lowest linalool ratio in fresh leaves was 48.23% at 00:00. It was determined that the anethole ratios in flowers were lower than those in the leaves. While this rate of fresh flowers was 19.77% at 00:00, it was determined as 7.79% at 06:00. In dry flowers, the anethole ratio was 21.43% at 00:00 and 19.99% at 06:00. Anethole ratio was not found in the components of both fresh and dry flowers at the other two harvest times (12:00 and 18:00). The eugenol ratios of fresh flowers were recorded as 12.76% at 06:00, 11.16% at 12:00, 13.60% at 18:00 and 3.97% at 00:00, respectively. In dry flowers, while eugenol decreased compared to fresh flowers, the ratio of α-muurolol increased in dry flowers.

When the variation of the components of essential oils obtained from the fresh leaves of the G4 genotype according to the harvest hours was examined, the main components were methyl cinnamate with 62.02% and linalool with 24.24% (Fig. 5). In other harvest hours, methyl cinnamate ratio decreased. These rates were 43.27% at 12:00, 15.52% at 18:00 and 34.79% at 00:00, respectively. There was an increase in the linalool ratio compared to the methyl cinnamate ratio. Linalool rates were 31.70% at 12:00, 34.36% at 18:00 and 34.59% at 00:00. In addition, while the anethole rate was 1.0% at 06:00 in the G4 genotype, these rates were determined as 8.98, 25.45 and 12.32% at 12:00, 18:00 and 00:00, respectively.

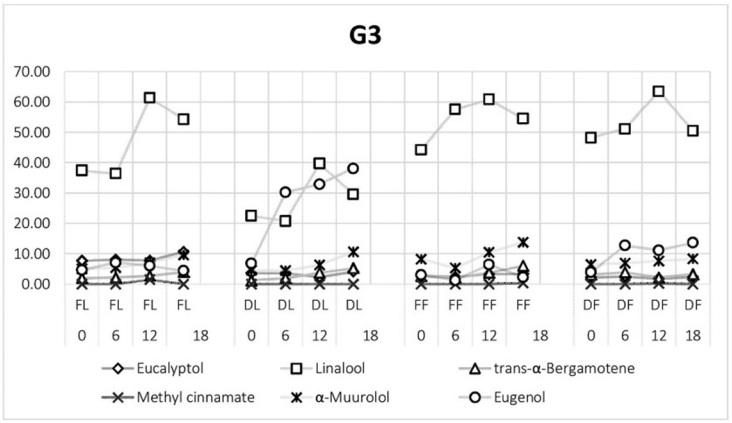

Fig. 4. Diurnal variability of essential oil components of the G3 genotype.

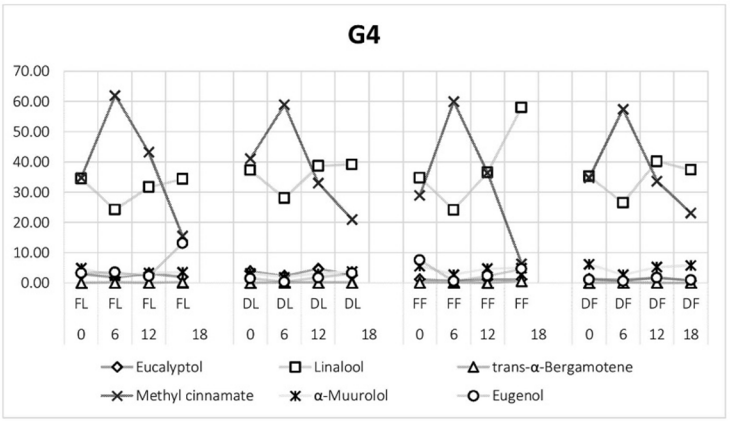

Fig. 5. Diurnal variability of essential oil components of the G4 genotype.

In the components of essential oils obtained from dry leaves, there was a decrease in methyl cinnamate ratios and an increase in linalool ratios compared to fresh leaves. At 06:00, the main ingredients were 58.94% methyl cinnamate and 28.08% linalool. At 12:00, 18:00 and 00:00, methyl cinnamate rates were 33.06, 21.06 and 41.18%, respectively. While the linalool rate was 38.71% at 12:00, it was 39.27% at 18:00. At 00:00, the rate of linalool was 37.32%. The anethole rates, which were 2.73% at 06:00, increased at 12:00 and 18:00 and were recorded as 10.74 and 22.49%, respectively. At 00:00, it decreased again and became 5.47%.

Considering the methyl cinnamate ratios in the fresh flowers of the G4 genotype, at 06:00, it was 60.01%. It was observed that the rates of this component were the lowest at 18:00 with 6.36%. At 18:00, the rate of linalool was the highest with 58.13%. The lowest linalool rate was found at 06:00 with 24.22%. As a result of the decrease in methyl cinnamate ratio, linalool increased. Anethole ratio was less than that in leaves.

The methyl cinnamate ratios in the components of essential oils obtained from dry flowers were 57.48% at 06:00, 33.68% at 12:00, 23.11% at 18:00 and 34.77% at 00:00. Linalool rate was the highest at 12:00 with 40.28%, while the lowest rate was at 06:00 with 26.52%.

In the essential oil components of the fresh leaves of the G5 genotype, the main component was eugenol with 51.79% at 06:00, 49.16% at 12:00, 59.11% at 18:00, 49.87% at 00:00. The highest linalool rate was 25.57% at 12:00, and the lowest was 21.21% at 18:00. Eucalyptol ratio varied between 8.07 and 10.34% (Fig. 6).

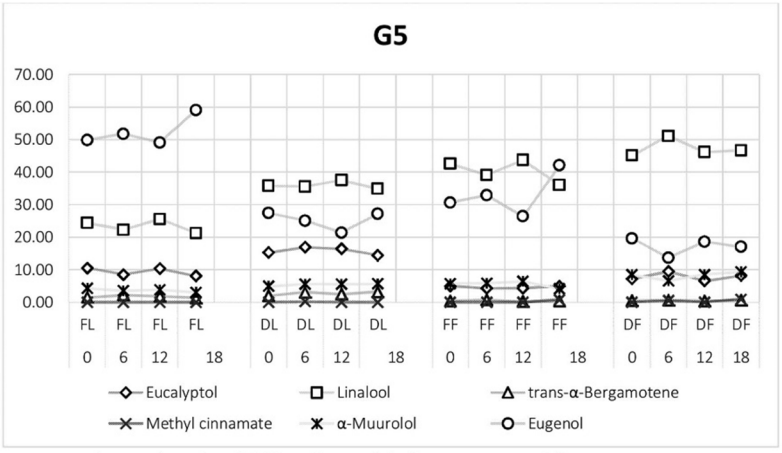

Fig. 6. Diurnal variability of essential oil components of the G5 genotype.

In the components of dry leaves, the ratio of linalool was higher than the ratio of eugenol of wet leaves. Linalool rates from sunrise to night were 35.59, 37.53, 34.86 and 35.80%, respectively. The highest eugenol ratio was 27.41% at 00:00, while the lowest was 21.42% at 12:00. Eucalyptol ratio in dry leaves was higher than that of in fresh leaves. The highest rate was at 06:00 with 16.95%, and the lowest rate was at 18:00 with 14.48%.

The main components of fresh and dried flowers were linalool and eugenol. Linalool ratios in fresh flowers were 39.15% at 06:00, 43.77% at 12:00, 36.02% at 18:00 and 42.59% at 00:00. The highest eugenol rates were found at 18:00 with 42.10%, and the lowest at 12:00 with 26.44%. Eucalyptol ratios decreased compared to leaves.

The highest linalool rate of dry flowers was 51.17% at 06:00, and the lowest rate was 45.14% at 00:00. Eugenol rates were 13.67% at 06:00, 18.63% at 12:00, 17.05% at 18:00 and 19.67% at 00:00.

Harvest hours had no effect on essential oil ratios in any genotype. Yet, the changes among these ratios in terms of plant parts were significantly different. The main components, linalool and eugenol, differed in each genotype according to plant parts and different times of the day. Also, in another study, essential oil ratios were not affected by harvest hours, but the changes in their contents were more pronounced and it has been reported that the essential oil ratio and content of basil leaves change between young and mature leaves (Chang *et al*. 2009). However, the diurnal

variations of essential oil ratios were significantly different in the *O. gratissimum* L. species (Kpadonou-Kpoviessi 2012). Similarly, the change in the essential oil components of this species was remarkable. The decrease of 1,8-Cineol in contrast to the increase in eugenol at noon (de Vasconcelos *et al*. 1999) indicated a similar content change in some genotypes used in the present study. The G1 genotype was consistent with the study reporting that basil essential oil content was higher at noon (Padalia *et al.* 2017), but essential oil content in dried flowers was higher in the morning. The essential oil ratios in fresh and dry leaves and flowers of all genotypes differed from each other according to the time of day. The variation of basil essential oil components according to whether the flowers and leaves are dry or fresh was observed quite clearly in G1, G2, G3 and especially G5 genotypes. Daily changes in essential oil ratio and content may differ not only between species belonging to *Ocimum* genus, but also chemo-types belonging to *O. basilicum* species (Padalia *et al.* 2017). Similarly, present study promoted that daily changes of essential oil ratio and component of *O. basilicum* plant is significant. This result indicated that harvesting time of this plant is important in a day.

The essential oil ratios in fresh leaves and flowers are lower than those in dry ones. Therefore, the necessity of using dried plants for high essential oil ratio has shown itself again. However, in this case, the change in essential oil components should be considered. The G5 genotype had the highest essential oil ratios per hour and plant part, with a few exceptions (Table 1).

While the effect of diurnal variability on essential oil ratios did not appear, it caused very significant differences on essential oil components. While the main component of the G1, G2 and G3 genotypes was linalool, eugenol accumulation was observed in the dried leaves. The increase and decrease of linalool and methyl cinnamate in all plant parts in the G4 genotype were sharply evident according to the time of day. In the G5 genotype, the similar variation was more pronounced among plant parts, not among hours. G5 genotype can be considered as a breeding material with its high essential oil content and essential oil components that vary widely according to plant parts and time of day. G1, G2 and G3 genotypes should be evaluated due to their stability and their potential to be brought into the industry should be considered.

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