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CHANGING OF AGRO MORPHOLOGICAL TRAITS, ESSENTIAL OIL CONTENT AND COMPONENTS IN *Thymus daenensis* Celak UNDER WATER DEFICIT STRESS CONDITIONS

Jalal KHORSHIDI, Majid SHOKRPOUR* and Vahide NAZERI

University of Tehran, Faculty of Agricultural Sciences and Engineering,
Department of Horticultural Sciences and Landscape, Karaj, Iran.
*Corresponding author, M. Shokrpour, E-mail: shokrpour@ut.ac.ir

ABSTRACT. *Thymus daenensis Celak. is an endemic medicinal plant in Iran, with high essential oil content and rich source of thymol. In this study effect of water deficit stress (normal irrigation, withholding irrigation in start of flowering and withholding irrigation in vegetative stage) on agro morphological traits, essential oil content and components were investigated. Results indicated that measured agro morphological traits with the exception of leaf and flower weight did not significantly affected by severity of stress. The highest (0.31 g) and the lowest (0.15 g) of leaf and flower weight was observed in normal irrigation and withholding irrigation in start of flowering treatment, respectively. Essential oil content and constituents with the exception of α-thujene did not affected by water deficit stress. The highest of essential oil content (4.7 %) was observed in withholding irrigation in start of flowering treatment. Under all treatments, oxygenated monoterpenes and thymol were the main class of compounds and the major constituent of essential oil, respectively. Thymus daenensis Celak. indicated a good tolerance to water deficit and had a good biomass and essential oil content in these conditions and so can be a suitable choice for culture in arid and semi-arid regions.*

KEY WORDS: *endemic, irrigation, thymol, oxygenated monoterpenes.*

INTRODUCTION

Water stress is one of the most important abiotic stresses affect growth and productivity of plants. Plants responds differently to water stress regarding to genus, species and even variety (Xu et al. 2010). Productivity of plants under water limited condition differs depends on stress intensity, developmental stage and duration of stress (Jaleel et al. 2008). There are various mechanisms such as morphological, physiological and phytochemical variations to survive under water stress conditions (Bray 1993). Changes in chlorophyll and carotenoids contents and secondary metabolites including terpenes, phenols, alkaloids and cyanogenic glycosides are affected by water stress in plants (Anjum et al. 2003; Khalid 2006; Selmar 2008). Understanding the response of plants to water stress could be helpful to choose preferable genotypes for culturing in these conditions (Martinez et al. 2007). Iran is located in arid and semi-arid regions of the world with 250 mm annual average rainfall (Saiedfar & Mahmoudi 2013). It is expected that the plant species growing under the arid climate have a great adaptation potential to water limited environments. Many of the medicinal plants with high economical increment are low water consuming plants and are proper choices for domesticating and introducing to agriculture systems. There are abundant studies on the effects of water stress on biomass and secondary metabolites of medicinal plants but very limited studies have made on endemic medicinal plant of *Thymus daenensis* Celak. Jordan et al. (2003) studied the effects of four irrigation levels on *Thymus hyemalis* and reported that the applied irrigation levels had no significant differences in essential oil yields. Eman et al. (2008) evaluated different irrigation intervals (3, 5, 7 and 10 days) on plant growth, essential oil yield and the main constituents of oil in *Thymus vulgaris* L. Their results showed that the highest dry matter weight and thymol percentage obtained in plants irrigated every 3 and 10 days, respectively. Study the effect of drought stress on dry matter and essential oil quality in *Thymus zygis* subsp. *gracilis* showed that maximum plant dry matter, essential oil yield and thymol concentration achieved under moderate watering level (Sotomayor et al. 2004). Another study on *Thymus vulgaris* L. indicated that water stress decreased plant height, dry and fresh weight and also led to increase in thymol percentage and proline content (Babaee et al. 2010). *Thymus daenensis* Celak. an endemic species in Iran (Jamzad 2009) has a proper biomass, high essential oil yield and thymol percentage and also good adaptation to different climates (Barazandeh & Bagherzadeh 2007). Due to high thymol metabolite in its essential oil, it is known as a valuable medicinal plant which is utilized in pharmaceutical, alimentary and hygienic industries. The raw material of the plant is supplied from the wild so it is extremely under overthrow danger. Study on the effects of different factors on biomass and secondary metabolites are necessary for domesticating and preservation of this plant. In this study evaluated effect of three irrigation regimes on essential oil content and components and also on agro morphological traits of *Thymus daenensis* Celak.

MATERIALS AND METHODS

Seed gathering, plant preparation and transplanting

Seeds of *Thymus daenensis* Celak. were collected from natural habitat (Arak province) with geographical characteristics: altitude 2032 m, latitude 33° 59' 35", longitude 49° 32' 56". Seeds sowed in pots containing peat and sand (1:1) and then were kept in the greenhouse located in agricultural and natural resources campus, university of Tehran, Karaj, Iran. Average daily temperature and relative humidity of greenhouse were 20°C and 70%, respectively. Pots were irrigated one time daily for 4 min with an automatic misting system. About four months after seeds sowing in greenhouse, seedlings were ready for transplant to the main field (in this stage seedlings had 8 leaves, 30 April 2013). Soil of field analyzed before planting and also diagram of monthly mean temperature and rainfall of experimental region were obtained from nearest weather station (Table 1 and Figure 1). In the field, seedlings planted with 50 cm space on rows and 50 cm space between rows.

Table 1. Physical and chemical characteristics of field soil.

EC (ds/m)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
1.42	7.5	2.34	23.2	2.28

Soil texture	Lime (%)	Sand (%)	Clay (%)	Silt (%)
Clay Loam	8.7	36	30	34

pH	N (%)	C (%)	P (mg/kg)	K (mg/kg)
7.7	0.09	0.64	53.1	390

Experimental design, water deficit treatment and harvesting of plants

The experiment was run as randomized block design with three replications. Nine seedlings were planted in each plot. Seedling irrigated regularly (once per week) until reached to 12 leaves stage. Three irrigation treatments were including: normal

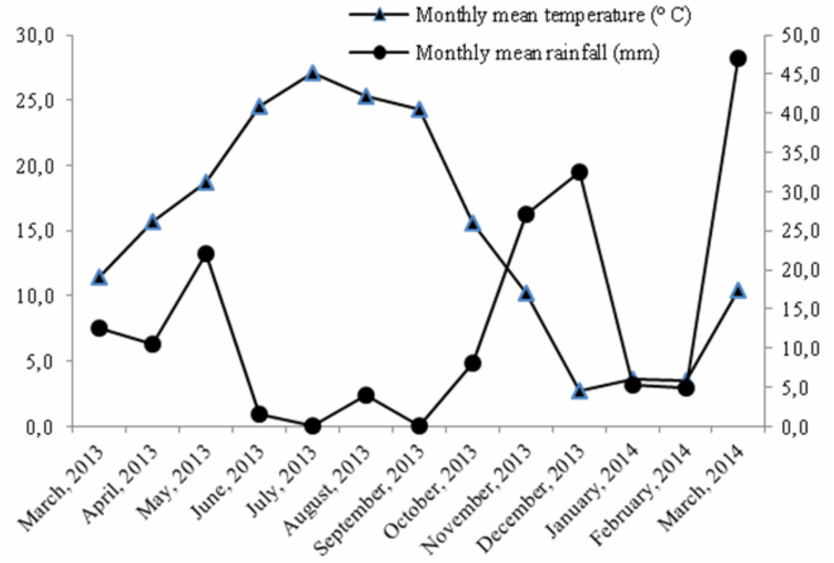

Figure 1. Monthly mean temperature and rainfall in experimental region.

irrigation (once per week irrigation until full bloom, 110 days after planting), withholding irrigation in start of flowering (90 days after planting) and withholding irrigation in vegetative stage (12 leaves stage, 70 days after planting). Fifteen plants from each treatment (5 plants from each replicate belonging to each treatment) were harvested at the full bloom stage (about 3.5 months after transplanting, 16 July 2013) and dried in room temperature (24 °C) and then were measured dry matter yield and their agro-morphological traits that mentioned in Table 2 and Table 3. The number of essential oil glands per leaf was determined using the visually method at ×60 magnification (Figure 2).

Essential oil isolation, GC and GC/MS analysis

Plant samples powdered and essential oils were obtained by conventional hydro distillation method (by Clevenger) for 3 hours (Rustaiee 2009; Rustaiee et al. 2010). Essential oils dehydrated using anhydrous sodium sulfate and yield of essential oils were calculated (v/w). Essential oils kept in dark glass and stored at 4 °C until analysis. Analyses of essential oils were done by GC and GC-Mass. Gas chromatography carried out using TRACE GC with DB-5 column (30 m length, 0.25 mm diameter and 0.25 µm film thickness); Oven temperature was kept at 60 °C for 1 min initially and then raised with rate of 5 °C/min to 250 °C and kept for 10 min in this temperature. Injector and detector temperature were 250 and 280 °C, respectively. N_2 was used as carrier gas with flow rate 1.1 mm/min. Peaks area percent were used for obtaining quantitate data. Gas chromatograph was coupled to a quadruple mass spectrometer with ionization temperature 200 °C, ionization

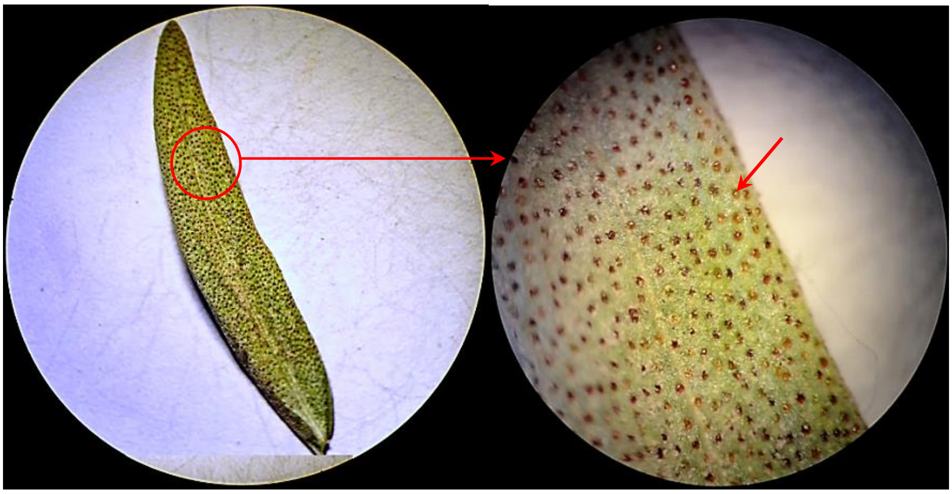

Figure 2. Essential oil glands of *Thymus daenensis* Celak.(×60 magnification).

voltage 70 eV and used Helium as carrier gas with flow rate 1.1 ml/min. Retention indices were obtained for all components using a homologous series of n-alkanes injected in conditions equal to samples ones. Identification of essential oil components was accomplished based on comparison of their retention times with those of authentic standards.

Analyses of data were done using SPSS 21. Duncan's multiple range test at $p \leq 0.05$ were used for mean comparison of data.

RESULTS AND DISCUSSION

Effect of water deficit stress on agro morphological traits

Results indicated that water deficit did not effect on some of agro morphological traits such as length of flower stem, length of inflorescence, diameter of inflorescence, number of flower in inflorescence, length of flower, number of internode, length of leaf, width of leaf, leaf area, length of internode, diameter of stem, stem weight and dry matter yield. In the other hand, leaf and flower weight was significantly affected by water deficit stress (Table 2). Our results were in contrast with previous findings which obtained in other medicinal and aromatic plants (Delfine et al. 2005; Dunford & Vasquez 2005; Khalid 2006; Bettaieb et al. 2009; Babaee et al. 2010; Alkire et al. 1993; Farooqi et al. 1999; Eman et al. 2008; Baghalian et al. 2011). The highest of stem weight and also leaf and flower weight were obtained in normal irrigation, whereas the highest of length of internode and diameter of stem were observed in withholding irrigation in start of flowering and withholding irrigation in vegetative stage, respectively. However dry matter yield decreased with the severity of stress, but decrease in dry matter with the severity of stress was not significant. The highest and the lowest dry matter yield were obtained in normal irrigation and withholding irrigation in vegetative stage, respectively (Table 3). Water deficit stress influence cell deviation and elongation, which consequently decreases stem height, leaf area and plant biomass (Dadrasan et al. 2015). The reduction in leaf area in plants under water deficit stress occurs to decrease water loss through transpiration (Jahansouz et al. 2014). In water deficit conditions, uptake of nutrient elements decreases, which reduces the dry matter yield (Diaz-Lopez et al. 2012). It seems this plant has a good tolerance to water stress and can be suitable growth and biomass production in these conditions. This finding that Thyme species are adapted species to drought conditions is agreement with Jordan et al. (2006) findings in study conducted on *Thymus vulgaris* L. Correlation between agro morphological traits, dry matter yield and essential oil content was showed in Table 4. We noted that there is no strong correlation between dry matter yield with others agro morphological traits. This result is in consistent with Aflakian et al. (2012) who demonstrated that was not significant correlation between dry matter yield in *Thymus daenensis* Celak. with stem length, inflorescence length and number of flower in inflorescence. But found a positive correlation (r= 0.486 at $p< 0.05$) between essential oil content and length of internode. So, length of internode could be considered as an indicator for the indirect selection and breeding of this plant. Also we observed a strong negative correlation between numbers of essential oil glands in leaf with length of flower stem (r= -0.439 at $p< 0.05$), stem diameter (r= -0.624 at $p< 0.05$) and leaf area (r= -0.617 at $p< 0.05$). This result was in contrast with findings of Azizi et al. (2012) who reported that there was a strong positive correlation (r= 0.585 at $p< 0.05$) between stem diameter with essential oil content in *Origanum vulgare* L. Essential oil glands in *Thymus daenensis* Celak. are more often located on flowers and leaves (Rustaiee 2009).We noted that larger leaves had fewer numbers of essential oil glands. Whereas stem of this plant have a fewer number of essential oil glands compared to flowers and leaves, so plants with higher stem length and diameter had fewer essential oil glands. Agro-morphological traits with a high correlation to the biomass, essential

Table 2. Analysis of variance for essential oil content and agro morphological traits in *Thymus daenensis* Celak. under different water deficit stress

Mean of square																	
Length of flower stem (cm)	Length of inflorescence (mm)	Diameter of inflorescence (mm)	Number of flower in inflorescence	Length of flower (mm)	Number of internode	Length of internode (mm)	Diameter of stem (mm)	Length of leaf (mm)	Width of leaf (mm)	Leaf area (mm^2)	Stem weight (g)	Leaf and flower weight (g)	Dry matter yield (g per plant)	Gland number ($number/mm^2$)	Essential oil (%)	d.f.	Source of variation
6.14 ^{ns}	5.37 ^{ns}	3.54 ^{ns}	1805.4 ^{ns}	0.067 ^{ns}	3.05 ^{ns}	29.1 ^{ns}	0.078 ^{ns}	2.39 ^{ns}	0.082 ^{ns}	38 ^{ns}	0.009 ^{ns}	0.019 *	14.92 ^{ns}	44.09 ^{ns}	0.046 ^{ns}	2	Treatment
0.83 ^{ns}	1.25 ^{ns}	1.56 ^{ns}	605.6 ^{ns}	0.145 ^{ns}	5.45 ^{ns}	3.96 ^{ns}	0.014 ^{ns}	2.06 ^{ns}	0.198 ^{ns}	14.89 ^{ns}	0.001 ^{ns}	0.007 ^{ns}	19.09 ^{ns}	17.43 ^{ns}	0.142 ^{ns}	2	Block
3.59	11.19	1.03	695.3	0.23	7.19	14.84	0.027	0.98	0.157	49.5	0.002	0.001	12.63	13.79	0.198	4	Error
11.1	13.2	9.8	30.7	9.4	21.6	33.4	15.1	8.1	13.4	18.9	42.0	36.7	32.3	18.0	8.3	-	Coefficient of variation

ns and *: not significant and significant at $p < 0.05$, respectively

Table 3. Different irrigation treatments effects on essential oil content and agro morphological traits of *Thymus daenensis* Celak.

Trait	Treatment		
	Normal irrigation	withholding irrigation in start of flowering	withholding irrigation in vegetative stage
Length of flower stem (cm)	18.61 ± 3.2	15.8 ± 2.4	16.6 ± 2.5
Length of inflorescence (mm)	21.71 ± 6.7	17.3 ± 4.5	20.6 ± 6.4
Diameter of inflorescence (mm)	14.1 ± 3.6	12.4 ± 1.8	14.4 ± 1.6
Number of flower in inflorescence	126.2 ± 63.6	77.4 ± 41.7	97.2 ± 36.2
Length of flower (mm)	4.3 ± 0.54	4.2 ± 0.45	4.5 ± 0.80
Number of internode	12 ± 2.3	10.0 ± 3.7	11.3 ± 2.0
Length of internode (mm)	9.7 ± 3.1 b	15.4 ± 5.5 a	10.3 ± 3.4 b
Diameter of stem (mm)	1.3 ± 0.19 ab	1.09 ± 0.23 b	1.4 ± 0.30 a
Length of leaf (mm)	15.1 ± 2.1	15.2 ± 2.06	16.74 ± 2.2
Width of leaf (mm)	2.7 ± 0.54	2.9 ± 0.76	3.02 ± 0.39
Leaf area (mm^2)	32.6 ± 9.2	28.9 ± 10.5	36.03 ± 11.6
Stem weight (gr)	0.2 ± 0.07 a	0.11 ± 0.07 b	0.11 ± 0.03 b
Leaf and flower weight (gr)	0.31 ± 0.14 a	0.15 ± 0.07 b	0.22 ± 0.07 ab
Dry matter yield (gr per plant)	13.53 ± 7.5	12.8 ± 9.05	9.3 ± 4.4
Gland number (number/mm^2)	23.07 ± 5.9 b	30.4 ± 5.5 a	24.9 ± 5.8 ab
Essential oil content (%)	4.5 ± 0.7	4.7 ± 0.87	4.5 ± 0.48

Means in each row followed by same letters are not significantly different at $p \leq 0.05$

Table 4. Correlation between agro morphological traits, dry matter yield and essential oil content.

	Length of flower stem (cm)	Length of inflorescence (mm)	Diameter of inflorescence (mm)	Number of flower in inflorescence	Length of flower (mm)	Number of internode	Length of internode (mm)	Diameter of stem (mm)	Length of leaf (mm)	Width of leaf (mm)	Essential oil (%)	Leaf and flower weight (gr)	Stem weight (gr)	Leaf area (mm2)	Gland number (number/mm^2)
Length of inflorescence (mm)	.318														
Diameter of inflorescence (mm)	-.187	.562**													
Number of flower in inflorescence	.164	.797**	.641**												
Length of flower (mm)	-.078	.440*	.649**	.272											
Number of internode	.528**	.345	.063	.264	-.022										
Length of internode (mm)	-.357	-.397*	-.268	-.355	-.127	-.837**									
Diameter of stem (mm)	.347	.199	.028	.002	.021	.144	-.328								
Length of leaf (mm)	.084	.156	.053	-.035	.117	-.142	.001	.452*							
Width of leaf (mm)	-.136	-.063	-.261	-.152	-.168	-.290	.156	.408*	.482*						
Essential oil (%)	-.182	-.068	.067	.148	-.026	-.341	.486*	-.305	-.141	-.070					
Leaf and flower weight (gr)	.148	.145	.294	.383*	.119	.259	-.257	.050	-.211	-.372	.047				
Stem weight (gr)	.245	-.080	-.179	.087	-.379	.163	-.040	.194	-.194	-.144	.200	.664**			
Leaf area (mm2)	.229	.030	-.286	-.059	-.285	-.070	-.037	.698**	.699**	.669**	-.049	-.194	.109		
Gland number (number/mm^2)	-.439*	-.166	.041	-.147	.095	-.262	.321	-.624**	-.353	-.275	.322	-.125	-.143	-.617**	
Dry matter yield (gr per plant)	.301	.281	.013	.186	-.169	.057	.058	-.043	-.237	-.089	.101	-.236	.069	-.116	-.011

** and *: Significantly at $p< 0.01$ and $p< 0.05$, respectively oil content and components could provide a useful tool for the indirect selection in breeding of medicinal plants before extraction and analysis of essential oils.

Changes in glands number and essential oil content affected by water deficit stress

The major content of essential oil in *Thymus daenensis* Celak. is produced in essential oil glands which are located in different organs. Microscopic study indicates that major of these glands exist on leaves, followed by flowers and stem (Rustaiee 2009). It seems increase in gland number cause in increase of essential oil content. We noted that water deficit stress affected number of these glands, but this effect was not significant. The highest and the lowest essential oil glands were observed in withholding irrigation in start of flowering and normal irrigation, respectively (Table 3). Indeed, gland number increased with moderate stress but decreased with the severity of stress from moderate to severe. Similar to number of essential oil glands, water deficit stress did not significantly effect on essential oil content. This result is in line with the results of Jordan et al. (2003) in *Thymus hymalis* and Khazaie et al. (2008) in *Thymus vulgaris* L. and *Hyssopus officinalis* who reported that water deficit stress had no significant effect on essential oil content. But our result was in contrast with results of Bahreininejad et al. (2014) in *Thymus carmanicus*, Vazin (2013) in *Cuminum cyminum*, Baher et al. (2002) in *Satureja hortensis* L. and Laribi et al. (2011) in *Carum carvi* who showed that essential oil percentage increased with the severity of water stress. However similar to essential oil gland number, the highest essential oil content was obtained in withholding irrigation in start of flowering, but these changes were not significant. Essential oil content of *Pelargonium graveolens* L. grown under moderate drought stress was higher than plants grown under well water irrigation and severe drought stress (Amiri et al. 2017). Overall, in most medicinal plants, moderate stress increases the essential oil content, but sever stress with negative influence on photosynthesis and primary metabolite production, reduces essential oil yield (Farsi et al. 2017).

Effect of water deficit stress on essential oil components

GC and GC/MS chromatogram of *Thymus daenensis* Celak. essential oil has been shown in Figure 3. In totally, thirty compounds were identified in *Thymus daenensis* Celak. essential oil, which thymol was the major

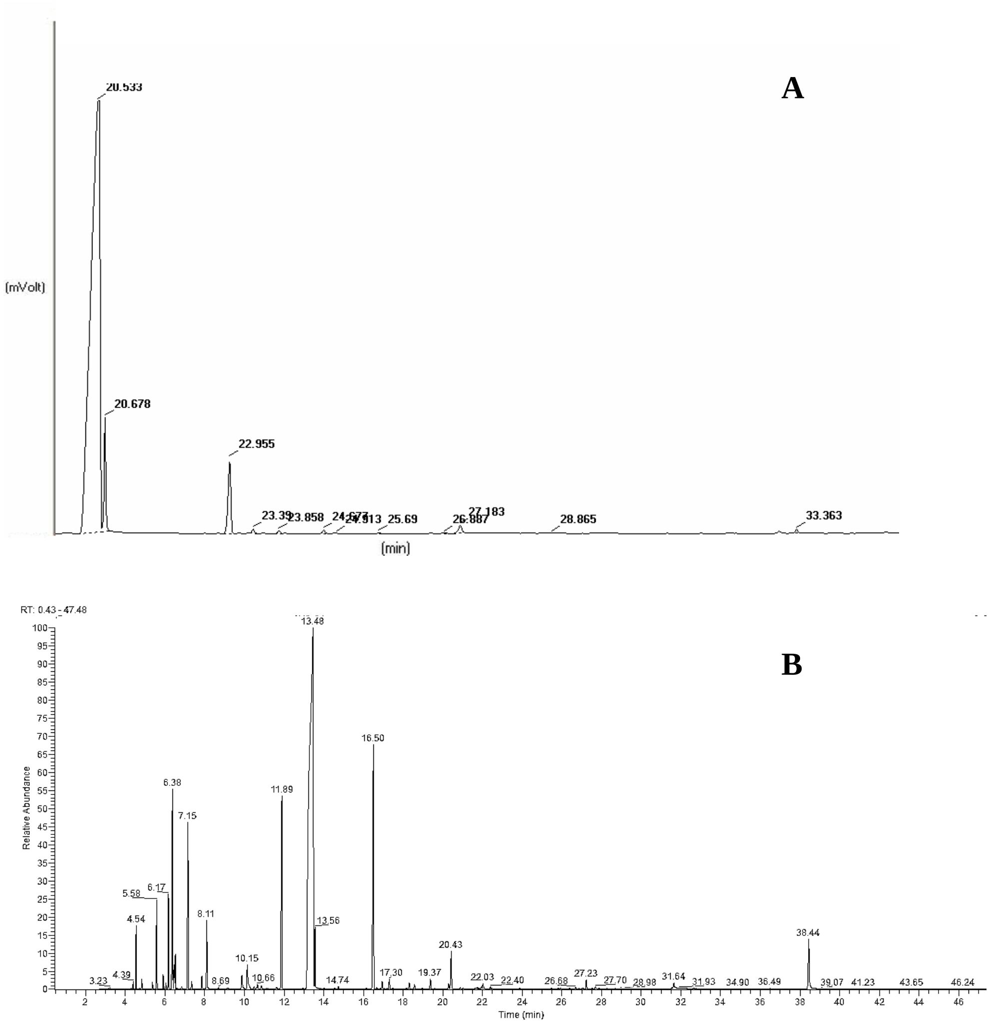

Figure 3. GC (A) and GC/MS (B) chromatogram of *Thymus daenensis* Celak. essential oil.

compound with 64 to 73% under withholding irrigation in start of flowering and normal irrigation, respectively (Table 5). Other compounds were noted such as carvacrol, p-cymene, trans-caryophyllene and γ-terpinene. Our results were agreement with some of other studies (Sajjadi & Khatamsaz, 2003; Barazandeh & Bagherzadeh, 2007; Rustaiee et al. 2010) who reported that thymol was the major constituent of essential oil in this plant species. Also we noted that all constituents with the exception of α-thujene did not affected by water deficit stress, significantly. The highest (1.03 %)

Table 5. Essential oil components of *Thymus daenensis* Celak. as affected by different irrigation treatments.

Sig.	Treatment			RI	RT	Component	Number
	withholding irrigation in vegetative stage	withholding irrigation in start of flowering	Normal irrigation				
*	0.73 ± 0.08	1.03 ± 0.17	0.79 ± 0.03	926	8.76	α-Thujene	1
ns	0.63 ± 0.07	0.63 ± 0.07	0.76 ± 0.01	934	9.01	α-Pinene	2
ns	0.33 ± 0.15	0.20 ± 0.08	0.44 ± 0.20	949	9.56	Camphene	3
ns	0.19 ± 0.03	0.20 ± 0.06	0.20 ± 0.02	978	10.39	β-Pinene	4
ns	1.20 ± 0.46	1.35 ± 0.65	1.57 ± 0.16	990	10.61	β-Myrcene	5
ns	0.21 ± 0.03	0.23 ± 0.06	0.25 ± 0.04	1006	11.22	α-Phellandrene	6
ns	0.85 ± 0.23	1.26 ± 0.24	1.10 ± 0.01	1017	11.53	α-Terpinene	7
ns	**5.31 ± 0.55**	**4.56 ± 1.43**	**4.19 ± 0.30**	1026	11.89	p-Cymene	8
ns	0.14 ± 0.02	0.33 ± 0.31	0.14 ± 0.02	1029	12.02	D-Limonene	9
ns	0.63 ± 0.13	0.68 ± 0.48	0.34 ± 0.13	1032	12.13	1,8-Cineole	10
ns	**2.36 ± 1.72**	**5.99 ± 2.63**	**4.11 ± 0.82**	1059	12.8	γ-Terpinene	11
ns	0.51 ± 0.08	0.44 ± 0.15	0.28 ± 0.07	1069	13.56	cis-Sabinene hydrate	12
ns	0.16 ± 0.02	0.08 ± 0.07	0.16 ± 0.02	1090	13.6	α-Terpinolene	13
ns	0.54 ± 0.32	0.28 ± 0.17	0.26 ± 0.05	1101	14.24	Linalool	14
-	0.01 ± 0.01	0.02 ± 0.03	tr	1171	16.6	Borneol	15
ns	1.03 ± 0.31	0.84 ± 0.14	1.28 ± 0.55	1182	16.76	4-Terpineol	16
-	0.01 ± 0.02	0.39 ± 0.59	tr	1203	17.91	α-Terpineol	17
ns	0.23 ± 0.11	2.87 ± 1.40	1.47 ± 2.32	1249	18.2	Carvacrol methyl ether	18

Continued on the next page

Table 5. (Continued)

Sig.	Treatment			RI	RT	Component	Number
	withholding irrigation in vegetative stage	withholding irrigation in start of flowering	Normal irrigation				
ns	**72.41 ± 3.77**	**64.93 ± 9.30**	**73.25 ± 0.47**	1309	20.5	Thymol	19
ns	**5.75 ± 1.48**	**5.52 ± 8.06**	**4.41 ± 3.12**	1312	20.61	Carvacrol	20
ns	**4.26 ± 1.99**	**5.94 ± 4.03**	**3.59 ± 0.61**	1427	22.96	trans-Caryophyllene	21
ns	0.04 ± 0.04	0.16 ± 0.08	0.13 ± 0.07	1444	23.39	Aromandendrene	22
ns	0.15 ± 0.07	0.17 ± 0.10	0.11 ± 0.01	1458	23.86	α-Humulene	23
ns	0.04 ± 0.04	0.15 ± 0.08	0.12 ± 0.06	1498	24.69	Ledene	24
ns	0.15 ± 0.14	0.09 ± 0.04	0.01 ± 0.01	1509	24.92	β-Bisabolene	25
ns	0.11 ± 0.03	0.46 ± 0.46	0.35 ± 0.43	1544	25.7	(E)-α-Bisabolene	26
-	tr	0.03 ± 0.02	0.01 ± 0.01	1584	26.89	Spathulenol	27
ns	1.76 ± 1.86	0.94 ± 0.98	0.40 ± 0.05	1590	27.2	Caryophyllene oxide	28
-	tr	0.01 ± 0.02	0.01 ± 0.02	1661	28.88	α-Cadinol	29
ns	0.26 ± 0.04	0.18 ± 0.13	0.15 ± 0.06	1696	33.38	Bis(2-ethylhexyl) phthalate	30
	13.25 ± 3.57	16.98 ± 6.40	14.33 ± 1.83			Monoterpene hydrocarbons	
	79.98 ± 6.02	74.85 ± 19.69	80.67 ± 6.51			Oxygenated monoterpenes	
	4.75 ± 2.31	6.97 ± 4.79	4.31 ± 1.19			Sesquiterpenes hydrocarbons	
	1.76 ± 1.86	0.98 ± 1.02	0.42 ± 0.08			Oxygenated sesquiterpenes	
	0.26 ± 0.04	0.18 ± 0.13	0.15 ± 0.06			Others	

RT: Retention time, RI: Retention index, *: Difference is significant at the $p \leq 0.05$, ns: non-significant at the $p \leq 0.05$. Tr: trace (quantity < 0.01 %)

and the lowest (0.73 %) of α-thujene were observed in withholding irrigation in start of flowering and withholding irrigation in vegetative stage, respectively. Similar to our findings, Bahreininejad et al. (2014) reported that thymol content of *Thymus carmanicus* was not significantly affected by water stress. But in contrast with our results, Aziz et al. (2008) and also Letchamo & Gosselin (1995) demonstrated that thymol content of *Thymus vulgaris* L. increased with the severity of stress. The main class of compounds was oxygenated monoterpenes under all of treatment levels, followed by monoterpene hydrocarbons (Table 5). The highest monoterpene hydrocarbons (16.98 %) and sesquiterpenes hydrocarbons (6.97 %) were identified in withholding irrigation in start of flowering, whereas the highest quantity of oxygenated monoterpenes (80.67 %) and oxygenated sesquiterpenes (1.76 %) were identified in normal irrigation and withholding irrigation in vegetative stage, respectively (Figure 4). It has been suggested that changes in main class of compounds can be different related to severity of stress. Some of these compounds decreased with the severity of stress, but some of others increased with raising stress level. We noted a strong negative correlations between thymol and carvacrol (r= -0.759 at *p*< 0.05) and also between thymol and trans-caryophyllene (r= 0.959 at *p*< 0.05). These findings were similar to results of Yavari (2009) in

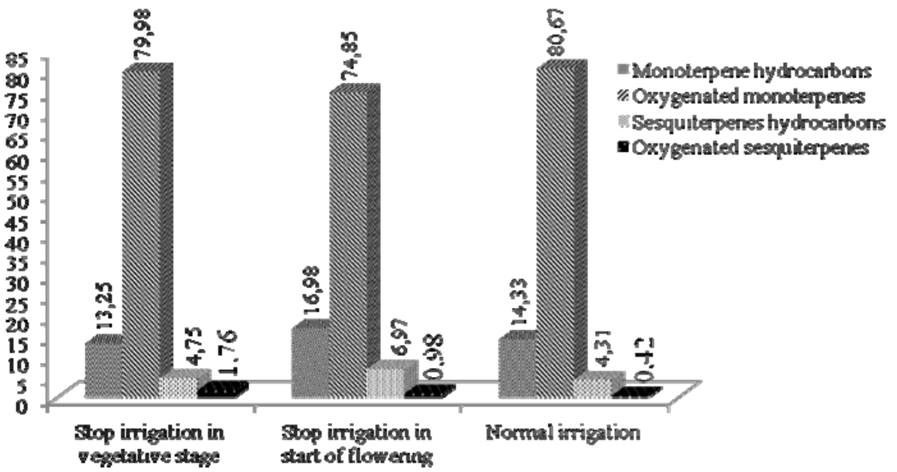

Figure 4. Comparison of essential oil components in *Thymus daenensis* Celak. under different irrigation treatments.

Thymus migricus Klokov & Des –Shost and Rustaiee (2009) in *Thymus daenensis* Celak. Thymol is an isomer of carvacrol and therefore increase in thymol content is associated with decrease in carvacrol and decrease in thymol content is giving rise in carvacrol content. Whereas thymol is a monoterpene and trans-caryophyllene is a sesquiterpene with same precursor but with two different biosynthesis pathways, so negative correlation between these two components can be correct.

CONCLUSION

Our results indicated that *Thymus daenensis* Celak. is a tolerant plant species to water deficit stress, with high essential oil content and also a rich source of thymol. Agro morphological traits with the exception of leaf and flower weight did not significantly affected by severity of stress. The highest of dry matter yield and essential oil content were obtained in normal irrigation and withholding irrigation in start of flowering treatments, respectively. As well as there was no significant correlation between yield and other traits. Essential oil constituents with the exception of α-thujene did not affected by severity of stress in this plant species. The main class of compounds and the major constituent of essential oil in all of treatment were oxygenated monoterpenes and thymol, respectively. On base these results, it has been suggested that this plant species is a suitable choice for culture in arid and semi-arid regions.

REFERENCES

Aflakian, S., Zenali, H., Madah Arefi, H., Enteshari, S., Kaveh, S. (2012): Study of yield and yield components of *Thymus daenensis* Celak. ecotypes. Iranian Journal of Medicinal and Aromatic Plants 28(2): 187-197.

Alkire, B.H., Simon, J.E., Palevtich, D., Putievsky, E. (1993): Water management for midwestern peppermint (*Mentha piperita* L.) growing in highly organic soils, Indiana, USA. Acta Horticulturae 344: 544-556.

Amiri, R., Nikbakht, A., Rahimmalek, M., Hosseini, H. (2017): Variation in the essential oil composition, antioxidant capacity, and physiological characteristics of *Pelargonium graveolens* L. inoculated with two species of mycorrhizal fungi under water deficit conditions. Journal of Plant Growth Regulation 36(2): 502-515.

Anjum, F., Yaseen, M., Rasul, E., Wahid, A., Anjum, S. (2003): Water stress in barley (*Hordeum vulgare* L.) effect on chemical composition and chlorophyll contents. Pakistan Journal of Agricultural Sciences 40: 45-49.

Aziz, E.E., Hendawy, S.F., Ezz El-Din, A.A., Omer, E.A. (2008): Effect of soil type and irrigation intervals on plant growth, essential oil yield and constituents of *Thymus vulgaris* plant. American-Eurasian Journal of Agricultural & Environmental Sciences 4: 443-450.

Azizi, A., Hadian, J., Gholami, M., Friedt, W., Honermeier, B. (2012): Correlations between genetic, morphological, and chemical diversities in a germplasm collection of the medicinal plant *Origanum vulgare* L. Chemistry & Biodiversity 9: 2784- 2801.

Babaee, K., Dehaghi, M.A., Sanavi, A., Jabbari, R. (2010): Water deficit effect on morphology, prolin content and thymol percentage of thyme (*Thymus vulgaris* L.). Iranian Journal of Medicinal and Aromatic Plants 2(26): 239-251.

Baghalian, K., Abdoshah, Sh., Khalighi-Sigaroodi, F., Paknejad, F. (2011): Physiological and phytochemical response to drought stress of german chamomile (*Matricaria recutita* L.). Plant Physiology and Biochemistry 49: 201-207.

Baher, Z.F., Mirza, M., Ghorbanli, M., Rezaii, M. B. (2002): The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. Flavour and Fragrance Journal 17: 275–277.

Bahreininejad, B., Razmjoo, J., Mirza, M. (2014): Effect of water stress on productivity and essential oil content and composition of *Thymus carmanicus*. Journal of Essential Oil-Bearing Plants 17(5): 717-725.

Barazandeh, M., Bagherzadeh, K. (2007): Evaluation of essential oil chemical components of *Thymus daenensis* Celak. collected from four regions in Esfahan province. Journal of Medicinal Plants 6(3): 15-19.

Bettaieb, I., Zakhama, N., Aidi Wannes, W., Marzouk, B. (2009): Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. Scientia Horticulturae 120: 271–275.

Bray, E.A. (1993): Molecular responses to water deficit. Plant Physiology 103(4): 1035-1040.

Dadrasan, M., Chaichi, M.R., Pourbabaee, A.A., Yazdani, D., Keshavarz-Afshar, R. (2015): Deficit irrigation and biological fertilizer influence on yield and trigonelline production of fenugreek. Industrial Crops and Products 77:156–162.

Delfine, S., Loreto, F., Pinelli, P., Tognetti, R., Alvino, A. (2005): Isoprenoids content and photosynthetic limitations in rosemary and spearmint plants under water stress. Agriculture, Ecosystems & Environment 106: 243–252.

Diaz-Lopez, L., Gimeno, V., Simón, I., Martinez, V., Rodriguez-Ortega, W.M., Garcia-Sanchez, F. (2012): *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. Agricultural Water Management 105:48–56.

Dunford, N.T., Vasquez, R.S. (2005): Effect of water stress on plant growth and thymol and carvacrol concentrations in mexican oregano grown under controlled conditions. Journal of Applied Horticulture 7: 20–22.

Eman, E., Aziz, S.T., Hendavwi, E., Omer, E.A. (2008): Effect of soil type and irrigation interval on plant growth, essential oil yield and constituents of *Thymus vulgaris* plant. American-Eurasian Journal of Agricultural & Environmental Sciences 4(4): 443-450.

Farooqi, A.H.A., Fatima, S., Ansari, S.R., Sharma, S. (1999): Effect of water stress on growth and essential oil metabolism in *Cymbopogon martini* (Plamerosa) cultivars. Journal of Essential Oil Research 11: 491496.

Farsi, M., Abdollahi, F., Salehi, A., Ghasemi, S. (2017): Study of physiological characteristics of marjoram (*Origanum majorana*), as a medicinal plant in response to zinc levels under drought stress conditions. Journal of Environmental Stresses in Crop Sciences 10(4): 559-570.

Jahansouz, M.R., Afshar, R.K., Heidari, H., Hashemi, M. (2014): Evaluation of yield and quality of sorghum and millet as alternative forage crops to corn under normal and deficit irrigation regimes. Jordan Journal of Agricultural Sciences 10: 699-714.

Jaleel, C.A., Manivannan, P., Lakshmanan, G.M.A., Gomathinavaam, M., Panneerselvam, R. (2008): Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. Colloids and Surfaces B: Biointerfaces 61(2): 298-303.

Jamzad, Z. (2009): Thymes and savorys in Iran. Research Institute of Forests and Rangelands, Iran.

Jordan, M.J., Martinez, R.M., Cases, M.A., Sotomayor, J.A. (2003): Watering level effect on *Thymus hyemalis* Lange essential oil yield and composition. Journal of Agricultural and Food Chemistry 51(18): 5420-5427.

Jordan, M.J., Martınez, R.M., Goodner, K.L., Baldwin, E.A., Sotomayor, J. A. (2006): Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition. Industrial Crops and Products 24: 253-263.

Khalid, K.A. (2006): Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* sp.). International Agrophysics 20(4): 289-296.

Khazaie, H.R., Nadjafi, E., Bannayan, M. (2008): Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). Industrial Crops and Products 27: 315-321.

Laribi, B., Kouki, K., Sahli, A., Mougou, A., Marzouk, B. (2011): Essential oil and fatty acid composition of a Tunisian caraway (*Carum carvi* L.) seed ecotype cultivated under water deficit. Advances in Environmental Biology 5: 257-264.

Letchamo, W., Gosselin, A. (1995): Effects of HPS supplemental lighting and soil water levels on growth, essential oil content and composition of two thyme (*Thymus vulgaris* L.) clonal selections. Canadian Journal of Plant Science 75: 231-238.

Martinez, J.P., Silva, H., Ledent, J.F., Pinto, M. (2007): Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). European Journal of Agronomy 26: 30–38.

Rustaiee, A.R. (2009): Effect of climate conditions on morphological and phytochemical characteristics of *Thymus daenensis* Celak. M.Sc. Thesis, University of Tehran, Iran.

Rustaiee, A.R., Khorshidi, J., Tabatabaei, M.F., Omidbaigi, R., Sefidkon, F. (2010): Essential oil composition of *Thymus daenensis* Celak. during its phenological cycle. Journal of Essential Oil Bearing Plants 13(5): 556-560.

Saiedfar, S., Mahmoudi, M. (2013): Effect of drought stress on antioxidant enzymes activity in *Agastache foeniculum* Pursh Kuntze. Plant Ecosystem 34: 21-30.

Sajjadi, S.E., Khatamsaz, M. (2003): Composition of the essential oil of *Thymus daenensis* Celak. subsp. *lancifolius* (Celak) Jalas. Journal of Essential Oil Research 15: 34-35.

Selmar, D. (2008): Potential of salt and drought stress to increase pharmaceutical significant secondary compounds in plants. Landbauforschung Volkenrode 58:139-144.

Sotomayor, J.A., Martinez, R.M., Garcia, A.J., Jordan, M.J. (2004): *Thymus zygis* subsp *gracilis*: watering level effect on phytomass production and essential oil quality. Journal of Agricultural and Food Chemistry 52(17): 5418-5424.

Vazin, F. (2013): Water stress effect on cumin (*Cuminum cyminum* L.) yield and oil essential components. Scientia Horticulture 151: 135-141.

Xu, Z., Zhou, G., Shimizu, H. (2010): Plant responses to drought and dewatering. Plant Signaling & Behavior 5: 649-654.

Yavari, A. R. (2009): Evaluation of morphological, molecular, ploidy level and phytochemical diversity of *Thymus migricus* Klokov & Des –Shost in Iran. M.Sc. Thesis, University of Tehran, Iran.