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Investigation morphological and physiological response of *Thymus vulgaris* L. to drought stress

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Abstract

This study was performed to evaluate the effect of drought stress on quality and quantity yield of *Thymus vulgaris* under field and laboratory condition in Karaj, Iran. The study was conducted as randomized complete block design with five treatments and three replications. Treatments were included 100% moisture (control), 80% (optimum irrigation), 60% (deficit stress), 40% (fairly high stress) and 20% (high stress) of field capacity. Before plant harvesting, morphological traits were recorded. Then plants were cut from about 3cm above ground and aerial organs yield was evaluated after drying at 60-70°C. Essential oil was taken by using Clevenger and water distillation method during 2.5h and its yield was calculated. Soluble sugars, proline, sodium, potassium, magnesium, calcium and iron were measured. Results indicated that drought stress significantly affected plant height, flowering shoot yield, oil percent, oil yield, thymol percent, carvacrol percent, amount of chlorophyll a, chlorophyll b, proline, soluble sugars, sodium, magnesium, iron and RWC ($\alpha \leq 0.01$). Mean comparisons showed that the highest flowering shoot yield belonged to 80% of field capacity with 1728kg/ha. The highest (2.22%) and the lowest (0.74%) oil percent were observed in 20 and 100% of field capacity, respectively. 80, 60 and 40% of field capacity had the maximum of oil yield with 19.26, 18.478 and 17.309kg/ha, respectively. The maximum of thymol percent belonged to 80 (42.37%), 60 (42.52%) and 40% (41.4%) of field capacity. The highest magnesium amount observed in 80 (0.74ppm) and 60% (0.63ppm) of field capacity. 100 and 80% of field capacity showed the highest iron with 0.53 and 0.67ppm, respectively.

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Introduction

Thymus vulgaris in variety form is used in food, pharmaceutical, health and beauty industries. Among the abiotic stresses, drought stress is the most important agronomic problem that makes decreased in agronomic crops yield which are constant or periodic exposed to it (Chadar and Obul Reddy, 2008). Drought stress limits plants growth and fertility especially in arid and semi-arid regions (Erdem *et al.*, 2001; Yang *et al.*, 2009, Zaidi *et al.*, 2014). Plants by morphological, physiological and metabolic changes in all of their organs answer drought stress (Cellier *et al.*, 1998). Environmental factors and water deficit as the most important physiology and biochemistry aspects of plant can influence plants active substances (Petropoulos *et al.*, 2008). In medicinal plants, drought stress increases their active substances and it was reported in some plants such as *Cymbopogon winterianus juwit* (Fatima *et al.*, 2000), *Hypericum brasiliense* L. (Khalid, 2006), *Calendula officinalis* L. (Taherkhani *et al.*, 2011) and *Silybum marianum* L. (Vazque, 2010). According to Abbaszadeh and his colleagues' study (Abbasazadeh *et al.*, 2009), different irrigation levels (100 (control), 80, 60, 40 and 20% of field capacity) significantly affected shoot yield, oil yield, oil percent, leaf yield, plant height, tiller number, stem diameter and yield of *Melissa officinalis* L. (α≤0.01). The highest plant height and shoot yield were observed in control treatment. 40% of field capacity showed the highest oil yield. The highest oil percent and stem diameter belonged to 20% of field capacity treatment (Abbasazadeh *et al.*, 2009). A study on *Nigella sativa* L. indicated that drought stress caused decreased in plant dry matter, plant height, oil and seed yield (Rezapour *et al.*, 2011). According to three drought stress levels (90, 70 and 50% of field capacity) on *Matricaria chamomilla*, the highest essential oil yield and percent was obtained from 70% of field capacity (Arazmju *et al.*, 2010). Also water deficit stress was not significantly affected plant height, sub stem number, mass, fresh and dry weight, root length and vegetative body yield of *Mentha piperita* (Petropoulos *et al.*, 2008) and *Tagetes minuta* L. (Tyler, 2009). Abbaszadeh and his colleagues were reported that the effect of different irrigation treatments (100, 80, 60, 40 and 20% of field capacity) were significant for chlorophyll a, chlorophyll b, proline, soluble sugars, RWC and essential oil yield (α≤0.01), and for totall chlorophyll (α≤0.05). The highest amount of chlorophyll a and totall chlorophyll belonged to control treatment. 20% irrigation of field capacity showed the highest amount of chlorophyll b. The highest proline accumulation, soluble sugars and RWC were observed in 20, 60 and 100% of field capacity, respectively.

According to extensive use of *Thymus vulgaris* and importance of water deficit problem, this project was conducted to evaluate different drought stress levels on morphological and physiological traits of the plant.

Material and methods

This project was conducted to investigate the effect of drought stress on quality and quantity yield of *Thymus vulgaris* under field and laboratory condition in Karaj, Iran in 2012 - 2013.

Study design

The study was conducted as randomized complete block design with five treatments and three replications. Treatments were included 100% moisture (control), 80% (optimum irrigation), 60% (deficit stress), 40% (fairly high stress) and 20% (high stress) of field capacity.

Field preparation

Dimensions of each plot, ridges distance from each other and plants distance on the line were 4m × 2m, 40cm and 40cm, respectively. Blocks and plots distance from each other were 3m and 2.5m, respectively. After ground preparing, seeds were planted in the main field in autumn.

Necessary tending before the treatments application

Thinning was done fifth week after planting. Plants were regularly watered from planting time until cold period once a week. Necessary tending such as weeding was equally taken for all plots during the growth period. Fight against weeds was manually performed through several stages during growth period.

Application and traits measuring methods

The treatments were applied in spring with weighted method and TDR. Before plant harvesting, morphological traits were recorded. Then plants were cut from about 3cm above ground and aerial organs yield was evaluated after drying at 60-70°C. Essential oil was taken by using Clevenger and water distillation method during 2.5h and its yield was calculated. Samples of young leaves were harvested to chlorophyll extract. Also in this stage, necessary samples were harvested to soluble sugars, proline, sodium, potassium, magnesium, calcium and iron extract. Proline and soluble sugars were measured with Irrigoyen and his colleagues method (Irrigoyen *et al.*, 1992) and Levitt formula (Levitt, 1980), respectively. For elements measuring of flowering shoots, Ghazanshahi method was used (Ghazanshahi, 1997).

Statistical analysis

Obtained data were analyzed by SAS statistical program and means were compared by the Duncans multiple range test.

Results

Analysis of variance showed (Table 1) that drought stress significantly affected plant height, flowering shoot yield, essential oil percent, essential oil yield, thymol percent, carvacrol percent, amount of chlorophyll a, chlorophyll b, proline, soluble sugars, sodium, magnesium, iron and RWC (α≤0.01).

Table 1a. Analysis of variance of the effect of different drought stress levels on some traits of *Thymus vulgaris* in field condition, 2013.

SOV	Df	Mean Squares (MS)								
		Plant height	Flowering shoot yield	Oil percent	Oil yield	Thymol	Carvacrol	Chlorophyll a	Chlorophyll b	Totall chlorophyll
Block	2	6.6	71542	0.12	45	12.6	0.7	1.17	1.08	3.4
Drought stress	4	230**	832086**	1.05**	61.2**	82**	0.69ns	1.34**	1.3**	0.08ns
Error	8	12.9	2368	0.02	2	7.8	0.46	0.12	0.13	0.18
CV(%)	-	13	4	9	9	7.2	25	20	24	13

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 1b. Analysis of variance of the effect of different drought stress levels on some traits of *Thymus vulgaris* in field condition, 2013.

SOV	Df	Mean Squares (MS)							
		Proline	Soluble sugars	Sodium	Potassium	Calcium	Magnesium	Iron	RWC
Block	2	3.4	22	1.4	3.8	0.95	0.02	0.19	273
Drought stress	4	6.9**	6.33**	1.01**	0.03ns	0.09ns	0.07**	0.09**	1967**
Error	8	0.07	0.23	0.07	0.32	0.06	0.005	0.009	51
CV(%)	-	7.8	6.4	20	32	25	13	21.8	10

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

morphological response

Comparison results indicated (Table 2) that the highest plant height belonged to 80% of field capacity with 36.2cm. Results showed that 100 and 60% of field capacity had not significant difference with 80% of field capacity. Also plant height was decreased reached to 19.7 and 16.8cm with increasing drought stress up to 40 and 20% of field capacity, respectively.

Comparison results of flowering shoot yield showed that 80% of field capacity had the highest flowering shoot yield with 1728kg/ha. According to the study results, the lowest flowering shoot yield (412kg/ha) observed in 20% of field capacity.

Table 2a. Effect of different drought stress levels on some traits of *Thymus vulgaris* in field condition, 2013.

Drought stress levels	Plant height (cm)	Flowering shoot yield (kg/ha)	Oil percent (%)	Oil yield (kg/ha)	Thymol (%)	Carvacrol (%)	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Totall chlorophyll (mg/l)
1	34.8a	1515b	0.74d	11.385b	35.62b	2.37a	2.3a	0.73c	3.05a
2	36.2a	1728a	1.11c	9.262a	42.37a	3.4a	2.3a	0.91bc	3.2a
3	29.4a	1375c	1.34c	18.478a	42.52a	3.01a	1.86ab	1.56ab	3.42a
4	19.7b	917d	1.87b	17.309a	41.4a	2.26a	1.25bc	2a	3.25a
5	16.8b	412e	2.22a	9.255b	30.63b	2.5a	0.79c	2.24a	3.02a

Means in a column followed by the same letter are not significantly different at P≤0.01.

1, 100% of field capacity; 2, 80% of field capacity; 3, 60% of field capacity; 4, 40% of field capacity; 5, 20% of field capacity

Table 2b. Effect of different drought stress levels on some traits of *Thymus vulgaris* in field condition, 2013.

Drought stress levels	Proline (mg/l)	Soluble sugars (mg/l)	Sodium (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Iron (ppm)	RWC (%)
1	1.9d	6.03d	0.64b	1.67a	0.98a	0.53bc	0.53ab	95a
2	1.74d	6.2d	0.76b	1.88a	1.25a	0.74a	0.67a	96a
3	3.18c	7.27c	1.5a	1.7a	1.05a	0.63ab	0.44bc	76.7b
4	4.15b	8.4b	1.62a	1.67a	0.9a	0.43cd	0.34cd	58.6c
5	5.3a	9.4a	1.99a	1.69a	0.8a	0.35d	0.23d	35.7d

Means in a column followed by the same letter are not significantly different at P≤0.01.

1, 100% of field capacity; 2, 80% of field capacity; 3, 60% of field capacity; 4, 40% of field capacity; 5, 20% of field capacity

Essential oil changes

The highest (2.22%) and lowest (0.74%) essential oil percent belonged to 20 and 100% of field capacity, respectively. The maximum of essential oil yield observed in 80 (19.26kg/ha), 60 (18.478kg/ha) and 40% (17.309kg/ha) of field capacity. Treatments of free (100% of field capacity) and high drought stress (20% of field capacity) had the lowest essential oil yield with 11.38 and 9.25kg/ha, respectively (Table 2).

Results indicated (Table 2) that the maximum of thymol percent belonged to 80 (42.37%), 60 (42.52%) and 40% (41.4%) of field capacity. In terms of carvacrol percent, there was no significant difference between treatments and the highest carvacrol percent was mathematically obtained from 80% of field capacity with 3.14%.

Physiological response

Comparison results of chlorophyll a showed that 100, 80 and 60% of field capacity had the maximum of chlorophyll a with 2.3, 2.3 and 1.86mg/l, respectively. The maximum of chlorophyll b belonged to 60 (1.56mg/l), 40 (2mg/l) and 20% (2.24mg/l) of field capacity. 100 and 80% of field capacity had the lowest amount of chlorophyll b with 0.73 and 0.91mg/l, respectively. Comparison results of total chlorophyll indicated that there was no significant different between treatments but the highest amount of total chlorophyll was observed in 60% of field capacity with 3.42mg/l. The highest and lowest proline amount belonged to 20 (5.3mg/l) and 100% (1.9mg/l) of field capacity, respectively. 20% of field capacity treatment showed the highest soluble sugars with 9.4mg/l.

Sodium comparison results indicated that 20 (1.5%), 40 (1.62%) and 60% (1.99%) of field capacity had the maximum of sodium. Comparison results of potassium showed that there was no significant difference between treatments. The maximum of magnesium was observed in 80 (0.74%) and 60% (0.63%) of field capacity. Comparison results of iron stated that 100 and 80% of field capacity had the maximum of iron with 0.53 and 0.67ppm, respectively. The maximum of RWC was observed in 100 (95%) and 80% (96%) of field capacity.

Correlation between measured traits

Results of correlation between traits showed (Table 3) that flowering shoot yield had significant positive correlation with plant height, chlorophyll a, magnesium and RWC (α≤0.01), and with thymol percent and iron amount (α≤0.05). The results of study indicated that flowering shoot yield had significant negative correlation with essential oil percent, chlorophyll b, proline, soluble sugars and sodium amount. Essential oil percent showed significant positive correlation with chlorophyll b, proline and soluble sugars, and significant negative correlation with plant height, chlorophyll a, calcium, iron and RWC.

Table 3. Effect of correlation between measured traits.

Traits	Flowering shoot yield	Essential oil percent
Flowering shoot yield	1	
Essential oil percent	-0.82**	1
Plant height	0.89**	-0.85**
Thymol	0.53*	-0.40ns
Carvacrol	0.38ns	-0.11ns
Chlorophyll a	0.66**	-0.77**
Chlorophyll b	-0.79**	0.60*
Proline	-0.90**	0.69**
Soluble sugars	-0.50**	0.61*
Sodium	-0.54*	0.82**
Potassium	0.05ns	-0.06ns
Calcium	0.39ns	-0.15ns
Magnesium	0.85**	-0.55*
Iron	0.60*	-0.51*
RWC	0.94**	-0.80**

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Discussion

Drought stress effects on plants are very complicated and multiply. Drought stress causes change in the phytohormones balance, stimulate ABA[1] increasing in leaf and reduce the mass of auxin, gibberellins and cytokinin (Fiqueired *et al.*, 2008). Plants photosynthetic pigments mainly decrease to catch light and produce energy. Drought stress has reducing capability of tissue chlorophyll and carotenoids concentrations and this reduction is mainly occur with producing ROS[2] in tilacoides (Jaleel *et al.*, 2009). In plants under drought stress, between cellular space and water amount decrease in their body to water enter with more force from soil into the plant and this cause decrease in relative water amount under drought stress (Khorshidi *et al.*, 2002).

In this study, firstly, all of measured traits in 100% of field capacity condition were lower than 80% of field capacity due to lack of oxygen uptake by roots, activity reduction of aerobic microorganisms and lack of access to sufficient food or the plant was under much water stress. Secondly, it was observed that 80% of field capacity was the best growth condition in terms of dry matter production of *Thymus vulgaris* and 80, 60 and 40% of field capacity were appropriate treatments for essential oil production. Considering that obtaining high active substances is the ultimate goal of medicinal plant, so using the fairly high stress treatment (40% of field capacity) can be used as the best production condition of *Thymus vulgaris*. Due to lower dry matter production in this treatment, costs of harvesting, transportation, drying and extracting active substances is lower than the other treatment and would be affordable and economically. Thirdly, it was observed that plant height and flowering shoot yield were decreased with increasing drought stress. Maybe reduction of relative water and some nutrients absorption are reasons for plant height and dry matter reduction.

[1] Abscisic acid

[2] Reactive Oxigen Species

Several studies have also demonstrated reduction effect of drought stress on morphological traits and yield of different plants. So that Rezapour and his colleagues in a study on *Nigella sativa* L. reported that drought stress caused decrease in plant dry matter, plant height, essential oil and seed yield but essential oil percent was increased by stress escalation (Rezapour *et al*., 2011). Evaluation drought stress on *Melissa officinalis* L. was indicated that drought stress caused 3MPa recution of plant water potential, 34% reduction of leaf water content, stomata closing and thus cause decreased in carbon dioxide absorption and plant yield (Munne and Alegre, 2000). Arazmju and his colleagues investigation about effect of three drought stress levels (50, 70 and 90% of field capacity) on *Matricaria chamomilla* indicated that the highest essential oil yield and percent were obtained from 70% of field capacity condition (Arazmju *et al*., 2010). According to Ahmadiyan and his colleagues results about effect of irrigation 50, 70 and 90% of field capacity on *Matricaria chamomilla*, 70% of field capacity treatment had the highest essential oil yield, while intense stress caused decreased in the trait (Ahmadiyan *et al*., 2011).

In *Thymus vulgaris* observed that some osmotic regulators inside the plant, like soluble sugars and proline, increase with stress intensity increasing. Osmotic regulation with storage of soluble solutes by cells is a process that can reduce cell water potential without pressure potential reduction (Chougan, 2004). Abbaszadeh and his colleagues reported that drought stress implementation on *Melissa officinalis* cuase incraesing in proline. It showes that the plant increase proline and soluble sugars amount for drought tolrence, while in non-stress condition proline and soluble sugars are produced in low low amount (Abbaszadeh *et al*., 2007). Plant yield is a function of the variety and optimal agriculture that is performed during growth season in the farm. In each district by selectibg the best and appropriate cultivar, a planting and harvesting system wich leads to the highest yield per area unit can be predicted. According to Abbaszadeh and his collaegues results on *Melissa officinalis*, the highest amount of chllorophyll a and total chlorophyll was obtained in free stress condition and both of them became low with stress increasing. Cholorophyll b almost had the opposite position and its amount increased with stress increasing (Abbaszadeh *et al*., 2007).

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