

## EFFECTS OF HARVESTING HOUR ON ESSENTIAL OIL CONTENT AND COMPOSITION OF *THYMUS VULGARIS*

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### Abstract

Diurnal variations in both fresh and dry leaf essential oils of *Thymus vulgaris* were evaluated in this study. *Thymus vulgaris* was harvested hourly starting at 6:00 and ending at 17:00. Fresh and dry leaf essential oil were extracted by hydrodistillation for 3 hours and analyzed by GC-MS. The results indicated that essential oil content significantly changed according to the hour of the day. The maximum oil yield was obtained by harvesting at the early hours of the day, which was found superior to oil yield corresponding to the late hours of the day. Thymol,  $\gamma$ -terpinen, p-cymene and carvacrol were the dominant essential oil components of both fresh and dry leaves of *T. vulgaris*. Except thymol, slight variations in essential oil composition of fresh and dry leaf of *T. vulgaris* were detected among harvesting hours. The results showed that the best harvesting hours of *T. vulgaris* for higher thymol concentrations were between 6:00 and 11:00.

### Rezumat

În acest studiu a fost evaluată variabilitatea diurnă asupra uleiurilor esențiale din *Thymus vulgaris* atât proaspăt cât și uscat. *Thymus vulgaris* a fost recoltat la fiecare oră începând de la 6:00 și până la 17:00. Uleiurile esențiale obținute din plantele proaspete și uscate au fost extrase prin hidrodistilare timp de 3 ore și analizate prin GC-MS. Rezultatele au indicat că, o schimbare semnificativă a conținutului de ulei volatil are loc în funcție de ora la care a fost recoltată planta. Randamentul maxim de ulei a fost obținut prin recoltarea la primele ore ale zilei, randamentul fiind superior randamentului corespunzător orelor târzii ale zilei. Timolul,  $\gamma$ -terpinena, p-cimenul și carvacrolul au fost componentele predominante în uleiurile esențiale obținute din plantele proaspete și uscate de *T. Vulgaris*. Exceptând timolul, orele de recoltare au influențat foarte puțin compoziția uleiurilor esențiale din plante de *T. vulgaris* proaspete și uscate. Rezultatele au arătat că cel mai bun interval orar de recoltare a *T. vulgaris* pentru a obține un randament bun de timol a fost între orele 6:00 și 11:00.

**Keywords:** *Thymus vulgaris*, essential oil, diurnal variability, GC-MS

## Introduction

*Thymus vulgaris*, a pleasant smelling perennial medicinal and aromatic herbaceous plant belonging to the *Lamiaceae* family, widely used in the Western Mediterranean countries [12, 22, 34].

As a mixture of monoterpenes, major essential oil components of *T. vulgaris* are thymol, p-cymene,  $\gamma$ -terpinene and carvacrol [3, 15, 18, 32, 34]. In addition to monoterpenes, terpenoids, flavonoid aglycones, flavonoids glycosides, and phenolic acids were also found in *Thymus* spp. [33]. *T. vulgaris* exhibits multiple biological activities including anti-inflammatory [7], immunomodulatory [2], antispasmodic [5], antioxidant [1], antibacterial [13, 19], antifungal [3], antiviral [8], antihelminthic [27], carminative [11], and free radical scavenging properties [14]. Traditionally, the leaves of *T. vulgaris* and its essential oil have been used in food industry for the flavour, aroma and preservation. Currently, an increasing interest has been expressed for *T. vulgaris* due to its relatively broad biological activities gradually discovered. The recent researches in medicine and pharmacy have focused their aim in developing new drugs and biomaterials based on essential oils or plant extracts, this field being a new future direction for treating different pathologies [2, 9, 10, 22, 24, 25, 30, 31].

The biosynthesis of the secondary metabolites, although controlled genetically, is affected strongly by temperature, humidity of the air surrounding, wind velocity, intensity of light, and photoperiod. Seasonal variation of *T. vulgaris* essential oil has been widely studied. However, diurnal variation of *T. vulgaris* essential oil has not been extensively studied yet. The purpose of the present study was to determine diurnal essential oil variation and to determine the best harvesting hour of *T. vulgaris*.

## Materials and Methods

Two years old *T. vulgaris* plants, grown in the research area of Mustafa Kemal University (Antakya, Hatay, Turkey, 36°19'29"N, 36°11'44"E), were harvested at one-hour intervals starting from 6:00 to 17:00 in 16, 17 and 18 June 2012. Harvested plant samples were divided in two groups. Group one was freshly studied immediately after harvesting and group two was dried in the shade at 26 °C for one week to determine diurnal essential oil content and component variations of fresh

and dry leaf of *T. vulgaris*. In each group, approximately 100 g fresh material was sampled for analysis.

The fresh and dried samples were subjected to steam distillation for 3 h using a Clevenger type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at  $-20^{\circ}\text{C}$  until gas chromatography-mass spectrometry (GC-MS) analysis. The oil percentage was expressed as w/v with respect to the dry matter of the initial material.

#### *GC-MS analysis*

Analysis of the essential oil was carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 60 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70eV. Mass range  $m/z$  50-650 amu. Data acquisition was scan mode. MS transfer line temperature was  $250^{\circ}\text{C}$ , MS Ionization source temperature was  $220^{\circ}\text{C}$ , the injection port temperature was  $220^{\circ}\text{C}$ . The samples were injected with 250 split ratio. The injection volume was 1  $\mu\text{L}$ . Oven temperature was programmed to  $50^{\circ}\text{C}$  to  $220^{\circ}\text{C}$  at  $3^{\circ}\text{C}/\text{min}$ . The structure of each compound was identified by comparison of their mass spectrum (Wiley) using the Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of *n*-alkane standard solutions C8-C20 (Fluka, product no. 04070) and C21-C40 (Fluka, product no. 04071).

#### *Statistical Analysis*

All experiments were conducted in a completely randomized design with three replicates. The statistical analysis of experimental data was determined using a general linear model procedure of SAS for Windows (Version 8.02, SAS Institute, Cary, NC, USA), applying the one way analysis of variance (ANOVA). Mean separation was calculated according to Tukey's test ( $P \leq 0.05$ ).

### **Results and Discussion**

Essential oil content of fresh leaf significantly varied among the harvesting hours ( $F=11.83$ ,  $p<0.01$ ) (Table I).

**Table I**  
Diurnal essential oil content variations of fresh and dry leaf of *T. vulgaris*

| Harvesting hour | Temperature (°C) | Humidity (%) | Essential oil content (%)* |              |
|-----------------|------------------|--------------|----------------------------|--------------|
|                 |                  |              | Fresh leaf                 | Dry leaf     |
| 6:00 AM         | 20.5             | 50           | 0.90±0.02 ab               | 2.20±0.02 a  |
| 7:00 AM         | 21.5             | 50           | 0.82±0.02 abc              | 2.18±0.02 a  |
| 8:00 AM         | 22.5             | 56           | 0.85±0.02 abc              | 2.05±0.02 ab |
| 9:00 AM         | 24.5             | 55           | 0.70±0.01 cd               | 1.70±0.02 cd |
| 10:00 AM        | 29.8             | 48           | 0.70±0.01 cd               | 1.90±0.02 bc |
| 11:00 AM        | 29.9             | 43           | 0.75±0.02 bcd              | 1.95±0.02 b  |
| 12:00PM         | 33.0             | 26           | 0.95±0.02 a                | 2.10±0.03 ab |
| 13:00 PM        | 32.9             | 29           | 0.70±0.01 cd               | 1.90±0.02 bc |
| 14:00 PM        | 30.1             | 28           | 0.85±0.02 abc              | 2.00±0.03 ab |
| 15:00 PM        | 34.3             | 32           | 0.65±0.01 d                | 1.70±0.02 cd |
| 16:00 PM        | 31.4             | 37           | 0.90±0.02 ab               | 2.05±0.03 ab |
| 17:00 PM        | 25.4             | 43           | 0.60±0.02 d                | 1.65±0.01 d  |
| Tukey's (0.05)  |                  |              | 0.168                      | 0.202        |

Means in each column followed by the same letter do not differ significantly using the Tukey test ( $P < 0.05$ )

\*The values ( $\pm$ SEM) are expressed as means of three different determinations.

Essential oil content of fresh *T. vulgaris*, harvested hourly, varied between 0.60 and 0.95%. The lowest fresh leaf essential oil content was obtained from the 17:00 harvest with 0.60%, while the highest was obtained from the 12:00 harvest with 0.95%. When dry leaf essential oil contents of *T. vulgaris* were considered, essential oil contents significantly varied among harvesting hours ( $F=22.20$ ,  $p < 0.01$ ). The highest essential oil content was obtained from the 6:00 harvest with 2.20%, while the lowest was obtained from the 17:00 harvest with 1.65 % followed by 10:00 and 15:00 harvesting hours. Rumińska *et al.* [28] reported that, essential oil content of *T. vulgaris* was between 0.7 and 3.5%. Essential oil content in our study were within the limits of Letchamo *et al.* [18], Shalaby and Razin [29], Jackson and Hay [16], Asllani and Toska [4], Hudaib *et al.* [15], and Badi *et al.* [6].

Clearly seen from the Table I, both fresh and dry leaf essential oil contents of *T. vulgaris* were significantly affected from the harvesting hours. The lowest essential oil contents were obtained during the hottest noon hours of the day (Table I). Essential oil content variation at diurnally harvested coriander (*Coriandrum sativum*) was recorded by Ramezani *et al.* [10, 26].

The relative amounts of each component were determined by the GC-MS analysis (Table II and III).

Table II. Diurnal essential oil component variations of fresh *T. vulgaris* leaf

| Components             | RT    | RI   | FRESH HERBA      |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
|------------------------|-------|------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                        |       |      | 6:00             | 7:00             | 8:00             | 9:00             | 10:00            | 11:00            | 12:00            | 1:00             | 2:00             | 3:00             | 4:00             | 5:00             |
|                        |       |      | AM               | AM               | AM               | AM               | AM               | AM               | PM               | PM               | PM               | PM               | PM               | PM               |
|                        |       |      | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              | (%)              |
| $\alpha$ -Pinene       | 3.63  | 1027 | 0.85             | 0.72             | 0.74             | 0.73             | 0.90             | 0.80             | 0.62             | 0.63             | 0.65             | 0.62             | 0.68             | 0.69             |
| $\alpha$ -Thujene      | 3.69  | 1031 | 1.17             | 0.91             | 0.96             | 1.04             | 1.34             | 1.20             | 0.92             | 0.85             | 0.88             | 0.91             | 0.97             | 0.77             |
| Camphene               | 4.34  | 1072 | 0.68             | 0.71             | 0.65             | 0.61             | 0.67             | 0.56             | 0.42             | 0.54             | 0.51             | 0.55             | 0.46             | 0.78             |
| $\beta$ -Pinene        | 5.13  | 1111 | 0.25             | 0.23             | 0.23             | 0.23             | 0.25             | 0.25             | 0.20             | 0.19             | 0.18             | 0.20             | 0.22             | 0.19             |
| Sabinene               | 5.43  | 1126 | 0.09             | 0.07             | 0.09             | 0.08             | 0.12             | 0.12             | 0.08             | 0.04             | 0.14             | 0.22             | 0.50             | 0.05             |
| $\beta$ -Myrcene       | 6.48  | 1169 | 1.43             | 1.24             | 1.31             | 1.34             | 1.52             | 1.32             | 1.06             | 1.14             | 1.08             | 1.03             | 1.22             | 1.09             |
| $\delta$ -4-Carene     | 6.88  | 1183 | 1.05             | 0.89             | 1.06             | 1.02             | 1.20             | 1.06             | 0.86             | 0.93             | 0.91             | 1.33             | 1.20             | 0.93             |
| Bornylene              | 7.40  | 1200 | 0.41             | 0.39             | 0.41             | 0.40             | 0.41             | 0.37             | 0.29             | 0.33             | 0.32             | 0.39             | 0.37             | 0.32             |
| Eucalyptol             | 7.59  | 1208 | 0.72             | 0.88             | 0.78             | 0.58             | 0.83             | 1.08             | 0.90             | 0.69             | 0.57             | 0.57             | 0.72             | 0.63             |
| $\beta$ -Phellandrene  | 7.67  | 1211 | 0.07             | 0.63             | 0.11             | 0.58             | 0.14             | 0.87             | 0.67             | 0.10             | 0.11             | 0.19             | 0.16             | 0.11             |
| $\gamma$ -Terpinene    | 8.82  | 1250 | 10.51 $\pm$ 0.06 | 8.94 $\pm$ 0.05  | 11.17 $\pm$ 0.08 | 9.25 $\pm$ 0.01  | 11.72 $\pm$ 0.05 | 9.84 $\pm$ 0.04  | 7.91 $\pm$ 0.05  | 8.88 $\pm$ 0.04  | 7.38 $\pm$ 0.03  | 8.03 $\pm$ 0.03  | 8.85 $\pm$ 0.03  | 8.71 $\pm$ 0.03  |
| p-Cymene               | 9.65  | 1275 | 21.80 $\pm$ 0.10 | 20.90 $\pm$ 0.09 | 21.20 $\pm$ 0.12 | 19.57 $\pm$ 0.08 | 21.37 $\pm$ 0.10 | 17.29 $\pm$ 0.06 | 15.19 $\pm$ 0.07 | 14.36 $\pm$ 0.05 | 15.37 $\pm$ 0.05 | 15.13 $\pm$ 0.08 | 14.70 $\pm$ 0.06 | 14.74 $\pm$ 0.05 |
| $\alpha$ -Terpinolene  | 10.02 | 1285 | 0.04             | 0.04             | 0.05             | 0.07             | 0.07             | 0.07             | 0.03             | 0.07             | 0.10             | 0.26             | 0.13             | 0.08             |
| l-Octen-3-ol           | 16.13 | 1453 | 0.51             | 0.67             | 0.45             | 0.71             | 0.45             | 0.45             | 0.42             | 0.46             | 0.42             | 0.36             | 0.44             | 0.42             |
| Cis-Sabinene hydrate   | 16.54 | 1464 | 1.04             | 1.03             | 0.94             | 0.96             | 1.03             | 1.01             | 0.87             | 0.99             | 0.88             | 0.90             | 0.86             | 0.80             |
| Camphor                | 18.31 | 1508 | 0.42             | 0.37             | 0.36             | 0.32             | 0.44             | 0.40             | 0.40             | 0.23             | 0.34             | 0.59             | 0.39             | 0.71             |
| Trans-Sabinene hydrate | 19.69 | 1546 | 0.31             | 0.30             | 0.31             | 0.34             | 0.31             | 0.63             | 0.89             | 0.75             | 1.30             | 2.24             | 1.79             | 0.61             |
| Linalool               | 19.87 | 1550 | 2.12             | 2.06             | 1.92             | 1.65             | 1.70             | 1.69             | 1.30             | 1.35             | 1.44             | 1.30             | 1.12             | 1.19             |
| Linalyl acetate        | 20.24 | 1560 | 0.12             | 0.23             | 0.19             | 0.08             | 0.06             | 0.11             | 0.17             | 0.12             | 0.22             | 0.31             | 0.21             | 0.13             |
| Endobornyl acetate     | 20.85 | 1575 | 0.24             | 0.23             | 0.21             | 0.17             | 0.21             | 0.17             | 0.12             | 0.20             | 0.17             | 0.23             | 0.15             | 0.20             |
| Caryophyllene          | 21.35 | 1588 | 1.32             | 1.45             | 1.55             | 2.02             | 1.40             | 1.78             | 1.98             | 2.59             | 2.41             | 3.21             | 4.04             | 2.47             |
| Thymyl methyl ether    | 21.62 | 1594 | 0.77             | 0.11             | 0.63             | 1.24             | 0.20             | 0.04             | 0.25             | 0.16             | 0.22             | 1.21             | 0.25             | 0.62             |
| Terpinene-4-ol         | 21.74 | 1597 | 0.50             | 0.54             | 0.68             | 0.63             | 0.54             | 1.13             | 1.44             | 1.16             | 1.81             | 3.11             | 1.75             | 1.06             |
| Carvacrol methyl ether | 22.00 | 1604 | 0.15             | 0.32             | 0.23             | 0.51             | 0.21             | 0.10             | 0.18             | 0.24             | 0.17             | 0.21             | 0.25             | 0.36             |
| $\beta$ -Selinene      | 24.02 | 1660 | 0.05             | 0.60             | 0.09             | 0.11             | 0.08             | 0.09             | 0.18             | 0.16             | 0.15             | 0.16             | 0.23             | 0.20             |
| Borneol                | 25.33 | 1694 | 1.51             | 2.16             | 1.79             | 1.70             | 1.42             | 1.30             | 1.00             | 1.85             | 1.50             | 1.91             | 1.36             | 1.96             |
| Farnesol               | 26.34 | 1722 | 0.04             | 0.05             | 0.05             | 0.05             | 0.07             | 0.46             | 0.15             | 0.32             | 0.31             | 1.16             | 0.75             | 0.52             |
| Caryophyllene oxide    | 34.79 | 1966 | 0.74             | 0.68             | 0.76             | 0.73             | 0.62             | 0.47             | 0.58             | 0.63             | 0.33             | 0.68             | 0.50             | 0.60             |
| Thymol                 | 41.73 | 2216 | 44.95 $\pm$ 0.14 | 46.71 $\pm$ 0.20 | 44.12 $\pm$ 0.18 | 44.26 $\pm$ 0.20 | 43.72 $\pm$ 0.01 | 45.95 $\pm$ 0.25 | 41.02 $\pm$ 0.10 | 40.14 $\pm$ 0.25 | 34.21 $\pm$ 0.10 | 26.81 $\pm$ 0.08 | 30.42 $\pm$ 0.12 | 27.51 $\pm$ 0.08 |
| Carvacrol              | 42.54 | 2237 | 3.52 $\pm$ 0.01  | 4.56 $\pm$ 0.03  | 4.08 $\pm$ 0.02  | 5.52 $\pm$ 0.03  | 4.84 $\pm$ 0.03  | 4.90 $\pm$ 0.02  | 8.58 $\pm$ 0.06  | 14.01 $\pm$ 0.05 | 17.08 $\pm$ 0.06 | 23.15 $\pm$ 0.07 | 22.80 $\pm$ 0.06 | 29.49 $\pm$ 0.07 |

Table III. Diurnal essential oil component variations of dry *T. vulgaris* leaf

| Components             | RT    | RI   | DRY HERBA  |            |            |            |             |             |             |            |            |            |            |            |
|------------------------|-------|------|------------|------------|------------|------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|
|                        |       |      | 6:00<br>AM | 7:00<br>AM | 8:00<br>AM | 9:00<br>AM | 10:00<br>AM | 11:00<br>AM | 12:00<br>PM | 1:00<br>PM | 2:00<br>PM | 3:00<br>PM | 4:00<br>PM | 5:00<br>PM |
| $\alpha$ -Pinene       | 3.63  | 1027 | (%)        | (%)        | (%)        | (%)        | (%)         | (%)         | (%)         | (%)        | (%)        | (%)        | (%)        | (%)        |
| $\alpha$ -Thujene      | 3.69  | 1031 | 0.54       | 0.78       | 0.70       | 0.66       | 0.14        | 0.46        | 0.46        | 0.62       | 0.65       | 0.82       | 0.67       | 0.59       |
| Camphene               | 4.34  | 1072 | 0.97       | 1.02       | 0.93       | 0.83       | 0.18        | 0.99        | 0.71        | 0.94       | 0.96       | 1.30       | 1.18       | 1.66       |
| $\beta$ -Pinene        | 5.13  | 1111 | 0.34       | 0.63       | 0.56       | 0.30       | 0.05        | 0.26        | 0.27        | 0.42       | 0.41       | 0.54       | 0.44       | 0.52       |
| Sabinene               | 5.43  | 1126 | 0.19       | 0.23       | 0.22       | 0.28       | 0.07        | 0.17        | 0.15        | 0.23       | 0.20       | 0.29       | 0.21       | 0.17       |
| $\beta$ -Myrcene       | 6.48  | 1169 | 0.19       | 0.04       | 0.03       | 0.10       | 0.08        | 0.47        | 0.14        | 0.44       | 0.47       | 0.10       | 0.30       | 0.00       |
| $\delta$ -4-Carene     | 6.88  | 1183 | 1.28       | 1.24       | 1.23       | 1.32       | 0.36        | 0.97        | 0.94        | 1.24       | 1.15       | 1.75       | 1.31       | 1.03       |
| Bornylene              | 7.40  | 1200 | 1.77       | 1.13       | 1.04       | 1.34       | 0.41        | 1.68        | 1.12        | 1.26       | 1.26       | 1.74       | 1.92       | 0.94       |
| Eucalyptol             | 7.59  | 1208 | 0.44       | 0.38       | 0.36       | 0.38       | 0.11        | 0.40        | 0.30        | 0.41       | 0.38       | 0.46       | 0.44       | 0.29       |
| $\beta$ -Phellandrene  | 7.67  | 1211 | 0.43       | 0.68       | 0.80       | 0.77       | 0.28        | 0.48        | 0.40        | 0.99       | 0.68       | 1.41       | 0.47       | 0.56       |
| $\gamma$ -Terpinene    | 8.82  | 1250 | 0.25       | 0.15       | 0.11       | 0.13       | 0.00        | 0.24        | 0.16        | 0.12       | 0.16       | 0.19       | 0.25       | 0.11       |
| $p$ -Cymene            | 9.65  | 1275 | 0.08       | 0.05       | 0.06       | 0.06       | 0.07        | 0.06        | 0.04        | 0.05       | 0.04       | 0.06       | 0.05       | 0.07       |
| $\alpha$ -Terpinolene  | 10.02 | 1285 | 0.12       | 0.15       | 0.14       | 0.15       | 0.10        | 0.14        | 0.12        | 0.14       | 0.16       | 0.13       | 0.10       | 0.14       |
| 1 Octen 3 ol           | 16.13 | 1453 | 0.32       | 0.46       | 0.51       | 0.18       | 0.21        | 0.30        | 0.37        | 0.48       | 0.47       | 0.54       | 0.43       | 0.30       |
| Cis-Sabinene hydrate   | 16.54 | 1464 | 0.96       | 0.84       | 0.89       | 0.39       | 0.51        | 0.94        | 0.85        | 1.02       | 0.96       | 1.44       | 0.97       | 0.57       |
| Camphor                | 18.31 | 1508 | 0.22       | 0.26       | 0.25       | 0.21       | 0.05        | 0.23        | 0.25        | 0.26       | 0.25       | 0.34       | 0.28       | 0.50       |
| Trans-Sabinene hydrate | 19.69 | 1546 | 1.67       | 1.06       | 0.60       | 0.21       | 0.88        | 1.04        | 1.95        | 2.00       | 1.04       | 1.54       | 1.97       | 1.53       |
| Linalool               | 19.87 | 1550 | 1.42       | 1.49       | 1.65       | 1.25       | 0.46        | 1.22        | 1.24        | 1.65       | 1.46       | 1.22       | 1.57       | 1.12       |
| Linalyl acetate        | 20.24 | 1560 | 0.31       | 0.19       | 0.12       | 0.03       | 0.22        | 1.83        | 0.61        | 0.23       | 0.32       | 0.27       | 0.33       | 0.11       |
| Endobornyl acetate     | 20.85 | 1575 | 0.20       | 0.25       | 0.19       | 0.12       | 0.13        | 0.20        | 0.18        | 0.15       | 0.15       | 0.21       | 0.18       | 0.16       |
| Caryophyllene          | 21.35 | 1588 | 1.99       | 2.14       | 1.60       | 2.44       | 2.69        | 3.06        | 3.36        | 2.81       | 2.50       | 2.56       | 2.78       | 2.72       |
| Thymyl methyl ether    | 21.62 | 1594 | 0.56       | 1.68       | 0.95       | 0.24       | 0.00        | 0.04        | 0.18        | 0.26       | 0.14       | 0.25       | 0.07       | 0.33       |
| Terpinene 4-ol         | 21.74 | 1597 | 2.89       | 0.74       | 0.89       | 0.58       | 1.35        | 1.67        | 2.81        | 1.68       | 2.16       | 2.34       | 2.94       | 2.99       |
| Carvacrol methyl ether | 22.00 | 1604 | 0.31       | 0.22       | 0.39       | 0.18       | 0.11        | 1.02        | 0.20        | 0.07       | 0.08       | 0.18       | 0.47       | 0.14       |
| $\beta$ -Selinene      | 24.02 | 1660 | 0.11       | 0.11       | 0.07       | 0.24       | 0.41        | 0.22        | 0.22        | 0.16       | 0.43       | 0.20       | 0.14       | 0.19       |
| Borneol                | 25.33 | 1694 | 0.68       | 1.64       | 1.51       | 0.75       | 1.06        | 1.62        | 1.31        | 0.77       | 1.23       | 1.72       | 1.60       | 1.30       |
| Farnesol               | 26.34 | 1722 | 0.31       | 0.38       | 0.16       | 0.15       | 0.32        | 0.63        | 1.83        | 0.46       | 0.13       | 0.19       | 0.43       | 0.95       |
| Caryophyllene oxide    | 34.79 | 1966 | 0.49       | 0.52       | 0.66       | 0.85       | 0.88        | 0.45        | 0.54        | 0.44       | 0.54       | 0.12       | 0.48       | 0.69       |
| Thymol                 | 41.73 | 2216 | 0.56       | 0.24       | 0.25       | 0.82       | 0.22        | 0.25        | 0.24        | 0.26       | 0.24       | 0.18       | 0.15       | 0.24       |
| Carvacrol              | 42.54 | 2237 | 0.38       | 0.10       | 0.12       | 0.68       | 0.10        | 0.15        | 0.10        | 0.11       | 0.11       | 0.14       | 0.11       | 0.12       |

More than 30 components, representing about 99.8% of the oil, were identified in both fresh and dry leaf essential oil, but most of them constituted less than 1%. Only 5 or 6 components detected in the fresh leaf essential oil of *T. vulgaris* were found in concentrations greater than 2%:  $\gamma$ -terpinene, p-cymene, linalool, thymol and carvacrol. More than 19 components were present in amounts less than 1% (Table II and III). Among the essential oil components, thymol was the major component (>30%) while p-cymene was the second major component (>9%) of the essential oil of *T. vulgaris* harvested hourly between 6:00 and 17:00. Carvacrol was the third major component (>3%). Other terpenes were present only in very low amounts or traces. Among major essential oil components,  $\gamma$ -terpinene and p-cymene did not greatly varied. But carvacrol has greatly varied considering the harvesting hours. The highest carvacrol rates were obtained from the late harvesting hours of the day. Essential oil compositions in the fresh and dry leaf of *T. vulgaris* were within the limits previously reported by Kluczyńska, [17] and Lis, [20]. Seasonal essential oil component variations of *T. vulgaris* were well documented. The highest level of thymol and carvacrol was observed in summer after the blooming stage [21]. The lowest essential oil content and the highest carvacrol content from the vegetative phase [23].

The concentrations of the major essential oil components in fresh and dry leaf varied hourly. Among the major essential oil components at different harvesting hours, only thymol content showed great variation. Particularly, the highest values of thymol were measured in the early hours of the day while the highest carvacrol rates were obtained during the late hours of the day in both the fresh and dry leaf of *T. vulgaris* (Figure 1-2).

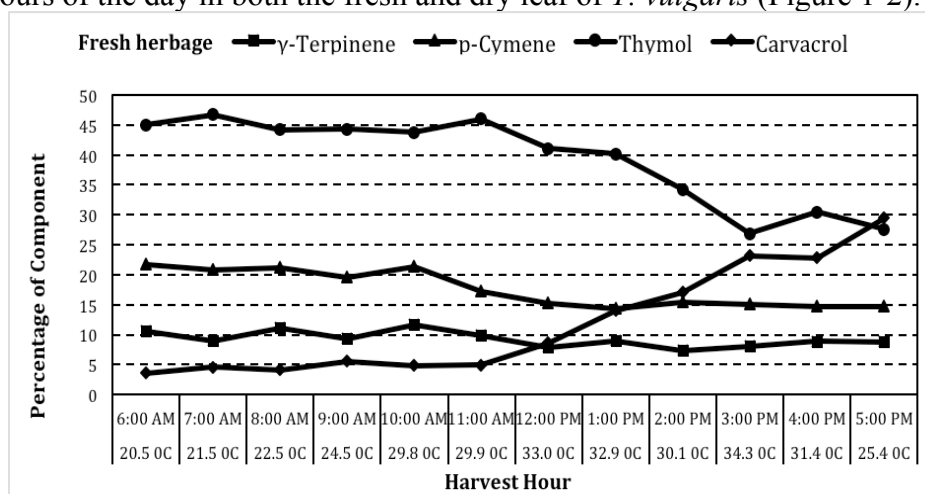
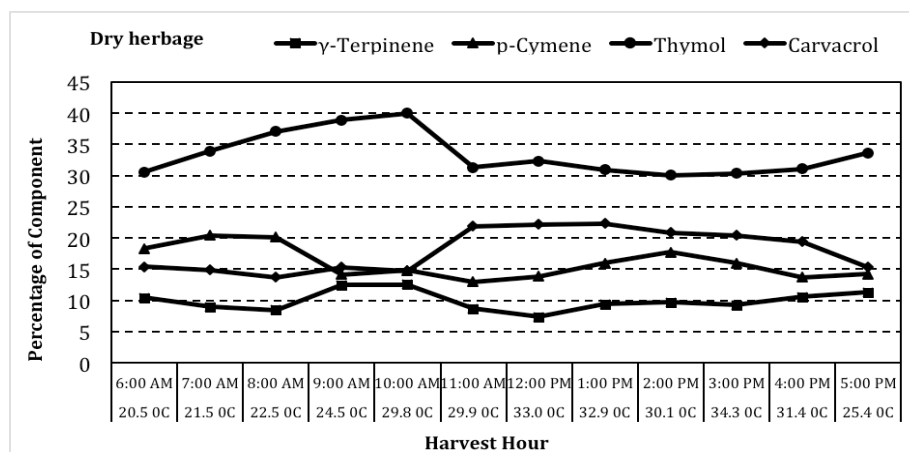


Figure 1

Diurnal variations of  $\gamma$ -terpinene, p-cymene, thymol and carvacrol in fresh leaf

**Figure 2**

Diurnal variations of  $\gamma$ -terpinen, p-cymen, thymol and carvacrol in dry leaf

In addition to the major essential oil component concentrations, the concentrations of the minor essential oil components in fresh and dry leaf also varied hourly, but the variations for most of the essential oil components among the harvesting hours were not significant ( $p > 0.05$ ).

### Conclusions

Currently, *Thymus vulgaris* is cultivated in Turkey for culinary purposes and essential oil production in small scale. Diurnal differences in essential oil content were observed. Fresh and dry leaves of *T. vulgaris* had greater oil content in early morning and late afternoon, than the hot noon hours of the day. The main compounds present in of the essential oils found in fresh and dry leaves are thymol,  $\gamma$ -terpinene, p-cymene and carvacrol. It can be concluded from the present study that the best harvesting hours of *T. vulgaris* for higher thymol content were between 6:00 and 11:00 AM.

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