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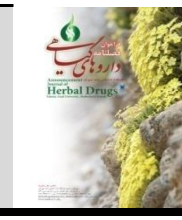

The influence of reduced irrigation on herbage, essential oil yield and quality of *Thymus vulgaris* and *Thymus daenensis*

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ABSTRACT

Background & Aim: Drought stress, as a main abiotic stress, has a significant influence on growth and metabolic activities of plant species. In this study, the influence of reduced irrigation on dry herbage, essential oil yield, and chemical components of two thyme species were investigated.

Experimental: This study was done in an experimental greenhouse, I.A.U., Shahrekord Branch, Iran at 2012 in a CRD with three replications. Three irrigation levels were I_1 (irrigated in field capacity or control), I_2 (slight drought stress or irrigation in 75% field capacity), and I_3 (mild drought stress or irrigation in 50% field capacity). Two thyme species included *Thymus daenensis* and *T. vulgaris*. The essential oils obtained by hydro-distillation and were analyzed by Gas Chromatography-Mass Spectrometry.

Results & Discussion: Results indicated that irrigation levels had a significant effect on many morphological and physiological characteristics, including dry matter, plant height, and leaf area index (LAI). In addition, levels of irrigation affected on oil yield and some major constituents in the essential oils. Carvacrol, γ-terpinene, and *p*-cymene contents in the essential oils were significantly increased under stressed conditions, whereas thymol amount was significantly reduced under stressed conditions.

Recommended applications/industries: According to the results of this study, drought stress reduces the essential oil yields and dry herbage in both species of thyme.

1. Introduction

Drought is the most common adverse environment, which limits crop production in different parts of the world special in Iran that is considered as dry and semi dry country. Drought stress has considerable adverse impact on growth and metabolic activities of plant species. For medicinal and aromatic crops, drought may cause significant changes in their metabolites yield and compositions (Ghasemi Pirbalouti *et al*., 2013a). Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis (Flexas & Medrano, 2002). Results of a study by Baher et al. (2002) indicted that greater soil water stress decreased plant height and total fresh and dry weight of savory (*Satureja hortensis*). Results another study by Razmjoo et al. (2008) also showed that water stress caused a significant reduction in plant height, the number of branches and flowers, peduncle length, head diameter, fresh and dry flower weight and essential oil content of German chamomile (*Matricaria chamomilla* L.). Bahreininejad et al. (2008) reported that water stress reduced growth, herbage production, chlorophyll and carotenoid content in *Thymus daenensis*, while increased proline, K+, essential oil content and irrigation water use efficiency based on essential oil yield (IWUEeso).

The genus *Thymus* L. belongs to the family Lamiaceae, which many species are herbaceous perennials and small shrubs in the world. The Mediterranean region has been identified as the center of the genus (Cronquist, 1988). The aerial parts and volatile constituents of thyme are commonly used as a medicinal herb. *Thymus* species are commonly used as herbal tea, flavoring agents (condiments and spices) and medicinal purposes (Stahl–Biskup & Saez, 2002). Thyme with the common Persian name of 'Avishan or Azorbe', consists of 14 species which grow wild in many regions of Iran. *Thymus daenensis* is a main endemic subspecies of Iran and grows in high altitudes in Zagros Mountains range, western and southwestern Iran. This species is an important medicinal and aromatic species in Iran (Nickavar *et al*., 2005; Ghasemi Pirbalouti *et al*., 2013b). Infusion and decoction of aerial parts of *Thymus* species are used to produce tonic, carminative, digestive, antispasmodic, anti–inflammatory, and expectorant and for the treatment of colds in Iranian traditional medicine (Nickavar *et al*., 2005; Ghasemi Pirbalouti, 2009).

Few studies have been done to investigate the effects of reduced irrigation on the accumulation of secondary metabolites in medical plants. This study was performed to evaluate the effect of various levels of irrigation on herbage, essential oil yield, and quality of *T. daenensis* and *T. vulgaris*.

2. Materials and Methods

2.1. Treatments

Plastic pots with a top diameter of 20 cm and a depth of 35 cm were filled with 70% natural soil obtained from a filed (silty clay texture, pH = 7.23, OC = 0.81%, E.C = 0.49 dS/m, total N = 0.01%, available P = 11.20 g/kg, available K = 694 g/kg, Fe = 1.11 mg/kg, Mn = 6.51 mg/kg, and Zn = 0.48 mg/kg) and 30% peat moss (Fig 1.). *T. daenensis* (local) and *T. vulgaris* (F1) seeds were obtained the Pakan Seed Company, Isfahan, Iran. In winter 2011 in plastic greenhouse conditions, ten seeds were sown in each plastic pot and after six weeks were thinned to two healthy seedlings per pot. The pots transferred to the filed in Shahrekord (latitude 32° 20´ N, longitude 50° 51´ E, altitude 2061 m above sea level), southwestern Iran on second week of May 2012. Type of study area climate by Emberger's climatology method is cold and semiarid and semi humid with temperate summer and very cold winter by Karimi's climatology method.

Experimental treatments were arranged as a 3 × 2 factorial in a CRD with four replications. Factor A, irrigation levels included I_1 (irrigated in field capacity or control), I_2 (slight drought stress or irrigation in 75% field capacity when 75% of maximum total available soil water was depleted from before flowering until complete bloom), and I_3 (mild drought stress or irrigation in 50% field capacity when 50% of maximum total available soil water was depleted from before flowering until complete bloom). Two thyme species (Factor B) were *T. daenensis* and *T. vulgaris*.

Fig 1. Experimental pots of two thyme species

2.2. Measurements

The aerial parts of *T. daenensis* and *T. vulgaris* were harvested at full flowering stage (July 2012), and following data was collected: plant height, herbage dry weight and leaf are index (LAI). Harvested tissues were dried inside for six days at room temperature (25 ± 5 °C), and the ground to fine a powder using Moulinex food processor. Essential oil content based on oil yield (ml / 100 g dry matter), and chemical compositions of the essential oils were estimated. The essential oil was extracted from 100 g of sample of tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100 °C for three hours in a Clevenger-type apparatus according to British Pharmacopoeia

2.3. Identification of the oil components

The essential oils were analyzed using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP–5MS 5% phenylmethylsiloxane capillary column (30.00 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60 °C for 4 min initially, and then raised at the rate of 4 °C / min up to 260 °C. Injector and detector temperatures were set at 290 °C and 300 °C, respectively. Helium was used as a carrier gas at a flow rate of 2 ml / min, and 0.1 µl samples were injected manually in the split mode. Peaks area percents were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI–MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 200 °C. Constituents were identified by comparison of their KI (Kovats index) relative to C5-C24 *n*-alkanes obtained on a nonpolar DB-5MS column by comparison of the KI, provided in the literature, by comparison of the mass spectra with those recorded by the Willey (ChemStation data system). The individual constituents were identified by retention indices and compared with constituents known from the literature (Adams, 2007).

2.4. Statistical analysis

Simple and interaction effects of experimental factors were derived from two-way analysis of variance (ANOVA) based on the GLM procedure of the SAS statistical package (SAS/STAT® v.9.2. SAS Institute Inc., Cary, NC). The significance of differences among treatment means was tested using Duncan's multiple range test (DMRT) at $p \leq 0.05$.

3. Results and discussion

3.1. Morpho-physiological traits

In this study, results indicated that levels of irrigation influenced ($p \leq 0.01$) on plants height, LAI, and herbage dry weight (Table 1). Water deficit resulted in reduced plant height, LAI, and dry weights of herb in two species of thyme (Table 1). Similarly, results of a study by Bahreininejad et al. (2013) indicated that plant height, leaf area and total dry matter in *T. daenensis* were significantly reduced under drought stress. In present study, there was significant interaction effect (irrigation × species) on herbage dry weight. The highest herbage dry weight was achieved by *T. daenensis* under normal irrigation (Table 1).

Table 1. Interaction effects on morphological and physiological characteristics of two thyme species

Treatments	Plants height (cm)	LAI	Herbage dry weight (g)
Normal Irrigation × *T. daenensis* (B_1C_1)	25.89ab	783.6a	36.18 b
Normal Irrigation × *T. vulgaris* (B_1C_2)	26.22a	64.89 d	50.64 a
Slight drought stress × *T. daenensis* (B_2C_1)	22.33ab	629.4 b	24.56 cd
Slight drought stress × *T. vulgaris* (B_2C_2)	21.89ab	51.20 d	31.69 bc
Mild drought stress × *T. daenensis* (B_3C_1)	20.89 b	272.2 c	16.71 d
Mild drought stress × *T. vulgaris* (B_3C_2)	25.67ab	32.29 d	25.16 cd
ANOVA	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

3.2. Essential oil yield and quality

The color of oils extracted from both species in all treatments was yellow. Results indicated that there was a significant difference ($p \leq 0.05$) irrigation effect on essential oil yield (Fig 2.). The highest oil yield was obtained from slight drought stress treatment (irrigation in 75% field capacity). Bahreininejad et al. (2013) reported an increase in essential oil content of *T. daenensis* under water stress. In addition, Simon et al. (1992) reported that water stress increased essential oil accumulation via higher density of oil glands due to the reduction in leaf area. In this study, there was no significant irrigation × species interaction effect on oil yield.

3.3. Chemical compositions of oil

The chemical compositions identified by GC–MS, are presented in Table 2. GC–MS analysis resulted in identification of 32 constituents of the oil composition. Their sum constituted the bulk of the oils and ranged from 90% up to 99% of total oil. The analysis of the essential oils detected five major compounds, viz. thymol, carvacrol, ɣ–terpinene, *p*–cymene, and β-caryophyllene for both thyme species (Table 2). The results indicated that the essential oils extracted from two thyme species contained oxygenated monoterpenes and hydrocarbons monoterpenes. Our results confirm earlier reports that major volatile constituents obtained from the aerial parts of thyme species (especially *T. daenensis* and *T. vulgaris*) were thymol, carvacrol, *p*–cymene, γ–terpinene and β–caryophyllene (Sajjadi & Khatamsaz, 2003; Naghdi–Badi *et al*., 2004; Nickavar *et al.*, 2005; Bahreininejad *et al.*, 2013; Ghasemi Pirbalouti *et al*., 2013a,b,c).

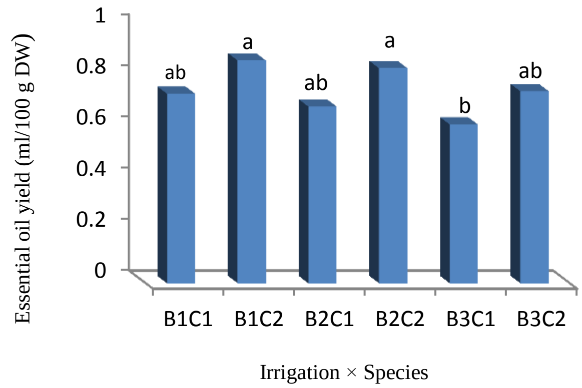

Fig 2. Interaction effects on essential oil yield of two thyme species

Table 2. Interaction effects on the main constituents of the essential oil from two thyme species

Treatments	α-Thujene	α-Pinene	β-Myrcene	α-Terpinene	*p*-Cymene	1,8-Cineole	γ-Terpinene	Borneol	Thymol	Carvacrol	β-Caryophyllene
A1C1	0.88 d*	1.46 a	1.60 a	2.20 bc	8.11 c	2.06 a	6.91 d	2.99 a	54.09 b	4.75 a	3.72 ab
A1C2	1.44 a*	1.09 c	1.49 abc	2.68 a	14.36 a	0.90 c	20.58 a	1.66 d	38.94 d	2.67 c	1.31 c
A2C1	0.88 d	1.43 a	1.54 ab	1.88 c	7.93 c	1.86 a	6.18 de	2.05 b	58.70 a	4.30 ab	3.45 ab
A2C2	1.28 b	1.01 c	1.33 bc	2.52 ab	12.67 b	0.74 c	18.64 b	1.75 cd	43.15 c	2.84 c	1.14 c
A3C1	1.06 c	1.44 a	1.20 d	0.76 d	8.46 c	1.20 b	5.31 e	3.03 a	56.22ab	4.89 a	3.95 a
A3C2	1.31 ab	1.24 b	1.26 cd	2.30 abc	12.02 b	1.29 b	15.26 c	2.00 bc	44.16 c	3.99 b	1.71 bc

Statistical results of this study indicated that levels of irrigation had a significant on the main components. Amounts of carvacrol, γ-terpinene, *p*–cymene, and β–caryophyllene in the oils increased under reduced irrigation, but amount of thymol decreased under water deficit stress. In another study (Ghasemi Pirbalouti *et al*., 2013a) also increased amounts of carvacrol and β–caryophyllene and decreased percentage of thymol in the essential oil from *T. daenensis* under reduced irrigation. Results of a study by Bahreininejad et al. (2013) demonstrated that percentage of thymol increased under moderate and severe water stress in the essential oil from *T. daenensis.* In present study, there was significant irrigation × species interaction effect on percentage of thymol. Decreased thymol content in the essential oil of *T. vulgaris* was higher than *T. daenensis* under water deficit conditions.

4. Conclusion

Results of present study indicated that levels of irrigation had a significant effects on herbage dry weight, plant height, leaf area index (LAI), essential oil yield and many compositions in the essential oils from the aerial parts of *Thymus vulgaris* and *T. daenensis* grown at Shahrekord climate, southwestern Iran. Percentages of carvacrol, γ-terpinene, and *p*-cymene in the oils were significantly increased under reduced irrigation conditions, whereas percentage of thymol was significantly reduced under stressed conditions. Shortage of water in arid and semi–arid parts of this region where annual precipitation is less than 350 mm with almost no rainfall during the summer is a prominent limiting factor of *Thymus* production. Good management and adoption of suitable practices will improve economic *Thymus* production.

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