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Original Article

Essential Oil Composition of *Thymus* × *citriodorus* (Pers.) Schreb. at Different Harvest Stages

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Abstract

Thymus is represented by 39 species (60 taxa) in Turkey and the rate of its endemism is 45% in Turkey. The herb is widely used for several purposes. Its essential oil compositions and biologically active substances are affected by the phenological stages. Therefore, the aim of this study is to evaluate the variations in the essential oil composition of *Thymus* × *citriodorus* (Pers.) Schreb. growing in Southeastern Anatolia Region of Turkey at different phenological stages (pre-flowering, full flowering, post flowering). The oils were obtained by hydrodistillation of air-dried samples. The yields of oils (w/w %) at different phenological stages was respectively 2.0% at pre-flowering state, 1.9% at flowering stage, and 1.3% at post-flowering stage. *T.* × *citriodorus* was determined mainly by using GC/MS. A total of 21 compounds, representing 93.9% in the pre-flowering stage, 98.8% in the flowering stage and 98.91% in post-flowering of the total oil, were identified. Monoterpene hydrocarbons (terpinolene) and oxygenated monoterpenes (α-terpineol) were observed to have the highest percentage in *T.* × *citriodorus* oil. The major compounds of the oil were terpinolene, α- terpineol, linalool, bornyl acetate and borneol. The highest percentage of terpinolene was determined to be 71% in flowering stages.

Keywords: harvest time, GC-MS, lemon thyme, monoterpenes, phenological stage

Introduction

The genus *Thymus* L. (Labiatae) consists of approximately 215 species of herbaceous perennials and subshrubs and the Mediterranean region is the gene center of this plant (Nickavar *et al.*, 2005; Agili 2014). *Thymus* is represented by 39 species (60 taxa) in Turkey, and the rate of its endemism is 45% (Avci, 2010). *Thymus citriodorus* (Pers.) Schreb. known as lemon thyme is a hybrid of *Thymus vulgaris* and *Thymus pulegioides* (Bagdat *et al.*, 2011). Among *Thymus* species, lemon thyme (*Thymus* × *citriodorus)* a perennial subshrub medicinal plant, is native to southern Europe and is cultivated in the Mediterranean region. The plant is characterized by a strong fragrance which varies from lemon to orange. The herbage of *Thymus* species has been widely used for herbal tea, carminative, antiseptic, treating colds, an antioxidant material, and natural substance preservation of foods. Studies have also revealed antibacterial and antifungal properties of the essential oils (Karaman *et al.*, 2001; Sharafzadeh and Bahmani, 2014). Medicinal properties of thyme species come mainly from its essential oil. Primary constituents of lemon thyme essential oil are geraniol, neral, 3-octanone, and borneol (Omidbaigi *et al.*, 2005; Bağdat *et al.*, 2011; Wu *et al.*, 2013).

Many factors determine the content and composition of essential oils in plants such as soil mineral fertilization, light intensity, climatic conditions, growing location, developmental stages, and genetic structure (Farhat *et al.*, 2016). The optimal harvest periods vary depending on climatic conditions of the region where the plant is growing (Ozguven and Tansi, 1998). Plant ontogeny is also one of the most important factors affecting essential oil accumulation in essential oil plants (Sangwan *et al.*, 2001). Omidbaigi *et al.* (2009) reported that the most suitable time for harvesting of lemon thyme (*Thymus* × *citriodorus*) to obtain high essential oil production was at fruit set stage. However, harvesting time should be recommended according to its location and environmental conditions (Badi *et al.*, 2004). The aim of this study was therefore to evaluate the cultivation of *Thymus* × *citriodorus* and to find out variations in essential oil components of this plant due to different developments stages in semi-arid conditions of Southeastern Anatolia Region of Turkey.

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Materials and Methods

Field studies

The field experiment was conducted at the Department of Field Crops, Faculty of Agriculture, Dicle University, Turkey (37°53'N, 40°16'E, 680 m above sea level) under the ecological conditions of Diyarbakir in 2011. The soil of the field was sandy-loam with pH 7.6, organic matter of 1.2%, phosphorus of 1.62%, and potassium of 8.16%. The climate of the region is dry and hot in summer and long term cold in winters with irregular precipitation. *Thymus* × *citriodorus* seeds were purchased from Garden seeds B.V., Enkhuizen, and Holland. In the third week of January 2011, seeds were sown in a greenhouse conditions. Seedlings were transplanted with 50 cm row spacing and 30 cm plant distance in first week of May, 2011 when they reached at 10-15 cm plant height. Irrigation and cultivation practices were done as needed. The plant was harvested in different developmental stages, viz. pre-flowering, flowering and post-flowering stages in the first week of May (plants at the vegetative stage), in the fourth week of May (flowering) and in the second week of June (plants in full blooming) in 2012. The aerial parts were harvested in the morning from 2 year old cultivated plants by randomized collection of 10 individuals for each developmental stage. The plants were cut at a 10 cm height above soil level and dried in a shaded area. The essential oil of all air-dried samples (20 g) was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus. Each essential oil was dried over anhydrous sodium kept at 4 °C until chromatographic analyses.

GC/MS analysis

GC-MS analyses were realized using Agilent GC-6890II series coupled with Agilent 5975C Mass Spectrometer. The GC was equipped with HP-88 capillary column (100 m × 250 µm × 0.20 µm film thickness) coated with 88%-cyanopropyl aryl-polysiloxane. Temperature was set from 70 °C (1 min) to 230 °C (20 min) at 10 °C/min. The injection temperature was 250 °C. Injection volume was 1.0 µL. Carrier gas was He. Injection volume was 1.0 µL. Carrier gas: He. Injection mode: split (20:1). MS interface temp: 250 °C; MS mode: EI; detector voltage: 70 eV; mass range: 35-400 m/z; scan speed (amu/s). The components of the oil were identified by mass spectra with those of pure authentic samples and NIST08, Willey7n.1, and HPCH1607 libraries reference compounds. Retention indices were calculated from gas chromatograms by using logarithmic interpolation between n-alkanes. The homologous series of n-alkanes C7-C40, Supelco, USA were used as standard. Retention indices were calculated as HP-88 capillary column (Kizil *et al*., 2015). All samples were repeated three times for GC/MS analysis. Percentage of each essential oil compound was the mean of three experiments ± SD.

Results and Discussion

The essential oil yields (w/w %) based on the dry weight of the plant, in different harvesting times of developmental growth stages were found as 2.0% at pre-flowering stage, 1.9% at flowering stage, and 1.3% at post-flowering. While the highest oil yield was obtained from pre-flowering stage, while the lowest one was obtained from post-flowering stage (Table 1). The oil yield decreased towards the stage of seed maturity. Similar results reported by Omidbaigi *et al.* (2009), who studied *Thymus* × *citriodorus* from Iran, indicated that the highest essential oil content was obtained as 2.21% at beginning of flowering stage. Moreover, the other essential oil content of aerial parts of the plants harvested at full flowering and fruit set stages was 1.45 and 1.66%, respectively. A similar tendency of oil content among phenological stages was also reported by Badi *et al.* (2004) for *Thymus vulgaris*. In other studies, Bagdat *et al.* (2011) reported that the essential oil content of *T. citriodorus* was identified between 1.30 and 1.43%. Kizil and Toncer (2013) determined that the essential oil content of lemon thyme was 0.9%.

Table 1 shows the essential oil components obtained from three harvest stages of *T. citriodorus* and twenty one compounds identified are listed according to their retention indices were found in the oils (Fig. 1). The majority of these compounds were 93.91% at the pre-flowering stage, 98.82% at the flowering stage, and 98.91% at the post-flowering stage. The essential oil was characterized by a high rate of monoterpene hydrocarbons (61.03-72.39%), followed by oxygen containing monoterpenes (23.27-33.69%). Monoterpene hydrocarbons (terpinolene) and oxygenated monoterpenes (α-terpineol) were shown to have the highest percentage in *Thymus* × *citriodorus* oil (Table 1).

The major components of *Thymus* × *citriodorus oils* were determined as terpinolene, α- terpineol, linalool, bornyl acetate, and borneol (Table 1). It was determined that main component was terpinolene (59.0-71.0%) which appeared to be unstable during the plant growth cycle. The highest content of terpinolene as main component (71.0%) was obtained at the flowering stage. The lowest one (59.0%) was obtained at the post-flowering stage. α-terpineol was another main component of the oil. While the highest rate of α-terpineol was found as 29.56% at the post-flowering stage, the lowest rate of α-terpineol was determined as 20.03% at the flowering stage. Linalool content of the oil ranged from 1.35% to 3.47%. The highest linalool (3.47%) was determined in the essential oil of harvested plant at the pre-flowering stage. Bornyl acetate and borneol content, the other main components, varied between 0.99% and 1.94% and between 0.62% and 1.24%, respectively. Moreover, the highest level of borneol and bornyl acetate was obtained at the post-flowering stage (Table 1). Omidbaigi *et al*., (2009) determined that geraniol was the major component (54.2-72.5%) in *Thymus* × *citriodorus* and obtained the highest geraniol content (72.5%) in the essential oil at the pre-flowering stage and the lowest geraniol content (54.2%) at the fruit development stage.

Stahl-Biskup and Holthuijzen (1995) reported that geraniol was the main compound (more than 60%) and also detected geranyl acetate (1.0%), geranyl butyrate (0.8%), nerol (2.8%), and citronellol (0.3%) in *Thymus* × *citriodorus*. Bağdat *et al.* (2011) also indicated that the main component of essential oil of *T. citriodorus* growing in central Anatolia was 'geraniol'. Wu *et al.* (2013) found that the main components of *T. citriodorus* oil were borneol (28.82%), thymol (14.43%), 3, 7-*dimethyl*-1, 6-octadiene-3-ol (8.26%), 1-methyl-4-[alpha-hydroxy-isopropyl] cyclohexene (8.23%), and terpenes camphor (5.1%).

In this study, major essential oil component of *T. citriodorus* was determined as terpinolene, which has not been previously reported as main component in *T. citriodorus* essential oil.

Table 1. Chemical composition of *Thymus × citriodorus (*Pers.*) Schreb.* and essential oil yield (%) according to different development stages

No.	Components	RI*	RT**	Percentage composition		
				Pre-flowering stage	Flowering stage	Post-flowering stage
1	Sabinene	1216	12.35	0.12±0.12	0.49±0.63	0.24±0.00
2	Limonene	1267	12.86	0.44±0.13	0.57±0.19	0.75±0.02
3	γ- Terpinene	1316	13.40	0.20±0.04	0.14±0.02	0.32±0.00
4	m-Mentha-1,8-diene, (+)	1350	13.79	0.10±0.00	0.08±0.00	0.10±0.00
5	1.8-Cineole	1365	13.96	0.43±0.01	0.38±0.00	0.52±0.01
6	*p*-Cymene	1399	14.37	0.11±0.03	0.11±0.00	0.62±0.00
7	1-Octen-3-ol acetate	1506	15.62	0.13±0.00	0.23±0.01	0.31±0.01
8	1-Octen-3-ol	1561	16.28	-	0.15±0.01	0.08±0.00
9	β-Bourbonene	1613	16.91	0.08±0.01	0.21±0.002	0.08±0.00
10	Linalool	1682	17.72	3.47±0.01	1.61±0.02	1.35±0.03
11	Caryophyllene-E	1772	18.75	0.57±0.02	0.68±0.03	0.53±0.01
12	Borneol	1849	19.43	1.11±0.00	0.62±0.06	1.24±0.00
13	Terpinolene	1925	20.38	61.20±1.26	71.0±1.76	59.0±0.02
14	α-Terpineol	1949	20.60	22.84±0.22	20.03±0.08	29.56±0.06
15	Bornyl acetate	1996	21.03	1.34±0.00	0.99±0.09	1.94±0.00
16	α-Terpinyl acetate	2057	21.57	0.30±0.08	0.14±0.08	0.23±0.00
17	Trans-Carveol	2114	22.07	0.19±0.01	0.21±0.04	0.26±0.00
18	Geraniol	2157	22.42	-	0.05±0.00	0.06±0.01
19	Thymol	2544	25.62	-	0.04±0.01	0.87±0.02
20	Caryophyllene oxide	2561	25.76	0.73±0.07	0.42±0.02	0.58±0.01
21	Carvacrol	2618	26.26	0.55±0.01	0.67±0.04	0.27±0.00
	Grouped Components					
	Monoterpene hydrocarbons			62.17	72.39	61.03
	Oxygen-containing monoterpenes			28.27	23.27	33.69
	Sesquiterpene hydrocarbons			0.65	0.89	0.61
	Oxygen-containing sesqiterpenes			0.73	0.42	0.58
	Monoterpene phenols			0.55	0.71	1.14
	Other			1.54	1.14	1.86
	Total %			93.91	98.82	98.91
	Essential oil yield (%)			2.0	1.9	1.3

* Retention Index; ** Retention Time

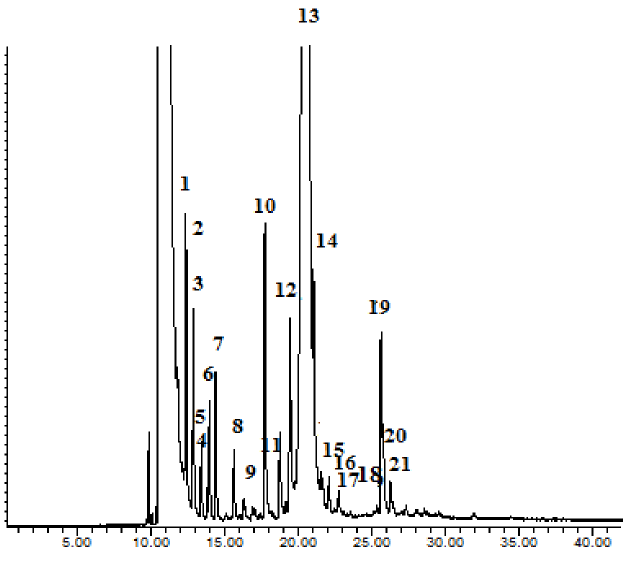

Fig. 1. Typical chromatogram of *Thymus citriodorus* essential oil components. 1-Sabinene, 2-Limonene, 3-γ- Terpinene, 4-m-Mentha-1,8-diene, (+), 5-1.8-Cineole, 6-p-Cymene, 7-1-Octen-3-ol acetate, 8-1-Octen-3-ol, 9-β-Bourbonene, 10-Linalool, 11-Caryophyllene-E,12-Borneol, 13-Terpinolene, 14- α-Terpineol, 15-bornyl acetate, 16-α-Terpinyl acetate, 17-trans-Carveol, 18-Geraniol, 19-Thymol, 20- Caryophyllene oxide, 21-Carvacrol

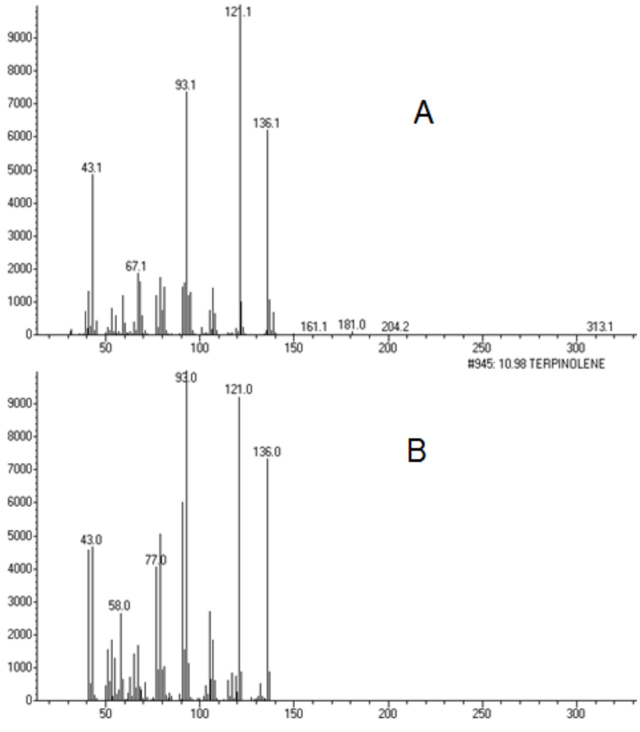

Fig. 2. Comparison of the mass spectrum (A) of obtained from peak 13 in Fig 1, (B) terpinolene spectrum obtained from mass spectral library with the resolved spectra

GC-MS analysis of which gave rise to four major signal at m/z 121, 136,93,43, also almost the same fragmentations as those of terpinolene were observed (Fig. 2A-B). This was confirmed by comparing GC retention times with pure terpinolene standart. The observed variations in the essential oil content and composition of *T. citriodorus* might be associated with variations in response to changing weather conditions at different phenological stages and infraspecific chemical variability for the species of the genus *Thymus* (Corticchiato *et al*., 1998; Jordan *et al*., 2006). Loziene *et al*. (2003) reported both infra-specific and intra-population chemical polymorphisms in *Thymus*.

Terpinolene is also a constituent of many essential oils e.g. *Citrus*, *Mentha*, *Juniperus*, *Myristica* species (Civjan, 2012). Choi *et al*. (2000) reported that terpinolene showed high radical scavengers on DPPH. Although terpinolene, α-Terpineol and borneol occur particularly in oils from Pinaceae and in citronella family, and has woody and fruity odor, the other important compound, linalool, is described in literature as floral, citric, fresh and sweet (Elsharif *et al*., 2015; Surburg and Panten, 2016).

Many chemotypes have been reported for *Thymus* species. Oh *et al*. (2008) reported that essential oil components of *Thymus* species contained commonly monoterpene hydrocarbons (α-pinene, camphene, β-myrcene, and terpinolene), oxygenated monoterpenes (*cis*-sabinene hydrate, camphor, borneol, α-terpineol, and thymoquinone), a monoterpene phenol (thymol), monoterpene phenol precursors (*p*-cymene and γ-terpinene), a monoterpene phenol derivative (thymol methyl ether) and sesquiterpenes (β-caryophyllene and β-bisabolene). The researchers also stated that the grouping of compounds has an important meaning as responsible for the characteristic aroma of *Thymus.* Asbaghian *et al.,* (2011) reported that the essential oil components of *T. caucasicus* was characterized by 1,8-cineol (21.5%), thymol (12.6%), beta-fenchyl alcohol (8.7%), nerolidol (7.8%), terpinolene (7.2%), α--pinene (7.0%), and myrcene (6.8%). In the oil of *T. kotschyanus*, carvacrol (24.4%), β-caryophyllene (14.5%), γ-terpinene (12.4%), α-phellandrene (10.8%), *p*-cymene (9.8%), and thymol (6.8%) were the predominant compounds; whereas, the main components of *T. vulgaris* oil were thymol (43.8%), *p*-cymene (15.2%), germacrene-D (11.7%), terpinolene (3.4%), carvacrol (3.2%), β-caryophyllene (2.8%), and α-thujene (2.2%). Behravan *et al*. (2007) reported that thymol (71.07%), terpinolene (13.08%), and cymene (10.20%) were the main component in *Thymus vulgaris*. Moreover, Dob *et al*. (2006) identified linalool as main component in *Thymus algeriensis* oil.

Conclusions

As a conclusion, it was found that the highest essential oil yield was obtained during the flowering period (2.0%), and α-terpinolene defined as main component, the highest level of it was obtained harvested plants from flowering stage as 71.0%. Harvesting of *T.* × *citriodorus* should be done in flowering plants to achieve better essential oil yield and quality.

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