JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Watering Level Effect on *Thymus hyemalis* Lange Essential Oil Yield and Composition

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Thymus hyemalis Lange (chemotype thymol) was cultivated as an experimental crop under different watering level conditions in order to achieve 80, 60, 40, and 20% of the local potential evapotranspiration (Eto). Two harvesting periods were considered, winter and spring. As a consequence of the great variability among plants, essential oil yield percentages did not show statistically significant differences among seasons and different levels of water supply. Capillary GC-MS analysis of the essential oils permitted the detection of 84 volatile components. Among them, 54 are described for the first time as volatile constituents of the chromatographic profile of this *Thymus* species. Winter harvesting showed high concentrations in thymol percentage (25.92 ± 4.39), the 40% Eto watering level being the best with respect to obtaining the optimum quality of this essential oil. However, in spring this thyme species needs a greater water supply (80% Eto) to achieve the same amount of thymol in the essential oil (29.20 ± 2.83). From this, it was concluded that winter harvesting could be used for the extraction of the essential oil, with a low level of water supply, whereas spring harvesting could be employed for collection of leaves as a food condiment.

KEYWORDS: Thyme; *Thymus hyemalis*; essential oil; water supply; yield; volatile components

INTRODUCTION

The genus *Thymus* is composed of ∼150 species, distributed throughout Europe, Asia, Africa, and Greenland. It is widely extended in the Iberian peninsula, most species being endemic.

Ecological and botanical interest in these species has been remarked upon by several researchers (*1*−*3*). Because of its great floristic and phytogenic richness, the southeastern Iberian peninsula has the richest spontaneous aromatic plant flora in Europe (*4*). This situation has allowed for the establishment of an important production and exporting industry based on the harvest of wild aromatic plants. This type of harvesting supposes a great commercial and ecological inconvenience, as a consequence of the great heterogeneity among the chemical compositions of the final products and a lack of control of the level of production. On the other hand, when harvesting is performed by pulling up the whole plant, physical damage is caused to the soil with subsequent erosion risks (*2*).

Aromatic plants and their essential oils rich in aromatic and flavoring chemicals are used in cosmetics and pharmaceutical products and as flavor ingredients in food products (*5*, *6*). The antifungal and antibacterial activities present in *Thymus* genus essential oil have been demonstrated by several researchers (*7*−*14*). Theantioxidant activity of the essential oil against the thermal autoxidation of lard has been demonstrated (*15*), and it has been shown that a dietary supplementation of thyme essential oil could address the unfavorable antioxidant−prooxidant balance that occurs with age (*16*).

Thymus hyemalis Lange, winter thyme, is an endemic shrub over the southeastern Iberian peninsula, mainly in and around Alicante, Murcia, and Almeria. This plant is normally present in siliceous and calcareous extensions, from sea level to 400−700 m above sea level. It is able to resist long dry periods, but its winter flowering condition (November through May) does not allow it to grow in areas having very cold weather.

The chemical variability of the essential oil from wild *T. hyemalis* of the southeastern Iberian peninsula has been reported (*2*, *17*−*23*). These researchers stated that thymol, carvacrol, borneol, and linalool were the chemotypes most abundant in this area. Recent studies from Sáez (*3*) have shown a complex chemical composition of some *Thymus* species in the southeastern Iberian peninsula, as a consequence of the edaphic and bioclimatological conditions, the ecological variability leading to a chemical variability.

Due to the high demand for thyme essential oil and the great variability that it exhibits, the next step in our investigation was to cultivate different species of the genus *Thymus* under different

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10.1021/jf034335m CCC: $25.00
Published on Web 07/17/2003

water supply conditions. As far as we know, *Thymus hyemalis* Lange has not been cultivated before as a commercial crop. For this reason, the goal of this study was to determine how the watering level affects the yield and composition of the *T. hyemalis* essential oil in order to be able to provide information on optimum cultivation conditions to commercial growers.

MATERIALS AND METHODS

Crop Experimental Design. This study was performed in an experimental area of the IMIDA (Murcian Institute of Investigation and Agricultural Development) at Torreblanca (37° 47′ N, 0° 54′ W, and 30 m above sea level) in the region of Murcia (Spain). Soil texture in the first 30 cm of the cultivation area can be defined as clayey, with a composition of sand (14.41%), silt (33.98%), and clay (51.61%). This soil shows a field capacity of 39% (by volume) and a wilting point of 21%. Semiarid climatic conditions are characterized by an annual average temperature of 18.2 °C and an average rainfall of 308.3 mm/year.

To study the effect of the irrigation level on thyme essential oil production, an assay having four blocks and four experimental replications was designed.

Each replication area had two lines of drip irrigation polythene tubes and a total of 140 plants, receiving different amounts of water. These amounts were calculated to achieve 80, 60, 40, and 20% of the area evapotranspiration (Eto). Actual values achieved corresponded to 83, 66, 47, and 34% of the Eto in winter and 77, 61, 42, and 29% in spring. The annual Eto of the cultivation area was ~1200 mm.

Cultivated land was covered with black plastic to ensure that the water received by the plants corresponded to the chosen watering levels.

Plant Material. Commercial seeds of *T. hyemalis* from Almería (Spain) were germinated and the plantlets grown under greenhouse conditions for 3 months. All of the plants were transplanted in the experimental area in May 2000 and received an initial watering.

Two harvesting periods were considered, February and May (2002), when this species is between the phenological stages of full bloom and the beginning of fructification. Four plants from each watering treatment were harvested, making a total of 16 plants per season. Before the essential oil extraction, plant material was dried, in a forced-air dryer, at 35 °C for 48 h, until it reached a constant weight.

Essential Oil Extraction. Aerial parts of individual plants were steam distilled for 3 h using a Clavenger-type system. The oil volume was measured directly in the extraction buret. Samples were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis. Yield percentage was calculated as volume (milliliters) of essential oil per 100 g of plant dry matter.

Gas Chromatography. Samples of 0.1 μL were subjected to analysis by capillary gas chromatography. A Hewlett-Packard 5890 gas chromatograph (GC) (Palo Alto, CA), equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm HP-5 (cross-linked phenylmethyl siloxane) column with 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA), was used for this study. The FID and the injector were maintained at 280 and 250 °C, respectively. Helium was used as carrier gas, the flow through the column was 1 mL/min, and the split ratio was set to 100:1. The column was maintained at 60 °C for 4 min, increased to 64 °C at a rate of 1 °C/min, then increased to 155 °C at a rate of 2.5 °C/min, and finally raised from 155 to 250 °C at a rate of 5 °C/min.

For the identification of the compounds, retention times and retention index were confirmed with commercially available standard compounds (Acros, Fisher Scientific S.A., and Sigma Aldrich Química S.A.).

Mass Spectrometry Analysis. The identification of volatile components in thyme essential oil was also made by gas chromatography−mass spectrometry (GC-MS). For this portion of the work, a Hewlett-Packard 5890 series II Plus gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 μm film thickness, was used. The GC was linked to a Hewlett-Packard model 5972 mass spectrometry detector. The chromatographic conditions were identical to those used for GC analysis.

Qualitative and Quantitative Analysis. The individual peaks were identified by retention times and retention index (relative to C_6-C_{17} *n*-alkanes), compared with those of known compounds and by comparison of mass spectra using the NBS75K library (U.S. National Bureau of Standards, 1986) and spectra obtained from the standard except for tricyclene, α-thujene, verbenene, thujol, pinocarvone, and spathulenol, which were tentatively identified considering only the NBS75K library spectra. Percentage compositions of samples were calculated according to the area of the chromatographic peaks.

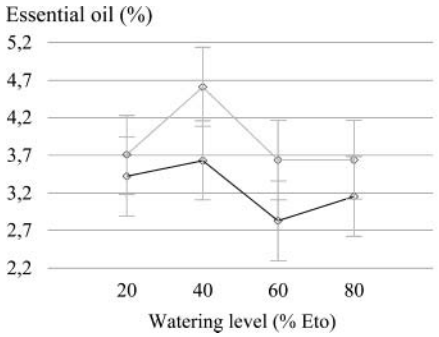

Figure 1. Essential oil yield percentage of *T. hyemalis* Lange under different water supplies in both harvesting seasons [(light line) winter; (heavy line) spring].

Statistical Analysis. For comparison of the mean values of each component in the essential oils, Student's *t* test was used.

RESULTS AND DISCUSSION

Colloquially, *T. hyemalis* is known as winter thyme. However, this thyme has the physiological characteristic of exhibiting two flowering stages per year (winter and spring) when it is watered and harvested in winter. For this reason, in the present study, these two harvesting periods have been considered.

Essential Oil Content. Essential oil yields obtained from winter and spring harvestings did not show statistically significant differences among percentages calculated on a dry matter basis. Similar results were obtained from the watering effect study, because the supplementation of water did not change the yield percentage of essential oil. No statistically significant differences were detected among values (**Figure 1**). This behavior was not observed by Letchamo et al. (*24*) in *Thymus vulgaris* L., because the essential oil yield is influenced by light intensity and water supply. For these researchers, the content of essential oil decreased significantly ($p < 0.01$) when the plants were supplemented with different levels of water under natural light growing conditions. The species *T. vulgaris* has been studied by several researchers. It is a homogeneous thyme with less variability among individual plants (*25*), unlike *T. hyemalis*, a species that, according to our knowledge, has been previously cultivated only by Sotomayor (*2*) and presents a great variability of composition among plants.

Oil yields (v/w) ranged from 3.64 ± 0.43 to 4.61 ± 1.15% in winter and from 2.83 ± 0.46 to 3.63 ± 0.60% in spring (**Figure 1**). These yields are richer than others reported by several authors in this and other thyme varieties. Thus, Sotomayor (*2*) reported the essential oil yield percentage obtained from a domestic crop of *T. hyemalis* during three years of harvests (from 1991 to 1993). For this work under dry land conditions, the oil yield ranged from 0.3 to 0.7% (v/w) calculated on a dry weight basis. Climatic conditions related to the annual rainfall of the cultivation area used by Sotomayor (*2*) could be compared to the 20% of Eto applied in our study. These differences between yields could be attributed to the different sources of wild seeds (Cartagena and Almeria) and to differing maximum and minimum annual temperatures in the two areas. According to Letchamo et al. (*26*), environmental conditions

Table 1. *T. hyemalis* Essential Oil Composition, Winter 2002[a]

component	RI[b]	watering level			
		80% Eto	60% Eto	40% Eto	20% Eto
hydrocarbons					
m-xylene	867	0.02 ± 0.01 ab	0.03 ± 0.02 b	0.01 ± 0.00 a	0.01 ± 0.01 a
tricyclene[d]	929	0.03 ± 0.04 a	0.02 ± 0.01 a	0.02 ± 0.02 a	0.03 ± 0.04 a
α-thujene[d]	940	0.95 ± 0.08 **a**	1.34 ± 0.65 a	0.96 ± 0.13 a	0.58 ± 0.06 a
α-pinene	949	0.58 ± 0.40 a	0.84 ± 1.07 a	1.32 ± 0.75 a	1.68 ± 1.66 a
camphene	969	1.11 ± 1.32 a	0.44 ± 0.19 a	0.50 ± 0.71 a	1.26 ± 1.45 a
verbenene[d]	976	0.05 ± 0.00 a	0.04 ± 0.01 a	0.06 ± 0.02 a	0.06 ± 0.03 a
sabinene	1001	0.22 ± 0.26 a	0.18 ± 0.16 a	0.23 ± 0.26 a	0.31 ± 0.31 a
β-pinene	1007	0.24 ± 0.27 a	0.30 ± 0.16 a	0.16 ± 0.12 a	0.26 ± 0.23 a
myrcene	1026	0.75 ± 0.37 a	0.90 ± 0.03 a	0.95 ± 0.18 a	0.74 ± 0.08 a
α-phellandrene	1042	0.15 ± 0.01 a	0.19 ± 0.02 a	0.19 ± 0.03 a	0.15 ± 0.03 a
Δ_3-carene	1048	0.06 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a	0.05 ± 0.01 a
α-terpinene	1058	1.28 ± 0.01 a	1.83 ± 0.54 a	2.00 ± 0.75 a	1.54 ± 0.70 a
p-cymene	1068	26.67 ± 13.72 a	24.29 ± 2.83 a	25.41 ± 5.46 a	22.28 ± 6.92 a
limonene	1073	0.87 ± 0.12 a	0.85 ± 0.32 a	0.93 ± 0.74 a	1.28 ± 0.75 a
ocimene	1086	0.02 ± 0.02 a	0.01 ± 0.02 a	0.03 ± 0.05 a	0.02 ± 0.02 a
γ-terpinene	1109	10.81 ± 2.18 a	13.37 ± 2.53 a	17.58 ± 4.96 a	12.85 ± 4.19 a
terpinolene	1141	0.16 ± 0.07 a	0.29 ± 0.23 a	0.25 ± 0.21 a	0.35 ± 0.27 a
α-copaene	1378				
(*E*)-caryophyllene	1419	0.80 ± 0.28 a	1.23 ± 0.48 a	1.08 ± 0.80 a	0.91 ± 0.71 a
calerene	1432		tr[c] a	tr a	0.01 ± 0.01 a
aromadendrene	1440	0.06 ± 0.05 a	0.10 ± 0.08 a	0.05 ± 0.03 a	0.08 ± 0.06 a
α-humulene	1457	0.04 ± 0.01 a	0.05 ± 0.03 a	0.04 ± 0.03 a	0.09 ± 0.12 a
alloaromadendrene	1465	0.09 ± 0.02 a	0.08 ± 0.03 a	0.08 ± 0.04 a	0.12 ± 0.15 a
valencene	1510	0.16 ± 0.06 a	0.27 ± 0.15 a	0.34 ± 0.26 a	0.76 ± 0.46 a
δ-cadinene	1553	0.04 ± 0.02 a	0.13 ± 0.05b	0.08 ± 0.06 ab	0.02 ± 0.03 a
alcohols					
1-octen-3-ol	1009	0.26 ± 0.06 a	0.18 ± 0.14 a	0.27 ± 0.17 a	0.12 ± 0.10 a
3-octanol	1031	0.11 ± 0.07 a	0.04 ± 0.07 a	0.02 ± 0.03 a	0.18 ± 0.19 a
2-octanol	1037	tr ab		tr bc	0.01 ± 0.00 c
thujol[d]	1095	0.21 ± 0.27 a	0.04 ± 0.03 a	0.44 ± 0.79 a	0.06 ± 0.04 a
(*E*)-sabinene hydrate	1116	0.62 ± 0.18 a	1.29 ± 1.52 a	2.00 ± 3.06 a	1.78 ± 2.62 a
octanol	1123				
(Z)-sabinene hydrate	1151	0.17 ± 0.07 a	1.74 ± 2.77 a	0.29 ± 0.24 a	0.43 ± 0.67 a
linalool	1152	2.19 ± 0.05 a	1.58 ± 1.38 a	0.89 ± 0.60 a	8.63 ± 8.88 a
fenchol	1163	0.06 ± 0.05 a	0.03 ± 0.05 a	0.08 ± 0.06 a	0.02 ± 0.02 a
(*E*)-pinocarveol	1186	0.03 ± 0.01 a	0.15 ± 0.12 ab	0.03 ± 0.01 a	0.20 ± 0.080 b
(Z)-verbenol	1188	0.16 ± 0.06 a	0.13 ± 0.03 a	0.18 ± 0.05 a	0.05 ± 0.10 a
(*E*)-verbenol	1193	0.81 ± 0.30 ab	0.46 ± 0.30 a	0.92 ± 0.20 ab	1.18 ± 0.31 b
isoborneol	1203	0.01 ± 0.00 a	tr a	0.01 ± 0.00 a	0.01 ± 0.01 a
borneol	1213	3.09 ± 3.99 a	0.38 ± 0.43 a	1.39 ± 2.43 a	3.76 ± 4.40 a
terpinen-4-ol	1220	0.47 ± 0.18 a	1.48 ± 1.77 a	1.52 ± 1.89 a	2.31 ± 3.78 a
p-cymen-8-ol	1227	0.29 ± 0.02 b	0.11 ± 0.09 a	0.22 ± 0.06 ab	0.24 ± 0.05 b
α-terpineol	1231	4.71 ± 6.35 a	0.57 ± 0.29 a	0.59 ± 0.38 a	0.69 ± 0.56 a
carveol	1252	0.34 ± 0.48 a	0.48 ± 0.40 a	0.75 ± 0.70 a	0.06 ± 0.02 a
nerol + citronellol	1260	0.04 ± 0.02 a	0.02 ± 0.01 a	0.04 ± 0.05 a	0.03 ± 0.02 a
geraniol	1280	tr a	tr a	tr a	tr a
isoeugenol	1451	0.02 ± 0.01 a	0.07 ± 0.09 a	0.03 ± 0.03 a	0.03 ± 0.02 a
spathulenol[d]	1640	0.25 ± 0.11 a	0.28 ± 0.08 a	0.32 ± 0.10 a	0.46 ± 0.13 a
aldehydes					
nonanal	1157	0.03 ± 0.00 a	0.04 ± 0.03 a	0.08 ± 0.05 a	0.02 ± 0.02 a
citronellal	1201		tr a		tr a
decanal	1243	0.02 ± 0.01 a	0.03 ± 0.02 a	0.02 ± 0.01 a	0.13 ± 0.23 a
neral	1270	0.02 ± 0.02 a	0.03 ± 0.01 a	0.04 ± 0.02 a	0.04 ± 0.03 a
perilla aldehyde + geranial	1291	0.05 ± 0.03 a	0.04 ± 0.03 a	0.05 ± 0.07 a	0.07 ± 0.04 a
ketones					
3-nonanone	1139	0.02 ± 0.03			
β-thujone	1158	0.01 ± 0.01 a	0.01 ± 0.02 a	0.01 ± 0.01 a	0.01 ± 0.01 a
camphor	1192	0.29 ± 0.03 a	0.26 ± 0.25 a	0.40 ± 0.21 a	0.28± 0.08 a
menthone	1200	0.01 ± 0.01 a		tr a	tr a
pinocarvone[d]	1208	0.02 ± 0.03 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.00 a
dihydrocarvone	1235		0.04 ± 0.04 a	0.05 ± 0.02 a	0.04 ± 0.01 b
verbenone	1245	2.22 ± 0.68 a	1.29 ± 1.41 a	3.69 ± 1.44 a	1.96 ± 1.16 a
carvone	1271	0.69 ± 0.02 a	0.02 ± 0.02 a	0.48 ± 0.59 a	0.27 ± 0.30 a
thymoquinone	1276	0.02 ± 0.01 a	0.03 ± 0.04 a	0.02 ± 0.01 a	0.02 ± 0.01 a
α-ionone	1426	tr a	tr a	tr a	tr a
β-ionone	1497	0.01 ± 0.00 a	tr a	tr a	tr a
esters					
benzyl acetate	1214	0.05 ± 0.01 a	0.10 ± 0.09 a	0.05 ± 0.01 a	0.05 ± 0.01 a
ethyl caprylate	1238	0.05 ± 0.03 a	0.03 ± 0.03 a	0.03 ± 0.02 a	0.02 ± 0.03 a
linalyl acetate	1281	tr a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.02 b
bornyl acetate	1302	0.04 ± 0.05 a	0.10 ± 0.12 a	0.07 ± 0.15 a	0.65 ± 0.92 a
terpenyl acetate	1353	tr a	tr a	0.01 ± 0.02 a	tr a
citronellyl acetate	1356	0.04 ± 0.05 a	0.13 ± 0.20 a	0.03 ± 0.03 a	0.14 ± 0.11 a

Table 1 (Continued)

component	RI[b]	watering level			
		80% Eto	60% Eto	40% Eto	20% Eto
esters (continued)					
neryl acetate	1366				0.01 ± 0.01 a
(*E*)-mirtanyl acetate	1381	tr a		tr a	tr a
geranyl acetate	1386	0.02 ± 0.02 a	0.07 ± 0.11 a	0.01 ± 0.01 a	0.005 ± 0.005 a
butyl caprylate	1387				
phenols					
thymol methyl ether	1265	0.06 ± 0.01 a	0.38 ± 0.49 a	0.13 ± 0.16 a	0.87 ± 1.71 a
(*E*)-anethole	1303	0.11 ± 0.15 a		0.09 ± 0.05 a	
thymol	1308	29.55 ± 4.86 b	31.87 ± 3.05 b	25.92 ± 4.40 ab	19.21 ± 4.56 a
carvacrol	1314	1.57 ± 0.20 a	1.74 ± 0.18 a	1.28 ± 0.41 a	1.13 ± 0.42 a
eugenol	1358	0.02 ± 0.00 a	0.04 ± 0.04 a	0.08 ± 0.13 a	0.01 ± 0.00 a
epoxides					
(*Z*)-linalool oxide	1123		0.01 ± 0.01 a		0.03 ± 0.03 a
(*E*)-linalool oxide	1140	0.02 ± 0.03 a			0.17 ± 0.34 a
(*Z*)-limonene oxide	1173	0.24 ± 0.01 a	0.22 ± 0.19 a	0.43 ± 0.25 a	0.45 ± 0.13 a
caryophyllene oxide	1650	0.19 ± 0.02 a	0.23 ± 0.06 a	0.24 ± 0.15 a	0.34 ± 0.17 a
ether					
cineole	1076	2.32 ± 3.24 a	3.30 ± 3.20 a	1.66 ± 2.33 a	1.89 ± 3.73 a

[a] Values within rows with common letters were not significantly different (*, $p < 0.05$); ± standard deviation. [b] Kovats index (HP-5). [c] tr, traces (<0.01%). [d] Tentative identification.

in which the plants are grown and the genetic constitution of the cultivars greatly influence both oil yield and composition of thyme.

Salgueiro et al. (*27*) studied the essential oil composition and variability of four populations of *Thymus lotocephalus* G. López and R. Morales and one population of *Thymus* × *mourae* Paiva and Salgueiro, two endemic taxa from Portugal. Average yields of essential oil of air-dried aerial parts were 1.1 and 1% (v/w), respectively. For Echeverrigaray et al. (*28*) oil yields calculated from the dried plant material ranged from 0.47 to 0.64% for commercial thyme cultivars of *T. vulgaris* from the United States, Spain, France, Italy, and Brazil. Hudaib et al. (*25*) performed a GC-MS evaluation of *T. vulgaris* oil composition variations during the vegetative cycle. Oil yields were calculated for fresh plant material. The oldest plants exhibited an oil yield of 0.15% (v/w). The young plants, collected just before the end of the vegetative cycle, provided the best oil yield (1.2%).

Chemical Composition. Related to the essential oil composition, the chromatographic analysis of the volatile profile of *T. hyemalis* essential oil allowed the identification of a total of 84 volatile components, including 25 hydrocarbons, 23 alcohols, 11 ketones, 10 esters, 6 aldehydes, 5 phenols, 4 epoxides, and 1 ether (**Tables 1** and **2**).

One of the most recent publications about the chemical composition of wild *T. hyemalis* essential oil is that of Sáez (*23*). This author reported a total of 32 volatile components and stated that *T. hyemalis* chemotype thymol is widespread in southeastern Spain and that it is found in most of the plant communities where *T. hyemalis* is predominant. Sotomayor (*2*) studied the volatile profile of the essential oil of this species under domestic cultivation. For this author, a total of 30 components represented the major volatile composition of this thyme oil. Thymol concentration varied from 24.7 to 15.4% in the winters of 1991 and 1993, respectively.

In the present work, 54 new components are described for the first time as volatile constituents of the essential oil in this thyme variety and chemotype including *m*-xylene, tricyclene, verbenene, α-phellandrene, Δ_3-carene, α-copaene, calerene, aromadendrene, α-humulene, alloaromadendrene, valencene, δ-cadinene, 1-octen-3-ol, 3-octanol, 2-octanol, thujol, octanol, (*Z*)-sabinene hydrate, fenchol, (*E*)-pinocarveol, (*E*)-verbenol, isoborneol, *p*-cymen-8-ol, carveol, nerol, spathulenol, nonanal, citronellal, decanal, perilla aldehyde, 3-nonanone, β-thujone, menthone, pinocarvone, dihydrocarvone, carvone, thymoquinone, α-ionone, β-ionone, benzyl acetate, ethyl caprylate, bornyl acetate, terpenyl acetate, citronellyl acatate, neryl acetate, (*E*)-mirtanyl acetate, butyl caprylate, thymol methyl ether, (*E*)-anethole, eugenol, (*Z*)-linalool oxide, (*E*)-linalool oxide, and (*Z*)-limonene oxide. All of these components have been previously reported to be present in other *Thymus* species such as *T. vulgaris* L., *T. zygis* L., *T. pulegioides* L., *T. serpyllum*, subspec. *villous*, *T. satureioides* Cass., *T. praecox* ssp. *areticus*, *T. broussonetii*, *T. maroccanus*, *T. pallidus*, *T. granatensis* Boiss., *T. orospedanus*, *T. chamaedris*, *T. carnosus* Boiss. *T. serpylium* L. var. *mongolicus* Ronn., *T. quinquecostatus* Celak, *T. funkii*, *T. aestivus*, *T. baeticus*, *T. camphoratus*, and *T. sibthorpii* Benth. (*29*).

The plant oil was characterized by a high percentage of monoterpene phenol (thymol). In addition, the oil was characterized by high levels of the precursor monoterpene hydrocarbons *p*-cymene and γ-terpinene, the concentrations of which were not found to vary in accordance with the variations in their corresponding phenol products (thymol and carvacrol). According to Saez (*23*), this can be attributed to the high intraspecific variability present among plants of this species.

To simplify the analysis of the results, and taking into account that statistically significant differences were found for some components between harvesting periods (**Table 3**), it is necessary to make separate studies concerning the effect of the watering level on the essential oil composition in both seasons.

Winter Harvesting. One of the aspects that needs to be considered before the analysis of the results is the great variability of the oil composition among plants under the same cultivation conditions (**Table 1**). As a consequence of this, the effect of the watering level on the essential oil composition is negligible for most of the volatile components under study. Taking into account this consideration, only *m*-xylene, δ-cadinene, 2-octanol, (*E*)-pinocarveol, (*E*)-verbenol, *p*-cymen-8-ol, linalyl acetate, and thymol had modified percentage concentrations in the essential oil with water supplementations.

Regarding terpenic hydrocarbons, *p*-cymene, γ-terpinene, α-thujene, α-pinene, camphene, α-terpinene, limonene, myrcene, and valencene were the hydrocarbons identified at higher concentrations.

Table 2. *T. hyemalis* Essential Oil Composition, Spring 2002[a]

component	watering level			
	80% Eto	60% Eto	40% Eto	20% Eto
hydrocarbons				
m-xylene[d]	0.01 ± 0.01 a	0.03 ± 0.01 a	0.01 ± 0.01 a	0.03 ± 0.00 a
tricyclene	0.01 ± 0.01 a	0.01 ± 0.00 a	0.03 ± 0.03 a	0.01 ± 0.00 a
α-thujene[d]	1.04 ± 0.23 a	1.18 ± 0.87 a	1.30 ± 0.43 a	0.63 ± 0.03 a
α-pinene	2.47 ± 2.36 a	0.34 ± 0.07 a	1.46 ± 0.87 a	2.24 ± 2.17 a
camphene	0.35 ± 0.18 a	0.29 ± 0.10 a	1.06 ± 1.01 a	0.35 ± 0.03 a
verbenene[d]	0.05 ± 0.02 a	0.03 ± 0.02 a	0.09 ± 0.06 a	0.05 ± 0.02 a
sabinene	0.14 ± 0.11 a	0.40 ± 0.16b	0.49 ± 0.33bc	0.83 ± 0.03 c
β-pinene	0.27 ± 0.16 a	0.20 ± 0.06 a	0.3 ± 0.23 a	0.16 ± 0.02 a
myrcene	0.90 ± 0.36 a	1.27 ± 0.79 a	1.07 ± 0.40 a	0.79 ± 0.06 a
α-phellandrene	0.19 ± 0.03 a	0.18± 0.07 a	0.17 ± 0.04 a	0.16 ± 0.01 a
Δ_3-carene	0.07 ± 0.01 a	0.05 ± 0.03 a	0.06 ± 0.02 a	0.04 ± 0.00 a
α-terpinene	1.33 ± 0.55 a	1.26 ± 0.50 a	1.51 ± 0.50 a	1.69 ± 0.00 a
p-cymene	27.29 ± 8.48 a	27.62 ± 10.25 a	28.94 ± 6.71 a	22.49 ± 0.41 a
limonene	1.61 ± 0.69 a	1.34 ± 0.48 a	1.79 ± 0.84 a	2.18 ± 0.64 a
ocimene	0.04 ± 0.05 a	0.11 ± 0.20 a	0.06 ± 0.07 a	0.04 ± 0.05 a
γ-terpinene	9.42 ± 4.59 a	9.24 ± 2.64 a	10.99 ± 4.12 a	9.06 ± 0.96 a
terpinolene	0.20 ± 0.03 a	0.35 ± 0.19 a	0.37 ± 0.18 a	0.54 ± 0.03 a
α-copaene		tr[c] a	tr a	
(*E*)-caryophyllene	0.72 ± 0.24 a	0.76 ± 0.65 a	0.88 ± 0.373 a	0.31 ± 0.12 a
calerene	0.01 ± 0.01 a	tr a	tr a	
aromadendrene	0.16 ± 0.08 a	0.32 ± 0.14 a	0.36 ± 0.08 a	0.30 ± 0.12 a
α-humulene	0.02 ± 0.02 a	0.03 ± 0.03 a	0.01 ± 0.02 a	0.01 ± 0.00 a
alloaromadendrene	0.01 ± 0.02 a	0.13 ± 0.06 b	0.03 ± 0.04 a	0.12± 0.03 b
valencene	0.27 ± 0.08 a	0.32 ± 0.18 a	0.32 ± 0.05 a	0.41 ± 0.02 a
δ-cadinene	0.02 ± 0.04 a	0.05 ± 0.06 a	0.01 ± 0.02 a	0.05 ± 0.08 a
alcohols				
1-octen-3-ol	0.13 ± 0.13 a	0.20 ± 0.15 a	0.13 ± 0.08 a	0.15 ± 0.06 a
3-octanol		0.02 ± 0.03 a	tr a	
2-octanol			tr a	tr b
thujol	0.17 ± 0.23 a	0.52 ± 0.91 a	0.38± 0.49 a	0.18± 0.20 a
(*E*)-sabinene hydrate	0.67 ± 0.26 a	2.76 ± 2.55 a	3.32 ± 3.41 a	15.42 ± 0.33 b
octanol	tr			
(*Z*)-sabinene hydrate	0.17 ± 0.06 a	4.03 ± 4.58 a	3.19 ± 4.78 a	1.05 ± 0.23 a
linalool	3.09 ± 2.69 a	1.87 ± 1.64 a	1.55 ± 1.37 a	1.54 ± 0.04 a
fenchol	0.01 ± 0.01 a	0.03 ± 0.04 a	0.03 ± 0.07 a	0.152 ± 0.048 b
(*E*)-pinocarveol	0.14 ± 0.09 a	0.22 ± 0.02 a	0.23 ± 0.11 a	0.20 ± 0.04 a
(*Z*)-verbenol	0.03 ± 0.06 a		0.04 ± 0.06 a	0.12 ± 0.00 a
(*E*)-verbenol	0.88 ± 0.61 a	0.64 ± 0.18 a	1.50 ± 0.75 a	1.04 ± 0.32 a
isoborneol	tr a	0.01 ± 0.00 a	0.01 ± 0.01 a	0.01 ± 0.00 a
borneol	0.43 ± 0.64 a	0.28 ± 0.13 a	3.68 ± 4.05 a	0.18 ± 0.01 a
terpinen-4-ol	0.51 ± 0.15 a	1.34 ±1.12 a	3.05 ± 2.84 a	4.14 ± 0.21 a
p-cymen-8-ol	0.18 ± 0.04 a	0.14 ± 0.10 a	0.25 ± 0.04 a	0.24 ± 0.06 a
α-terpineol	0.61 ± 0.29 a	7.91 ± 9.45 a	1.98 ± 2.51 a	1.54 ± 0.06 a
carveol	0.29 ± 0.52 a	0.01 ± 0.02 a	0.04 ± 0.03 a	0.05 ± 0.02 a
nerol+citronellol	0.03± 0.02 a	0.02 ± 0.03 a	0.03 ± 0.01 a	0.01 ± 0.01 a
geraniol	tr a	0.01 ± 0.01 a	0.01 ± 0.01 a	
isoeugenol	0.01 ± 0.01 a	0.01 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.00 a
spathulenol**	0.33 ± 0.06 a	0.29 ± 0.11 a	0.36 ± 0.10 a	0.46 ± 0.08 a
aldehydes				
nonanal	0.02 ± 0.01 a	0.03 ± 0.03 a	0.05 ± 0.04 ab	0.11 ± 0.01 b
citronellal	tr a		tr a	tr a
decanal	tr a	0.01 ± 0.01 a	0.02 ± 0.02 a	0.01 ± 0.01 a
neral	0.02 ± 0.02 a	0.01 ± 0.01 a	0.02 ± 0.02 a	0.03 ± 0.03 a
perilla aldehyde + geranial	0.14 ± 0.11 a	0.21 ± 0.02 a	0.15 ± 0.04 a	0.13 ± 0.04 a
ketones				
3-nonanone	tr a			
β-thujone	0.01 ± 0.03 a	0.02 ± 0.02 a	tr a	0.03 ± 0.00 a
camphor	0.86 ± 0.56 a	0.38 ± 0.14 a	0.78 ± 0.44 a	0.74 ± 0.09 a
menthone		tr a	tr a	0.01 ± 0.01 a
pinocarvone[d]	0.01 ± 0.01 a	0.03 ± 0.02 a	0.03 ± 0.03 a	0.01 ± 0.01 a
dihydrocarvone	0.13 ± 0.09 a	0.06 ± 0.02 a	0.14 ± 0.07 a	0.20 ± 0.08 a
verbenone	2.97 ± 2.04 a	1.47 ± 1.95 a	4.31 ± 3.17 a	4.68 ± 1.54 a
carvone	0.27 ± 0.34 a	0.43 ± 0.38 a	0.70 ± 0.63 a	0.07 ± 0.05 a
thymoquinone	0.03 ± 0.01 a	0.03 ± 0.03 a	0.03 ± 0.03 a	0.03 ± 0.05 a
α-ionone	0.01 ± 0.00 b	0.01 ± 0.01 b	tr ab	
β-ionone	0.02 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.01 a	tr a
esters				
benzyl acetate		0.10 ± 0.03 b	0.08 ± 0.02 b	0.07 ± 0.01 b
ethyl caprylate	0.04 ± 0.07 a			
linalyl acetate	0.06 ± 0.05 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.01 a
bornyl acetate	0.09 ± 0.02 a	0.10 ± 0.04 a	0.11 ± 0.01 a	0.08 ± 0.02 a
terpenyl acetate	tr a	0.03 ± 0.05 a	0.01 ± 0.02 a	
citronellyl acetate	tr a	0.01 ± 0.01 a	0.01 ± 0.00 a	0.01 ± 0.01 a

Table 2 (Continued)

component	watering level			
	80% Eto	60% Eto	40% Eto	20% Eto
esters (continued)				
neryl acetate	0.03 ± 0.03 a	0.01 ± 0.01 a	tr a	
(*E*)-mirtanyl acetate	tr a	0.01 ± 0.01 a	0.01 ± 0.00 a	
geranyl acetate	0.03 ± 0.05 a	0.02 ± 0.02 a	0.01 ± 0.01 a	tr a
butyl caprylate	0.01 ± 0.01 a			tr a
phenols				
thymol methyl ether	0.53 ± 0.59 a	0.18 ± 0.34 a	0.13 ± 0.10 a	0.48 ± 0.67 a
(*E*)-anethole				
thymol	29.20 ± 2.83 b	17.15 ± 9.30 a	14.45 ± 3.42 a	16.55 ± 2.53 a
carvacrol	2.05 ± 0.27 a	1.39 ± 0.85 a	0.98 ± 0.25 a	1.27 ± 0.36 a
eugenol	0.07 ± 0.04 a	0.12 ± 0.04 a	0.07 ± 0.06 a	0.07 ± 0.03 a
epoxides				
(*Z*)-linalool oxide		0.02 ± 0.04 a	0.01 ± 0.01 a	
(*E*)-linalool oxide	0.01 ± 0.01 a			
(*Z*)-limonene oxide	0.58 ± 0.76 a	0.27 ± 0.15 a	0.57 ± 0.44 a	0.71 ± 0.07 a
caryophyllene oxide	0.25 ± 0.07 a	0.27 ± 0.13 a	0.33 ± 0.08 a	0.19 ± 0.08 a
ether				
cineole	2.06 ± 2.38 a	1.41 ± 1.68 a	1.69 ± 2.04 a	0.61 ± 0.83 a

[a] Values within rows with common letters are not significantly different (*$p < 0.05$); ± standard deviation. [b] Kovats index (HP-5). [c] tr, traces (<0.01%). [d] Tentative identification.

Table 3. Components with Statistically Significant Differences between Seasons

component	watering level			
	80% Eto	60% Eto	40% Eto	20% Eto
m-xylene	different			
α-thujene	different			
aromadendrene			different	different
alloaromadendrene	different			
δ-cadinene			different	
(*E*)-sabinene hydrate				different
(*E*)-pinocarveol			different	
(*Z*)-verbenol		different	different	
fenchol				different
p-cymen-8-ol	different			
terpinen-4-ol		different		
decanal			different	
neral		different		
perilla aldehyde + geranial				different
β-thujone			different	
camphor				different
benzyl acetate			different	
ethyl caprylate			different	
linalyl acetate		different	different	
terpenyl acetate		different	different	
(*E*)-anethole			different	
eugenol				different
thymol		different	different	

For *m*-xylene and δ-cadinene, there were no statistically significant differences between the concentrations at the maximum and minimum levels of water supply. The 60% Eto watering treatment produced the highest concentrations for these two hydrocarbons. This behavior cannot be extrapolated to the rest of the terpenic hydrocarbons as a consequence of the great variability in the essential oil quantitative composition of the plants.

Regarding alcohols, four alcoholic compounds showed statistically significant differences among treatments, 2-octanol, (*E*)-pinocarveol, (*E*)-verbenol, and *p*-cymen-8-ol having increased concentrations at the lowest level of watering.

2-Octanol was not detected in oils of plants watered at 60% Eto and did not exhibit statistically significant differences with the 80% water supplement. (*E*)-Pinocarveol showed at the 20% watering level a concentration higher than with 40 or 80% but no higher than at 60%. For (*E*)-verbenol and *p*-cymen-8-ol maximum yields were achieved at 20% Eto, but they showed statistically significant differences only at the 60% watering level.

Aldehydes did not exhibit statistically significant differences among watering treatments for any of the components identified. Only citronellal was not identified in the samples watered to 80 or 40% Eto. As a consequence of the great variability among plants, we cannot affirm that this fact is due to a watering level effect.

Regarding ketones, this chemical group did not show statistically significant differences among treatments for most of the components identified. Only dihydrocarvone showed significant differences among the 20% watering level and the rest of the water supplements, its concentration being lowest at 20% Eto. For this component, the highest concentrations were present for the 60% and 40% Eto treatments, because dihydrocarvone was not found in samples receiving the 80% water supplement.

3-Nonanone was detected only in plants receinving 80% Eto. Lower levels of water meant that it could not be identified in the volatile profile of the essential oil.

In the analysis of esters, linalyl acetate showed an effect of the watering level on its concentration. Maximum yield was achieved at the lowest water supply. Although no statistically significant differences were found for the rest of the esters identified, this behavior can be extrapolated to the others esters, as occurs with neryl acetate, because this compound has been identified only in plants receiving a water supplement of 20% Eto.

Related to the group of phenols identified, thymol is considered to be one of the most important determinants of thyme essential oil quality. This component represents between 29% at 80% Eto and 19% at 20% Eto of the oil composition in thyme, there being statistically significant differences between concentrations. An important point that needs to be considered is that there were no significant differences among the 80, 60, and 40% Eto watering levels. From this result, we can deduce that a supply of 40% of the Eto would be enough to obtain a good yield.

Epoxides and the single ether identified (cineole) showed behaviors similar to the rest of the components identified. Once again, the great variability in plant composition prevented determination of the effect of the watering supply on the essential oil quality.

Spring Harvesting. During this season, as in winter, *T. hyemalis* essential oil quality was characterized by a great variability (**Table 2**). Sabinene and alloaromadendrene were the terpenic hydrocarbons exhibiting statistically significant differences among watering treatments. In both cases, the presence of these components increased at the lowest watering level. For sabinene, watering equivalent to 20% of the Eto produced a concentration that was significantly higher than at 60 or 80% Eto but was not higher than with 40% Eto. Alloaromadendrene presented a similar behavior, but there were no significant differences between the 60 and 20% Eto treatments.

Regarding alcohols, 2-octanol, (*E*)-sabinene hydrate, and fenchol had higher concentrations at the lowest watering level, especially (*E*)-sabinene hydrate, which increased its concentration almost 25 times compared to the highest water supplement.

Aldehydes and ketones, in general, did not have modified concentrations at different watering levels. Nonanal and α-ionone behaved differently: nonanal had its higher concentration at the lowest watering level, whereas α-ionone was not detected at 20% Eto.

Benzyl acetate was not detected in samples from the watering level at 80% of the Eto, exhibiting statistically significant differences compared to the other watering treatments. Among the phenols, it is interesting to remark that thymol was detected at its highest concentrations in samples with a higher supplement of water, as in the winter season. (*E*)-Anethole was not identified in the essential oil at this harvesting. No more statistically significant differences were detected in the analysis of these essential oils. Probably, the variability in the composition of the essential oil again did not allow the determination of the effect of the water supplementation on the essential oil quality.

An interesting point was to study whether the composition of the essential oil changed with the season of harvesting. For this a statistical analysis of the concentrations at both harvests at different watering levels was performed (**Table 3**). From this we can deduce that a greater number of components reached their highest concentrations at the lowest watering supplements (20−40% Eto). Thus, in winter *T. hyemalis* essential oil was richer in δ-cadinene, (*Z*)-verbenol, decanal, β-thujone, ethyl caprylate (not observed in spring), terpenyl acetate, (*E*)-anethole (not identified in spring), and thymol. Meanwhile, in spring, the composition of the essential oil was characterized by higher contents of aromadendrene, (*E*)-sabinene hydrate, (*E*)-pinocarveol, fenchol, perilla aldehyde + geranial, camphor, benzyl acetate, linalyl acetate, and eugenol. On the other hand, an increased water supply brought about a decrease in concentration for most of the volatile components identified. Winter essential oil with a supplement of 80 or 60% Eto showed the greatest concentrations of alloaromadendrene, (*Z*)-verbenol (60% Eto, not identified in spring oil), *p*-cymen-8-ol, and neral. Also, winter oil with these supplements of water was richer in α-thujene, linalyl acetate, and terpinyl acetate.

From these results and taking into account only the components that define the quality of this chemotype (thymol), winter harvesting produced a better quality essential oil than spring harvesting. Regarding the watering level required to obtain the optimum quality of essential oil in winter, a supplement that achieves 40% Eto would be enough. This is in agreement with the essential oil yield percentage trends, because this water supply obtained the highest yields for some of the plants. However, in spring a higher watering level (80% Eto) was required to reach the same production.

According to these results we can conclude that winter harvesting with a low level of water supplement could be used for the extraction of the essential oil. This would be advantageous to commercial growers because of the semiarid climate conditions of southeastern Spain. Spring harvesting could be designated for the collection of leaves as a food condiment.

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Received for review April 3, 2003. Revised manuscript received June 17, 2003. Accepted June 22, 2003. We thank the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) for providing Project SC00-052-C7-3, under which this work has been accomplished.

JF034335M