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Diurnal Changes on Content and Composition of *Thymbra Spicata* L. Essential Oil

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Abstract: Diurnal variations in fresh and dry herbage for preparing essential oil of *Thymbra spicata* were studied. Plants were harvested hourly starting at 6 a.m. and ending at 5 p.m. Fresh and dry herbage essential oil was extracted by hydrodistillation for 3 h and analyzed by GC-MS. The results indicated that essential oil content changed according to the hour of the day. Increases of essential oil content were determined in the early morning and late afternoon harvests. But, the increases were not statistically significant. Carvacrol, γ-terpinen and cymol were the dominant essential oil components of both fresh and dry herbage of *T. spicata*. Small variations in essential oil composition of fresh and dry herbage of *T. spicata* were detected among harvesting hours. The best hour of harvest to obtain the essential oils with the highest active ingredients of *T. spicata* are the cooler hours of the day.

Key words: *Thymbra spicata*, essential oil, diurnal variability, GC-MS, dry herbage

INTRODUCTION

Thymbra spicata var. *spicata*, a perennial medicinal and aromatic herbaceous plant is widely distributed in the rocky areas of the Mediterranean, Aegean and Southeastern Anatolia regions of Turkey (Davis, 1982; Tanker and Ilisulu, 1984). *T. spicata* is reminiscent of the taste and fragrance of commercial oregano and thyme oils due to its harsh phenolic character. Traditionally, the dried young shoots are used as herbal tea for common cold, asthma, colic, bronchitis, coughs, diarrhea (Baser, 2001) and boiled parts of the plant are used for topical application on the wounds (Akin *et al*., 2010) and its essential oil is externally used to treat rheumatism (Baser, 2001) in the Mediterranean and Southeastern part of Turkey. Fresh shoots are used as a vegetable for main salad ingredients, especially in the Eastern Mediterranean part of Turkey. In addition to medicinal and food ingredients usage, its essential oil has antibacterial (Muller-Riebau *et al*., 1997; Unlu *et al*., 2006), antifungal (Yegen *et al*., 1992), insecticidal (Sarac and Tunc, 1995a, b) and herbicidal properties (Azirak and Karaman, 2008). The chemical composition of *T. spicata* essentialoil was extensively studied. The main compounds of *T. spicata* essential oil are carvacrol, α-terpinene, p-cymene, α-pinene, myrcene, α-terpinene, β-terpinene, γ-terpinene, 1,8-cineol, p-cymene and thymol (Hanci *et al*., 2003; Ozel *et al*., 2003; Kizil, 2010). The major essential oil constituent, carvacrol, of *T. spicata* was reported to have antifungal (Yegen *et al*., 1992), antimicrobial (Kilic, 2006) and antioxidant (Kosar *et al*., 2003) activities. Essential oils are complex mixtures of numerous chemical compounds including phenols, terpenes, alcohols and aldehydes. Essential oil of *T. spicata* is composed mainly of carvacrol, camphene, linalool, borneol, α-pinene, γ-terpinene and γ-caryophyllene (Kizil, 2010; Baser *et al*., 1996; Inan *et al*., 2011). It is well known that many environmental factors such as temperature, humidity of the air surrounding the leaf, wind velocity, intensity of light and photoperiod affect essential oil content and compositions of aromatic plants.

Many studies have been conducted to determine essential oil content, composition and usage of *T. spicata*, however, information about harvesting hours on essential oil content and composition of *T. spicata* is limited. The objectives of this study were to determine diurnal variations in essential oil content and composition of fresh and dry *T. spicata* herbage and to determine best harvest hours for essential oil production and essential oil quality.

MATERIALS AND METHODS

Material plant: About 3 years old *T. spicata* plants, grown in the research area of Mustafa Kemal University (Antakya, Hatay, Turkey, 36°19'29"N, 36°11'44"E) were

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harvested at 1 h intervals starting from 6 a.m. to 5 p.m. on 16, 17 and 18 June, 2012. Harvested plant samples were divided in two groups. Group one was freshly studied immediately after harvest and group two was dried in the shade at 26°C for 1 week to determine diurnal essential oil content and component variations of fresh and dry herbage of *T. spicata*. In each group, approximately 100 g fresh material was sampled for analysis.

Essential oil obtaining: 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°C until Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The oil percentage was expressed as w/v with respect to dry matter of the initial material.

GC-MS analysis: Analysis of the essential oil carried out by using Thermo Scientific ISQ Single Quadrupole Gas Choromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm ×30 m, i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL min^{-1}; ionization energy was 70 eV. Mass range m/z 1.2-1100 amu. Data acquisition was scan mode. MS transfer line temperature was 250°C, MS Ionization source temperature was 220°C, the injection port temperature was 220°C. The samples were injected with 250 split ratio. The injection volume was 1 μL. Oven temperature was programmed to from 50-220°C at 3°C min^{-1}. The structure of each compound was identified by comparison of their mass spectrum (Wiley) data was handled through using of Xcalibur Software program. The Retention Indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product No. 04070) and C_{21}-C_{40} (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). Differences between means were tested through LSD and values of $p<0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Essential oil content of fresh herbage did not significantly vary among harvesting hours (Table 1). Essential oil content of fresh *T. spicata*, harvested hourly, varied between 0.72 and 90%. The lowest fresh herbage essential oil content was obtained from 12 p.m. harvest with 0.70% while the highest was obtained from 6 a.m. and 17 p.m. harvests with 0.90%. When dry herbage essential oil contents of *T. spicata*, harvested hourly were considered, essential oil contents did not significantly varied among harvesting hours. The highest essential oil content was obtained from 5 p.m. harvest with 3.04% while the lowest was obtained from 1:00 p.m., harvest with 2.55% fallowed by 11:00 a.m. and 2:00 p.m., harvesting hours. In current study, the essential oil content obtained from dry herbage of *T. spicata* are higher than that of previously reported by Akgul and Kivanc (1989) (1.5%) and Hanci *et al.* (2003) 1.57% and within the range of Tansi (1991) (2.48-2.64%) and Tumen *et al.* (1994). Clearly as seen from the Table 1, both fresh and dry herbage essential oil content of *T. spicata* was affected from harvesting hours. The lowest essential oil contents were obtained from the hottest noon hours 11 a.m., 12 p.m., 13 p.m. and 13 p.m., harvests (Table 1). Essential oil variations in the fresh and dry herbage of *T. spicata* were less than the essential oil variations recorded for different plant parts and different developmental stage of the plants (Inan *et al*., 2011; Akgul and Kivanc, 1989; Tansi, 1991; Tumen *et al.*, 1994; Toncer and Kizil, 2005). Essential oil content variation at diurnally harvested coriander (*Coriandrum sativum*) was recorded by Ramezani *et al.* (2009).

The relative amounts of each component were determined by the GC-MS analysis (Table 1 and 2). About >28 components, representing about 99.8% of the oil were identified in both fresh and dry herbage essential oil but most of them constituted <1%. The only 4 components detected in the fresh herbage essential oil of *T. spicata* in concentrations >2% were β-myrcene α-terpinene, cymol, trans caryophyllene and carvacrol. About >21 components were present in amounts <1%.

Table 1: Diurnal essential oil content variations of fresh and dry *T. spicata* herbage

Harvesting hour	Temperature (°C)	Humidity (%)	Essential oil content (%)	
			Fresh herbage	Dry herbage
6:00 a.m.	20.5	50	0.90	2.92
7:00 a.m.	21.5	50	0.85	3.05
8:00 a.m.	22.5	56	0.82	2.88
9:00 a.m.	24.5	55	0.78	2.86
10:00 a.m.	29.8	48	0.75	2.80
11:00 a.m.	29.9	43	0.73	2.75
12:00 p.m.	33.0	26	0.72	2.61
1:00 p.m.	32.9	29	0.75	2.55
2:00 p.m.	30.1	28	0.80	2.77
3:00 p.m.	34.3	32	0.85	2.78
4:00 p.m.	31.4	37	0.86	2.97
5:00 p.m.	25.4	43	0.90	3.04
LSD/0.05%	-	-	NS	NS

Table 2: Diurnal essential oil component variations of fresh *T. spicata* herbage

Essential oil			Harvest hour ----------Area (%)----------											
Component	RT	RI	6:00 a.m.	7:00 a.m.	8:00 a.m.	9:00 a.m.	10:00 a.m.	11:00 a.m.	12:00 p.m.	1:00 p.m.	2:00 p.m.	3:00 p.m.	4:00 p.m.	5:00 p.m.
α-pinene	3.64	1028	0.30±0.1	0.34±0.2	0.48±0.0	0.44±0.1	0.45±0.0	0.30±0.0	0.45±0.0	0.46±0.0	0.37±0.1	0.39±0.1	0.35±0.2	0.36±0.1
α-phellandrene	3.70	1032	1.00±0.4	1.13±0.7	1.62±0.1	1.45±0.1	1.39±0.1	0.97±0.0	1.36±0.1	1.41±0.1	1.21±0.5	1.18±0.1	1.16±0.7	1.18±0.3
Camphene	4.35	1072	0.07±0.0	0.07±0.0	0.09±0.0	0.09±0.0	0.11±0.0	0.07±0.0	0.10±0.0	0.12±0.0	0.08±0.0	0.10±0.0	0.08±0.0	0.07±0.0
β-pinene	5.14	1112	0.13±0.0	0.14±0.0	0.18±0.0	0.17±0.0	0.15±0.0	0.13±0.0	0.16±0.0	0.16±0.0	0.16±0.0	0.14±0.0	0.14±0.0	0.12±0.0
Sabinene	5.45	1127	0.18±0.0	0.19±0.0	0.21±0.0	0.19±0.0	0.19±0.0	0.15±0.0	0.21±0.0	0.21±0.0	0.21±0.1	0.16±0.0	0.17±0.1	0.18±0.0
Δ-3-carene	6.09	1154	0.08±0.0	0.08±0.0	0.10±0.0	0.11±0.0	0.09±0.0	0.09±0.0	0.10±0.0	0.10±0.0	0.08±0.0	0.08±0.0	0.08±0.0	0.09±0.0
β-myrcene	6.50	1170	1.42±0.1	1.34±0.4	1.52±0.2	1.55±0.0	1.53±0.0	1.30±0.0	1.49±0.1	1.54±0.1	1.42±0.3	1.39±0.0	1.36±0.4	1.39±0.2
α-terpinene	6.89	1184	2.42±0.2	2.23±0.5	2.41±0.5	2.44±0.2	2.63±0.1	2.32±0.0	2.64±0.3	2.75±0.1	2.55±0.2	2.03±0.2	2.26±0.6	2.60±0.3
Dl-limonene	7.41	1201	0.24±0.0	0.23±0.0	0.26±0.0	0.27±0.0	0.25±0.0	0.24±0.0	0.26±0.0	0.29±0.0	0.24±0.0	0.22±0.0	0.23±0.1	0.23±0.0
β-phellandrene	7.68	1211	0.18±0.0	0.18±0.0	0.22±0.0	0.22±0.0	0.21±0.0	0.20±0.0	0.21±0.0	0.23±0.0	0.19±0.0	0.20±0.0	0.19±0.0	0.19±0.0
γ-terpinene	8.84	1251	24.61±1.8	20.54±2.8	20.16±4.1	21.14±2.0	23.63±2.5	22.42±0.2	24.22±5.4	25.51±2.1	25.18±0.3	17.54±3.8	20.28±4.5	25.38±2.5
Cis-ocimene	9.08	1258	0.10±0.0	0.09±0.0	0.10±0.0	0.09±0.0	0.09±0.0	0.09±0.0	0.09±0.0	0.10±0.0	0.09±0.0	0.09±0.0	0.09±0.0	0.10±0.0
Cymol	9.67	1276	4.50±0.2	5.12±1.5	8.33±5.0	6.99±1.5	7.10±0.7	6.10±0.3	8.32±4.2	9.93±4.4	8.83±6.3	5.85±3.4	5.21±0.7	4.75±0.5
α-terpinolene	10.04	1286	0.08±0.0	0.07±0.0	0.06±0.0	0.06±0.0	0.06±0.0	0.06±0.0	0.07±0.0	0.07±0.0	0.03±0.0	0.06±0.0	0.03±0.0	0.07±0.0
Oct-1-en-3-ol	16.14	1453	0.21±0.1	0.29±0.1	0.27±0.0	0.28±0.1	0.32±0.0	0.22±0.1	0.18±0.1	0.23±0.0	0.23±0.0	0.20±0.1	0.18±0.0	0.20±0.0
Trans sabinene hydrate	16.56	1464	0.19±0.0	0.21±0.1	0.31±0.1	0.23±0.1	0.19±0.0	0.21±0.1	0.20±0.0	0.23±0.0	0.21±0.0	0.23±0.1	0.28±0.0	0.19±0.1
Cis sabinene hydrate	19.70	1546	0.10±0.0	0.13±0.0	0.16±0.0	0.14±0.0	0.13±0.0	0.15±0.0	0.13±0.0	0.14±0.0	0.13±0.0	0.16±0.0	0.13±0.0	0.11±0.0
Linalool	19.88	1551	0.10±0.0	0.15±0.0	0.12±0.0	0.17±0.0	0.11±0.0	1.23±1.6	0.06±0.1	0.06±0.1	0.04±0.1	0.11±0.1	0.12±0.0	0.10±0.0
Trans caryophyllene	21.36	1588	2.65±1.4	2.06±0.1	1.45±0.7	1.89±0.4	1.99±0.0	1.07±1.2	1.83±0.3	2.11±0.3	1.90±0.0	1.77±0.3	1.96±0.0	2.36±0.2
4-terpineol	21.77	1598	0.20±0.0	0.25±0.0	0.28±0.1	0.26±0.0	0.26±0.0	0.18±0.1	0.25±0.0	0.26±0.0	0.25±0.0	0.26±0.1	0.26±0.0	0.22±0.0
α-humulene	24.04	1660	0.11±0.0	0.09±0.0	0.04±0.1	0.10±0.0	0.09±0.0	0.11±0.0	0.09±0.0	0.10±0.0	0.10±0.0	0.08±0.0	0.10±0.0	0.11±0.0
Isoborneol	25.33	1694	0.15±0.1	0.15±0.0	0.20±0.0	0.15±0.0	0.18±0.0	0.28±0.1	0.26±0.1	0.18±0.0	0.19±0.0	0.21±0.0	0.20±0.1	0.15±0.1
Bicylog -ermacrene	26.41	1724	0.33±0.1	0.29±0.1	0.19±0.1	0.27±0.1	0.42±0.2	0.23±0.3	0.44±0.3	0.43±0.2	0.42±0.3	0.13±0.1	0.29±0.0	0.44±0.0
D-carvone	26.52	1727	0.04±0.1	0.03±0.0	0.10±0.0	0.06±0.0	0.05±0.0	0.00±0.0	0.06±0.0	0.03±0.0	0.0±0.0	0.10±0.0	0.07±0.0	0.09±0.1
Caryophyllene oxide	34.82	1967	0.16±0.0	0.16±0.1	0.18±0.1	0.22±0.1	0.24±0.1	0.22±0.1	0.28±0.2	0.33±0.2	0.35±0.3	0.14±0.0	0.14±0.0	0.12±0.0
Spathulenol	39.50	2122	0.09±0.0	0.09±0.0	0.07±0.0	0.05±0.1	0.12±0.0	0.12±0.0	0.12±0.1	0.14±0.1	0.11±0.1	0.05±0.1	0.10±0.0	0.05±0.1
Thymol	41.75	2217	0.19±0.0	0.28±0.0	0.28±0.1	0.28±0.0	0.30±0.0	0.28±0.0	0.21±0.0	0.33±0.0	0.29±0.1	0.31±0.1	0.25±0.1	0.22±0.1
Carvacrol	42.56	2238	58.78±2.5	63.48±6.0	60.19±0.2	60.28±4.4	57.20±2.2	60.45±0.1	55.74±2.3	51.89±6.4	54.60±6.9	66.38±0.2	63.94±7.2	58.20±3.2

Table 3: Diurnal essential oil component variations of dry *T. spicata* herbage

Essential oil			Harvest hour ----------Area (%)----------											
component	RT	RI	6:00 a.m.	7:00 a.m.	8:00 a.m.	9:00 a.m.	10:00 a.m.	11:00 a.m.	12:00 p.m.	13:00 p.m.	14:00 p.m.	15:00 p.m.	16:00 p.m.	17:00 p.m.
α-pinene	3.64	1028	0.61±0.1	0.43±0.1	0.66±0.0	0.54±0.1	0.55±0.2	0.50±0.1	0.59±0.0	0.51±0.1	0.81±0.2	0.38±0.0	0.60±0.0	0.72±0.2
α-phellandrene	3.70	1032	1.87±0.1	1.36±0.2	1.98±0.1	1.59±0.2	1.65±0.4	1.43±0.2	1.65±0.0	1.49±0.5	1.98±0.1	1.12±0.0	1.83±0.1	1.84±0.1
Camphene	4.35	1072	0.12±0.0	0.09±0.0	0.13±0.0	0.13±0.1	0.11±0.0	0.10±0.0	0.15±0.0	0.08±0.0	0.22±0.1	0.07±0.0	0.10±0.0	0.11±0.0
β-pinene	5.14	1112	0.18±0.0	0.15±0.0	0.21±0.0	0.19±0.0	0.19±0.0	0.15±0.0	0.19±0.0	0.16±0.0	0.22±0.0	0.12±0.0	0.21±0.0	0.19±0.0
Sabinene	5.45	1127	0.14±0.0	0.12±0.1	0.10±0.0	0.13±0.0	0.12±0.0	0.18±0.1	0.12±0.0	0.09±0.0	0.12±0.0	0.16±0.1	0.11±0.0	0.13±0.0
Δ-3-carene	6.09	1154	0.10±0.0	0.09±0.0	0.15±0.0	0.10±0.0	0.11±0.0	0.08±0.0	0.11±0.0	0.08±0.0	0.13±0.0	0.07±0.0	0.09±0.0	0.12±0.0
β-myrcene	6.50	1170	1.72±0.2	1.64±0.1	1.70±0.1	1.53±0.0	1.58±0.1	1.39±0.4	1.51±0.1	1.49±0.5	1.96±0.4	1.23±0.1	1.67±0.0	1.71±0.0
α-terpinene	6.89	1184	2.75±0.1	2.42±0.0	2.46±0.4	2.25±0.3	2.54±0.2	2.24±0.7	2.29±0.2	2.39±0.6	2.94±0.0	2.00±0.0	2.61±0.0	2.85±0.4
Dl-limonene	7.41	1201	0.28±0.0	0.27±0.0	0.30±0.0	0.27±0.0	0.28±0.0	0.25±0.0	0.29±0.0	0.25±0.0	0.37±0.1	0.25±0.0	0.27±0.0	0.29±0.0
β-phellandrene	7.68	1211	0.25±0.0	0.22±0.0	0.24±0.0	0.22±0.1	0.22±0.0	0.23±0.0	0.23±0.0	0.19±0.1	0.28±0.1	0.19±0.1	0.24±0.0	0.24±0.0
γ-terpinene	8.84	1251	21.89±0.0	21.17±0.7	18.96±3.8	17.29±4.9	20.97±2.0	17.60±7.6	19.30±3.7	19.55±2.9	20.84±6.2	15.04±2.1	21.13±1.1	23.84±4.7
Cis-ocimene	9.08	1258	0.09±0.0	0.09±0.0	0.09±0.0	0.08±0.0	0.10±0.0	0.08±0.0	0.08±0.0	0.08±0.0	0.09±0.0	0.06±0.0	0.10±0.0	0.08±0.0
Cymol	9.67	1276	7.62±0.9	7.01±2.2	11.63±4.7	9.27±1.2	10.12±3.1	7.44±3.0	12.60±4.1	8.10±2.4	10.64±1.8	4.99±0.0	8.66±0.5	7.63±1.9
α-terpinolene	10.04	1286	0.04±0.0	0.07±0.0	0.00±0.0	0.09±0.1	0.04±0.1	0.08±0.1	0.04±0.0	0.09±0.0	0.11±0.2	0.14±0.1	0.03±0.0	0.00±0.0
Oct-1-en-3-ol	16.14	1453	0.25±0.1	0.22±0.0	0.23±0.1	0.30±0.1	0.33±0.0	0.22±0.1	0.25±0.1	0.21±0.0	0.21±0.1	0.14±0.0	0.27±0.0	0.27±0.0
Trans sabinene hydrate	16.56	1464	0.21±0.0	0.29±0.0	0.32±0.0	0.29±0.2	0.23±0.1	0.57±0.4	0.21±0.0	0.31±0.1	0.21±0.0	0.65±0.4	0.28±0.0	0.19±0.0
Cis sabinene hydrate	19.70	1546	0.12±0.0	0.07±0.1	0.19±0.0	0.49±0.5	0.13±0.0	2.35±3.1	0.14±0.0	0.28±0.2	0.21±0.1	2.66±3.3	0.16±0.0	0.12±0.0
Linalool	19.88	1551	0.13±0.0	0.12±0.0	0.12±0.0	0.13±0.0	0.12±0.0	0.21±0.2	0.08±0.1	0.12±0.1	0.10±0.1	0.15±0.0	0.13±0.0	0.11±0.0
Trans caryophyllene	21.36	1588	1.8±0.4	3.01±1.0	0.55±0.6	1.71±0.3	1.94±0.2	1.57±1.3	1.83±0.4	3.03±1.6	1.21±0.3	3.27±0.8	1.76±0.2	1.86±0.3
4-terpineol	21.77	1598	0.28±0.0	0.32±0.1	1.06±0.9	0.84±0.8	0.31±0.1	3.18±4.1	0.29±0.0	0.51±0.3	0.58±0.4	0.33±0.4	0.32±0.0	0.28±0.0
α-humulene	24.04	1660	0.05±0.1	0.12±0.0	0.15±0.2	0.10±0.0	0.13±0.0	0.22±0.1	0.29±0.4	0.31±0.3	0.30±0.2	0.20±0.0	0.11±0.0	0.00±0.0
Isoborneol	25.33	1694	0.15±0.0	0.16±0.0	0.17±0.1	0.47±0.5	0.16±0.0	0.67±0.7	0.27±0.1	0.25±0.1	0.29±0.2	0.82±0.8	0.20±0.0	0.46±0.4
Bicylog-ermacrene	26.41	1724	0.20±0.0	0.09±0.1	0.13±0.2	0.75±0.6	0.30±0.0	0.38±0.2	0.21±0.1	0.22±0.1	1.60±1.6	0.62±0.9	0.27±0.0	0.24±0.2

Table 3:Continue

Essential oil component	RT	RI	Harvest hour (Area (%)) 6:00 a.m.	7:00 a.m.	8:00 a.m.	9:00 a.m.	10:00 a.m.	11:00 a.m.	12:00 p.m.	13:00 p.m.	14:00 p.m.	15:00 p.m.	16:00 p.m.	17:00 p.m.
D-carvone	26.52	1727	0.00±0.0	0.08±0.0	0.13±0.2	0.07±0.1	0.00±0.0	0.03±0.0	0.00±0.0	0.02±0.0	0.03±0.0	0.07±0.0	0.07±0.1	0.00±0.0
Caryophyllene oxide	34.82	1967	0.21±0.1	0.19±0.1	0.24±0.0	0.29±0.0	0.31±0.1	0.25±0.0	0.36±0.2	0.32±0.3	0.40±0.2	0.24±0.0	0.19±0.0	0.25±0.1
Spathulenol	39.50	2122	0.12±0.0	0.0±0.0	0.05±0.1	0.10±0.0	0.13±0.0	0.14±0.0	0.09±0.1	0.14±0.0	0.14±0.0	0.14±0.1	0.08±0.1	0.07±0.1
Thymol	41.75	2217	0.42±0.0	0.27±0.1	0.46±0.0	0.71±0.6	0.46±0.2	5.88±7.6	0.60±0.3	0.67±0.7	3.56±4.2	0.68±0.3	0.44±0.2	0.59±0.3
Carvacrol	42.56	2238	56.60±0.3	58.57±3.6	55.87±2.1	59.30±0.5	55.27±8.1	54.05±8.4	53.04±3.4	57.64±2.2	51.25±3.9	58.79±8.2	56.96±2.6	50.63±1.9

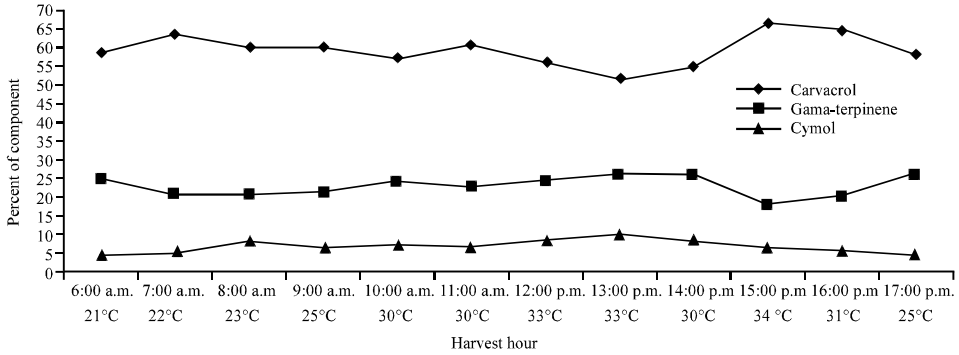

Fig. 1: Diurnal variations of carvacrol, γ-terpinen and cymol in fresh herbage

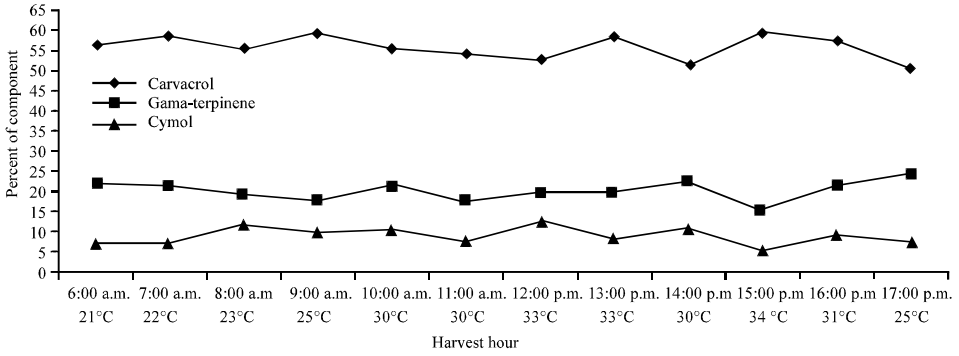

Fig. 2: Diurnal variations of carvacrol, γ-terpinen and cymol in dry herbage

(Table 2 and 3). Among the essential oil components, carvacrol was the major component (>50%) while α-terpinene was the second major component (>17%) of the essential oil of *T. spicata* harvested hourly between 6 a.m. and 5 p.m. Other terpenes were present only in very low amounts or traces. Contrary to the earlier researches, higher amount of cymol in fresh and dry herbage of *T. spicata* essential oil was detected in the current study (Ozel *et al*., 2003; Kilic, 2006; Inan *et al*., 2011). High content of carvacrol is responsible for the main odor and flavor characteristics of the essential oil. Carvacrol was the most encountered component in *T. spicata* essential oil (Tumen *et al*., 1994; Baser, 2002; Baydar *et al*., 2004). The concentrations of the major essential oil components in fresh and dry herbage varied hourly but the variations among the harvesting hours were not significant. Particularly, the lowest values of carvacrol were measured at 12:00, 1:00 and 2:00 p.m. in both the fresh and dry herbage of *T. spicata* (Fig. 1 and 2). In contrast to carvacrol, the concentrations of γ-terpinen and cymol increased at 12.00, 1.00 and 2.00 p.m. In addition to major essential oil component concentrations, the concentrations of the minor essential oil components in fresh and dry herbage also varied hourly but the variations among the harvesting hours were not significant.

CONCLUSION

Currently, *Thymbra spicata* is grown in Turkey for culinary and salad ingredients due to its essential oil content and compositions in the small scale. Diurnal

differences in essential oil content were not observed. However, fresh and dried leaves of *T. spicata* had greater oil content in early morning and late afternoon than the hottest noon hours of the day. The main compounds of the essential oils found in fresh and dry herbage are carvacrol (>50%), γ-terpinen (>17%) and cymol (>5%). It is also concluded from the present study that the best time for *T. spicata* harvesting is the coolest hours of the day especially early morning and late afternoon.

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