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Additional Information

# **Title page**

# Effect of Drought Stress on Essential Oil Composition of *Thymus vulgaris* L. (Chemotype 1, 8-cineole) from wild populations of Eastern Iberian Peninsula

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#### Abstract

1,8-cineole defines a typical chemotype of *Thymus vulgaris* L. in Iberian Peninsula. This compound has a wide range of potentially useful bioactive properties. In order to study the influence of drought stress in the essential oil (EO) composition of this chemotype, sixty plants from six wild populations of Eastern Iberian Peninsula were distilled and analyzed by GC and GC/MS. The harvest dates (May and August) were selected in such a way that the typical summer drought in Mediterranean climates was the critical factor affecting EO composition.

Despite the high intrapopulational variability, significant increases of 1,8-cineole were found after the drought period (21-8 % - 43.2%, in May, up to 42.6 % - 68.5 % in August). On the other hand, individuals from one of the populations showed different profiles rich in linalool and camphor or sesquiterpenoid compounds as  $\alpha$ -cadinol.

Keywords: *Thymus vulgaris*, essential oil, 1,8-cineole, drought stress, intrapopulational variability.

# Introduction

Thyme (*Thymus vulgaris* L.) is a perennial Labiatae widely extended throughout the Mediterranean countries. Given its antiseptic, antifungical and antioxidant properties (1-6) of its essential oil (EO), it can be considered a valuable and promising natural resource with an increasing demand by pharmaceutical, cosmetic and food industries (7, 8). From the ecological point of view, thyme is a species highly adapted to a great diversity of environmental conditions, from the sea level up to 2000 m a.s.l. Indeed, as reported by Jordan *et al.* (1), thyme is one of the most successful species when it comes to colonize eroded lands as consequence of forest degradation. Its blooming stage takes place from March to June, according the particular climatic features. In general, it is precisely the period in which the highest EO yield is achieved (9, 10).

With regard to chemical polymorphism, common thyme shows a relative low number of chemotypes, unlike other thymus species (11). In a first approach, two types of chemotypes can be distinguished: (a) phenolic, whose major compounds are thymol and carvacrol, typical of milder and drier Mediterranean environment; and (b) non-phenolic, which are characterized by the major occurrence of cyclic and acyclic aliphatic oxygenated monoterpenes, adapted to a wider range of habitats, even to extreme climates (12). As reported by Thomson *et al.* (13), four of these non-phenolic chemotypes were found in Southern France and defined by the predominance of geraniol,  $\alpha$ -terpineol, thujan-4-ol and linalool. In addition, another non-phenolic chemotype rich in 1, 8-cineole is endemic of Iberian Peninsula (8, 14, 15) although its occurrence in the French Southern has also been reported (16).

The bioactivity of 1,8-cineole is solidly founded in many researches based on species whose EO is rich in this compound: antifungical activity in EO of *Thymus mastichina* (2), as larvicidal in *Eucalyptus globulus* (17), antimicrobial with synergic activity with limonene (18) and antiseptics such as clorhexidine digluconate (19), etc. Likewise, insecticide and repellent properties are referred by Nerio *et al.* (20) in essential oil of *Ocimum kenyense*, the same way that against *Ceratitis capitata* with *Lavandula spica* EO (21) or *Aphis gosypii* with *Eucaliptus globulus* (22).

This way, to optimize the 1, 8-cineole content in thyme EO has been a relevant target for investigations focused on the different factors affecting yields and chemical profiles, such as plant organ, seasonal variations, water and fertilizer supplies, crop management features, etc. Specifically, water stress has been found to have a noticeable influence on yield and chemical profile of thymus essential oils (23-25). Concerning 1,8-cineole proportions, Leicach et al. (26) have reported an increase of 1,8-cineole and oxygenated sesquiterpenes in Eucaliptus camaldulensis, at the same time that a decrease of non-oxygenated monoterpenes was observed. As pointed out by Velasco-Negueruela & Pérez-Alonso (27), it is a well-known fact that humidity and precipitation regulate oxidation of linalyl pyrophosphate to 1, 8-cineole. The most xeric conditions active it, regulating osmotic pressure in stomata avoiding water loss by evapotranspiration. Nevertheless, no data are available about these changes in wild populations of 1,8-cineole thyme chemotype, given that only studies in crop conditions with irrigation have been conducted (1). Given that Mediterranean climate is characterized by a typical summer drought, thyme wild populations can be considered as suitable 'natural laboratories' to test the effect of drought on essential oils. In Eastern Iberian Peninsula, the first half of August is especially appropriate for this purpose, as, after two months with very scarce rainfall, the typical storms at the end of summer have not yet happened. This way, the goal of this research is to study the effect of summer drought on chemical profile of Spanish thyme chemotype, rich in 1, 8-cineole.

#### **Experimental**

#### **Plant material**

#### Location and sampling

Five randomly selected individual plants keeping a distance of around 10 m at least were collected in each one of the six selected locations (Table 1). The sampling was carried out in two moments related to the most representative phenological stages: (1) full flowering, at the first week of May 2013 and (2) post fruiting stage, in the most dry period (first week of August 2013). The plants were cut at approximately 10 cm height, in such a way that the most lignified stems were not collected in order to make easier the further sprouting. All voucher specimens

were kept at the herbarium of Mediterranean Agroforestal Institute at the Polytechnic University of Valencia (Spain) (VALA 9519 to 9528)

As reported by Ozgüven & Tansi (28) in thyme crops, soil type and chemotype structure are correlated. For this reason, the sampling places were selected in such a way that they have geomorphological variety (Table 1). Likewise, they were also distributed in supramediterranean and termomediterranean zones in order to evaluate the effect of summer drought in different climatic conditions (29, 30).

		High Jiloca basin		Sur	roundings of Vale	ncia
	Supra	amediterranean cl	imate	Term	omediterranean cl	limate
Coordinates <sup>1</sup>	Calamocha (Sta. Bárbara)	Calamocha (Lechago)	Calamocha (Navarrete)	Xeraco (Font de l'Ull)	Bugarra (Crta. Gestalgar)	Serra (Camí del Castell)
Altitude <sup>1</sup>	40° 56' 36,3 " N 1° 22' 10,2 " W 1050	40° 57' 17,5 " N 1° 16' 40,5 " W 920	40° 55' 10,1 " N 1° 14' 31,1 " W 977	40° 56' 36,3 " N 15' 30,48" W 209	40° 57' 17,5 " N 46' 37,3 " W 194	40° 55' 10,1 " N 25' 23,7" W 387
Average yearly temp. (°C) <sup>2</sup>	10.8	10.8	10.8	18.0	16.1	15.3
Average yearly precip. (mm) <sup>2</sup>	414	414	414	485	406	469
Geomorphological features <sup>3</sup>	Cuarzites	Siliceous conglomerates, limonites and red clays	Gypsums and gipsy limonites	Limestone with dolomites	Clays variegated with gypsums	Sandstone

# Table 1 Climatic and geomorphological features of sampling locations

1. Source: Google Earth

2. Data source: <u>http://es.climate-data.org</u>

3. Data source: Instituto Geológico y Minero de España. http://info.igme.es/cartografia/magna50.asp#PDFs

# Essential oil distillation

The fresh material (leaves, flowers or fruits) removed from the stems belonging to each individual plant (10-15 g) was subjected to hydrodistillation for 1.5 h in a Clevenger type apparatus. The essential oil was swept away with 5 mL of dichloromethane. After removing aqueous phase, the essential oil extract was dried with anhydrous sodium sulphate and kept at -18°C in sealed vials until chromatographic analysis.

# Meteorological data

Meteorological data over this period (Fig.1, 2) were obtained from the nearest meteorological station for each location above-mentioned: Calamocha, Gandía (Xeraco), Líria (Bugarra) and Bétera (Serra)

### Analysis of the essential oils

The analysis of samples was carried out by GC and GC/MS. A Clarus 500 GC (Perkin-Elmer Inc.) chromatograph equipped with a FID detector and capillary column ZB-5 (30 m x 0.25 mm i.d. x 0.25  $\mu$ m film thickness) was used for quantitative analysis. The injection volume was 1  $\mu$ L. The GC oven temperature was programmed from 50°C to 250°C at a rate of 3°C min<sup>-1</sup>. Helium was the carrier gas (1.2 mL min<sup>-1</sup>). Injector and detector temperatures were set at 250°C. The percentage composition of the essential oil was computed from GC peak areas without correction factors by means of the software Total Chrom 6.2 (Perkin-Elmer Inc., Wellesley, PA)

Analysis by GC-MS was performed using a Clarus 500 GC-MS (Perkin-Elmer Inc.) with the same capillary column, carrier and operating conditions described above for GC analysis. Ionization source temperature was set at 200°C and 70 eV electron impact mode was employed. MS spectra were obtained by means of total ion scan (TIC) mode (mass range m/z 45-500 uma). The total ion chromatograms and mass spectra were processed with the software Turbomass 5.4 (Perkin-Elmer Inc.).

Retention indices were determined by injection of C8–C25 *n*-alkanes standard (Supelco<sup>®</sup>) under the same conditions. The essential oil components were identified by comparison of their mass spectra with those of computer library NIST MS Search 2.0 and available data in the literature (31). The identification of the following compounds were confirmed by comparison of their experimental RI with those of authentic reference standards (Sigma-Aldrich<sup>®</sup>):  $\alpha$ -pinene,  $\beta$ pinene, camphene, myrcene, camphor, terpinolene, borneol, terpinen-4-ol, bornyl acetate and linalool.

#### Statistical analysis

The statistical analyses were carried out by means of analysis of variance (ANOVA) using Statgraphics 5.1. Software. As the raw data were expressed as % peak areas, they were subjected to  $\arcsin[\text{square root}(\%/100)]$  transformation and previous homocedasticity test. Then, Tukey's HSD multiple-range test at P<0.05 was used to consider significant differences in average values of the components among the sampling dates.

#### **Results and Discussion**

#### Climate results

Average daily temperatures and accumulated precipitation over the period between both samplings are displayed in figures 1, 2. Regarding temperature, it shows the typical seasonal change increasing during May and June to become stable in July and August.

Except for Xeraco, with an unusual dry spring, the typical spring rainfalls took place in the rest of locations. In the other close to coast locations (Bugarra and Serra), the rainfall was practically negligible from end of June on. On the contrary, in supramediterranean places the convective summer storms provided a bit of rainfall during summer months.

Figure 1. Average daily temperatures between both samplings. Data source: www.accuweather.com

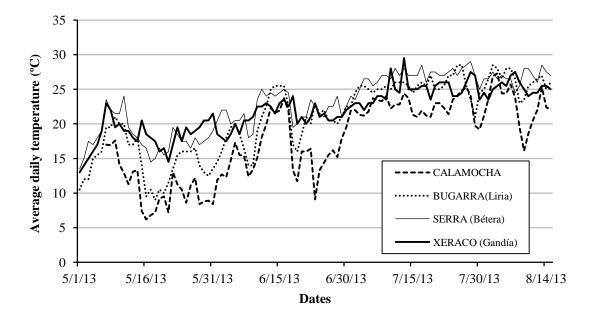
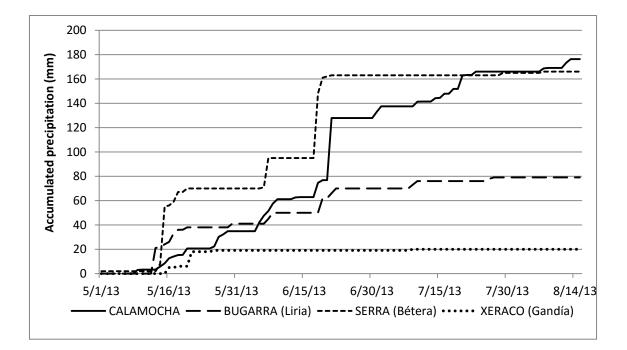
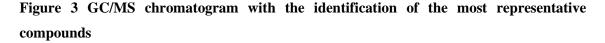


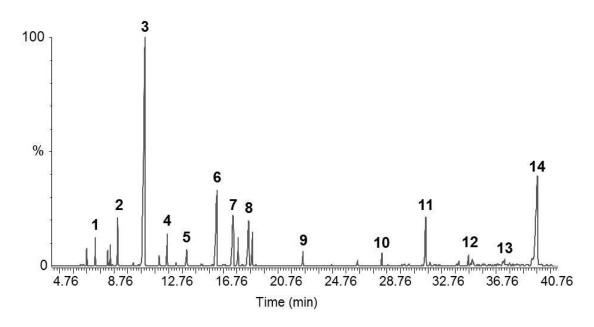
Figure 2. Accumulated precipitation between samplings. Data source: www.accuweather.com



# **Chemical profiles**

Six wild thyme populations belonging to different edaphoclimatic conditions have been studied in the present work. The typical profile of 1,8-cineole chemotype is shown in figure 3. It was observed for five of the studied locations (Tables 2, 3) but samples from Xeraco could not be considered belonging to this chemotype. Indeed, seven of the ten plants coming from this location were found rich in linalool (48.3-80.6 %), such as the chemotype referred by Thompson et al. (14) in Southern France. The main components in the other individuals were  $\alpha$ cadinol and camphor (Table 4).





1,8-cineole was the major compound accounting for 21.8 - 43.2 % in May (full blooming) and 42.6 - 68.5 % in August (after drought period) in the other five populations (Tables 2, 3). This increase was strong and statistically significant except for one termomediterranean location (Bugarra) (Table 3). A similar although less pronounced change was already reported by Salas et al. (32) in a wild population of a phenolic thymus (*Thymus praecox* ssp. *penyalarensis*).

Other compounds showed a relative high proportion although with noticeable intrapopulational variability: camphor (0.3 - 18.8 %), borneol (0.9 - 9.9 %), camphene (0.1 - 6.9 %), linalool (0.5 %)-6.7 %), myrcene (t -5.8 %),  $\beta$ -pinene (t -3.7 %). The sesquiterpenic fraction, mainly the oxygenated one, presented a high variability in which some compounds achieved relative high proportions: an unidentified oxygenated sesquiterpene (0 – 15.9 %),  $\tau$ -cadinol (t – 10.0%), elemol (t - 5.7 %) and spathulenol (t - 4.4 %). The main sesquiterpene hydrocarbons were bicyclogermacrene (0 - 8.6 %) and  $\beta$ -caryophyllene (t - 5.2 %). In general, no significant changes were found in these compounds between both sampling dates. Only significant differences were noted in camphor and borneol for some locations, but no regular tendencies could be observed. However, if the compounds sharing camphene skeleton are considered as a whole, some regularities were detected: a decrease in August is observed in 1,8-cineole chemotype for termomediterranean places whereas it remains stable in supramediterranean ones. Specifically, sesquiterpene hydrocarbons in 1,8-cineole samples accounted for 4.5 - 14.1% in May and 1.4 - 4.8 % in August, showing a statistically significant decrease (Tables 2, 3). Oxygenated sesquiterpenes showed a similar behavior: a significant and more pronounced decrease was observed from May (5.9 - 25.9 %) to August (1.2 - 11.3 %). Qualitative and quantitative significant changes in essential oil profiles due to water deficit have been also found in other studies concerning phenolic Thymus daenensis and Thymus carmanicus (24, 33) and other species as Cymbopogon nardus (34) or Salvia officinalis (35).

				Nav	arrete	امر	hago	Santa	Bárbara
							-		
1	2	. 3		May	August	May	August	May	August
Compounds <sup>1</sup>	RI exp <sup>2</sup> .	RI lit. <sup>3</sup>	Identification <sup>4</sup>			_			
tricyclene	925	927	GC/MS, RI	0.1 ±0.1	-	t5	_6	-	$0.1 \pm 0.1$
lpha-thujene	929	930	GC/MS, RI	t	0.4 ±0.3	t	0.3 ±0.2	$0.2 \pm 0.3$	$0.6 \pm 0.1$
α-pinene	937	939	GC/MS, RI, std.	1.0 ± 0.6	1.8 ±0.7	t	1.7 ±0.7	1.1 ±0.9	0.7 ± 0.2
camphene	953	954	GC/MS, RI	2.9 ± 2.0	2.5 ± 1.8	1.3 ±1.0	1.9 ± 1.2	3.9 ± 3.5	5.9 ± 3.2
sabinene	975	975	GC/MS, RI	1.1 ± 0.5	3.0 ± 1.0	0.9 ±0.6	-	1.1 ±0.9	1.0 ± 1.0
α-pinene	979	979	GC/MS, RI, std.	1.7 ±0.7	3.7 ±1.2	1.2 ±0.7	2.8 ±0.6	1.7 ± 1.0	2.7 ±0.3
1-octen-3-ol	983	979	GC/MS, RI	-	t	-	-	-	-
myrcene	992	991	GC/MS, RI, std.	4.7 ± 2.7	5.0 ± 1.6	5.6 ± 3.6	4.3 ± 1.1	2.7 ± 3.4	2.0 ± 1.8
3-octanol	997	991	GC/MS, RI	-	-	-	-	t	-
$\alpha$ -terpinene	1021	1017	GC/MS, RI, std.	t	0.2 ±0.1	0.1 ±0.2	-	0.4 ±0.5	0.4 ± 0.4
<i>p</i> -cymene	1027	1025	GC/MS, RI, std.	0.4 ± 0.7	0.4 ±0.5	0.7 ±1.0	0.1 ±0.0	1.4 ± 2.8	2.8 ± 5.2
limonene	1032	1029	GC/MS, RI, std.	1.6 ± 0.7	1.3 ±1.9	1.1 ±0.7	0.3 ±0.2	0.7 ± 1.0	0.9 ± 1.4
1,8-cineol <sup>7</sup>	1035	1031	GC/MS, RI, std.	26.8 ± 8.6	50.5 ± 11.9 <sup>**</sup>	21.8 ± 9.1	55.1 ±8.7 <sup>**</sup>	24.5 ± 11.0	42.6 ± 13.6 <sup>*</sup>
(Z)-β-ocimene	1040	1037	GC/MS, RI, std.	-	-	0.2 ±0.1	-	-	-
(E)-β-ocimene	1050	1050	GC/MS, RI	1.9 ± 1.6	0.4 ±0.2	3.1 ±1.2	2.8 ± 1.0	0.6 ± 0.5	0.7 ± 0.4
γ-terpinene	1061	1060	GC/MS, RI, std.	0.5 ±0.6	0.9 ±0.7	1.4 ± 1.3	0.4 ±0.1	2.8 ± 4.7	2.0 ± 2.5
(Z)-sabinenhydrate	1072	1070	GC/MS, RI	0.7 ± 0.2	0.9 ±0.6	1.2 ±0.5	1.2 ±0.2	0.5 ±0.7	1.2 ± 0.8
terpinolene	1086	1089	GC/MS, RI, std.	t	0.1 ±0.0	t	t	0.2 ± 0.3	0.2 ±0.1
linalool	1099	1099	GC/MS, RI, std.	2.1 ± 1.9	1.2 ±0.5	6.7 ±7.5	3.7 ± 2.5	0.9 ± 1.2	1.2 ± 1.5

# Table 2. Thymus vulgaris essential oil composition of three populations from Teruel (mean values ± SD of five individuals)

lpha-campholenal	1127	1126	GC/MS, RI	-	-	t	t	t	0.1 ±0.1
camphor	1147	1146	GC/MS, RI, std.	3.2 ± 5.0	6.2 ± 3.1	6.8 ± 1.3	4.9 ± 1.2 <sup>*</sup>	8.8 ± 4.2	13.1 ±6.8
3-pinenone	1160	1162	GC/MS, RI	t	0.5 ± 1.0	-	-	-	t
2,6-Dimethyl-1,5,7-octatrien-3-ol	1162 <sup>7</sup>		GC/MS	0.1 ±0.3	-	-	-	-	-
borneol	1172	1169	GC/MS, RI	4.1 ± 1.7	2.3 ± 1.6	4.5 ± 3.0	2.5 ± 1.2	9.9 ± 5.8	7.5 ± 4.4
terpinen-4-ol	1181	1177	GC/MS, RI, std.	0.6 ±0.1	1.8 ± 1.8	1.0 ± 0.7	0.5 ± 1.0	0.3 ±0.6	0.0 ± 0.0
alpha-terpineol	1193	1195	GC/MS, RI	-	-	-	0.9 ± 0.2	-	0.5 ±0.6
hexenyl 3-methylbutanoate <(3Z)>	1233	1247	GC/MS, RI	-	-	-	-	-	t
linalyl acetate	1252	1257	GC/MS, RI	-	-	2.3 ± 3.3	0.2 ± 0.2	-	-
bornyl acetate	1283	1289	GC/MS, RI	0.9 ±0.5	0.2 ±0.2	2.2 ± 1.5	0.6 ±0.3	4.3 ± 3.6	0.5 ±0.3
thymol	1296	1299	GC/MS, RI, std.	-	-	-	-	t	-
$\delta$ -terpinyl acetate	1311	1317	GC/MS, RI	0.1 ±0.2	t	0.5 ± 0.3	0.8 ± 0.5	0.3 ±0.4	0.2 ± 0.4
δ-elemene	1329	1335	GC/MS, RI	-	-	t	-	-	-
$\beta$ -terpinyl acetate	1347	1359	GC/MS	$0.4 \pm 0.8$	t	2.1 ± 1.3	3.1 ± 1.9	1.6 ± 2.0	0.8 ± 1.8
isobornyl propanoate	1374	1384	GC/MS, RI	0.3 ±0.2	0.3 ±0.2	0.3 ±0.5	t	0.6 ± 0.3	0.3 ±0.4
$\beta$ -bourbonene	1379	1388	GC/MS, RI	0.1 ±0.1	0.1 ±0.1	0.1 ±0.2	0.1 ± 0.2	t	0.3 ±0.3
β-elemene	1386	1390	GC/MS, RI	-	-	-	t	-	0.2 ± 0.3
β-caryophyllene	1414	1419	GC/MS, RI	4.1 ±0.6	2.7 ± 2.3	4.1 ± 2.1	1.5 ± 1.1	5.2 ± 3.1	0.2 ±0.2
β-copaene	1426	1432	GC/MS, RI	-	t	-	t	-	0.5 ±0.7
aromadendrene	1433	1441	GC/MS, RI	-	-	-	-	t	t
α-humulene	1450	1454	GC/MS, RI	-	-	t	-	t	t
alloaromadendrene	1455	1460	GC/MS, RI	0.3 ±0.2	0.3 ±0.2	0.2 ±0.1	t	-	-
bornyl butanoate	1463	1470	GC/MS, RI	0.1 ±0.1	0.1 ±0.2	0.1 ±0.2	-	0.4 ±0.3	0.1 ±0.2
germacrene-D	1477	1485	GC/MS, RI	0.4 ±0.5	0.2 ±0.1	1.0 ± 1.5	0.2 ±0.1	0.6 ±0.5	0.0 ± 0.1
bicyclogermacrene	1492	1500	GC/MS, RI	7.6 ± 5.7	1.4 ±0.8	8.6 ± 5.1	1.4 ± 1.3	5.4 ± 4.1	0.4 ±0.6

γ-cadinene	1509	1513	GC/MS, RI	-	-	0.1 ±0.2	-	-	$0.1 \pm 0.1$
δ-cadinene	1515	1523	GC/MS, RI	0.2 ±0.4	0.1 ±0.1	t	t	t	$0.0 \pm 0.0$
elemol	1546	1549	GC/MS, RI	1.2 ± 2.4	2.3 ± 4.8	t	0.1 ±0.2	5.7 ± 6.0	0.8 ± 1.1
spathulenol	1571	1578	GC/MS, RI	4.4 ± 2.0	1.9 ±0.9	1.1 ±0.9	t	1.3 ± 1.6	0.4 ± 0.5
caryophyllene oxide	1576	1583	GC/MS, RI	-	-	0.4 ±0.1	1.4 ±1.0	1.0 ± 0.5	0.3 ± 0.5
cubenol <1,10-di-epi>	1610	1619	GC/MS, RI	t	t	-	-	-	-
caryophylla-4(12),8(13)-dien-5 $eta$ -ol	1638	1640	GC/MS, RI	-	t	t	-	-	-
τ-cadinol	1640	1640	GC/MS, RI	-	-	-	-	0.1 ±0.2	-
β-eudesmol	1649	1650	GC/MS, RI	0.5 ±0.3	0.2 ±0.4	0.8 ±1.0	-	0.1 ±0.1	0.1 ±0.2
$\alpha$ -cadinol	1651	1654	GC/MS, RI	1.2 ± 1.0	0.3 ±0.2	0.8 ±1.0	t	0.8 ±0.9	-
calamenen-10-ol <cis></cis>	1656	1660	GC/MS, RI	0.4 ±0.7	1.6 ± 3.0	t	t	0.6 ± 0.7	0.2 ± 0.3
elemol acetate	1668	1680	GC/MS, RI	-	-	-	-	-	0.4 ± 1.0
eudesma-4(15),7-dien-1-beta-ol	1678	1687	GC/MS, RI	0.3 ±0.4	0.3 ±0.6	t	-	1.0 ± 1.0	$0.1 \pm 0.1$
eudesm-7(11)-en-4-ol	1689	1700	GC/MS, RI	1.8 ± 4.1	0.1 ±0.2	0.2 ±0.3	-	1.7 ± 3.7	-
Unknow <sup>8</sup>	1704		GC/MS	15.9 ±8.4	4.2 ± 2.6	15.0 ± 9.2	1.6 ± 1.8	7.1 ± 3.2	0.4 ±0.5
Monoterpenes				15.9 ± 4.1	19.6 ± 3.7	16.4 ±4.5	14.8 ± 2.1	16.6 ± 7.6	20.0 ± 7.1
Oxygenated monoterpenes				39.3 ± 10.3	64.1 ± 12.6 <sup>**</sup>	49.5 ± 12.8	73.5 ± 9.5 <sup>**</sup>	52.1 ± 13.8	68.3 ± 16.0 <sup>*</sup>
Sesquiterpenes				12.5 ± 5.7	4.8 ± 2.4 <sup>*</sup>	14.1 ± 5.8	3.5 ±1.7 <sup>*</sup>	11.2 ± 5.2	$1.8 \pm 1.1^{**}$
Oxygenated sesquiterpenes				25.9 ± 9.9	11.2 $\pm 6.3^{*}$	18.6 ± 9.3	3.3 ± 2.1 <sup>**</sup>	19.4 ± 8.0	2.7 ± 2.0 <sup>**</sup>
Other				-	t	-	-	t	t
Total identified				76.8	95.5	83.6	93.4	92.3	91.4

Notes:

1. Elution order as determined on ZB-5 column.

2. Retention indices as determined on ZB-5 column using homologous series of n-alkanes.

3. Retention indices from Adams, R. P. (2007), except for those deliberately pointed out.

4. Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample.

5. -: no detected

6. t: traces (<0.1%).

7. Pairs of values within boxes show significant differences: \*(P<0.05), \*\* (P<0.01)

8. MS, 70 eV, 200°C, m/z(rel. int.): 77 (45), 91 (74), 93 (56), 105 (72), 131 (53), 145 (60), 159 (100), 187 (29), 202 (24), 220 (3)

			-	Xeraco		Xeraco (Che	m. Linalool)	Bug	jarra	Ser	rra	
			-	May	August	May	August	May	August	May	August	
Compounds <sup>1</sup>	RI exp <sup>2</sup> .	RI lit. <sup>3</sup>	Identification <sup>4</sup>									
tricyclene	925	927	GC/MS, RI	-	t	-	t	t	0.1 ±0.1	0.1 ±0.1	0.4 ±0.1	
$\alpha$ -thujene	929	930	GC/MS, RI	t	0.3 ± 0.4	t	$0.1 \pm 0.1$	0.2 ± 0.2	0.2 ±0.1	0.2 ±0.2	-	
$\alpha$ -pinene	937	939	GC/MS, RI, std.	0.4 ±0.5	1.9 ± 2.3	0.2 ±0.1	0.6 ± 0.7	1.8 ± 0.5	3.1 ± 0.4	1.5 ±0.7	2.1 ± 1.0	
camphene	953	954	GC/MS, RI	0.1 ±0.1	6.9 ± 7.6	0.1 ±0.1	1.7 ± 2.3	2.3 ± 2.1	1.3 ± 2.3	3.0 ± 1.6	3.3 ± 1.5	
sabinene	975	975	GC/MS, RI	-	t	-	-	0.4 ± 0.7	0.3 ±0.7	$1.4 \pm 0.4$	2.0 ± 0.6	
$\alpha$ -pinene	979	979	GC/MS, RI, std.	1.5 ± 2.5	t	0.4 ±0.3	t	2.1 ± 0.9	3.0 ± 0.4	1.9 ±0.5	3.1 ± 1.0	
1-octen-3-ol	983	979	GC/MS, RI	0.2 ± 0.4	0.5 ±0.5	0.2 ±0.5	0.2 ± 0.2	3.4 ± 1.3	-	-	-	
myrcene	992	991	GC/MS, RI, std.	0.7 ±0.4	t	0.7 ±0.5	t	5.7 ±1.8	3.4 ±1.5	5.8 ± 2.0	3.8 ±1.3	
3-octanol	997	991	GC/MS, RI	0.3 ±0.7	0.5 ± 0.4	0.4 ±0.8	0.3 ± 0.3	-	-	-	-	
$\alpha$ -terpinene	1021	1017	GC/MS, RI, std.	t	0.2 ±0.1	t	$0.1 \pm 0.1$	$0.0 \pm 0.1$	t	0.3 ±0.2	0.5 ±0.6	
<i>p</i> -cymene	1027	1025	GC/MS, RI, std.	0.1 ±0.3	-	t	-	$0.1 \pm 0.1$	t	2.0 ± 1.3	0.6 ± 1.4	
limonene	1032	1029	GC/MS, RI, std.	0.9 ±0.7	0.8 ± 0.9	0.7 ±0.4	0.3 ±0.3	1.0 ± 1.2	$0.4 \pm 0.4$	2.0 ± 1.3	1.0 ± 2.2	
1,8-cineol <sup>7</sup>	1035	1031	GC/MS, RI, std.	10.5 ±11.0	3.8 ± 2.2	12.9 ±11.0	2.3 ±1.0	43.2 ± 28.9	68.5 ± 19.3	30.5 ±10.3	50.1 $\pm$ 8.0 <sup>*</sup>	
( <i>Z</i> )-β-ocimene	1040	1037	GC/MS, RI, std.	0.3 ±0.6	t	0.3 ±0.7	t	-	-	-	-	
benzeneacetaldehyde	1042	1036	GC/MS, RI	-	-	-	-	-	-	t	-	
( <i>E</i> )-β-ocimene	1050	1050	GC/MS, RI	1.0 ± 1.7	0.3 ±0.2	0.3 ±0.6	$0.1 \pm 0.1$	1.4 ± 2.7	0.1 ±0.1	1.0 ± 0.7	0.3 ±0.5	
γ-terpinene	1061	1060	GC/MS, RI, std.	0.1 ±0.1	0.4 ± 0.4	0.1 ±0.1	0.2 ± 0.2	0.9 ± 1.4	0.5 ±0.2	1.0 ± 0.6	1.1 ±0.4	
(Z)-sabinenhydrate	1072	1070	GC/MS, RI	0.7 ± 0.5	1.1 ±0.6	0.8 ±0.4	1.5 ± 0.2	1.6 ± 1.0	0.9 ±0.2	3.5 ± 1.7	2.2 ± 0.8	
terpinolene	1086	1089	GC/MS, RI, std.	0.2 ±0.2	0.5 ±0.5	0.3 ±0.2	0.7 ± 0.5	t	t	0.2 ±0.1	0.2 ±0.1	
linalool oxide	1088	1087	GC/MS, RI	t	0.2 ± 0.4	t	0.3 ±0.5	$0.1 \pm 0.1$	t	-	-	

Table 3. Thymus vulgaris essential oil composition of three populations from Valencia (mean values ± SD of five individuals)

linalool	1099	1099	GC/MS, RI, std.	53.5 ± 32.3	43.1 ± 39.2	66.9 ±14.2	71.0 ± 12.4	1.5 ±1.4	0.5 ±0.4	2.4 ±0.7	0.9 ±0.5
octen-3-yl acetate <1->	1109	1110	GC/MS, RI	-	0.1 ±0.2	-	0.2 ±0.3	t	0.3 ±0.1	-	-
lpha-campholenal	1127	1126	GC/MS, RI	t	0.2 ±0.2	t	t	t	t	0.1 ±0.1	0.2 ± 0.0
(E)-pinocarveol (GC/MS)	1142	1139	GC/MS, RI	-	t	-	t	-	-	0.3 ±0.4	0.3 ± 0.2
camphor	1147	1146	GC/MS, RI, std.	0.5 ±0.6	18.8 ± 19.2	0.4 ±0.5	5.2 ± 5.8	5.7 ±7.9	3.6 ± 7.3	6.3 ± 4.0	0.3 ± 0.3 <sup>*</sup>
3-pinenone	1160	1162	GC/MS, RI	-	t	-	-	t	0.1 ±0.1	2.4 ± 5.3	7.8 ± 3.0
2,6-Dimethyl-1,5,7-octatrien-3-ol	1162		GC/MS	t	$0.1 \pm 0.1$	t	t	t	-	t	0.2 ± 0.1
borneol	1172	1169	GC/MS, RI	0.9 ±1.2	1.0 ± 2.0	1.0 ± 1.3	1.7 ± 2.6	5.1 ± 2.0	2.6 ± 0.3 <sup>*</sup>	3.3 ± 2.6	ť
terpinen-4-ol	1181	1177	GC/MS, RI, std.	0.4 ±0.3	7.9 ± 7.8	0.3 ±0.3	3.0 ± 5.0	-	-	1.9 ± 3.1	4.3 ± 1.7
alpha-terpineol	1193	1195	GC/MS, RI	0.1 ±0.2	1.4 ± 1.2	0.1 ±0.2	0.7 ±0.6	0.9 ± 0.4	1.1 ±0.7	2.7 ±0.9	2.7 ± 0.4
hexenyl 3-methylbutanoate <(3Z)>	1233	1247	GC/MS, RI	0.3 ±0.6	0.2 ± 0.2	0.3 ±0.6	t	-	-	-	-
linalyl acetate	1252	1257	GC/MS, RI	t	t	t	-	-	-	-	t
bornyl acetate	1283	1289	GC/MS, RI	t	0.6 ± 0.7	t	0.1 ±0.2	0.7 ± 0.5	t	1.3 ±0.6	$0.1 \pm 0.1$
$\beta$ -terpinyl acetate	1347	1359	GC/MS	t	-	0.1 ±0.2	-	0.1 ±0.2	0.1 ±0.2	-	-
isobornyl propanoate	1374	1384	GC/MS, RI	0.4 ±0.5	t	0.5 ±0.5	-	0.2 ± 0.2	-	0.2 ±0.1	0.2 ± 0.2
$\beta$ -bourbonene	1379	1388	GC/MS, RI	0.1 ±0.2	0.4 ±0.2	0.1 ±0.3	0.4 ±0.3	-	-	-	-
β-elemene	1386	1390	GC/MS, RI	t	t	-	t	t	0.2 ±0.2	-	-
β-caryophyllene	1414	1419	GC/MS, RI	0.9 ±0.9	0.1 ±0.1	1.1 ±0.9	t	0.9 ±1.4	0.1 ±0.3	2.4 ± 1.8	1.0 ± 0.8
$\beta$ -copaene	1426	1432	GC/MS, RI	0.1 ±0.3	0.7 ± 0.4	0.2 ±0.3	1.0 ± 0.3	0.7 ± 1.0	0.3 ±0.2	-	-
aromadendrene	1433	1441	GC/MS, RI	-	t	-	t	-	-	-	-
$\alpha$ -humulene	1450	1454	GC/MS, RI	-	t	-	0.1 ±0.1	t	t	0.2 ±0.1	t
alloaromadendrene	1455	1460	GC/MS, RI	0.3 ±0.4	0.2 ±0.1	t	0.1 ±0.0	0.2 ± 0.4	0.1 ±0.2	0.2 ±0.3	0.3 ±0.1
bornyl butanoate	1463	1470	GC/MS, RI	-	t	-	t	0.2 ± 0.2	t	-	-
germacrene-D	1477	1485	GC/MS, RI	2.1 ± 1.3	0.2 ± 0.1	2.4 ±1.3	0.3 ±0.1	0.3 ± 0.7	0.4 ±0.5	$0.0 \pm 0.1$	t
valencene	1490	1496	GC/MS, RI	0.9 ±0.9	-	1.1 ± 1.0	-	2.3 ± 1.9	0.1 ±0.3	1.7 ± 1.7	t

bicyclogermacrene	1492	1500	GC/MS, RI	0.2 ± 0.4	0.5 ±0.2	0.2 ±0.5	0.5 ± 0.2	-	-	1.6 ± 2.3	1.0 ±0.7
γ-cadinene	1509	1513	GC/MS, RI	2.2 ± 4.6	t	0.2 ±0.4	$0.1 \pm 0.1$	t	t	0.1 ±0.1	$0.1 \pm 0.0$
$\delta$ -cadinene	1515	1523	GC/MS, RI	0.2 ± 0.3	0.1 ±0.1	t	$0.1 \pm 0.1$	0.2 ± 0.3	t	0.2 ±0.3	0.2 ± 0.1
elemol	1546	1549	GC/MS, RI	1.2 ± 1.9	-	1.4 ± 2.1	-	-	-	-	-
spathulenol	1571	1578	GC/MS, RI	0.4 ± 0.3	3.0 ± 1.6	0.5 ±0.3	2.7 ± 2.1	0.7 ± 0.9	0.4 ±0.9	0.6 ±0.5	t
caryophyllene oxide	1576	1583	GC/MS, RI	0.4 ± 0.3	$0.8 \pm 0.4$	0.5 ±0.2	0.7 ± 0.4	-	-	0.9 ±0.8	3.6 ± 2.0
cubenol <1,10-di-epi>	1610	1619	GC/MS, RI	-	t	-	t	t	t	t	t
$\delta$ -cadinol	1638	1640	GC/MS, RI	2.1 ± 4.3	t	0.2 ±0.4	$0.1 \pm 0.1$	0.1 ± 0.2	t	t	t
τ-cadinol	1640	1640	GC/MS, RI	t	$0.1 \pm 0.1$	t	0.2 ± 0.1	0.1 ±0.1	t	-	t
$\beta$ -eudesmol	1649	1650	GC/MS, RI	-	-	-	-	0.1 ±0.1	0.1 ±0.2	-	-
$\alpha$ -cadinol	1651	1654	GC/MS, RI	10.0 ± 18.8	t	1.7 ± 1.9	t	0.5 ± 0.7	t	-	-
calamenen-10-ol <cis></cis>	1656	1660	GC/MS, RI	0.8 ± 1.2	0.3 ±0.4	0.3 ±0.3	0.5 ±0.5	0.2 ± 0.4	t	-	$0.1 \pm 0.1$
elemol acetate	1668	1680	GC/MS, RI	0.3 ± 0.3	-	0.2 ±0.4	-	0.3 ± 0.2	0.2 ±0.3	-	0.7 ±0.5
eudesma-4(15),7-dien-1-beta-ol	1678	1687	GC/MS, RI	$0.1 \pm 0.1$	t	t	0.1 ±0.2	$0.1 \pm 0.1$	t	-	t
eudesm-7(11)-en-4-ol	1689	1700	GC/MS, RI	0.1 ±0.2	-	0.2 ±0.2	-	t	t	-	-
Unknow <sup>8</sup>	1704		GC/MS	-	0.0 ±0.1	-	t	13.5 ± 14.1	-	12.4 ±4.6	2.6 ± 1.9
Monoterpenes				5.5 ± 3.3	11.6 ± 8.0	3.1 ± 1.2	3.9 ± 2.5	16.0 ± 4.5	12.5 ± 5.1	20.3 ± 3.4	18.3 ± 3.7
Oxygenated monoterpenes				67.2 ± 34.2	78.3 ± 44.5	83.2 ± 18.1	85.9 ±8.1	59.3 ± 30.1	77.7 ± 20.7	54.9 ± 13.1	69.3 ±8.8 <sup>*</sup>
Sesquiterpenes				6.9 ± 5.0	2.2 ±0.9	5.4 ± 2.0	$2.5 \pm 1.0^{*}$	4.5 ± 2.7	1.4 ±0.7 <sup>*</sup>	6.0 ± 3.4	2.4 ± 1.1
Oxygenated sesquiterpenes				15.6 ± 19.4	4.6 ± 2.3	5.2 ± 3.1	4.5 ± 2.9	15.8 ± 14.2	0.9 ±0.9	14.1 ± 4.7	7.3 ± 2.8
Other				0.8 ± 1.0	0.3 ±0.7	0.2 ± 1.1	0.7 ±0.8	2.1 ± 1.3	0.3 ±0.1	t	-
Total identified				96.0	97.9	97.5	98.4	84.3	93.9	82.2	94.5

Notes:

1. Compounds ordered by elution time from a ZB-5 column.

2. Retention indices as determined on ZB-5 column using homologous series of n-alkanes.

3. Retention indices from Adams, R. P. (2007), except for those expressly referred.

4. Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample.

5. -: no detected

6. tr: traces (<0.1%).

7. Pairs of values within boxes show significant differences \*(P<0.05), \*\* (P<0.01)

8. MS, 70 eV, 200°C, m/z(rel. int.): 77 (45), 91 (74), 93 (56), 105 (72), 131 (53), 145 (60), 159 (100), 187 (29), 202 (24), 220 (3))

								Xei	raco				
						May					August		
Compounds <sup>1</sup>	RI exp <sup>2</sup> .	RI lit. <sup>3</sup>	Identification <sup>4</sup>	1	2	3	4	5	1	2	3	4	5
tricyclene	925	927	GC/MS, RI	_5	-	-	-	-	0.2	-	0.10	-	0.1
α-thujene	929	930	GC/MS, RI	-	-	-	-	t <sup>6</sup>	1.0	-	0.46	-	0.2
α-pinene	937	939	GC/MS, RI, std.	1.3	0.3	0.1	0.3	0.1	5.7	0.2	2.1	0.1	1.4
camphene	953	954	GC/MS, RI	0.3	-	0.2	0.2	-	17.7	0.6	11.6	0.3	4.4
sabinene	975	975	GC/MS, RI	-	-	-	-	-	t	-	0.1	-	-
β-pinene	979	979	GC/MS, RI, std.	6.0	0.7	0.1	0.6	0.2	0.2	t	0.1	-	0.1
1-octen-3-ol	983	979	GC/MS, RI	-	-	-	0.9	-	1.3	0.1	0.9	-	0.4
myrcene	992	991	GC/MS, RI, std.	0.8	1.18	1.1	0.1	0.4	0.1	t	t	t	0.1
3-octanol	997	991	GC/MS, RI	-	-	-	1.6	-	1.1	0.3	0.6	0.1	0.6
α-terpinene	1021	1017	GC/MS, RI, std.	t	-	-	-	t	0.5	0.1	0.2	0.1	0.2
<i>p</i> -cymene	1027	1025	GC/MS, RI, std.	0.6	t	t	-	t	-	-	-	-	-
limonene	1032	1029	GC/MS, RI, std.	2.0	1.1	0.9	t	0.7	2.3	0.1	1.0	t	0.6
1,8-cineol	1035	1031	GC/MS, RI, std.	0.7	17.3	3.6	26.5	4.3	5.5	3.2	6.5	1.3	2.4
(Z)-β-ocimene	1040	1037	GC/MS, RI, std.	-	-	1.4	-	-	0.1	-	t	-	t
$(E)$ - $\beta$ -ocimene	1050	1050	GC/MS, RI	3.9	1.2	t	-	t	0.5	0.1	0.5	t	0.3

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# Table 4 Chemical profiles of individuals coming from Xeraco (Valencia)

γ-terpinene	1061	1060	GC/MS, RI, std.	0.3	-	0.13	t	0.2	0.92	t	0.4	-	0.4
(Z)-sabinenhydrate	1072	1070	GC/MS, RI	0.2	0.3	0.8	1.0	1.4	0.7	1.5	0.3	1.3	1.8
terpinolene	1086	1089	GC/MS, RI, std.	-	0.2	0.6	-	0.4	0.2	1.1	0.2	1.0	0.1
linalool oxide	1088	1087	GC/MS, RI	-	-	-	0.3	-	t	-	-	-	0.8
linalool	1099	1099	GC/MS, RI, std.	0.1	63.3	76.7	48.3	79.2	0.9	75.4	1.6	80.6	57.1
1-octenyl acetate	1109	1110	GC/MS, RI	-	-	-	-	-	-	-	-	-	0.5
α-campholenal	1127	1126	GC/MS, RI	-	t	t	-	-	t	-	0.6	-	0.2
(E)-pinocarveol (GC/MS)	1142	1139	GC/MS, RI	-	-	-	-	-	-	t	-	-	-
camphor	1147	1146	GC/MS, RI, std.	1.3	t	1.0	0.4	t	36.3	2.3	42.0	1.3	11.9
3-pinenone	1160	1162	GC/MS, RI	-	-	-	-	-	t	-	t	-	-
2,6-Dimethyl-1,5,7-octatrien-3-ol	1162		GC/MS	0.1	t	-	-	-	0.3	-	0.2	-	t
borneol	1172	1169	GC/MS, RI	0.3	t	0.5	2.9	0.6	t	4.6	0.1	0.4	t
terpinen-4-ol	1181	1177	GC/MS, RI, std.	0.7	-	0.6	-	0.6	13.2	0.2	17.5	-	8.8
alpha-terpineol	1193	1195	GC/MS, RI	-	-	-	0.4	-	3.0	0.4	2.1	0.2	1.4
(Z)-3-hexenyl valerate	1243	1247	GC/MS, RI	-	-	-	-	1.3	0.4	-	0.4	-	0.1
linalyl acetate	1252	1257	GC/MS, RI	t	-	-	-	0.1	t	-	-	-	-
bornyl acetate	1283	1289	GC/MS, RI	t	-	-	0.3	-	1.3	t	1.4	-	0.4
β-terpinyl acetate	1347	1350	GC/MS	-	-	-	-	0.4	-	-	-	-	-

isobornyl propanoate	1374	1384	GC/MS, RI	-	1.1	0.4	-	0.6	t	-	-	-	-
β-bourbonene	1379	1388	GC/MS, RI	-	-	-	0.6	-	0.2	0.3	0.6	0.7	0.2
β-elemene	1386	1390	GC/MS, RI	-	-	-	-	-	-	0.1	-	-	-
β-caryophyllene	1414	1419	GC/MS, RI	-	1.1	2.1	-	1.3	0.2	t	0.2	-	0.1
β-copaene	1426	1432	GC/MS, RI	-	-	-	0.7	-	0.3	0.8	0.4	1.3	0.8
aromadendrene	1433	1441	GC/MS, RI	-	-	-	-	-	-	-	-	0.1	-
α-humulene	1450	1454	GC/MS, RI	-	-	-	-	-	-	-	-	0.2	t
alloaromadendrene	1455	1460	GC/MS, RI	1.0	-	-	0.3	-	t	0.1	0.3	0.2	0.1
bornyl butanoate	1463	1470	GC/MS, RI	-	-	-	-	-	0.3	-	t	-	0.1
germacrene-D	1477	1485	GC/MS, RI	1.0	4.3	1.3	2.2	1.9	0.1	0.2	0.2	0.4	0.2
bicyclogermacrene	1492	1500	GC/MS, RI	0.2	0.7	1.3	-	2.2	-	-	-	-	-
γ-elemene	1490	1496	GC/MS, RI	-	-	-	1.0	-	0.5	0.4	0.4	0.8	0.3
γ-cadinene	1509	1513	GC/MS, RI	10.4	0.8	-	-	-	-	-	-	0.2	t
elemol	1546	1549	GC/MS, RI	-	-	4.4	-	1.3	-	-	-	-	-
spathulenol	1571	1578	GC/MS, RI	0.3	0.2	0.4	0.4	0.9	2.8	1.8	4.1	5.1	1.2
caryophyllene oxide	1576	1583	GC/MS, RI	-	0.4	0.8	0.2	0.4	0.6	0.5	1.2	1.2	0.4
α-cubenol	1610	1619	GC/MS, RI	-	-	-	-	-	-	-	t	t	t
δ-cadinol	1638	1640	GC/MS, RI	9.8	0.18	-	0.8	-	-	0.2	-	0.2	-

τ-cadinol	1640	1640	GC/MS, RI	-	-	0.23	-	-	t	0.32	t	t	0.1
α-cadinol	1651	1654	GC/MS, RI	43.5	3.2	-	3.6	-	-	-	t	0.1	-
cis-calamenen-10-ol	1656	1660	GC/MS, RI	2.9	0.1	0.3	0.7	0.1	-	0.2	0.3	1.1	0.2
elemol acetate	1668	1680	GC/MS, RI	0.3	-	0.8	-	0.2	-	-	-	-	-
eudesma-4(15),7-dien-1-beta-ol	1678	1687	GC/MS, RI	-	-	-	0.3	-	-	0.3	-	-	t
eudesm-7(11)-en-4-ol	1689	1700	GC/MS, RI	-	-	0.2	0.5	-	-	-	-	-	-
Monoterpenes				15.2	4.6	4.3	1.3	2.0	29.2	2.2	16.7	1.5	7.9
Oxygenated monoterpenes				3.2	82.0	83.5	8.0	87.1	61.3	87.6	72.2	85.1	84.7
Sesquiterpenes				12.6	6.9	4.7	4.6	5.4	1.4	1.8	2.0	3.7	1.8
Oxygenated sesquiterpenes				56.7	4.1	7.0	6.4	2.9	3.4	3.4	5.6	7.6	1.8
Other				-	-	-	2.5	1.3	2.8	0.4	1.9	0.1	1.6
Total identified				87.7	97.3	99.6	94.8	98.7	98.1	95.4	98.3	98.0	97.7

Notes:

1. Compounds ordered by elution time from a ZB-5 column.

2. Retention indices as determined on ZB-5 column using homologous series of n-alkanes.

3. Retention indices from Adams, R. P. (2007), except for those expressly referred.

4. Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample.

5. -: no detected.

6. t: traces (<0.1%).

As mentioned above, 1,8-cineole was not the major compound in samples from Xeraco. As it is detailed in Table 4, four individuals in May and three ones in August had linalool as a major compound, accounting for 48.31 - 80.64 %. No significant changes were found neither in linalool nor other compounds between sampling dates. Besides the individuals rich in linalool, one of the chemotypes reported by Thompson *et al.* (14) in Southern France, other one rich in cadinene skeleton ( $\alpha$ -cadinol: 43.5 %,  $\gamma$ -cadinene: 10.4 % and  $\delta$ -cadinol: 9.8 %) and other two which major compounds are camphor (36.3 – 42.0 %), terpinen-4-ol (13.2 – 17.5 %) and camphene (11.6 – 17.7 %) were found among Xeraco samples.

#### Conclusions

To summarize, despite the high intrapopulation variability, some clear and statistically significant differences were found. The most relevant one concerns to the main component (1,8-cineole) whose rate shows a high and significant increase during the drought period, the same way the oxygenated monoterpenes in whole. Other significant changes concern to sesquiterpenic fraction (both and hydrocarbon and oxygenated) which shows a significant decrease. With respect to previous works, the most relevant differences are related to negligible amounts of  $\alpha$ -terpineol acetate and the relative high proportion of camphene derivatives (camphor, mainly). Among the individuals sampled, other chemical profiles have been also detected as the one rich in linalool, or individuals in which the major compounds are sesquiterpenoids with cadinene skeleton and monoterpenoids rich in camphene and derivatives such as camphor and borneol. Unlike it happens in 1,8-cineole chemotype, these chemical profiles do not seem to change due to summer drought.

In conclusion, taking into account that 1,8-cineole is a valuable component of thyme chemotype, its increasing proportion owing summer drought suggests weighing up the convenience of applying a regulate water stress in thyme crop management in order to improve its proportion. Obviously, the impact on yield of this agricultural practice should be also considered.

#### References

 M. J. Jordán, R. M. Martínez, K. L. Goodnerb, E. A. Baldwinb and J.A Sotomayor, Seasonal variation of Thymus hyemalis Lange and Spanish Thymus vulgaris L. essential oils composition. Ind. Crop. Prod., 24(3), 253-263 (2006).

- C. Pina-Vaz, A. Gonçalves Rodrigues, E. Pinto, S. Costa-de-Oliveira, C. Tavares, L. Salgueiro and J. Martinez-de-Oliveira, *Antifungal activity of Thymus oils and their major compounds*. J. Eur. Acad. Dermatol. Venereol., 18(1), 73-78 (2004).
- H. Naghdi Badi, D. Yazdani, S.M. Ali, and F. Nazari, *Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme (Thymus vulgaris L)*. Ind. Crop. Prod., 19(3), 231-236 (2004).
- G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, and R. Bruni, *Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods*. Food Chem., **91(4)**, 621-632 (2005).
- M. D. Soković, J. Vukojević, P. D. Marin, D. D. Brkić, V. Vajs, and L. J. Van Griensven, *Chemical Composition of Essential Oils of Thymus and Mentha Species and their Antifungal Activities*. Molecules, 14(1), 238-249 (2009).
- M. A. A. Al Maqtari, S. M. Alghalibi, and E. H. Alhamzy, *Chemical composition and antimicrobial activity of essential* oil *of Thymus vulgaris from Yemen*. Turk. J. Biochem., 36(4), 342-349 (2011).
- L. A. Guerrero-Lagunes, L. D. M. Ruiz-Posadas, M. D. Rodríguez-Mendoza, M. Soto-Hernández and A. Castillo-Morales, *Efecto del cultivo hidropónico de tomillo (Thymus vulgaris L.) en la calidad y rendimiento del aceite esencial*. Revista Chapingo. Serie horticultura, **17(2)**, 141-149. (2011).
- O. Santana, R. Cabrera, A. González-Coloma, R. Sánchez-Vioque, M. De los Mozos-Pascual, M. F. Rodríguez-Conde and D. Herraiz, *Chemical and biological profiles of the essential oils from aromatic plants of agro industrial interest in Castilla-La Mancha* (Spain). Grasas Aceites, 63(2), 214-222 (2012).
- P. R. Venskutonis, *Harvesting and post-harvest handling in the genus thymus. In Thyme: the genus Thymus.* Edits. E. Stahl-Biskup and F. Sáez, CRC Press, Taylor & Francis Group, London (UK) (2003).
- M. P. Arraiza, M. P. Andrés, C. Arrabal and J. V. López, Seasonal Variation of Essential Oil Yield and Composition of Thyme (Thymus vulgaris L.) Grown in Castilla—La Mancha (Central Spain). J. Essen. Oil Res., 21(4), 360-362 (2009).
- E. Stahl-Biskup and F. Sáez, Essential oil chemistry of the genus Thymus. A global view. In Thyme: the genus Thymus. CRC Press. Edits. E. Stahl-Biskup and F. Sáez, CRC Press, Taylor & Francis Group, London (UK) (2003).
- S. Kulevanova, M. Ristić, T. Stafilov, K. Dorevski and T. Ristov, T. *Essential Oil Analysis of Some Taxa of Genera Thymus L. Environment Influences*. Bulletin of the Chemists and Technologists of Macedonia, 15(1), 33-38 (1996).

- 13. J. D. Thompson, J. C. Chalchat, A. Michet, Y. B. Linhart and B. Ehlers, *Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of Thymus vulgaris chemotypes.* J. Chem. Ecol., **29(4)**, 859-880 (2003).
- M. D. Guillén, and M. J. Manzanos, Study of the composition of the different parts of a Spanish Thymus vulgaris L. plant. Food Chem., 63(3), 373-383 (1998).
- J. Torras, M. D. Grau, J. F. López and F. X. C. de las Heras, Analysis of essential oils from chemotypes of Thymus vulgaris in Catalonia. J Sci. Food Agr., 87(12), 2327-2333 (2007).
- K. Keefover-Ring, J. D. Thompson and Y. B. Linhart, *Beyond six scents: defining a seventh Thymus vulgaris chemotype new to southern France by ethanol extraction*. Flavour Frag. J., 24(3), 117-122 (2009).
- 17. X. Y. Rueda, O. G. Pérez, O. G. and H. Meza, Actividad larvicida del aceite esencial foliar de Eucaliptus globulus contra Aedes aegypti Linnaeus. Bistua: Revista de la Facultad de Ciencias Básicas, 8(1), (2010).
- S. F. Van Vuuren and A. M. Viljoen, Antimicrobial activity of limonene enantiomers and 1, 8-cineole alone and in combination. Flavour Frag. J., 22(6), 540-544 (2007).
- E. R. Hendry, T. Worthington, T., B. R. Conway and P. A. Lambert, Antimicrobial efficacy of eucalyptus oil and 1, 8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J Antimicrob. Chemoth., 64(6), 1219-1225 (2009).
- 20. L. S. Nerio, J. Olivero-Verbel, J. and E. Stashenko, *Repellent activity of essential oils: a review*. Bioresource Technol., **101(1)**, 372-378 (2010).
- S. V. Clemente, G. Mareggiani, A. Broussalis and G. Ferraro, Actividad insecticida de 1,8-cineol sobre mosca de los frutos, Ceratitis capitata Wied. (Diptera: Tephritidae). Dominguezia, 23, 29-34 (2007).
- 22. G. Mareggiani, S. Russo and M. Rocca, Eucalyptus globulus (Mirtaceae) essential oil: efficacy against Aphis gossypii (Hemiptera: Aphididae), an agricultural pest. Rev Latinoam. Quím, **36**, 16-21 (2008).
- 23. S. Sharafzadeh and M. Zare, Effect of Drought Stress on Qualitative and Quantitative Characteristics of Some Medicinal Plants from Lamiaceae Family: A Review. Adv. Environ. Biol., 5(8), 2058-2062 (2011).
- 24. B. Bahreininejad, M. Mirza, and A. Arzani, *Essential oil variation in Thymus daenensis subsp. daenensis Cleak populations*. J. Essen. Oil Res., **22(1)**, 48-51 (2010).
- B. Bahreininejad, J. Razmjoo and M. Mirza, M. Effect of Water Stress on Productivity and Essential Oil Content and Composition of Thymus carmanicus. J. Essent. Oil Bear. Pl., 17(5), 717-725 (2014).

- 26. S. R. Leicach, A. M. Garau, A. B. Guarnaschelli, M. A. Yaber Grass, N. D. Sztarker and A. Dato, *Changes in Eucalyptus camaldulensis essential oil composition as response to drought preconditioning*. J. Plant Interact., 5(3), 205-210 (2010).
- M. J. P. Alonso and A. Velasco-Negueruela, Aceites esenciales de tomillos ibéricos: IV. Contribución al estudio quimiotaxonómico (Terpenoides) del género Thymus L. Botanica complutensis, (13), 115-134 (1986)
- M. Ozguven and S. Tansi, S. Drug yield and essential oil of Thymus vulgaris L. as in influenced by ecological and ontogenetical variation. Turk. J. Agric. For., 22(6), 537-542 (2009).
- 29. M. Costa, *Pisos bioclimáticos y series de vegetación en el área valenciana*. Cuadernos de Geografia, (**31**), 129-142 (1982).
- A.F. Cancio, S. S. Roscales, and E.M. Menendez. Estudio preliminar de la estructura interna del subtipo fitoclimático VI (IV) 1 de Allué (1990). Aplicaciones fitoclimáticas. Forest Systems, 8(3), 11-35 (2008).
- 31. R. P. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry* (No. Ed. 4). Allured publishing corporation, Carol Stream II. (2007).
- 32. J. B. Salas, T. R. Téllez, F. M. V. Pardo, M. J. Pérez-Alonso and M. A. C. Capdevila, *The essential oil of the protected species: Thymus praecox ssp. penyalarensis.* Acta Soc. Bot. Pol. 81(1), 23-27 (2012).
- B. Bahreininejad, J. Razmjoo and M. Mirza, *Effect of Water Stress on Productivity and Essential Oil Content and Composition of Thymus carmanicus*. J. Essent. Oil Bear. Pl., **17(5)**, 717-725 (2014).
- N. Sing Sangwan, A. H. Abad Farooqi and R. Singh Sangwan, R. Effect of drought stress on growth and essential oil metabolism in lemongrasses. New phytol., 128(1), 173-179 (1994).
- 35. I. Bettaieb, N. Zakhama, W. A. Wannes, M. E. Kchouk and B. Marzouk, *Water deficit effects on Salvia officinalis fatty acids and essential oils composition*. Sci. Hortic. Amsterdam, **120(2)**, 271-275 (2009).