

# VARIATION OF AGRONOMICAL CHARACTERISTICS AND ESSENTIAL OIL COMPONENTS OF ZAHTER (*Thymbra spicata* L. var. *spicata*) POPULATIONS IN SEMI-ARID CLIMATIC CONDITIONS

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#### ABSTRACT

*Thymbra spicata* L. var. *spicata* is grown naturally in semi-arid climatic conditions of Turkey. The seeds of wild populations collected from various regions were cultivated under arid climatic conditions of Diyarbakır in South East Anatolian region of Turkey. Components of hydrodistilled essential oils obtained from air dried aerial parts of *T. spicata* were identified and compared using gas chromatography/mass spectrophotometer. Fresh herbage yields of populations ranged 4925 kg ha<sup>-1</sup> to 13275 kg ha<sup>-1</sup>, dry herbage yield varied between 2035 to 4782 kg ha<sup>-1</sup> and dry leaf yield ranged 1347 kg ha<sup>-1</sup> to 3364 kg ha<sup>-1</sup>. Essential oil contents of *T. spicata* populations in dry herb ranged 2.25% to 4.65%. The total oil content of plants and the percentage contribution of the major oil constituents, carvacrol,  $\gamma$ -terpinene and *p*-cymene, varied remarkably among localities. The highest carvacrol content was found in the population collected from Diyarbakir-Dicle (97.9%). The results indicated that agronomic characteristics, essential oils and chemical compositions of *T. spicata* are affected by environmental, genetic and agronomic practices under cultivation conditions.

Keywords: Thymbra spicata L. var. spicata, populations, cultivation, essential oil, carvacrol

#### **INTRODUCTION**

The genus *Thymbra* L., belong to Lamiaceae family (syn Labiatae), vernacular "Zahter" and "Karabas kekik" is an economically significant plant. It includes dicotyledonous dwarf shrubs that mostly occur in arid or semi-arid warm and mountainous areas. It is intensively collected from wild, and is considered under threat (Kizil, 2010). *T. spicata* has a very large distribution area extending to almost all of the Eastern Mediterranean region countries. *T. spicata* var. *spicata* grows on calcareous, hillsides and open sunny places from 121 to 1249 m both under Mediterranean and Semi-arid climatic condition of Turkey (Kizil, 2010).

Type and quantity of essential oil compounds are generally subject to remarkable variability depending on the growing area, the growth stage and the part used for oil extraction. *T. spicata* contain 60-80% carvacrol in its essential oil (Kokkini and Vokou, 1989). Essential oils and leaves of *T. spicata* have different industrial uses for flavouring of several kinds of food products, liqueur production, perfumery, herbal teas, and spice for foods, antiseptic and antimicrobial agents in medicine. Both the raw material of medicinal and aromatic plants and their

products must meet the maintenance of high standards required on quality, safety and efficiency. This can only be fulfilled by cultivation of plants which are selected according to particular scale based on biological and chemical characteristics of plants. The diversity among plants selected from natural habitats could be used to form a basis for development of new cultivars (Kizil, 2013) after subjecting them to strong selective programs (Shi et al., 2008). Many abiotic factors like climatic, geographic conditions and biotic factors like growth stage of plants may severely affect essential oil yield, their composition and their biological properties. Thus, chemical polymorphism studies based on essential oil constituents comparison in relation to environmental factors are supposed to provide good information towards this end (Zouari et al., 2012).

The study aimed to compare and select most suitable *T. spicata* var. *spicata* populations from Southeast Anatolia and Mediterranean Regions of Turkey under semi-arid conditions of Diyarbakir for their agronomical characteristics and essential oil components for use in breeding programs.

## MATERIALS AND METHODS

## Field site description

The experiment was conducted in the experimental area of the Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey (latitude 37°53'N, longitude 40°16'E, altitude 680 m) with dominant semi-arid characteristics during 2009 and 2010 years. Diyarbakir climate is dry, hot in summer and long term cold in winters with irregular precipitation.

During field trial (2010), the average temperature (17.6°C) of second year was higher compared to long years temperature (15.8 °C). Precipitation of 2010 year (449.5 mm) was lower compared to precipitation (479.6 mm) for long years. Relative humidity of 2010 (55%) were also found lower compared to long years mean values (53.1%). The active growth period of *Thymbra spicata* was between March and June months, when Diyarbakır showed elevated temperature (State

Meteorology Institute, Diyarbakir). *T. spicata* grows well on calcareous light, dry and stony soils under natural conditions. Contrarily, the soil characteristics of the experimental area had 0.41% organic matter, 70% humidity, 1.7 kg ha<sup>-1</sup> phosphorus ( $P_2O_5$ ), 79.8 kg ha<sup>-1</sup> potassium ( $K_2O$ ), pH of 8.08 and electrical conductivity of 0.026 (mm hos/cm).

## Field studies

Seeds of *T. spicata* var. *spicata* belonging to 30 different locations (Figure 1) were sown in a seedbed nursery (December 2008), were transplanted to the fields when they attained a plant height of 10–15 cm (April 2009). Each plot (Location) was arranged as 5 rows, with  $70 \times 20$  cm plant density and plot size of  $14 \text{ m}^2$  ( $3.5 \text{ m} \times 4 \text{ m}$ ). During vegetation period, the plots were irrigated and weeded as and when required. No harvesting was done during the first year (2009), while the second year harvest was carried out on 2 - 4 June 2010, at flowering stage.

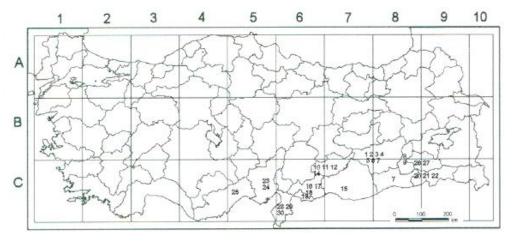


Figure 1. Thymbra spicata populations collected from 30 different localities.

Fresh herbage samples of each plot were manually harvested using pruning shears. Agronomic characteristics were determined taking samples from 10 plants in each plot. Fresh herbage samples of plants were dried under cool, dry, shaded, well ventilated place for 7-10 days. After drying, the leaves and stems were separated. Plant height (cm), canopy diameter of each plant (cm), spike length (cm), fresh herbage (kg ha<sup>-1</sup>), dry herbage (kg ha<sup>-1</sup>), dry leaf yield (kg ha<sup>-1</sup>) and essential oil content (% - v/w) were measured from two years old plants during 2010.

#### Essential oil extraction

Essential oil of 20 g dried leaf samples was extracted by hydro-distillation for 2.5 hours under continuous steam using a Clevenger-type apparatus (v/w). The isolated oils were stored in tightly closed vials at 4°C until analysis.

## Gas chromatography–mass spectrometry (GC/MS) analysis

GC-MS/FID analyses were performed using Agilent GC-6890II series coupled with Agilent 5975C Mass Spectrometer. The GC was equipped with HP-88 capillary

column (100 m x 250 µm x 0.20 µm film thickness) coated with 88%-cyanopropyl aryl-polysiloxane. It is high polarity column; it shows better separation than DB-23 of cis-trans isomers that has nearly identical selectivity as the CP-Sil 88, but with higher temperature limits (Anonymous, 2015). HP-88 column can effectively separate volatile oils in addition fatty oils due to the column length (100m) (Liu et al., 2007; Toncer et al., 2009; Toncer et al., 2010). Temperature programmed: from 70°C (1 min) to 230°C (20 min) at 10°C/min. The injection temperature: 250°C. Injection volume: 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: 35-400m/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. The ratios of compounds were evaluated according to FID results. Retention indices were computed from gas chromatograms by logarithmic interpolation between n-alkanes. The homologous series of n-alkanes C7-C40, Supelco, USA were used as standard. Retention indices calculated as HP-88 capillary column. Retention indices calculation evaluated first time with this column and discussed with different columns in literature. All samples were repeated three times for GC/MS analysis. This is the first report about HP-88 column used for essential oil composition of Zahter.

### Statistical analysis

For all examined characteristics, mean values were calculated using IBM SPSS computer software version18.0.0 (Statistical Package for the Social Sciences, 2008).

### **RESULTS AND DISCUSSION**

## Agronomical Characteristics

The observations on morphological characteristics of

*T. spicata* var. *spicata* obtained from field conditions are given Table 1. In general, collected plants of *T. spicata* var. *spicata* have vertical growth and little hairy and hairless type plant. The flowering date of populations varied between 02 May 2010 and 07 June 2010. The earliest flowering date was observed from Batman/Kozluk population and also the latest flowering date was recorded in the plants of Batman/Sason population.

Table 2 gives average values of plant height, canopy diameter and flower spike length from 30 different localities. Results showed that plant height varied significantly between populations of *T. spicata*. Plant height of the populations ranged from 22.6 cm to 44.6 cm. The maximum plant height was recorded on the Mersin/Tarsus population; the minimum plant height was recorded in the Batman/Sason population (Figure 2).

Population Num.	Name	Flowering date	Hair	Vertical/horizontal
INUIII.	Diyarbakir			
1	Cermik	21 May 2010	Yes	V
2	Cungus	26 May 2010	No	v
3	Dicle	20 May 2010 21 May 2010	Few	v
4	Hani	21 May 2010 21 May 2010	Yes	v V
5	Kulp	21 May 2010 21 May 2010	No	v V
6	Lice	21 May 2010 21 May 2010	Yes	v V
0	Mardin	21 May 2010	res	v
7		26 Mar. 2010	N-	37
7	Center	26 May 2010	No	V
0	Batman	0010	F	
8	Kozluk	02 May 2010	Few	V
9	Sason	07 June 2010	Few	V
	Adiyaman			
10	Center	26 May 2010	No	V
11	Sincik	26 May 2010	No	V
12	Kahta	31 May 2010	No	V
13	Kahta Road	31 May 2010	No	V
14	Gerger	01 June 2010	Few	V
	Sanliurfa			
15	Halfeti	26 May 2010	Yes	V
	Gaziantep	,		
16	Oguzeli	01 June 2010	Yes	V
17	Nizip	01 June 2010	Few	V
18	Center	03 June 2010	Few	V
	Kilis			
19	Center	28 May 2010	Few	V
1)	Sirnak	20 May 2010	1000	·
20	Uludere	28 May 2010	Few	V
20	Eruh to Sirnak 10 km	01 June 2010	No	v
<u>~1</u>	Adana	01 Julie 2010	110	v
22		01 June 2010	Yes	V
22 23	C.U. Kampus Pozanti		Much	v V
23		28 May 2010	witten	v
24	Mersin	01 1 2010	Б.	17
24	Tarsus	01 June 2010	Few	V
25	Siirt		37	
25	Cevizli	26 May 2010	Yes	V
26	Center	26 May 2010	Few	V
27	Eruh	01 June 2010	Yes	V
	Antakya			
28	Hassa	26 May 2010	Yes	V
29	Harbiye	26 May 2010	No	V
30	Senkoy	26 May 2010	Few	V

Table 1. Phenological and morphological properties of different T. spicata populations

Population	Plant height	Canopy diameter	Flower spike length
Num.	(cm)	(cm)	(cm)
1	32.6	52.4	4.88
2	39.6	64.6	4.10
2 3	33.8	61.2	4.68
4	34.6	62.6	4.44
5	34.0	63.0	4.42
6	34.4	56.0	3.88
7	35.8	60.8	4.32
8	27.9	23.4	2.23
9	22.6	29.2	2.07
10	29.0	50.8	4.25
11	32.0	59.6	3.34
12	37.4	65.4	2.34
13	32.4	56.4	2.40
14	32.6	52.0	2.78
15	34.2	62.8	3.34
16	36.0	49.6	2.80
17	41.6	60.4	3.38
18	36.6	66.4	3.44
19	40.4	69.0	4.34
20	38.4	58.8	5.10
21	36.8	65.0	4.72
22	42.2	40.6	3.28
23	32.6	52.4	2.54
24	44.6	68.2	3.26
25	32.0	54.8	4.26
26	37.4	54.4	3.72
27	39.8	43.8	2.49
28	42.4	51.6	3.60
29	39.9	44.4	2.31
30	36.4	46.6	2.09
Mean.	35.1	54.3	3.49
Min.	22.6	23.4	2.07
Max.	44.6	69.0	5.10
Sdev.	2.68	4.10	1.90

 Table 2. Some agronomical characteristics of different T. spicata populations in the field condition

Diyarbakir/1-Cermik, 2- Cungus, 3- Dicle, 4- Hani, 5- Kulp, 6- Lice, Mardin/7- Center, Batman/8- Kozluk, 9- Sason, Adiyaman/10- Center, 11-Sincik, 12- Kahta, 13- Kahta Road, 14- Gerger, Sanliurfa/15- Halfeti, Gaziantep/16- Oguzeli, 17- Nizip, 18- Center, Kilis/19- Center, Sirnak/20-Uludere, 21- Eruh to Sirnak 10 km, Adana/22- C.U. Kampus, 23- Pozanti, Mersin/24- Tarsus, Siirt/25- Cevizli, 26- Center, 27- Eruh, Antakya/28-Hassa, 29- Harbiye, 30- Senkoy

Plant height results of *T. spicata* populations were similar or higher than that reported (10-40 cm) by Davis (Davis, 1982). Variations in plant height between populations can be attributed to different edaphic and climatic factors. Our results about canopy diameter varied between 23.4 and 69.0 cm. Among the localities, the maximum canopy diameters were obtained from Kilis/center population, Mersin/Tarsus and Gaziantep/Center population as 69.0, 68.2 and 66.4 cm respectively. The minimum canopy diameter was obtained from Batman/Kozluk population as 23.4 cm. Flower spike length of different populations varied between 5.10 cm and 2.07 cm. The maximum values were obtained from the plants of Sirnak-Uludere and Diyarbakir-Cermik populations, while the minimum flower spike length was obtained from the plants of Batman-Sason population. Our results supported Flora of Turkey records with inflorensence reported as 1-8 cm in Davis (Davis, 1982; Thompson et al., 2004).

Ceylan et al. (2003) reported that differences in agronomical characters may occur because of the area in which the plants were collected and the fact that the plants were grown for the first year. Thompson et al. (2004) emphasize that the need to consider diverse genetic effects in order to fully evaluate the causes of performance variation among plant populations. Keskitalo et al. (2001) concluded that if the biodiversity of herbaceous species is to be examined, different factors need to be recognized such as dependency between the geographical origin, genetic, chemical and morphological variation.

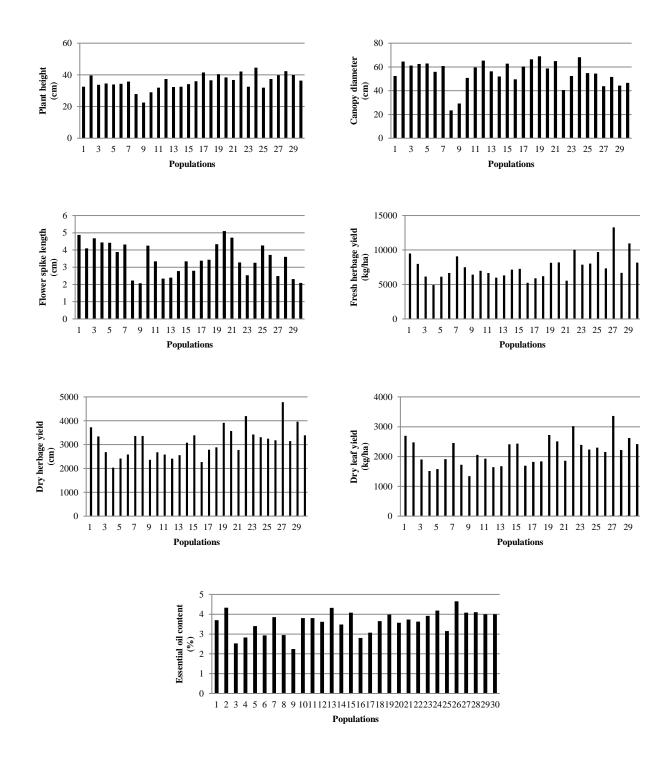


Figure 2. Investigated different agronomical characteristics of *T. spicata* populations.

There were wide variations for fresh herbage yield of populations. Fresh herbage yield of populations ranged 4925 kg ha<sup>-1</sup> to 13275 kg ha<sup>-1</sup>. Highest fresh herbage resulted by Siirt-Eruh population and the lowest ones were noted on Diyarbakir-Hani population. In concerning with fresh herb yield, dry herbage and dry leaf yields have followed a similar trend. The highest dry herbage yield (4782 kg ha<sup>-1</sup>) was recorded on Siirt-Eruh population, while the lowest dry herbage yield (2035 kg ha<sup>-1</sup>) was recorded on Diyarbakir-Hani population. The highest dry leaf yield was obtained from populations of Siirt-Eruh and

Adana-CU Campus populations as 3364 kg ha<sup>-1</sup> and 3018 kg ha<sup>-1</sup>, while, the lowest dry leaf yield was obtained from Diyarbakir-Hani and Batman-Sason populations as 1514 and 1347 kg ha<sup>-1</sup>, respectively (Table 3; Figure 2). Under irrigation or culture conditions, wild plants have given high herbage yield per hectare per annum (Anonymous 2013). Under dry land conditions the yields has vary considerably.

Essential oil content of *T. spicata* var. *spicata* populations in dry herb from 30 localities ranged 2.25% to 4.65% (Table 3). Significant differences in essential oil

content were observed among the populations. Taking into consideration mean of populations, the highest essential oil contents were obtained from Siirt-center (4.65%) and Diyarbakir-Cungus (4.33%) populations. The lowest oil

content (2.25%) was observed in Batman-Sason, followed by Diyarbakir-Dicle (2.53%) populations (Table 3; Figure 2).

Table 3. Fresh & dry herbage yield, dry leaf yield and essential oil content of different populations of *T. spicata* in the field conditions

Population	Fresh herbage	Dry herbage	Dry leaf yield	Essential oil content	
Num.	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(%)	
1	9496	3725	2700	3.70	
2	7985	3343	2475	4.33	
3	6143	2689	1904	2.53	
4	4925	2035	1514	2.83	
5	6132	2418	1579	3.40	
6	6675	2589	1914	2.93	
7	9089	3368	2457	3.85	
8	7492	3364	1724	2.95	
9	6421	2364	1347	2.25	
10	6993	2679	2061	3.80	
11	6675	2586	1932	3.80	
12	5993	2411	1646	3.62	
13	6321	2561	1675	4.32	
14	7161	3086	2411	3.48	
15	7275	3393	2432	4.08	
16	5257	2271	1696	2.80	
17	5893	2789	1821	3.07	
18	6207	2886	1839	3.65	
19	8161	3918	2725	3.98	
20	8207	3575	2511	3.57	
21	5543	2775	1861	3.73	
22	10032	4196	3018	3.63	
23	7864	3425	2389	3.92	
24	8032	3311	2236	4.18	
25	9696	3254	2300	3.15	
26	7328	3182	2154	4.65	
27	13275	4782	3364	4.08	
28	6678	3150	2218	4.10	
29	10953	3964	2621	4.00	
30	8182	3393	2421	4.00	
Mean.	7633.8	3134.3	2176.8	3.60	
Min.	4925	2035	1347	2.25	
Max.	13275	4782	3364	4.65	
Sdev.	929.1	234.7	197.2	1.41	

Diyarbakir/ 1-Cermik, 2- Cungus, 3- Dicle, 4- Hani, 5- Kulp, 6- Lice, Mardin/ 7- Center, Batman/ 8- Kozluk, 9- Sason, Adiyaman/ 10- Center, 11-Sincik, 12- Kahta, 13- Kahta Road, 14- Gerger, Sanliurfa/ 15- Halfeti, Gaziantep/16- Oguzeli, 17- Nizip, 18- Center, Kilis/19- Center, Sirnak/20-Uludere, 21- Eruh to Sirnak 10 km, Adana/22- C.U. Kampus, 23- Pozanti, Mersin/24- Tarsus, Siirt/25- Cevizli, 26- Center, 27- Eruh, Antakya/28-Hassa, 29- Harbiye, 30- Senkoy

Dogan et al. (1987) (1.3-1.5%), Tansi (1991) (2.25-2.20 %), Tumen et al. (1994) (0.5–3.4%), Muller-Riebau et al. (1997) (2.9-10.8%), Baser (2002) (1.0–3.4%), Hanci et al. (2003) (1.57%), Kilic (2006) (3.1%), Inan et al. (2011) (3.56%) and Barakat et al. (2013) (3.5-6.6%) report variable essential content of *T. spicata* var. *spicata*. These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage conditions.

Pirbalouti et al. (2013) reported that elevation and temperature provide better growing condition for oil content in *Thymus vulgaris*. Figueiredo et al. (2008) also stated that there are number of examples showing effects of geographic locations on variations of yield and composition of volatiles, for several species, the existence of distinct chemotypes/chemical races. Local landraces in medicinal and aromatic plants are an important source of variation and provides significant advantages in breeding of plants. Chemical variability of these plants also diversifies their use. In this study, seeds of original populations were collected from different isolated altitudes. In a previous study (Kizil, 2010) conducted on this species no significant change in the rate of essential oil from plant habitats related to the altitude was recorded. Essential oil content of the plants collected from low altitude showed higher percentage compared to those collected from high altitudes. While, it is also clear that certain values in plants, fresh and dry yield, essential oil, can be deliberately enhanced under controlled conditions of cultivation (Kizil, 2010; Palevitch, 1991).

#### Identified essential oil compounds

Essential oil composition of *T. spicata* var. *spicata* were determined by GC-MS and twenty three compounds, which identified are listed according to their retention

indices, were found in the oils (Table 4; Figure 3). The main identified constituents of the essential oils of the *T. spicata* var. *spicata* populations were calculated according to GC-FID detector results and represented 95.1% to 99.8% of the oils (Table 5). Carvacrol was the major component in the oil of all populations varying from 46.6 to 97.9% except one (Antakya-Senkoy population). In addition to carvacrol,  $\gamma$ -terpinene and *p*-cymene were predominant components in the oil (Figure 3). Five populations contain only small amounts of thymol varying from 0.4-37.5% (Table 5). Dogan et al. (1987) reported that thymol which is responsible, together with carvacrol for the phenolic characters of the oil and therefore was found in higher percentage in some samples.

Table 4. Essential oil composition, min-max and mean value of thirty *T. spicata* var. *spicata* populations according to GC - MS results

Compounds	RT	Min-max	Mean	RI	LRI
β-pinene	12.22	0.06-0.32	0.21	1101.93	1113*
myrecene	12.40	0.065-1.86	1.18	1220.33	$1187^{*}$
α-phellandrene	12.66	0.10-0.23	0.16	1246.05	$1290^{**}$
Delta-2-carene	12.85	0.06-2.80	1.16	1264.39	
γ-terpinene	13.44	0.40-21.83	12.23	1319.66	1293***
Terpinolene	13.82	0.06-0.21	0.15	1353.07	1323***
1.8 cineole	13.99	0.06-0.16	0.11	1368.49	1253***
<i>p</i> -cymene	14.41	0.46-22.7	9.83	1402.84	1303*
Cis-1-octen-3-ol	17.45	0.09-0.39	0.19	1658.62	1462***
Cumin alcohol	18.15	0.02-0.12	0.08	1719.40	
E-caryophyllene	18.77	0.70-2.19	1.22	1772.95	1732*1420*****
Trans-caryophyllene	18.91	0.12-0.80	0.38	1783.16	$1418^{****}$
Terpinen-4-ol	19.47	0.20-0.82	0.55	1835.91	1642*
α-humulene	19.80	0.02-0.10	0.8	1867.82	1709***
α-terpinolene	20.58	0.10-0.36	0.14	1945.93	1323***
Borneol	21.04	0.10-0.66	0.23	1997.31	1747*
Cis-dyhdrocarvone	21.29	0.06-0.12	0.09	2026.13	
Trans-dhydrocarvone	21.65	0.03-0.06	0.05	2067.12	
+ carvone	22.62	0.04-0.13	0.09	2181.10	
Cumin alcohol	25.46	0.05-0.44	0.19	2524.14	
Thymol	25.65	0.27-40.68	2.26	2547.01	2212***
Caryophyllene oxide	25.86	0.41-1.77	0.95	2571.88	$2068^{*}$
Carvacrol	26.33	14.34-91.77	67.08	2625.08	2246***

RT: Retention Time; Min-max: minimum and maximum values of 30 samples, Mean: mean value of 30 sample. RI: Retention Index LRI: retention indices reported from literature (\*Barakat et al. 2013; \*\* Pirbalouti et al. 2013; \*\*\*Figueiredo et al. 2008; \*\*\*\* Palevitch 1991; \*\*\*\*\*http://www.pherobase.com/database/kovats)

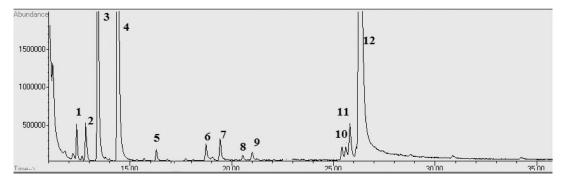
The highest content of carvacrol was found in Diyarbakir-Dicle population (97.9%), whereas the lowest content was found in Antakya-Senkoy population (12.7%). In contrast, oil of Antakya-Senkoy population having a different chemical composition namely thymol was the main compound (37.5%), followed by *p*-cymene (25.4%),  $\gamma$ -terpinene (17%) and carvacrol (12.7%). These variations in the components may be due to biosynthesis of the essential oils is affected by physiological variations (i.e. organ and leaf position), environmental conditions (i.e. harvest date and planting time), geographic variations and genetic factors and evolution (Figueiredo et al., 2008). Generally, when  $\gamma$ -terpinene and *p*-cymene is higher in populations, thymol ratio was lower than in others. A

biosynthetically related increase and decrease in the periodicity of the concentrations can be observed for *p*-cymene and  $\gamma$ -terpinene, while the other values showed no significant change (Muller-Riebau, 1997). Milos et al (2001), in their study with *Satureja cuneifolia*, are reported that thymol and carvacrol represent simply the isomeric monoterpenic phenols, which are biosynthesized through  $\gamma$ -terpinene and *p*-cymene. These differences among the populations may be caused by this biosynthetic pathway.

Our results verify previous studies that major essential oil components of *T. spicata* were carvacrol,  $\gamma$ -terpinene and *p*-cymene (Kizil, 2013; Barakat et al., 2013; Ravid and Putievsky, 1985; Baydar et al., 2004; Unlu et al.,

2009; Markovic et al., 2011). Tansi (1991) found that the main components of *T. spicata* oil were carvacrol (61.15-58.12 %) at the flowering stage in Adana . Tumen et al. (1994) reported that the major component in the oils but one was carvacrol (49.19–76.86%) in *T. spicata*, and thymol (50.71%) was the main component in the oil of one strain of *T. spicata* var. *intricata*. Barakat et al. (2013) found that the main components of *T. spicata* oil in growing wild in Lebanon were carvacrol (16.1–62.9%),  $\alpha$ -thujene (1.7–4.8%), myrcene (1.1–5.1%),  $\gamma$ -terpinene

(11.4–24.1%) and *p*-cymene (8.1–46.8%). In other study, of the 33 compounds identified in *T. spicata* oil, the main components were *p*-cymene (22.1%),  $\gamma$ -terpinene (21.4%), carvacrol (21.1%) and thymol (11.8%) (Akgul et al., 1999). Schulz et al. (2005) reported that percentage of carvacrol in the essential oil of *T. spicata* was 62.3% and 71.7%. Kilic (2006) also reported *T. spicata* essential oils were 60.3% carvacrol, 12.9%  $\gamma$ -terpinene, and 9.6% *p*-cymene as major compounds.



**Figure 3.** GC-MS chromatogram result. Peaks were identified as retention times and given respectively. 1: Myrcene, 2: Carene<Delta-2->, 3: Terpinene<Gamma->, 4: Cymene<Ortho->, 5: Cis-1-octen-3-ol, 6: Caryophyllene<E->, 7: Terpinen-4-ol, 8:  $\alpha$ -terpinolene, 9: Borneol, 10: Cumin alcohol, 11: Thymol, 12: Carvacrol

Table 5. Essential oil comp	osition of different T. spicata	var. <i>spicata</i> populations with	GC and FID detector

	<u>Compounds</u>									
Population Num.	Myrcene	Delta-2- carene	γ- terpinene	<i>p</i> -cymene	E- caryophyllene	Terpinen- 4-ol	Thymol	Caryophyllene oxide	Carvacrol	Total
1	1.6	1.4	8.1	6.5	1.1	0.4	-	0.6	80.1	99.8
2	-	-	3.2	1.5	1.1	0.6	-	0.5	92.8	99.7
3	-	-	-	-	1.3	0.3	-	0.3	97.9	99.8
4	1.6	2.0	15.0	11.2	1.5	0.4	-	0.8	67.1	99.6
5	1.8	2.1	14.5	11.2	1.6	0.5	-	-	68.0	99.6
6	1.6	1.8	9.5	8.1	2.0	0.6	-	-	76.1	99.7
7	1.1	1.4	12.8	7.0	0.9	0.4	-	0.9	74.8	99.3
8	2.4	2.7	21.9	11.4	1.92	0.5	-	-	58.7	99.02
9	2.3	2.6	20.2	9.1	2.0	0.2	-	-	62.4	98.8
10	2.0	2.2	17.0	10.7	1.5	0.5	-	0.8	65.0	99.7
11	2.2	2.4	18.1	11.0	0.8	0.4	-	0.7	63.8	99.4
12	1.7	1.3	13.2	20.2	0.5	0.7	-	1.0	61.0	99.6
13	2.2	2.4	19.2	12.0	0.8	0.4	-	0.7	61.7	99.4
14	1.7	1.9	15.2	8.3	1.6	0.4	0.4	0.8	69.2	99.5
15	2.0	2.5	26.8	18.2	2.0	0.2	-	1.2	46.6	99.5
16	1.6	1.7	16.5	10.0	0.6	0.2	6.0	1.2	61.9	99.7
17	2.2	2.4	18.7	11.1	1.8	-	-	0.9	62.5	99.6
18	1.6	1.7	16.8	10.2	0.5	0.2	6.0	1.2	61.3	99.5
19	2.0	1.4	11.0	18.8	0.6	0.8	-	0.9	59.6	95.1
20	1.7	1.8	12.2	7.3	1.8	0.4	-	0.7	73.7	99.6
21	2.2	2.3	20.3	12.4	0.8	0.5	-	-	61.2	99.6
22	2.2	2.5	25.8	18.7	1.6	0.5	-	1.1	47.1	99.5
23	1.9	2.3	22.4	15.2	1.9	0.3	-	1.0	54.4	99.4
24	4.6	2.3	0.7	0.9	0.8	-	-	1.4	89.1	99.8
25	-	-	1.8	1.2	0.7	0.7	-	-	94.8	99.2
26	1.9	2.0	14.3	9.7	1.6	0.4	-	0.7	68.9	99.5
27	1.3	1.3	13.7	24.2	0.5	0.8	-	1.4	54.3	97.5
28	1.5	1.7	13.9	9.4	2.0	0.5	-	1.0	69.5	99.5
29	2.1	2.5	23.4	15.3	0.9	0.5	5.0	1.2	48.5	99.4
30	1.8	2.1	17.0	25.4	1.8	0.4	37.5	0.7	12.7	99.4

Diyarbakir/1-Cermik, 2- Cungus, 3- Dicle, 4- Hani, 5- Kulp, 6- Lice, Mardin/7- Center, Batman/ 8- Kozluk, 9- Sason, Adiyaman/10- Center, 11-Sincik, 12- Kahta, 13- Kahta Road, 14- Gerger, Sanliurfa/15- Halfeti, Gaziantep/16- Oguzeli, 17- Nizip, 18- Center, Kilis/19- Center, Sirnak/20-Uludere, 21- Eruh to Sirnak 10 km, Adana/22- C.U. Kampus, 23- Pozanti, Mersin/24- Tarsus, Siirt/25- Cevizli, 26- Center, 27- Eruh, Antakya/28-Hassa, 29- Harbiye, 30- Senkoy

Although, the biosynthesis of secondary metabolites are genetically controlled they are strongly affected by environmental influences (Yanivie and Palevitch, 1982). Agricultural factors have a critical effect on quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment. Variations in essential oil rates and compositions may be due to on individual variability. Environmental factors, including cultivation, temperature, moisture, and soil, appear to have an impact on volatile compound production in medicinal and herb crops (Boira and Blanquer, 1998; Medina-Holguin et al., 2007). The results of Karousou et al (2005) partially supports our finding showing that single species growing in different habitat types have different oil composition and high carvacrol content and are associated to the dry dwarf-shrub formations of the lowlands.

Moreover, the occurrence of different oils of a species from different geographical origins may also be determined by the processing of the material after harvest (Figueiredo et al., 2008). Garcia and Sanz (2001) stated that plants concerning to the same species may represent several volatile composition when the differences cannot be related to their environmental properties; these plants may belong to different chemotypes.

#### CONCLUSIONS

Consequently, the Zahter species including Thymbra spicata is largely harvested from the wild populations in the World, especially in Turkey. Medicinal plant production through cultivation can reduce dependence on wild populations; reduce environmental degradation and loss of genetic diversity. The results of this study also showed that yield of dry weight of T. spicata var. spicata under culture condition was more, when it was compared to yield under wild conditions. T. spicata has given high herbage yield under taking cultivation conditions. Essential oil yield of cultivated plants was higher compared to wild plants. The highest dry herbage yield was obtained from population No. 22, 23, 19 and 1, essential oil yield from population No. 27, 2, 13, 25 and 28, and the highest carvacrol content also was obtained from population No. 2, 3 and 26 respectively. Thymbra spicata species could be used as carvacrol source due to its high essential oil contents.

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