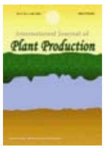

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Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*

B. Bahreininejad[a], J. Razmjoo[a,*], M. Mirza[b]

[a]*Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, 84156-83111, Isfahan, Iran.*
[b]*Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran.*
*Corresponding author. E-mail: krazmjoo@cc.iut.ac.ir

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Abstract

Thymus daenensis is a medicinal plant endemic to semi-arid regions of Iran. A field experiment using a randomized complete block design with four replications was conducted to evaluate the effect of 20, 50 and 80% soil water depletion on morpho-physiological traits, essential oil content and composition and water use efficiency of *T. daenensis* during 2010-2011. Water stress reduced growth, herbage production, chlorophyll and carotenoid content, while increased proline, K^+, essential oil content and irrigation water use efficiency based on essential oil yield ($IWUE_{eso}$). Thymol was the highest essential oil composition (63.3-73.5%) followed by carvacrol (3.6-16.0%), ρ-cymene (3.8-7.4%), γ-terpinene (3.3-4.7%), β-caryophyllene (2.8-4.0%) and borneol (1.4-3.4%), respectively. Thymol, ρ-cymene and γ-terpinene were increased, while the other compositions decreased under water stress. It is concluded that irrigation of *T. daenensis* based on 50% water depletion should be an appropriate choice for first growing season and 80% water depletion for the second growing season in semi-arid climatic conditions.

Keywords: *Thymus daenensis*; Water deficit; Growth; Essential oil.

Introduction

Arid and semi-arid regions of the world facing water shortage, therefore using new and more tolerate plant species with higher production is a vital necessity (Prohens et al., 2003; Razmjoo et al., 2008; Baghalian et al., 2011).

Generally, endemic plants are more adapted to environmental conditions than commercial agronomic crops. Shifting from current commercial agronomic crops to endemic plants which are more adaptable to environmental conditions would be a wise way for sustainable agriculture production.

Thymus daenensis subsp. *daenensis* Celak, a perennial dwarf shrub native plant to semi-arid zones of Iran, is considered as an aromatic and medicinal plant. The aerial parts of *T. daenensis* are commonly used as spices, condiments and flavoring agents (Rechinger, 1982; Zargari, 1990; Nickavar et al., 2005). Effects of irrigation regimes on growth, essential oil content and composition of other species of *Thymus* such as *T. hyemalis* (Jordan et al., 2003) and *T. vulgaris* (Lechamo and Gosselin, 1995; Khazaiei et al., 2008) have been reported. There has been a few published reports concerning the chlorophyll, cartenoid, proline and K^+ contents of medicinal and aromatic plants in general (Misra and Srivasta, 2000; Letchamo et al., 1995) and *T. daenensis,* in particular, under drought stress for which there is a need to discover as to how drought stress may influence such traits. This species is also considered as an endangered species in recent times due to overharvesting for traditional medicinal uses, rough grazing, climate changes and plowing of rangelands for agricultural purposes (Jalili and Jamzad, 1999; Norouzi, 2000; Rahimmalek et al., 2009). The objective of this experiment was to investigate the effects of water stress on growth, chlorophyll, carotenoid, proline, K^+ contents, essential oil yield, content and composition, of *T. daenensis* in a clay loam soil at Experimental Station of Isfahan Agricultural and Natural Resources Research Center located in a semi-arid region.

Materials and Methods

Plant material and field site description

Seeds of *T. daenensis* subsp. *daenensis* Celak, were collected from the region of Kohroyeh, Isfahan, Iran (31° 42’ N, 51° 41’ E and 1850 m above the mean sea level) in July 2008 and used in this study.

The experiments were conducted at the Experimental Station of Isfahan Agricultural and Natural Resources Research Center, located at Najaf-Abad (18 km west Isfahan, 32° 37’ N, 51° 28’ E and an altitude of 1612 m), during 2010-2011 using a randomized complete block design with four replications. This site was characterized by a semi-arid climate with a mean annual precipitation of 140 mm (mainly during the fall and winter) and an average temperature of 16 °C (Karimi, 1992; Yaghmaei et al., 2009). Precipitation was almost negligible (less than 2 mm) over the experiment period. The daily minimum and maximum air temperatures, average relative humidity, reference evapotranspiration based on the FAO Penman-Monteith equation (Allen et al., 1998) are presented in Figure 1.

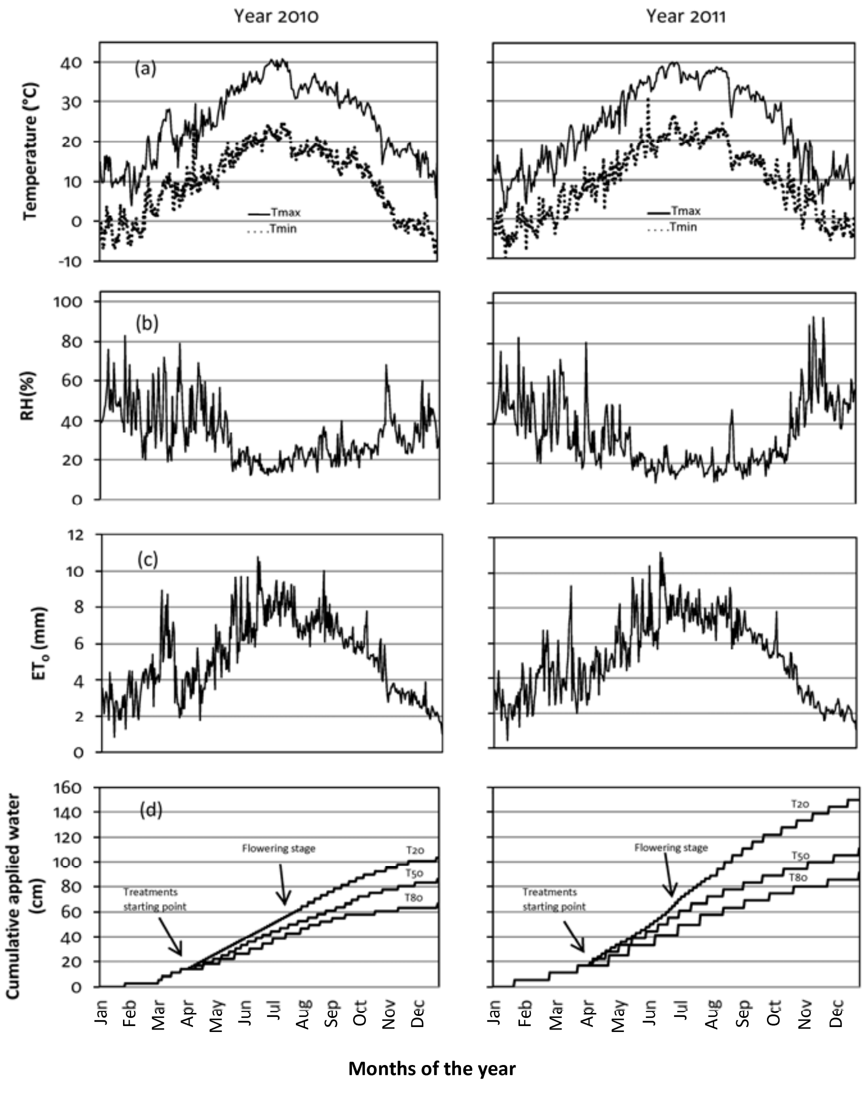

Figure 1. The daily minimum (T_{min}) and maximum (T_{max}) air temperatures (a); average relative humidity (b); reference evapotranspiration (ET_o); and cumulative amount of water applied for irrigation regimes (T_{20}, T_{50} and T_{80}) (d), for 2010 and 2011.

The soil characteristics were composed as the following: sand (45%), silt (24 %), clay (31%), pH (7.7), EC (2.8 dS m^{-1}), volumetric water content at field capacity (31%) and permanent wilting point (13.5%). Two parallel trials were established on the same plots. Trial one was conducted from March 2010 to February 2011. Trial two was conducted from March 2011 to February 2012. Seeds were sown in the nursery on 10 January 2010. The seedlings were transplanted to the field on 5 March 2010 in 4 equidistant rows with adjacent rows being 60 cm apart. The distance between plants in each row was 30 cm. Each experimental plot size was 5×2 m. Fertilizers were applied at a rate of 60, 50 and 25 kg ha^{-1} of P_2O_5, K_2O and N, respectively, incorporated uniformly to the soil before planting.

Irrigation treatments

Irrigation treatments were scheduled based on the maximum allowable depletion (MAD) percentage of the soil available water (SAW) between-0.03 to -1.5 MPa (Kramer and Boyer, 1995). The predefined treatments applied were 20, 50 and 80% MAD of ASW, representing the non-stressed control (T_{20}), moderate drought stress (T_{50}) and severe drought stress (T_{80}), respectively. A TDR probe (TDR Trase System, Model 6050X1; Soil Moisture, Santa Barbara, CA) was used to measure soil water content. The TDR probe readings (at the center of each plot to a soil depth of 20 cm for the first year and 40 cm for the second year) were taken two days after irrigation and continued up to one day prior to irrigation. Based on the previous studies on this species (Bahreininejad, 2007; Bahreininejad el al., 2010) root zone observation was made six times over growing season and revealed that the root growth in this species is mainly horizontally near to the soil surface. Also the root depth over April-July (treatment period) was about 20 cm for the first year and 40 cm for the second year.

No water stress was applied in the first month of growth cycle in order to limit plant mortalities. In this period, all experimental plots were irrigated when 50% of SAW was depleted. Irrigation treatments were imposed during the remaining three months of growing period. The experimental plots were irrigated when the respective MAD threshold values for each treatment were reached. The depth of irrigation was determined based on the soil water content and calculated using the following equations:

$$SAW = (\theta_{fc} - \theta_{pwp}) \times D \times 100 \tag{1}$$

$$I_d = SAW \times p \tag{2}$$

$$I_g = \frac{I_d \times 100}{E_a} \tag{3}$$

Where SAW is soil available water (cm); θ_{fc} and θ_{pwp} are the volumetric soil water content (%) at field capacity (0.03 MPa) and permanent wilting point (1.5 MPa), respectively; D is the soil layer depth (cm); I_d is the irrigation depth (cm); p is the fraction of SAW (20%, 50% and 80%) that can be depleted from the root zone; I_g is the gross depth of irrigation (cm) and E_a is the irrigation efficiency (%) averagely assumed as 65% (Sepaskhah, 2003; Tafteh and Sepaskhah, 2012). The applied irrigation water (based on Eq. (3)) at each irrigation event was measured by flow meters installed in the pipe outlet delivering water to the plots (cumulative amount of water applied for irrigation regimes are presented in Figure 1).

Morphological and physiological measurements

Plant height, leaf area and areal fresh and dry weights were determined at harvesting time (at full flowering stage, on 10 July 2010 and 25 June 2011). Plant height (cm) was measured from the soil surface to the tip of the tallest flowering stem. Plants were cut from 1 m^2 area just above the lignified parts of the stem, immediately weighed (fresh weight) and then dried at 40 ^oC in the dark until it reached a constant weight. The total leaf area of 1 m^2 area of harvested herbs was measured using a green leaf area meter (Model GA-5, Ogawa Seki Co. LTD, Tokyo, Japan).

Five randomly selected plants were dug from 1 m deep soil trenches and roots were washed with water. The root lengths and weigh were determined (after the tissues were dried at 40 ^oC until it reached a constant weight) at the end of growing season in each year.

Proline content was determined in fully expanded uppermost leaves at full flowering stage using the method of Bates et al. (1973).

Leaf chlorophyll (chlorophyll *a* and chlorophyll *b)* contents were measured using spectrophotometry (Model PD-303, Apel Co. Ltd, Japan) in fully expanded uppermost leaves at full flowering stage, based on the method described by Arnon (1949).

Irrigation water-use efficiency

Irrigation water use efficiency (IWUE) is defined as the ratio of the crop yield to irrigation water applied, including rainfall (Howell, 1994). The $IWUE_{dm}$ and $IWUE_{eso}$ (kg m^{-3}) were estimated by dividing dry matter (kg ha^{-1}) and essential oil yield (kg ha^{-1}), respectively using water applied for each irrigation level (m^3 ha^{-1}).

Phytochemical measurements

The aerial parts of plants were harvested at full flowering stage, dried at room temperature and stored until analysis inside paper bags in a cool and dark place. Essential oils were obtained from aerial parts of each sample (40 g dry matter) by hydrodistillation for 2 hours using a Clevenger-type apparatus. The oils were stored in sealed vials at 4 ^{o}C before analyses.

Samples of 0.1 µL were subjected to analysis by capillary gas chromatography (GC). The GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (60 m × 0.25 mm; 0.25 µm film thickness). Oven temperature was held at 40 ^{o}C for 5 minutes and then programmed to 280 ^{o}C at a rate of 4 ^{o}C min^{-1}. Injector and detector (FID) temperatures were 290 ^{o}C; Helium was used as carrier gas with a linear velocity of 32 cm s^{-1}.

The identification of volatile components in thyme essential oil was made by gas chromatography/mass spectrometry (GC-MS). The GC-MS analyses were carried out on a Varian 3400 column (60 m × 0.25 mm; 0.25 µm film thickness). Oven temperature was 40-220 ^{o}C at a rate of 3 ^{o}C min^{-1}; transfer line temperature was 240 ^{o}C; injector temperature was 230 ^{o}C; carrier gas was helium with a linear velocity of 31.5 cm s^{-1}; split ratio was 1:60; flow rate was 1.1 mL min^{-1}; ionization energy was 70 eV; scan time was 1 second; and mass range was 40-350 amu.

The components of the essential oil were identified by comparison of their mass spectra with those of a GC/MS system equipped with a DB-5 fused silica computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 1995). The retention indices (retention index is a concept used in gas chromatography to convert retention times into system-independent constants) were calculated for all volatile constituents using a homologues series of n-alkanes.

Statistical analysis

The recorded data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) for comparison of means using SAS (ver. 8.2) software.

Results and Discussion

Effect of water deficit on plant morphology

Plant height, leaf area and root length and dry weight reduced under water stress in both years and this effect was more pronounced with the severity of water stress (Table 1). Except plant height, other traits were higher in second year. That was expected since plants were produced more biomass and the plant roots were deeper in the second year. Our results were generally in line with Letchamo and Gosselin (1995) in *T. vulgaris*, Bettaieb et al. (2009) in *Salvia officinalis,* Davatgar et al. (2009) in rice and Laribi et al. (2009) in *Carum carvi*. The reductions in plant height, leaf area, root length and dry weight under water stress were perhaps due to the decline in the cell enlargement and more leaf senescence resulting from reduced turgor pressure (Shao et al., 2008).

Table 1. Water deficit effects on plant height, leaf area, root length and root dry weight of *Thymus daenensis*.

Water deficit levels	Plant height (cm)		Leaf area ($m\ m^{-2}$)		Root length (cm)		Root dry weight ($kg\ ha^{-1}$)	
	2010	2011	2010	2011	2010	2011	2010	2011
T_{20}	24.13^a	22.83^a	0.88^a	1.44^a	16.20^a	41.74^a	701.95^a	2103.8^a
T_{50}	23.89^a	20.78^a	0.36^b	1.03^b	14.74^a	35.20^b	448.97^b	882.0^b
T_{80}	18.16^a	20.45^a	0.16^c	0.92^b	12.22^b	27.72^c	351.67^b	452.4^c
Mean	22.06^a	21.35^a	0.46^b	1.13^a	14.39^b	34.89^a	500.86^b	1146.07^a

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at $P<0.05$ (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at $P<0.05$ (means of the treatments over each year).

Effect of water deficit on herbage production

Fresh and dry weight of aerial parts reduced as water stress level increased in both years (Table 2). In agreement with our results, Letchamo and Gosselin (1995) in *T. vulgaris*, Said-Al Ahl et al. (2009) in oregano and Houshmand et al. (2011) in chamomile also reported that drought stress reduced herbage yield of tested species.

Table 2. Water deficit effects on top fresh weight, dry matter weight, essential oil content and yield of *Thymus daenensis*.

Water deficit levels	Top fresh weight (kg ha^{-1})		Dry matter weight (kg ha^{-1})		Essential oil content (%)		Essential oil yield (kg ha^{-1})	
	2010	2011	2010	2011	2010	2011	2010	2011
T_{20}	4944.1^{a}	9259.3^{a}	1359.4^{a}	3383.5^{a}	1.63^{c}	2.19^{b}	22.16^{a}	74.23^{a}
T_{50}	2991.5^{b}	7122.5^{b}	1012.3^{b}	2391.0^{b}	2.36^{b}	2.57^{ab}	23.83^{a}	61.71^{a}
T_{80}	1340.0^{c}	6832.1^{b}	553.4^{c}	2081.2^{b}	2.55^{a}	3.22^{a}	14.11^{b}	64.93^{a}
Mean	3091.8^{b}	7738.0^{a}	975.0^{b}	2618.6^{a}	2.18^{b}	2.66^{a}	20.03^{b}	66.96^{a}

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at $P<0.05$ (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at $P<0.05$ (means of the treatments over each year).

Decrease in fresh and dry weight was as a result of reduction in plant height and leaf area as indicated in Table 1. Reduction in fresh and dry weight of the plant may also be due to a decrease in plant growth, photosynthesis and canopy structure during the water stress as reported by Shao et al. (2008). Moreover, the pronounced effect of decreased irrigation on overall growth of *T. daenensis* may be attributed to the lower availability of sufficient moisture around the root and thus a lesser proliferation of root biomass resulting in the lower absorption of nutrients and water leading to production of lower biomass (Singh et al., 1997).

Dry matter weight decreased 26% and 59% in 2010 and 29% and 39% in 2011 under moderate and severe water stresses, respectively. Top fresh and dry matter weight were significantly higher in second year (Table 2). These results suggested that the plants produced proportionally more dry matter weight in the second year, which was expected since plants produced deeper root in the second year. In general for attain to the highest dry matter weight, 20% soil water depletion is recommended.

Effect of water deficit on essential oil content and yield

Essential oil content increased while essential oil yield decreased under water stress (Table 2). It was reported that water stress increased essential oil accumulation via a higher density of oil glands due to the reduction in leaf area (Simon et al., 1992). In addition, the increase in essential oil concentration under water stress could be due to the fact that plants produce high terpene concentrations under water stress conditions due to a low allocation of carbon to the growth, suggesting a trade-off between growth and defense (Turtola et al., 2003).

Water deficit decreased oil yield of *T. daenensis* under moderate and severe water deficit, respectively (Table 2). Reduction in essential oil yield was due to reduction in herbage yield as indicated in Table 2. This indicated that essential oil yield is positively related to soil water content and herbage yield (Singh et al., 1997).

Effect of water deficit on proline and K^+ content

Proline content reduced under moderate water stress, but increased under severe water stress (Table 3). In agreement with our results, Wang et al. (2004) reported an increase in proline content of several plant species in water stress conditions. They further suggested that the accumulation of proline may play an important role in drought adaptation in the tested species.

Moderate water stress had no effect on K^+ content, but severe water stress increased K^+ content (Table 3). In plants coping with water stress, the accumulation of K^+ may be more important than the production of organic solutes during the initial adjustment phase. This is because osmotic adjustment through ion uptake like K^+ is more energy efficient (Hsiao, 1973). The underlying mechanism for maintaining adequate tissue K^+ levels under water stress seems to be dependent upon selective K^+ uptake and selective cellular K^+ compartmentalization and distribution in the shoots (Carden et al., 2003).

Table 3. Water deficit effects on concentrations of proline and K^+.

Water deficit levels	Proline (μ mol/g FW)		K^+ (%)	
	2010	2011	2010	2011
T_{20}	3.10^a	3.57^a	1.73^b	1.73^b
T_{50}	2.38^b	2.72^a	1.69^b	1.79^b
T_{80}	3.52^a	3.86^a	1.84^a	1.92^a
Mean	3.00^a	3.38^a	1.75^b	1.81^a

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at P<0.05 (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at P<0.05 (means of the treatments over each year).

Effect of water deficit on photosynthetic pigments content

Water stress decreased chlorophyll *a*, total chlorophyll and carotenoid contents of the leaf tissues (Table 4). Carotenoid content was affected more at moderate than severe water stress. Effect of irrigation regimes on chlorophyll *b* content was not significant but increased under moderate water stress while reduced under severe water stress levels. Photosynthetic pigments and proline are both synthesized from the same substrate. Therefore, an increase in the photosynthesis of proline leads to a decrease in the synthesis of photosynthetic pigments under water deficit (Paleg and Aspinal, 1981). Thus, the reduction in photosynthetic pigments in our study could be, at least in part, due to increase in proline content as indicated in Table 3.

Table 4. Water deficit effects on concentrations of leaf chlorophyll and carotenoid.

Water deficit levels	Chlorophyll *a* (µg ml^{-1})		Chlorophyll *b* (µg ml^{-1})		Chlorophyll total (µg ml^{-1})		Carotenoid (µg ml^{-1})	
	2010	2011	2010	2011	2010	2011	2010	2011
T_{20}	4.74^a	4.58^a	5.66^a	5.89^a	10.40^a	10.46^a	7.22^a	6.71^a
T_{50}	3.23^b	3.25^b	6.30^a	5.42^a	9.53^a	8.67^{ab}	4.86^b	4.42^b
T_{80}	3.16^b	2.64^b	5.26^a	5.07^a	8.42^a	7.70^b	6.05^{ab}	5.38^b
Mean	3.71^a	3.49^a	5.74^a	5.46^a	8.45^a	8.94^b	6.04^a	5.50^b

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at $P<0.05$ (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at $P<0.05$ (means of the treatments over each year).

Water use analysis

Average numbers of irrigation were 52, 26 and 17 and average yearly amount of water used were 12908, 9660 and 7888 m^3 for control, moderate and severe water stresses, respectively (Table 5). These results imply that *T. daenensis* used 25 and 39% more water under control as compared with moderate and severe water stress treatments, respectively. In addition numbers of irrigation were 28% and 41% higher under control as compared with moderate and severe water stresses, respectively. These results suggest that perhaps more water was lost through evapotranspiration under control. Our results were in agreement with those of Ucan et al. (2007) in sesame and Eiasu et al. (2009) in rose-scented geranium.

Table 5. Number of irrigation, water use, and irrigation water use efficiency (based on dry matter and essential oil yield) of *Thymus daenensis* under different water deficit levels.

Water deficit levels	Number of irrigation (yr^{-1})		Water use (m^3 ha^{-1})		$IWUE_{dm}$ (kg m^{-3})		$IWUE_{eso}$ (kg m^{-3})	
	2010	2011	2010	2011	2010	2011	2010	2011
T_{20}	60	44	10142^a	15674^a	0.134^a	0.216^a	0.0022^b	0.0047^b
T_{50}	31	20	8211^b	11109^b	0.123^a	0.215^a	0.0029^a	0.0056^b
T_{80}	20	14	6409^c	9367^c	0.086^b	0.222^a	0.0022^b	0.0069^a
Mean	37	26	8254^b	12050^a	0.118^b	0.217^a	0.0024^b	0.0056^a

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at $P<0.05$ (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at $P<0.05$ (means of the treatments over each year).

Number of irrigation in the second year was 36% lower than the first year (Table 5), that could be due to deeper roots in the second year. Whereas the amount of water used in the second year was 46% higher than the first year (Table 5), that could be as a result of higher leaf area in the second year which caused more evapotranspiration.

Average of two years, herbage and essential oil yield decreased 28%, 45%, 3% and 18% under moderate and severe water stress as compared with the control, respectively (Table 2). At the same time, $IWUE_{dm}$ decreased 3% and 12% while $IWUE_{eso}$ increased 23% and 32% under moderate and severe water stresses, respectively (Table 5). This suggested that the plants invested proportionally more of its photosynthetic resources into essential oil production per unit of water consumed. Water stress apparently increased essential oil content as indicated in Table 2 and it was sufficient to compensate for the yield loss due to reduced herbage yield. $IWUE_{dm}$ and $IWUE_{eso}$ were significantly higher in the second year. The increased in the $IWUE_{dm}$ and $IWUE_{eso}$ was more pronounced in moderate and severe water stress levels as compare to the control. These results showed that there is a high potential for increasing water use efficiency through deficit irrigation practice. Based on reduction in the number of irrigation, water used in a year, essential oil yield and $IWUE_{eso}$, the authors recommend 50% soil water depletion for the first growing season; however irrigation of *T. daenensis* based on 80% water depletion should be an appropriate choice for the second growing season.

Effect of water deficit on essential oil compositions

The average concentrations of thymol, carvacrol, ρ-cymene, γ-terpinene, β-caryophyllene and borneol were 66.41-70.48%, 6.72-11.69%, 5.05-6.17%, 3.67-4.22%, 3.72-3.92% and 1.68-3.07% for two years, respectively and they were the main compositions of essential oil of *T. daenensis*. Thymol content was from 63.29-66.83% under controlled condition during 2010 and 2011, respectively, whereas Bahreininejad et al. (2010) reported that thymol concentration of *T. daenensis* population was from 1.9-72%. According to their results, it can be assumed that this population of *T. daenensis* (Kohroyeh) belongs to the thymol chemotype category.

Thymol contents increased under moderate and severe water stress (Table 6). Our results are in agreement with those of Aziz et al. (2008) in *T. vulgaris* and Said-Al Ahl and Hussein (2010) in oregano. The ratio of thymol to other constituents, particularly, carvacrol, plays an important role for cosmetic, culinary and pharmaceutical purposes (Letchamo and Gosselin, 1995). Thus, the results indicated that quality of essential oil of *T. daenensis* may increase under drought stress.

Carvacrol content reduced under both moderate and severe drought stresses (Table 6). In line with our results, Letchamo and Gosselin (1995) and Aziz et al. (2008) in *T. vulgaris* and Jordan et al. (2003) in *T. heymalis* reported that carvacrol content of such plants reduced under water stress. Whereas, Baher et al. (2002) showed that carvarcol content of *Satureja hortensis* increased under moderate water stress while reduced under severe water stresses.

On average, the ρ-cymene content was not significantly affected by moderate water stress, however it increased under severe water stress (Table 6). Our results are in agreement with those of Baher et al. (2002) in *Satureja hortensis*.

The β-caryophyllene reduced under both moderate and severe water stress (Table 6). Our results were in line with results of Letchamo and Gosselin (1995) in *T. vulgaris*, Baher (2002) in *Satureja hortensis* and Said-Al Ahl et al. (2009) in oregano. These results are in contrast with those of Jordan et al. (2003) in *T. heymalis and* Said-Al Ahl and Hussain (2010) in oregano who reported that β-caryophyllene increased under moderate water stress while reduced under severe water stresses.

The variations of the results between this study and the studies of others on measured traits may be due to genetics (genus, species and accession), water stress levels and environmental factors.

Table 6. Changes of essential oil components of *Thymus daenensis* as influenced by water deficit.

Volatile compound	RI	Year	Components proportion (%) Water deficit levels T_{20}	T_{50}	T_{80}	Mean
α-Thujene	928	2010	0.53	0.34	0.58	0.48
		2011	0.47	0.56	0.63	0.55
α-Pinene	940	2010	0.49	0.39	0.59	0.49
		2011	0.39^{b}	0.41^{b}	0.54^{a}	0.45
Camphene	956	2010	0.27^{b}	0.27^{b}	0.51^{a}	0.35^{b}
		2011	0.29^{c}	0.39^{b}	0.55^{a}	0.41^{a}
β-Pinene	970	2010	0.55	0.64	0.80	0.66
		2011	0.54^{b}	0.84^{a}	0.88^{a}	0.75
Myrcene	985	2010	0.00	0.00	0.00	0.00^{b}
		2011	1.28^{a}	0.84^{b}	0.64^{c}	0.92^{a}
α-Terpinene	1022	2010	0.00	0.18	0.00	0.06
		2011	0.00	0.06	0.00	0.02
ρ-Cymene	1031	2010	3.84^{b}	3.99^{b}	7.32^{a}	5.05^{b}
		2011	5.93^{b}	5.20^{b}	7.37^{a}	6.17^{a}
1,8-cineole	1039	2010	0.66	0.90	1.25	0.94^{b}
		2011	0.95^{c}	1.22^{b}	1.95^{a}	1.37^{a}
γ-Terpinene	1062	2010	3.74	4.21	4.70	4.22^{a}
		2011	3.29	3.88	3.83	3.67^{b}
cis-Sabinene hydrate	1071	2010	0.59^{b}	0.54^{b}	1.05^{a}	0.73^{b}
		2011	0.73^{b}	0.72^{b}	1.22^{a}	0.89^{a}
Linalool	1102	2010	0.00	0.27	0.00	0.09
		2011	0.00	0.09	0.00	0.03
Camphor	1142	2010	0.00	0.08	0.00	0.03
		2011	0.00	0.03	0.00	0.01
Borneol	1170	2010	3.44^{a}	3.36^{a}	2.42^{b}	3.07^{a}
		2011	1.96	1.67	1.41	1.68^{b}
Methyl carvacrol	1249	2010	0.00	0.23	0.00	0.08
		2011	0.00	0.11	0.00	0.04
Thymol	1296	2010	63.29^{c}	66.07^{b}	69.88^{a}	66.41^{b}
		2011	66.83^{b}	71.14^{a}	73.48^{a}	70.48^{a}
Carvacrol	1308	2010	16.00^{a}	12.95^{b}	6.12^{c}	11.69^{a}
		2011	9.84^{a}	6.76^{b}	3.57^{c}	6.72^{b}
β-caryophyllene	1424	2010	4.03	3.76	3.37	3.72
		2011	5.29^{a}	3.68^{b}	2.80^{c}	3.92
Bicyclogermacrene	1500	2010	0.20	0.14	0.15	0.16^{b}
		2011	0.28	0.26	0.20	0.25^{a}
β-bisabolene	1514	2010	0.22^{a}	0.16^{b}	0.12^{c}	0.17^{b}
		2011	0.32^{ab}	0.41^{a}	0.16^{b}	0.30^{a}
Elemol	1551	2010	0.50^{a}	0.50^{a}	0.19^{b}	0.40
		2011	0.57^{a}	0.54^{a}	0.25^{b}	0.45
Spathulenol	1565	2010	0.16	0.19	0.20	0.18
		2011	0.13	0.33	0.28	0.25
Caryophyllene oxide	1581	2010	0.00	0.16	0.00	0.05
		2011	0.00	0.12	0.00	0.04

T_{20}: non-stressed control; T_{50}: moderate drought stress; T_{80}: severe drought stress.
Values followed by different superscripts (a-c) in the columns are significantly different at P<0.05 (means of the replicates).
Values followed by different superscripts (a-b) in the last row are significantly different at P<0.05 (means of the treatments over each year).

Conclusions

From the obtained results and taking into account that thymol is the main component that defines the quality of this species, *T. daenensis* produced good essential oil yield and content under field condition suggesting that domestication of this species is economically convincible. The main compositions of essential oil of *T. daenensis* were thymol, carvacrol, ρ-cymene, γ-terpinene, β-caryophyllene and borneol. Water stress reduced chlorophyll and carotenoid contents, root and shoot growth, and $IWUE_{dm}$ However, the contents of proline, K^+ and essential oil and $IWUE_{eso}$ of *T. daenensis* were increased. Increased essential oil content was sufficient to compensate for the yield loss due to the reduced herbage yield. The results show that irrigation of *T. daenensis* based on 50% water depletion should be an appropriate choice for the first growing season and 80% water depletion for the second growing season in semi-arid climatic conditions.

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