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Thymus zygis Subsp. *Gracilis*: Watering Level Effect on Phytomass Production and Essential Oil Quality

JOSE A. SOTOMAYOR, ROSA M. MARTÍNEZ, ANTONIO J. GARCÍA, AND MARÍA J. JORDÁN*

Murcian Institute of Investigation and Agricultural Development (IMIDA), C./Mayor s/n, 30150 La Alberca (Murcia), Spain

Thymus zygis subsp. *gracilis* (chemotype thymol) was evaluated on the basis of its phytomass production and essential oil quality. Three different watering levels were assayed to achieve 63, 44, and 30% of the local potential evapotranspiration (Eto). According to the statistical analysis, a water supplement equivalent to 44% Eto in this cultivation area was optimal for maximum plant dry matter production and essential oil yield. Capillary GC-MS analysis of the essential oil allowed the identification of 86 volatile components. Among them, 30 are described for the first time as volatile constituents of the essential oil in this thyme subspecies and chemotype. The watering level effect on essential oil composition was noticeable, because the application of a water supplement equivalent to the 63% Eto favored the production of an essential oil richer in low molecular weight components. However, the greatest thymol concentrations were obtained under the 30 and 44% Eto watering levels.

KEYWORDS: **Thyme; *Thymus zygis* subsp. *gracilis*; water supply; essential oil; volatile profile; phenol content**

INTRODUCTION

Thymus zygis subsp. *gracilis* (Boiss.) R. Morales (red thyme) has become the most commercial Spanish thyme because of the economical importance that the presence of thymol has in thyme essential oil quality. This species is a widespread endemic plant in the Iberian peninsula, with three subspecies recognized by Morales (*1*). Two of them can be found in southeastern Spain, namely subsp. *sylvestris* (Hoffmanns and Link) Brot ex Coutinho and subsp. *gracilis* (Boiss.) R. Morales (*2*). Studies carried out by this author about the essential oil variability of *T. zygis* growing wild in southeastern Spain showed that the most common chemotype of this thyme species was thymol, although a pure linalool chemotype was also recorded. Concentrations of thymol in the essential oil ranged from 29.93 to 71.84% for subsp. *gracilis* and from 25.45 to 34.18% in subsp. *sylvestris*. Moldão-Martins et al. (*3*) studied the seasonal variation in yield and composition of the essential oil of *T. zygis* subsp. *sylvestris* collected in northern Portugal. The yield of essential oil showed a maximum at the flowering stage (0.9–1.4%) and a minimum during the dormancy period (0.15%). Similar results were obtained with regard to the richness of the essential oil with respect to thymol; at the flowering period the essential oil was richer in thymol (23.8%) than during the postflowering stage (21%). Later, Moldão-Martins et al. (*4*, *5*) published the essential oil composition of this thyme species, extracted by supercritical fluid extraction (SFE) and the traditional steam distillation (SD). According to these authors, SFE allows the extraction of higher yields than the SD technique.

To date, almost all of the papers in the scientific literature are related to the essential oil composition and yield of wild *T. zygis* subsp. *sylvestris* or *T. zygis* subsp. *gracilis*, but Sotomayor et al. (*6*, *7*) published the results obtained from plantations of *T. zygis* subsp. *gracilis* grown under dry and irrigated conditions, respectively. In both cases, dry matter content (22.2–38.1%) and essential oil yield (0.8–1.50%), calculated on the basis of fresh plant material production, were reported. The results showed the positive effect of watering on the thyme crop yield.

Essential oils and volatile products of plant secondary metabolism have wide applications in medicine, food flavoring, and preservation as well as in fragrance industries. There are many publications related to the antibacterial and antifungal activities of thyme essential oil (*8*–*12*). It has been used as weed germination inhibitor (*13*). There is interest in using thyme essential oil for delaying the autoxidation of food lipids (*14*).

Several culinary herbs, including thyme, are important sources of dietary antioxidants (*15*). Different extracts from thyme leaves have shown the presence of a large number of flavonoids and vitamin E, compounds of great interest in the food industry due to their antioxidant activities (*16*).

As plants are not available throughout the year, production of extracts is interesting. The industry demand is not met with commercial thyme. Currently, in Spain, >1500 ton/year of dry leaves is exported to foreign countries (*17*). This necessitates the harvesting of wild plants to satisfy the external market.

* Author to whom correspondence has been addressed (e-mail mariaj.jordan@carm.es; telphone +34 968-366765; fax +34 968-366792).

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However, this type of harvesting imposes a great commercial and ecological inconvenience, due to the great heterogeneity among the chemical compositions of the final products and the lack of production control.

The aims of the present study are to evaluate the possibility of cultivating red thyme under drip irrigation conditions and to determine how irrigation affects the yield and quality of the essential oil and dry material production.

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 average rainfall of 308.3 mm/year.

The study consisted of the application of three theoretical watering levels to achieve 20, 40, and 60% of the corresponding evapotranspiration (Eto) for cultivation of Graminaceous species. These values were calculated according to the Penman−Monteith model, taking into account the cultivation period (1234 mm/year). Weekly rainfall and the watering levels programmed were considered in order to regulate the amount of water received by the crops. Thus, actual watering levels corresponded to 30, 44, and 63% Eto, equivalent to 371, 542, and 777 mm/year, respectively.

An assay with four blocks and four experimental replications was designed. Each replication area (10.26 m^2) had two lines of drip irrigation polythene tubes. Two lines of plants were transplanted in both sides of each polythene tube, constituting a total of 140 plants, with a density of 1365 plants/100 m^2.

Plant Material. Commercial seeds of *T. zygis* subsp. *gracilis* from Puerto Lumbreras (Murcia, 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. Watering level treatments were applied after the second harvest (July 2001) and carried on until the third harvest period (June 6, 2002), when this species was between the phenological stages of full bloom and the beginning of fructification.

Fresh plant material was weighed immediately after harvesting, and a sample of 3 kg was kept for the determination of dry matter production. For that, plant material was dried in a forced-air dryer at 35 °C for 48 h, until it reached a constant weight. Dry matter obtained per 100 m^2 of cultivation area was determined by extrapolation of dry matter weight from 3 kg of fresh plant material to the total production in 100 m^2 of cultivation area.

Essential Oil Extraction. Four plants from each watering treatment were harvested, making a total of 16 plants. Aerial parts of dry individual plants were steam distilled for 3 h using a Clevenger-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.

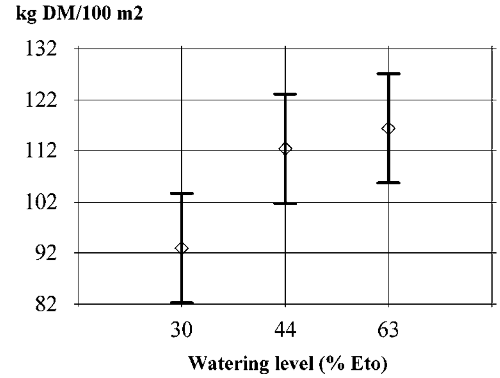

Figure 1. Plant dry matter production of *T. zygis* subsp. *gracilis* under different water supplies.

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

Mass Spectrometry Analysis. Gas chromatography−mass spectrometry (GC-MS) was used for the identification of volatile components in thyme essential oil. 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, γ-cadinene, 3-methyl-2-buten-1-ol, (*Z*)-Sabinene hydrate, (*E*)-verbenol, 3-heptanone, and pinocarvone, which were tentatively identified considering the NBS75K library spectra and their corresponding retention indices. Percentage compositions of samples were calculated according to the area of the chromatographic peaks.

Statistical Analysis. For comparison of the plant dry matter production, essential oil productions, and mean values of each component in the essential oils, Duncan's test was used.

RESULTS AND DISCUSSION

T. zygis subsp. *gracilis*, according to the scientific references consulted, has been cultivated previously by only Sotomayor (*6*), in dry land conditions, and by only Sotomayor et al. (*7*), under an experimental irrigation design.

The lack of essential oil and plant dry matter production from wild red thyme necessitates the adaptation of this species to domestic cultivation. On the basis of this, this work has been accomplished to achieve the optimum yield and quality production.

Plant Dry Matter Production. According to the results shown in **Figure 1**, yields of plant dry matter ranged from 93 ± 16 kg/100 m^2, at the lowest watering level (30% Eto), to 116 ± 10 kg/100 m^2 for the 63% Eto water supplement.

As expected, the application of different watering levels to *T. zygis* crops affected the dry matter production of this species. The lowest watering level (30% Eto) had a smaller yield than 63% Eto, but according to the statistical analysis it did not show differences with respect to the 44% Eto (112 ± 13 kg/m^2) water supply. This could be attributed to the great intraspecific variability observed among cultivation areas, as a consequence of using seeds from wild plants, which have not been previously subjected to any type of selection.

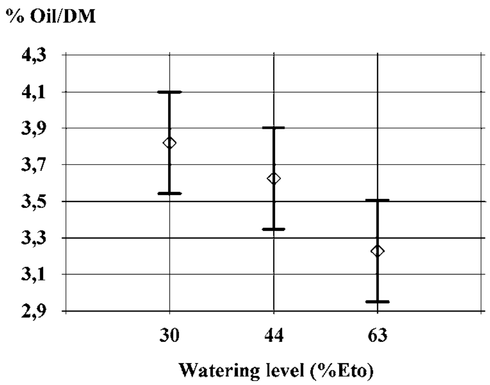

Figure 2. Essential oil yield percentage of *T. zygis* subsp. *gracilis* under different water supplies.

Agronomic assays carried out by Sotomayor et al. (*7*), related to the essential oil and dry matter production of three *Thymus* species cultivated under irrigation conditions (60% Eto), revealed for *T. zygis* subsp. *gracilis* a plant dry matter yield of 21.20 kg/100 m^2. This result corresponds to the first harvest of the shrubs in the current experimental work. Differences in production can be attributed to the development stage of the plants, because 116 ± 10 kg/100 m^2 was obtained after three years and three harvests in this cultivation area. Nevertheless, studies related to the dry matter production of this species under dry land conditions, reported by Sotomayor (*6*), showed an average plant dry matter production of 20.07 kg/100 m^2 after three years of cultivation practices. These results confirm the necessity of watering the crop, because an increase in the water supplement improves the plant dry matter production.

On the other hand, similar results are obtained when red thyme yields were compared with others obtained with *Thymus vulgaris* L., such as those published by Rometsch (*18*). This researcher studied the ecology and cultivation assessment of *T. vulgaris* L. in the Canton Valais, the driest region of Switzerland. For this, three different ecotypes were planted on 13 experimental plots of various meso- and microclimatic conditions. The results showed plant dry matter productions of 202, 99.8, and 166.3 kg/100 m^2 for German, Val d'Aosta, and CH90 ecotypes, respectively. Dry matter yields from Italian (Val d'Acosta) and Swiss (CH90) ecotypes were similar to those obtained in the present study from red thyme at different water supplements.

Rey (*19*) published the dry matter production of *T. vulgaris* var. Varicó after five harvestings, during three years of cultivation. For this variety, a production of 170 kg/100 m^2 was obtained. This work was performed in Arbaz (Switzerland), where the annual rainfall is greater than that of the semiarid conditions in which our study was carried out.

It is reasonable to think that the production of dry matter is correlated to the amount of water received by the shrubs, as shown in **Figure 1**. Although taking into account the great intraspecific variability existing among plants, differences cannot be detected between the 44 and 30% Eto and between the 44 and 63% Eto water treatments, respectively. Considering these results and the semiarid climate conditions in which these plants were cultivated (southeastern Spain), a water supplement equivalent to 44% Eto of the cultivation area would be enough to obtain an optimum plant dry matter production.

Essential Oil Yield. According to the results shown in **Figure 2**, essential oil production was enhanced by semiarid conditions. The watering level corresponding to 30% Eto had the greatest yield with (3.82 ± 0.40)% (relative to plant dry matter), showing statistically significant differences from the highest watering level treatments (3.23 ± 0.40% for 63% Eto), but no differences were detected compared to the 44% Eto treatment (3.62 ± 0.20%). Regarding the tendencies in the production of essential oils (**Figure 2**), it seems that increases in oil yield are associated with the decrease in the amount of water added. The production of essential oil could be associated with a response of the plant to a hydric stress. Nevertheless, no statistically significant differences were detected between 44 and 63% Eto watering level treatments, due to the great heterogeneity among plants. These results agree with those published by Letchamo and Gosselin (*20*), which affirmed that the content of *T. vulgaris* L. essential oil decreased significantly ($p < 0.01$) when the plants were supplemented with different levels of water under natural light growing conditions. Similar studies were carried out by Jordán et al. (*21*), related to the watering level effect on the essential oil yield and composition of *T. hyemalis* L. cultivated under the same conditions and cultivation area as in the present work. In this case, no statistically significant differences were detected among Eto watering level treatments. Once again, the great intraspecific variability among wild plants did not allow the study of the real effect of this parameter on the essential oil production.

Sotomayor (*6*, *7*) published the results obtained from plantations of *T. zygis* subsp. *gracilis* under dry and irrigated (60% Eto) land conditions, respectively. In both cases, yields of essential oil were expressed (0.8−1.0%) on the basis of fresh plant material production. These differences in production, compared to those obtained in the current experimental work, are probably due to the different growing stages in which the plants were collected. In this way, plants raised under dry land conditions cannot reach a correct development and less matter is produced. On the other hand, relative to the irrigation mode, as we expressed above, this result corresponds to the first year of plantation, in which the shrubs were harvested before reaching the flowering stage.

Moldão-Martins et al. (*3*) studied the seasonal variation in yield and composition of *T. zygis* subsp. *sylvestris* essential oil from Portugal. According to these authors, this species reaches the maximum essential oil yield at the flowering stage (0.9−1.4%). By comparison of these results with those obtained in the present work, *T. zygis* subsp. *gracilis* had double this production of essential oil, even considering the yield obtained under the maximum level of watering.

All of the information that can be found related to the essential oil production of cultivated *Thymus* genus concerns *T. vulgaris* L., including the work of Rometsch (*18*), who showed the essential oil production of three different ecotypes that have been already defined in the dry matter production section of the present work. Results showed an ecotype from Italy (Val d'Acosta) with an essential oil production on a dry weight basis (4.2%) superior to that obtained from red thyme, followed by the German (3.6%) and Swiss (2.5%) ecotypes. According to Letchamo and Gosselin (*22*), the environmental conditions in which plants are grown and the genetic constitutions of the cultivars greatly influence both oil yield and composition of thyme.

At an agronomic level, the production of essential oil is normally expressed as liters of oil per cultivation area, as shown in **Figure 3**. In this case, it is necessary to consider the fact that the water supplementation level is directly proportional to dry matter production and indirectly proportional to essential oil yield. Because these changes are compensatory with respect to the essential oil yield per cultivation area, no statistically

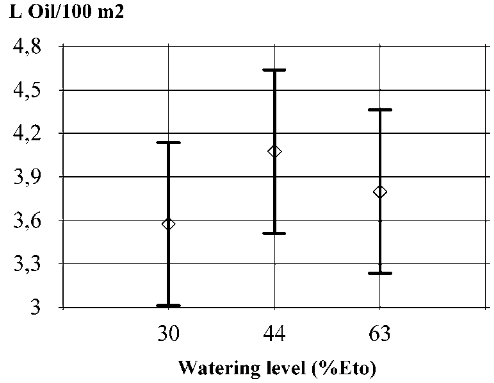

Figure 3. Essential oil yield percentage per cultivation area of *T. zygis* subsp. *gracilis* under different water supplies.

significant differences were detected among watering level treatments. From these results, and considering tendencies in yield production (**Figures 1** and **3**), a water supply equivalent to 44% Eto would be enough to produce optimum dry matter and essential oil yields per cultivation unit.

Essential Oil Volatile Profile. GC analysis of red thyme essential oil allowed the identification of a total of 86 volatile components, including 24 hydrocarbons, 22 alcohols, 5 aldehydes, 13 ketones, 9 esters, 8 phenols, 4 epoxides, and 1 ether (**Table 1**). Thymol was the major component quantified, defining the chemotype of this thyme species. This phenol is considered to be the component that defines the essential oil quality of thyme, because many chemical and pharmaceutical properties have been related to this component (*12*).

Currently, few publications can be found related to the essential oil composition of this thyme species. Sáez (*2*) studied the essential oil variability of *T. zygis* subsp. *gracilis* growing wild in southeasthern Spain. According to this author, a total of 36 components appeared to constitute the volatile profile of this thyme, which showed two different chemotypes, thymol and linalool. Related to the thymol chemotype, the concentration of this component ranged from 29.93 to 71.84%. Similar results were obtained by Sotomayor (*6*), who analyzed the essential oil production and quality of domestic *T. zygis* subsp. *gracilis* cultivated under dry land conditions. The chromatographic analysis resulted in the identification of a total of 32 major volatile components, thymol being the component present at the highest concentration. This assay was carried out over three years (1991−1993), at the same cultivation area used in the current study, so dry land conditions can be comparable to a water supply equivalent to 30% Eto. Taking into account the quantitative volatile profile of the essential oil distillate from plants harvested at the phenological stage of full bloom and the beginning of fructification, thymol concentration ranged from 14.9 to 50.8%.

Nevertheless, in the present work lesser variability was detected in the thymol concentration, which under the equivalent edaphoclimatic conditions yielded 64.16 ± 0.67% (**Table 1**).

In addition, 30 components are described for the first time as volatile constituents of the essential oil in this thyme subspecies and chemotype [3-methyl-1-butanol, 3-methyl-2-buten-1-ol, 3-hexanone, 3-heptanone, (*Z*)-jasmone, methyl acetate, octyl acetate, isobornyl acetate, methyl eugenol, tricyclene, Δ_3-carene, bisabolene, (*Z*)-3-hexen-1-ol, hexanol, (*Z*)-verbenol, (*E*)-verbenol, nonanal, decanal, perilla aldehyde, 2-butanone, 3-octanone, pinocarvone, α-ionone, β−ionone, benzyl acetate, neryl acetate, butyl caprylate, eugenol, (*Z*)-linalool oxide, and (*Z*)-limonene oxide]. Some of these components have been described to be present in other *Thymus* species (*21*, *23*).

To facilitate the description of the watering level effect on the essential oil composition, volatile components have been grouped on the basis of their chemical structures. One aspect 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**). The watering level effect was not noticeable for most of the volatile components under study.

Hydrocarbons. Components quantified at the highest concentrations were *p*-cymene, γ-terpinene, myrcene, α-thujene, and (*E*)-caryophyllene. Among 24 components identifieds, 16 showed statistically significant differences among treatments. It is interesting to highlight that *p*-cymene, β-pinene, and Δ_3-carene showed their maximum concentrations at the 63% Eto water supply, showing significant statistically differences from the other watering treatments. However, according to the statistical analysis, most of the components that showed statistically significant differences among watering supplement were favored by a 30% Eto water supply. These included tricyclene, α-thujene camphene, myrcene, α-phellandrene, γ-terpinene, γ-cadinene, and δ-cadinene. Aromadendrene and α-humulene were the components favored by a 44% Eto water supply.

Alcohols. Among the 22 components identified in the essential oil of this thyme species, linalool, borneol, terpinen-4-ol, (*E*)-sabinene hydrate, 1-octen-3-ol, and 3-octanol were the components having the greatest concentrations. Related to the watering level effect on these compounds, the concentrations of a total of 12 components showed statistically significant differences. Components characterized by their ability to confer a fresh aroma to the essential oil were favored by a water supply equivalent to 63% Eto, such as 3-methyl-2-buten-1-ol, hexanol, 1-octen-3-ol, linalool, *p*-cymen-8-ol, and γ-terpineol. Nevertheless, for the rest of the alcohols under study, including (*E*)-pinocarveol, (*Z*)-verbenol, (*E*)-verbenol, borneol, and nerol + citronellol, their presences were favored by a watering level equivalent to 30% Eto.

Aldehydes. Geranial was the component quantified at the greatest relative concentration. A watering level effect on the essential oil aldehydic composition was noticeable for most of the components identified. In this way, a water supply equivalent to 63% Eto yielded the highest concentrations for these components, it being interesting that geranial was not detected at the lowest watering level applied.

Ketones and Esters. A similar behavior was observed for these groups of components, because the highest concentrations for most of the components that showed statistically significant differences among concentrations were detected at the water supply equivalent to 63% Eto. Compounds including 3-hexanone, 3-hepatanone, 3-octanone, benzyl acetate, terpenyl acetate, and butyl caprylate were detected at their maximum concentrations under this water supply.

Phenols. These components are characterized by their definition of the essential oil quality of the thyme essential oil. Thymol is the component that defines the chemotype in this thyme variety. Its concentration ranged from 64.16% ± 0.67 to 54.69% ± 1.37 at the 30 and 63% Eto watering level treatments, respectively. In this case, the 30% Eto water supply yielded the maximum concentrations for most of the phenols whose concentrations showed statistically significant differences, except for (*E*)-anethole, which seems to need more watering in order to be detected at greater than trace amounts.

Table 1. Watering Level Effect on *T. zygis* Essential Oil Composition[a]

component	RT[b]	RI[c]	watering level 63% Eto	44% Eto	30% Eto
terpenic hydrocarbons					
tricyclene[d]	8.45	929	0.02 ± 0.01a	0.01 ± 0.00b	tr[a,b]
α-thujene[d]	8.74	940	1.12 ± 0.07a	0.95 ± 0.04b	1.00 ± 0.17ab
α-pinene	9.08	949	0.74 ± 0.02a	0.60 ± 0.04a	0.57 ± 0.06a
camphene	9.84	969	0.37 ± 0.13a	0.16 ± 0.04b	0.25 ± 0.15ab
verbenene[d]	10.09	976	0.01 ± 0.00a	tr[a]	tr[a]
sabinene	11.08	1001	0.01 ± 0.01a	0.01 ± 0.00a	0.01± 0.01a
β-pinene	11.31	1007	0.18 ± 0.01a	0.16 ± 0.01b	0.14 ± 0.02c
myrcene	12.14	1026	1.01 ± 0.10a	1.11 ± 0.15a	1.30 ± 0.12b
α-phellandrene	12.83	1042	0.10 ± 0.02a	0.13 ± 0.02b	0.14 ± 0.03b
Δ_3-carene	13.12	1048	0.09 ± 0.00a	0.08 ± 0.00 b	0.08 ± 0.00b
α-terpinene	13.58	1058	1.01 ± 0.08a	1.18 ± 0.27a	1.22± 0.22a
p-cymene	14.05	1068	22.41 ± 0.42a	16.79 ± 4.59b	14.21 ± 0.88b
limonene	14.28	1073	0.51 ± 0.02a	0.48 ± 0.03ab	0.45 ± 0.02b
(*Z*)-β-ocimene	14.88	1086	0.01 ± 0.00ab	tr[a]	tr[b]
(*E*)-β-ocimene	15.52	1097	0.01 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.01a
γ-terpinene	16.08	1109	1.99 ± 0.60a	3.33 ± 0.76b	2.99 ± 0.39a,b
terpinolene	17.87	1141	0.16 ± 0.03a	0.14 ± 0.02a	0.15 ± 0.01a
(*E*)-caryophyllene	37.22	1419	0.93 ± 0.10a,b	1.19 ± 0.47a	0.62 ± 0.16b
aromadendrene	38.25	1440	0.03 ± 0.03a	0.43 ± 0.13b	0.22 ± 0.15c
α-humulene	38.99	1457	0.04 ± 0.01a	0.13 ± 0.06ab	0.16 ± 0.12b
alloaromadendrene	39.41	1465	0.03 ± 0.02a	0.04 ± 0.03a	0.02 ± 0.01a
γ-cadinene[d]	42.12	1537	0.08 ± 0.01a	0.03 ± 0.02b	0.06 ± 0.04ab
δ-cadinene	42.59	1553	0.16 ± 0.05a	0.06 ± 0.02b	0.11 ± 0.07ab
(*Z*)-α-bisabolene	43.56	1572	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a
alcohols					
3-methyl-1-butanol	3.33	767	0.01 ± 0.01a	0.01 ± 0.01a	tr[a]
3-methyl-2-buten-1-ol[d]	3.94	789	0.02 ± 0.01a	0.01 ± 0.01b	tr[b]
(*Z*)-3-hexen-1-ol	5.89	852	0.03 ± 0.03a	0.04 ± 0.04a	0.05 ± 0.00a
hexanol	6.29	865	0.04 ± 0.01a	tr[b]	0.02 ± 0.02b
1-octen-3-ol	11.50	1009	0.47 ± 0.06a	0.26 ± 0.05b	0.19 ± 0.07c
3-octanol	12.41	1031	0.15 ± 0.08a	0.13 ± 0.07a	0.17 ± 0.14a
(*E*)-sabinene hydrate	16.51	1116	0.67 ± 0.26a	0.59 ± 0.10a	0.49 ± 0.02a
(*Z*)-sabinene hydrate[d]	18.53	1151	0.11 ± 0.03a	0.08 ± 0.04a	0.09 ± 0.00a
linalool	18.64	1152	3.35 ± 0.75a	1.36 ± 0.10b	2.48 ± 0.68c
(*E*)-pinocarveol	20.90	1186	tr[a]	0.01 ± 0.01b	tr[a,b]
(Z)-verbenol	21.12	1188	0.01 ± 0.00ab	0.02 ± 0.00a	0.01 ± 0.00b
(*E*)-verbenol[d]	21.56	1193	0.01 ± 0.01a	tr[b]	0.01 ± 0.01a
isoborneol	22.21	1203	tr[a]	tr[a]	0.01 ± 0.00a
borneol	22.60	1213	0.74 ± 0.23a	0.32 ± 0.13b	0.60 ± 0.41ab
terpinen-4-ol	23.34	1220	0.62 ± 0.14a	0.63 ± 0.06a	0.62 ± 0.06a
p-cymen-8-ol	23.91	1227	0.08 ± 0.05a	0.02 ± 0.01b	0.02 ± 0.00b
α-terpineol	24.18	1231	0.08 ± 0.04a	0.09 ± 0.05a	0.06 ± 0.04a
γ-terpineol	24.59	1237	0.04 ± 0.03a	tr[b]	tr[b]
nerol + citronellol	26.47	1260	0.03 ± 0.02ab	tr[a]	0.05 ± 0.02b
geraniol	28.14	1280	0.01 ± 0.01a	tr[a]	tr[a]
spathulenol	45.21	1640	0.07 ± 0.08a	0.13 ± 0.10a	0.07 ± 0.04a
aldehydes					
nonanal	18.95	1157	0.02 ± 0.01a	0.01 ± 0.00a	0.01 ± 0.00a
decanal	25.16	1243	0.02 ± 0.00a	0.02 ± 0.01a	0.02 ± 0.00a
neral	27.16	1270	0.01 ± 0.00a	tr[b]	tr[c]
geranial	28.94	1291	0.43 ± 0.03a	0.43 ± 0.18a	tr[b]
perialdehyde	29.07	1303	0.02 ± 0.01a	tr[b]	tr[b]
ketones					
2-butanone	2.33	729	tr[a]	tr[a]	tr[a]
3-hexanone	4.36	795	0.01 ± 0.01a	tr[b]	tr[b]
3-heptanone[d]	6.96	888	0.01 ± 0.00a	tr[b]	tr[b]
3-octanone	11.87	1019	0.37 ± 0.16a	0.16 ± 0.03b	0.13 ± 0.05b
β-thujone	18.94	1158	0.01 ± 0.01a	tr[a]	tr[a]
camphor	21.30	1192	0.02 ± 0.01a	0.01 ± 0.00a	0.02 ± 0.00a
pinocarvone[d]	22.51	1208	tr[a]	tr[a]	tr[a]
dihydrocarvone	24.58	1235	0.01 ± 0.02a	0.02 ± 0.00ab	0.06 ± 0.06b
verbenone	25.22	1245	0.02 ± 0.00a	0.01 ± 0.01a	0.01 ± 0.01a
thymoquinone	27.77	1276	0.02 ± 0.00a	0.02 ± 0.02a	tr[b]
(*Z*)-jasmone	36.18	1404	0.02 ± 0.00ab	0.03 ± 0.02b	0.01 ± 0.01a
α-ionone	37.67	1426	0.01 ± 0.01a	0.01 ± 0.01a	tr[a]
β-ionone	40.74	1497	0.02 ± 0.01a	0.01 ± 0.01a	0.01 ± 0.01a
esters					
methyl acetate	2.03	719	0.01 ± 0.00a	0.03 ± 0.04b	0.01 ± 0.01a
benzyl acetate	22.68	1214	0.05 ± 0.01a	0.05 ± 0.01a	0.02 ± 0.01b
octyl acetate	25.52	1248	0.01 ± 0.01a	tr[a]	tr[a]
bornyl acetate	29.78	1302	tr[a]	0.06 ± 0.05a	0.04 ± 0.07a
isobornyl acetate	29.83	1304	tr[a]	0.02 ± 0.04a	tr[b]

Table 1. (Continued)

component	RT[b]	RI[c]	watering level 63% Eto	44% Eto	30% Eto
esters (continued)					
terpenyl acetate	33.44	1353	0.01 ± 0.00a	tr[b]	tr[b]
citronellyl acetate	33.66	1356	0.01 ± 0.02a	tr[a]	0.01 ± 0.01a
neryl acetate	34.24	1366	0.01 ± 0.02a	tr[a]	tr[a]
butyl caprylate	35.57	1387	0.03 ± 0.01a	tr[b]	tr[b]
phenols					
thymol methyl ether	26.89	1265	0.01 ± 0.00a	0.02 ± 0.01a	0.02 ± 0.01a
carvacrol methyl ether	27.40	1272	0.06 ± 0.03a	0.08 ± 0.05a	0.51 ± 0.65a
(*E*)-anethole	30.06	1303	0.18 ± 0.04a	tr[b]	tr[b]
thymol	30.13	1308	54.69 ± 1.37a	61.63 ± 3.33b	64.16 ± 0.67b
carvacrol	30.78	1314	3.43 ± 0.09a	3.60 ± 0.09a	3.33 ± 0.55a
eugenol	33.88	1358	0.03 ± 0.03a	0.11 ± 0.04b	0.08 ± 0.04b
methyl eugenol	36.57	1408	0.01 ± 0.01a	0.01 ± 0.00a	tr[a]
isoeugenol	38.86	1451	tr[a]	0.04 ± 0.03a	0.02 ± 0.04a
epoxides					
(*Z*)-linalool oxide	16.94	1123	0.03 ± 0.02a	0.01 ± 0.01b	0.01 ± 0.02ab
(*E*)-linalool oxide	17.90	1140	0.01 ± 0.01a	tr[a]	0.01 ± 0.02a
(*Z*)-limonene oxide	20.69	1173	0.01 ± 0.01a	0.01 ± 0.00a	tr[a]
caryophyllene oxide	37.22	1650	0.30 ± 0.04a	0.34 ± 0.06a	0.19 ± 0.04b
ether					
cineole	14.45	1076	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a

[a] Values within rows with common letters were not significantly different (**P* < 0.05) ± standard deviation. [b] RT Retention times. [c] Kovat's Index (HP-5). ** tentative identification.

Epoxides and Ethers. For these components, taking into account the statistical analysis, only a decrease in the concentration of caryophyllene oxide at the lowest water supply stands out. The rest of the components, probably due to the intraspecific variability, did not show statistically significant differences among watering level treatments.

According to these results, watering level treatments affect the volatile profile of thyme essential oil composition. Thus, the application of a water supplement equivalent to 63% Eto favored the production of an essential oil richer in low molecular weight components, which are characterized by their ability to impart a fresh aroma to the essential oil. Conversely, for thymol, the major component quantified, its concentration was favored by treatments with lesser amounts of applied water. On the other hand, the concentrations of the precursors *p*-cymene and γ-terpinene have been found to vary in accordance with the variations in concentrations of their corresponding final phenol products (thymol and carvacrol). A water supplement equivalent to 63% Eto improved the presence of these two precursors in red thyme essential oil. From this, it can be concluded that 44% Eto is the optimum water supply with respect to achieving the best quantity and quality (on the base of thymol production) of essential oil and dry matter production of *T. zygis* subsp. *gracilis*. These results agree with those published by Piccaglia and Marotti (*24*), which affirmed that different climatic conditions had considerable effects on *T. vulgaris* L. essential oil composition. They reported that production of phenolic compounds increases in warm and dry environmental conditions. However, according to Jordán et al. (*21*), this effect was not noticeable for the *T. hyemalis* Lange phenolic content. For this species, thymol content was favored by an increase in the amount of water added. As a consequence of the great intraspecific variability among plants, no statistically significant differences were detected among the 80, 60, and 40% Eto watering levels, although 20% Eto showed statistically significant differences with respect to the 80 and 60% Eto water supplements.

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