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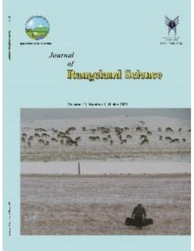

Research and Full Length Article:

Effects of Water Stress on Seedling Growth and Physiological Traits in Four Thyme Species

Ali Sepahvand[A], Ali Ashraf Jafari[B*], Fatemeh Sefidkon[B] and Sepideh kalatejari[C]

[A] PhD student, Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[B] Professors, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran. *(Corresponding author) Email: aajafari@rifr-ac.ir

[C] Assist. Prof., Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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Abstract. Drought stress is one of the most important factors limiting the survival, growth and production of various crops and medicinal plants in the different habitats of Iran. This study was conducted to evaluate the effects of drought stress on seedling growth and physiological traits in four native thyme species as (*Thymus kotschyanus, T. dianensis, T. lancifolius and T. pubescens*). A factorial experiment was conducted using thyme species as main factor and three levels of water stresses namely well-watered (90% of field capacity), mild (70% FC) and severe drought stress (40% FC) as the second factor based on a Completely Randomized Design (CRD) with 3 replications in 2018, Khorramabad, Iran. Data collected for 14 morpho-physiological traits of the seedlings. The result of analysis of variance showed significant effects of species and water stress levels for all of the traits ($p<0.01$). The species by water stress interaction effects were significant for all of the traits except, carbohydrates and peroxidase indicating that the thyme species had different responses to water stress. Results of means comparison between water stress levels showed that the values of root length, root dry weight, root volume and physiological traits, as: leaf electrolyte leakage, carbohydrates, proline content and catalase activity were increased by drought stress and the higher values were observed in 40% FC, indicating that by increasing drought stress both root growth parameters and many physiological traits were significantly increased in all of the species, but, the leaf relative water content (RWC) and leaf pigments decreased. Results of species by water stress showed that the higher values of root length were obtained in *T. daenensis* and *T. lacnifolius* in severe stress (40% FC), respectively. However, in the same species, the higher values of roots dry weights were obtained in the mild stress (70% FC), respectively. For physiological traits, the higher values of (RWC), leaf electrolyte leakage, carbohydrates content and leaf pigments were obtained in *T. lancifolius*, indicating that this species was more tolerated to drought stress than the other species. It was concluded that species of *T. daenensis* and *T. lacnifolius* had produced longer roots in the severe drought stress. However, *T. daenensis*, due to higher areal part production and essential oil yield in the field, was recommended for domestication and cultivation in dryland farming system and semi-stepic rangelands of Iran.

Key words: Drought, *Thymus pubescens,T. lacnifolius,T. daenenesis,T. kotschyanus*

Introduction

Thymus genus belongs to Lamiaceae family containing almost 215 species of perennial forbs and small shrubs native to the Mediterranean region and also grow in some parts of southern Europe, Africa and some parts of Asia (Stahl-Biskup and Saez, 2002). This genus is represented of Iranian flora by 18 species (Jamzad, 2009), four of which (*T. carmanicus, T. daenensis and T. lancifolius, T. persicus and T. trautvetteri*) are endemic of Iran (Rechinger, 1982).

The essential oil of Thyme is the world's ten most important essential oils, which have antibacterial, antifungal, antioxidant, and natural preservatives (James, *et al*., 1991). In Iran, the aerial part biomasses of thymes are more widely used as herbal tea, flavoring agents (condiment and spice) and medicinal plants (Stahl-Biskup, 2002). It also is used as tonic, carminative, digestive, antispasmodic, anti-inflammatory, antitussive, expectorant and for the treatment of colds in Iranian traditional medicine (Zargari, 1997). The antimicrobial properties are often due to their phenol content and presence of thymol and carvacrol in its oils (Bauer *et al*. 1997).

Thymes have abundant stem relatively short and woody which gives a pulvinate crown to this species along with robust and dense roots playing a key role in soil stabilization and also preventing from water erosion in mountainous and sharp slope regions (Moghimi, 2005).

In medicinal and aromatic plants, oil production and rate of growth are influenced by environmental factors such as drought stress (Sabih *et al.*, 1999). Increasing drought stress in thymes causing the reduce traits such as plant height, number of lateral shoots, dry and fresh weight of biomass and thymol, and in contrast, leading to increase the root volume, root dry weight and root length (Amini Dehaghi and Babaee, 2010 and Azimi *et al*., 2018). In a pot experiment, Moradi *et al.* (2015) found the highest values of fresh and dry weight, plant height and oil yield in *T. daenensis* in irrigation in 60% of Field Capacity (FC) and the lowest values were related to irrigation in 40%FC. In another experiment, Pourmeidani et al. (2017) found significant variation between accessions of *T. kotschyanus* to drought tolerance, so that, in the moderate stress (60%FC), the accessions of Qazvin, Abyek, and Uremia had higher oil productions than the other ones. Safikhani (2006) in severe drought (40% FC) in *Dracocephalum moldavica* found lower values of plant height, leaf area, internode length, shoot yield and essential oil yield as compared to mild and normal stress.

Proline accumulation is a common physiological response in many plants in response to drought stress. Proline as an amino acid is compatible solutes for cell osmotic adjustment and protection of cell components during dehydration (Zhang *et al*., 2009). Drought stress increases proline content in *Brassica napus* (Mirzaee, 2013), Sainfoin (Veisipoor *et al*, 2013), grape cuttings (Meng *et al*., 2014) and soybean (Ghorbanali and Niakan, 2007). Drought stress also increases soluble carbohydrate and protein content (Kabiri *et al*., 2018; Mirzaee, 2013; Meng *et al*., 2014; Ghorbanali and Niakan, 2007). In contrast, photosynthesis is limited by drought stress due to stomatal closure (Flexas *et al*., 2004; Chaves *et al*. 2009; Mafakheri, *et al*. 2010), consequently drought stress decreases the chlorophyll content in plant species (Bahreininejad *et al*., 2008; Alaei *et al*., 2013; Begum Paul 1993).

Many of important thyme species are currently harvested in their natural habitats in Iran. These can lead to the destruction of a large part of the germplasm of valuable species. Therefore, domestication and cultivation of important thyme species in dryland farming conditions are high priority here here in Iran. This study was aimed to assess responses of four thyme species for yield, morphological and physiological traits in greenhouse condition.

Material and Methods

Seeds of four species of *Thymus pubescens, T. daenensis, T. lacnifolius* and *T. kotschyanus* were collected from their natural habitat in Lourestan Province, Iran (Table 1). This experiment was conducted in the greenhouse condition. The soil compositions consisted of 25% clay, 25% decayed animal fertilizer and 50% sandy soil. Plastic pots in size of 20 and 25 cm in diameter were used. Pots had some holes for drainage. A bit of gravel was placed at the bottom of the pots to facilitate the discharge of water. The pots were filled with soils. Seeds were disinfected by Mancoseb fungicide with the 1:2000 ratios to prevent the fungal contamination. In each pots 10 seeds sown in January 2018 in Khorramabad, Iran. Pots were irrigated regularly until the seeds go to germination and seedlings were appeared. Then, the pots were kept outdoor under shelter during winter. In March 2018, the pots were arranged using a factorial experiment based on a completely randomized design (CRD) with three replications The first factor was four species of *Thymus pubescens*, *T. daenensis*, *T. lacnifolius* and *T. kotschyanus* (Table 1). The second factor was drought stress levels as control 90% Field Capacity (FC), 70% FC and 40% FC. To determine the amount of water requirements for pots, at the beginning of the experiment, the soil FC was determined by pots weighing method. To this end, water was gradually added to the dry soil in a pot. After saturation and the withdrawal of excess water, the pots were weighed again. The weight of pots (soil+ water) at 90% FC was 3000g. So, the weight of water at 90% FC was 500 g. With regard to irrigation, each treatment after the reduction of 20% and 50% (equal to 100 and 250 g) was irrigated for 70% and 40% FC after achieving weight pots in each treatment as 2900, 2750 g, irrigation was done. For this purpose, the pots were weighed every two days interval on the specified weight for each treatment, the amount of needed water was added to each pot. Irrigation treatments were continued for three months. Then, data collected for four morphological traits as: root length (cm), root dry weight (g/pot), root volume (cm^3) and, survival rates (from 1=the lowest to 10=the highest score) and 10 physiological traits in May 2018.

The Leaf pigments, chlorophyll a and b and carotenoid content were quantified according to the protocol of Lichtenthaler and Wellburn (1983). Proline content was determined using the method of Bates *et al.* (1973) and soluble sugars, measured using methods of Irigoyen *et al*. (1992). The leaf relative water content (RWC) determined according to the method of Ritchie *et al.* (1990) and the leaf electrolyte leakage percentage was measured according to Lutts *et al*. (1996) and meanwhile, the proxidase and catalase enzyme activities were measured using Elstner *et al.* (1995) method.

At the end of the experiments, the analysis of variance was carried out and the mean comparisons were made using Duncan's method for all traits. SAS and Excel software were used for statistical analyses.

Table 1. Information for thyme species used in the present research

Name of species	Species origin	Longitude N	Latitude E	Altitude masl	Annual Average Precipitation mm	Annual average Temperature °C
Thymus kotschyanus	Aleshtar -Peresk	48° 20' 56"	33°48'24"	1834		
Thymus pubescens	Bourojerd- Absardeh	48° 41' 08"	33°45'11"	1752		
Thymus lancifolius	Khramsbad-Zagheh	48° 40' 26"	33°29'11"	1973	588.4	18.4
Thymus daenensis	Khramsbad-Zagheh	48° 40' 26"	33°29'11"	1973	588.4	18.4

weight with values of 2.0 and 1.28 g/p were observed in mild 70% FC and severe stress 40% FC, respectively. For survivor rate, the higher and lower values were observed in 90% FC and 40% FC (Table 4).

The species by water stress interaction effects were significant for all root's related traits and survivor rates (Table 2), indicating that responses of four thyme species to drought stress were not similar. The root length of *T. daenensis* and *T. lacnifolius* had increased by 76% and 57% in severe stress (40% FC) than that for control (90% FC), respectively (Fig 1). Similarly, the highest root's dry weights of both species were obtained in mild stress (70%FC).

For root volume, *T. daenensis, T. kotschyanus* and *T. lacnifolius* in mid stress (FC 70%) had significantly 79%, 61% and 300% higher root volume than control (FC 90%), respectively. In severe stress, except for *T. kotschyanus*, that has had similar root weights at 70% FC and 40% FC, the values of other species decreased by increasing drought stress (Fig 1). The survival rate of all species except *T. pubescens* were dramatically decreased by drought stress. For *T. pubescens*, the higher value was observed in 70% FC that had no significant difference with the control (Fig 1).

Results

Morphological traits

The results of the analysis of variance showed significant effects of species, water stress and species by water stress for all of roots traits ($p<0.05$) and survivor rate (Table 2).

Results of means comparison between species showed that *T. lancifolius* and *T. kotschyanus* with average values of 12.75 cm and 11.06 cm had significantly longer roots than that for the other two species, respectively, and raked in the first class. For root dry weight, *T. dianensis* and *T. lancifolius* with average values of 2.40 and 2.32 g/pot had significantly the highest roots weight than that for the other species. For root volume, the higher and lower values of 1.49 and 0.69 cm^3 were obtained in *T .kotschyanus* and *T. pubescens*, respectively. For survivor rate, the *T. pubescens* and *T. lancifolius* with scores of 6.67 and 3.33 had a higher and lower values of survival rate, respectively, (Table 3).

Results of means comparison between water stress levels showed that the higher value of root length (11.35 cm) was obtained in severe drought stress (40% FC) that was significantly higher than other treatments, indicating that by increasing drought stress the root length was significantly increased. For root volume, the higher values of 1.31 and 1.15 cm^3 were observed in 70% FC and 40% FC, respectively and both of them ranked in class a. The higher and lower roots dry

Table 2. ANOVA of seedling growth traits in four thyme species under three levels of drought stress

Sources	DF	Root length (cm)	Root dry weight (g/p)	Root volume (cm^3)	Survivor rate (Score)
Species	3	186.4*	1.66 *	6.69 *	17.58**
Water stress	2	173.8*	1.88 *	4.34 *	68.25**
Species*stress	6	75.9*	1.23 *	2.41 *	5.58**
Error	24	12.47	0.16	1.77 *	1.29
Total	35				

*significant at the 0.05 probability level; ** significant at the 0.01 probability level.

Table 3. Means of seedling growth traits in four thyme species (Average over drought stresses)

Species name	Root length (cm)	Root dry weight (g/p)	Root volume (cm^3)	Survivor rate (Score)
T. dianensis	9.44 b	2.40 a	1.22 b	4.33 b
T. kotschyanus	11.06 a	1.41 b	1.49 a	4.67 b
T. lancifolius	12.75 a	2.32 a	1.07 b	3.33 c
T. pubescens	6.92 c	0.21 c	0.69 c	6.67 a

The means of the column with same letters were not significantly different based on Tukey;s method (p<0.05|).

Table 4. Means of seedling growth traits in three levels of water stresses (Average over species)

Water stress	Root length (cm)	Root dry weight (g/p)	Root volume (cm^3)	Survivor Rate (Score)
90% FC	9.48 b	1.48 ab	0.69 c	7.25 a
70% FC	9.09 b	2.00 a	1.31 a	4.5 b
40% FC	11.35 a	1.28 b	1.15 a	2.5 c

The means of the column with same letters were not significantly different based on Tukey's method (p<0.05|).

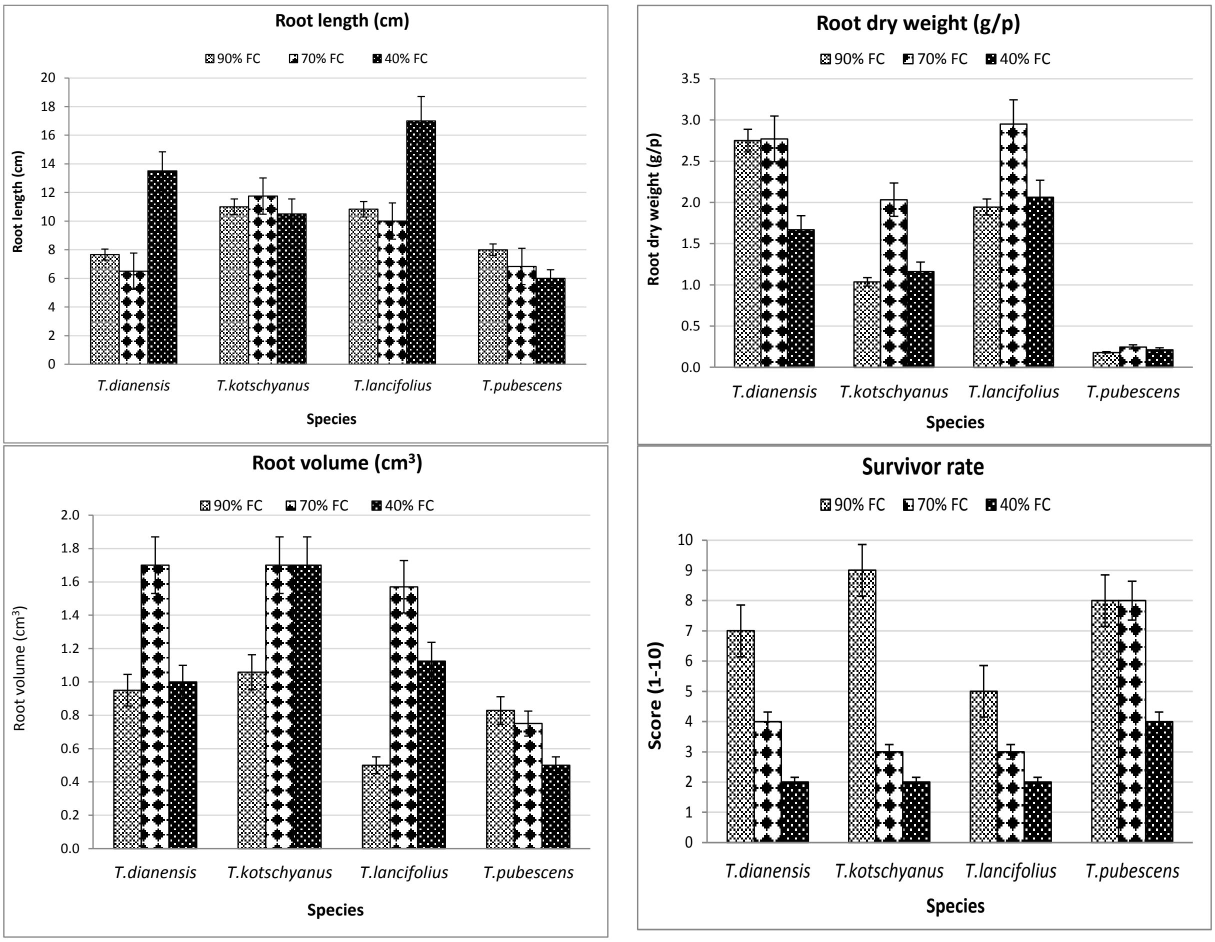

Fig 1. Means of germination traits and seedling growth in four thyme species under three levels of drought stress

Physiological traits

The results of the analysis of variance showed significant effects of species, water stress for all of physiological traits (p<0.01). The species by water stress interaction effects were significant for RWC, Leaf electrolyte leakage and catalase (p<0.01), and proline (p<0.05), (Table 5).

The result of mean comparisons showed, *T. lancifolius* and *T. pubescens* with average values of 51.65 and 53.87% had higher RWC, respectively, indicating their ability to retain more water in their leaves under drought stress (Table 6). The results of mean comparison between treatments showed that the values of RWC were 58%, 45% and 34% for FC 90%, FC 70% and FC40%, respectively (Table 7). Although all of species had similar trends for RWC, but the slope of decreases in *T. kotschyanus* were lower than the other species (Fig 2).

For leaf electrolyte leakage, the highest values of 59% and 57% were observed in *T. lancifolius* and *T. pubescens*, respectively (Table 6). In comparisons between drought stress levels, the values of 33%, 47% and 69% were observed in FC 90%, FC 70% and FC 40%, respectively, indicating that its values increased by increasing drought stress (Table 7). Result, showed that in all of species, the amount of leaf electrolyte increases was the lowest in FC 90%, but the slope of increasing were more in *T. daenensis* and *T. kotschyanus* than other species. It seems they have less membrane stability than other species (Fig 2).

For carbohydrates content, the highest and lowest values of 928 and 772 mgg^{-1}FW were obtained in *T. lancifolius* and *T. daenensis*, respectively (Table 6). In comparisons between drought stress levels, the highest value of 1151 mgg^{-1}FW was obtained in FC 40% that was significantly higher than the two other stress levels. Indicating the amount of carbohydrates increased with increasing drought stress, Due to the lack of species by water stress interaction, all four species had a similar trend of carbohydrate and the highest values was observed in FC 40% (Fig 2).

For proline content, the highest and lowest values of 4.07 and 1.75 mgg^{-1}FW were observed in *T. daenensis* and *T. lancifolius* species, respectively (Table 6). In comparing between three levels of drought stress, the highest and lowest amount of proline with 4.13 and 2.40 mgg^{-1}FW was obtained in FC 40% and FC 90%, respectively. In all species the proline values increased with increasing drought stress. However, due to species by water stress interaction effects, the response of species to drought stress were not similar, so that in FC 40%, the highest amount of proline with the value of 5.88 mg was obtained in *T. kotschyanus*, that was significantly higher than the control (Fig 2). However, in other species, although the trend was similar, and the lowest amount of proline was obtained in *T. lancifolius* species (Fig. 2).

For catalase enzyme activity the highest and lowest values of 5.61 and 4.44 mgg^{-1}FW were obtained in *T. kotschyanus* and *T. pubescens*, respectively (Table 6). In comparing between three levels of drought stress, the highest value of 8.19 mgg^{-1}FW was observed in FC 40% that was significantly higher than the two levels of drought stress, in the other words, the amount of this enzyme increased with increasing drought stress (Table 7). The result of species by drought stress interaction, showed that in all of species, the amount of catalase was the lowest in FC 90%, but their sl of increasing were different in species and the highest value of catalase with 9.86 mgg^{-1}FW was obtained in *T. kotschyanus* in the severe stress (FC 40%) (Fig 2).

For peroxidase enzyme activity, the highest and lowest values of 4.54 and 2.58 mgg^{-1}FW were obtained in *T. daenensis and T. pubescens*, respectively (Table 6). In comparisons between treatments, the highest and the lowest concentrations of this enzyme with average values of 5.11 and 1.99 mgg^{-1}FW were obtained in severe stress (FC 40%) and control, respectively. There was no significant effect of species by water stress interaction, therefore, the trend peroxidase activity were similar in all four species and enzyme activity increased by increasing drought stress (Fig 2).

Table 5. ANOVA of physiological traits in four thyme species under three levels of drought stress

Source of Variation	DF	Relative water contents	Leaf electrolyte leakage %	Carbohydrates mgg^{-1}FW	Proline mgg^{-1}FW	Catalase mgg^{-1}FW	Peroxidase mgg^{-1}FW
Species	3	544**	670**	380*	9.90**	2.28*	6.25**
Water stress	2	1633**	3175**	12284**	9.28**	95.63**	29.75**
Species*stress	6	175**	52**	222.5	1.52*	2.55**	1.65
Error	24	21.4	3.14	116.6	0.72	1.31	1.16
Total	35						

*Significant at the 0.05 probability level; ** significant at the 0.01 probability level.

Table 6. Means of physiological traits in four thyme species (Average over drought stresses)

Species name	Relative water content	Leaf electrolyte leakage %	Carbohydrates $mgg^{-1}FW$	Proline $mgg^{-1}FW$	Catalase $mgg^{-1}FW$	Peroxidase $mgg^{-1}FW$
T. dianensis	37.93 b	42.35 c	772.44 b	4.07 a	4.69 b	4.54 a
T. kotschyanus	41.26 b	45.35 b	831.42 ab	3.84 a	5.61 a	4.02 b
T. lancifolius	51.65 a	59.41 a	928.26 a	1.75 c	4.92 ab	3.6 b
T. pubescens	53.87 a	57.78 a	864.5 ab	3.00 b	4.44 b	2.58 c

The means of the column with same letters were not significantly different based on Tukey's method p<0.05|).

Table 7. Means of physiological traits in three levels of drought stresses (Average over four thyme species)

Water Stress	Relative water Content	Leaf electrolyte Leakage %	Carbohydrates $mgg^{-1}FW$	Proline $mgg^{-1}FW$	Catalase $mgg^{-1}FW$	peroxidase $mgg^{-1}FW$
90% FC	58.23 a	37.37 c	514.02 c	2.40 c	2.4 c	1.99 c
70% FC	45.36 b	47.16 b	882.07 b	2.97 b	4.16 b	3.97 b
40% FC	34.93 c	69.14 a	1151.37 a	4.13 a	8.19 a	5.11 a

The means of the column with same letters were not significantly different based on Tukey's method p<0.05|).

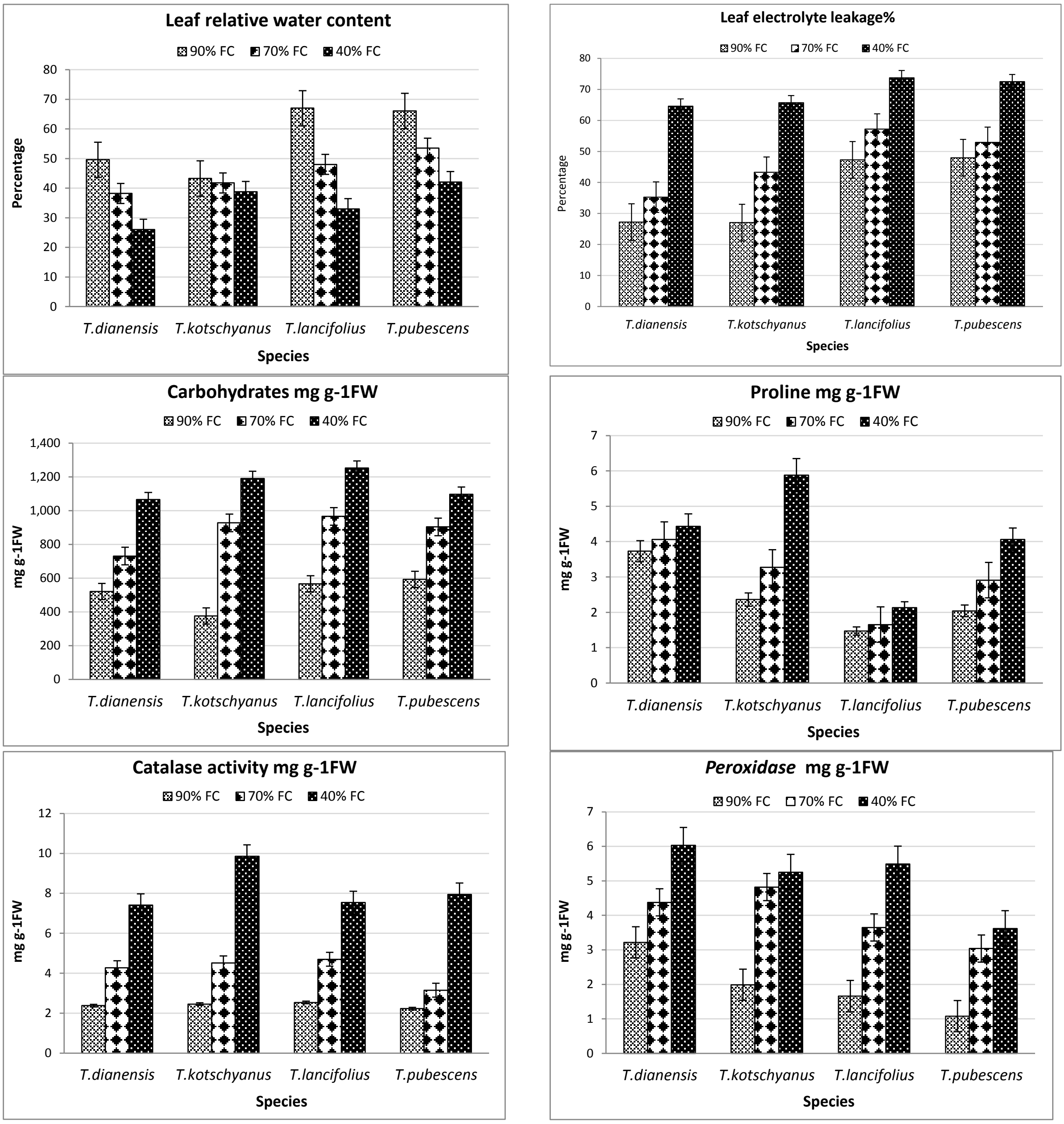

Fig 2. Means of physiological traits in four thyme species under three levels of drought stress

Leaf pigments

The results of the analysis of variance showed significant effects of species, for all of leaves pigments except chlorophyll b. The effects of water stress and species by water stress interaction were significant for all of leaves pigments (chlorophyll b, total chlorophyll and carotenoid), (Table 8).

The results of mean comparing showed that there was no significant difference between the four species for chlorophyll a. However, for chlorophyll b, total chlorophyll and carotenoid the higher values were obtained in *T. lancifolius* (Table 8).

In mean comparison between drought stress treatments, the highest and lowest values of chlorophyll a (4.92 and 3.07 mgg^{-1}FW), chlorophyll b (with 1.90 and 1.17 mgg^{-1}FW), total chlorophyll (with 6.97 and 4.31) and carotenoid (with 2.34 and 1.64) were obtained in normal (FC 90%) and severe (FC 40%) stress, respectively (Table 9). Due to the lack of species by water stress interaction, all four species had a similar trend for leaves pigment changes and the highest and lowest values always were observed in FC 90% and FC 40%, respectively (Fig 3).

Table 8. ANOVA of leaf pigments traits in four thyme species under three levels of drought stress

Source of Variation	DF	Chlorophyll a mgg-1FW	Chlorophyll b mgg^{-1}FW	Total chlorophyll mgg-1FW	Carotenoid mgg^{-1}FW
Species	3	0.31	0.34**	1.42*	0.31*
Water stress	2	9.61**	1.66**	21.8**	1.50**
Species*stress	6	0.47*	0.24**	1.70*	0.19*
Error	24	0.16	0.12	0.32	0.14
Total	35				

*significant at the 0.05 probability level; ** significant at the 0.01 probability level.

Table 9. Means of leaf pigments traits in four thyme species (Average over drought stresses)

Species name	Chlorophyll a mgg^{-1}FW	Chlorophyll b mgg^{-1}FW	Total chlorophyll mgg^{-1}FW	Carotenoid mgg^{-1}FW
T. dianensis	3.94 a	1.51 a	5.39 a	1.79 b
T. kotschyanus	3.72 a	1.23 b	5.06 b	1.99 ab
T. lancifolius	4.17 a	1.70 a	6.01 a	2.14 a
T. pubescens	3.88 a	1.52 a	5.63 ab	1.89 ab

The means of the column with same letters were not significantly different based on Tukey's method $p<0.05$|).

Table 10. Means of leaf pigments traits in three levels of drought stresses in (Average over species)

Stress	Chlorophyll a mgg^{-1}FW	Chlorophyll b mgg^{-1}FW	Total chlorophyll mgg^{-1}FW	Carotenoid mgg^{-1}FW
90% FC	4.92 a	1.90a	6.97 a	2.34 a
70% FC	3.7 b	1.41b	5.31 b	1.88 b
40% FC	3.07 c	1.17 c	4.31 c	1.64 c

The means of the column with same letters were not significantly different based on Tukey's method $p<0.05$|).

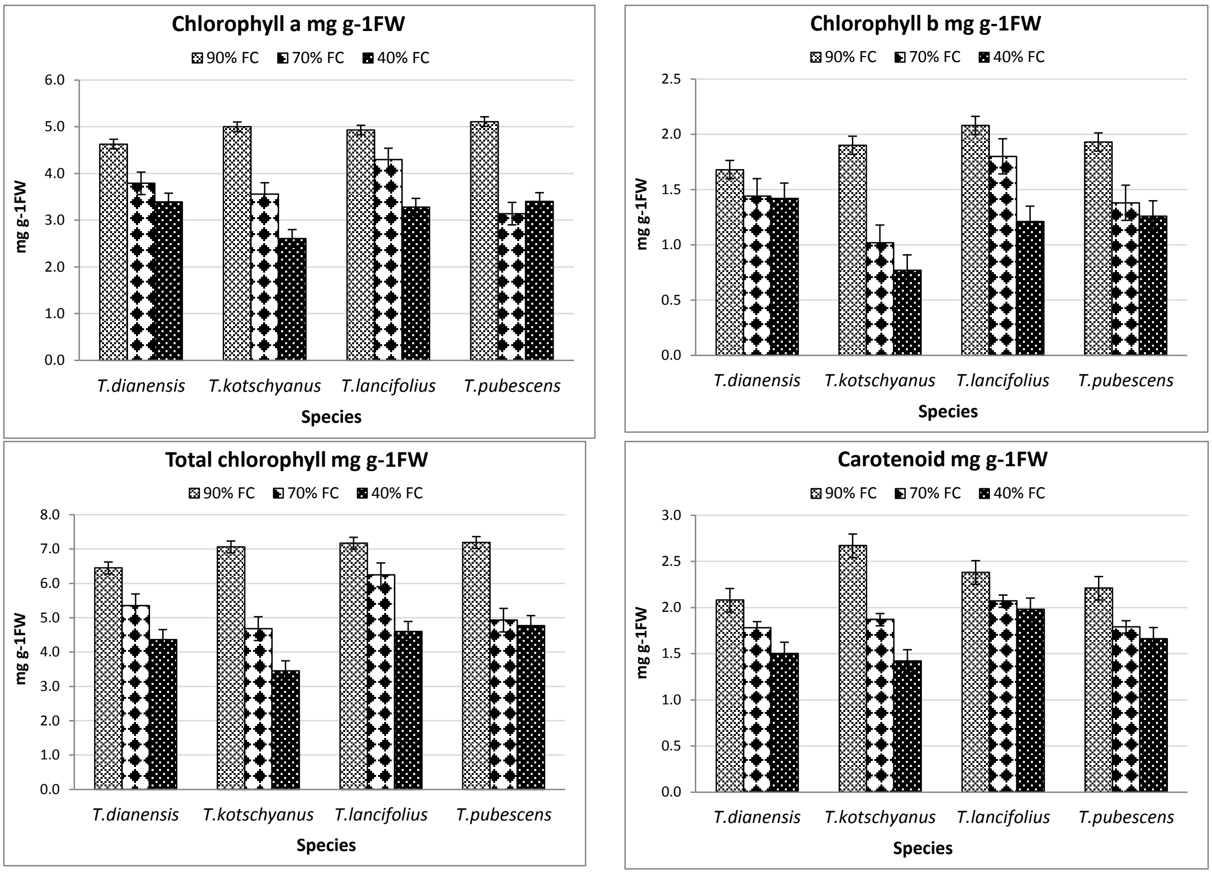

Fig 3. Means of leaf pigments traits in four thyme species under three levels of drought stress

Discussion

Drought stress is one of the most important factors limiting the growth of plants in their habitats. The purpose of this study was to evaluate the effects of water stress on the root growth and physiological traits. Results of means comparison between species showed that *T. lancifolius* and *T. kotschyanus*, for root length, *T. dianensis* and *T. lancifolius* for root dry weight, *T. kotschyanus* for root volume and *T. pubescens* for survival rate had higher mean values (Table 3). Results showed that in all of the species, the higher values of root length, root dry weight and root volume were obtained in severe drought stress (40% FC), indicating that by increasing drought stress the root lengths were significantly increased. However, duo to the species by water stress interaction effects, the responses of species were not similar, the root length of *T. daenensis* and *T. lacnifolius* increased by 76% and 57% in severe stress (40% FC) than that for control, respectively. Similarly, the same species had the highest root dry weights in the mild stress (70% FC). The highest root volume was obtained in *T. lacnifolius*, in the mid stress (FC 70%). Nouraei *et al.* (2013) using three ecotypes of *T. kotschyanus* found that the reduced water from normal to mild stresses increased aerial part yield, but severe stress reduces the yield and morphological traits in the ecotypes. Najafzadeh, *et al*., (2020) in a study of the effect of ecological factors on essential oil yield of five thyme species in the northwest of Iran, found the *T. kotschyanus* (Chenareh ecotype) as the best one for cultivation and using in pharmaceutical industries. In a pot experiment, Moradi *et al.* (2015) in *T. daenensis* found the highest seedling weight, in mild stress (FC 60%) and by severe drought (40% FC) its values sharply decreased. Longer roots can be an advantage for soil moisture absorption in drought stress and dryland farming. More root growth in severe stress, increases the plant's ability to absorb more water and nutrients. Ranjbar *et al.* (2015) also reported the root length increases in drought tolerance of *Thymus transcaucasicus*. Similar to our finding, Amiri Dehghi and Babaei (2010) reported that drought stress increases the length, weight and root volume of *Thymus vulgaris.*

For physiological traits the trends of osmotic solutes (proline and soluble sugars), Leaf electrolyte leakage) and antioxidant enzymes (peroxidase and catalase) were similar and drought stress significantly increased accumulation of both of them. But, the RWC and pigments decreased.

For RWC, *T. lancifolius* and *T. pubescens* with average values of 51.65 and 53.87% had higher RWC than other species, respectively, indicating their ability to retain more water in leaves under drought stress (Table 6). The RWC values were decreased by drought, but, the trend of decreases were not similar in species and the slope of decrease was lower in the *T. kotschyanus* than the other species (Fig 2). Similar to our results, drought stress decreases RWC in *Medicago laciniata* (Ghorbani Javid, *et al.* 2006), *Medicago sativa* (Yarnia *et al*. 2001) and *Anthemis* (Salehi *et al*., 2015). In a study, Kaiser (1989) reported that photosynthesis is rather insensitive to dehydration down to 50–70% RWC and in severe dehydration not only photosynthesis, but also dark CO_2 fixation and photorespiration are decreased. Plant recovery is possible by increasing concentrations of solutes in dehydrated cells. In Our study, although, the RWC had decreased down to 60% in severe stress (40% FC), but in all of the species this effect was reversed in the severe stresses.

For leaf electrolyte leakage, the highest values of 59% and 57% were observed in *T. lancifolius* and *T. pubescens*, respectively (Table 6). Drought stress significantly increased accumulation of leaf electrolyte in the species, but the intensities of increasing in *T. daenensis* and *T. kotschyanus* were higher than other species. It seems they have less membrane stability than other species (Fig 2).

For carbohydrates content, the highest and lowest values of 928 and 772 $mgg^{-1}FW$ were obtained in *T. lancifolius* and *T. daenensis*, respectively (Table 6). Due to the lack of species by water stress interaction, all four species had a similar trend of carbohydrate and the highest values was observed in FC 40% (Fig 2). Drought stress increases soluble carbohydrate activity in *Dracocephalum moldavica* (Kabiri *et al*., 2018), canola (Mirzaee, 2013), grape (Meng *et al.*, 2014), soybean (Ghorbanali and Niakan (2007). For proline content, the highest value of 4.07 $mgg^{-1}FW$ was observed in *T. daenensis*. The response of species to drought stress were not similar, so that the highest amount of proline was obtained in *T. kotschyanus* with 5.88 $mgg^{-1}FW$ in FC 40% (Fig 1). Proline accumulation is a common physiological response in many plants in response to drought stress. Similar to our results, drought stress increases proline content in *Brassica napus* (Mirzaee, 2013), Sainfoin (Veisipoor *et al*, 2013), grape cuttings (Meng *et al*., 2014) soybean (Ghorbanali and Niakan, 2007).

For catalase enzyme activity, the highest value of 9.86 $mgg^{-1}FW$ was obtained in *T. kotschyanus* in severe stress (FC 40%). For peroxidase, there was no significant species by water stress interaction effect, therefore, the trend of its activity in all four species were similar and enzyme activity increased by increasing drought stress (Fig 2). Türkan (2005) in *Phaseolus vulgaris* and *Phaseolus acutifolius* showed that the higher activity of antioxidant enzymes and it was related to resistance to drought stress.

For the chlorophyll a, there was no significant difference between the four species. However, For chlorophyll b, total chlorophyll and carotenoid the higher values were obtained in *T. lancifolius.* The trends of pigment decreases in all of species were similar and the highest many reports that photosynthesis is limited by drought stress due to stomatal closure (Flexas *et al.*, 2004; Chaves *et al*. 2009; Mafakheri, *et al*. 2010), consequently drought stress decreases the chlorophyll content in plant species (Bahreininejad *et al*., 2008; Alaei *et al*., 2013; Begum Paul 1993).

It was concluded that species of *T. daenensis* and *T. lacnifolius* had stronger roots in drought stress. However, *T. daenensis*, due to higher aerial part production and essential oil yield (up to 2%) in the field (Sepavand *et al*, 2020), was recommended for domestication and cultivation in dryland farming system and semi-stepic rangelands of Iran.

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اثرات تنش رطوبتی بر رشد گیاهچه و صفات فیزیولوژیکی چهار گونه آویشن(*Thymus spp.*) بومی ایران در شرایط گلخانه

علی سپهوند[الف] ، علی اشرف جعفری[ب]*، فاطمه سفیدکن[ب] و سپیده کلاته جاری[ج]

[الف] دانشجوی دکترای باغبانی، دانشکده کشاورزی و صنایع غذایی، دانشگاه آزاد اسلامی -واحد علوم و تحقیقات، تهران

[ب] استاد پژوهش، مؤسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، *(نگارنده مسئول)، پست الکترونیک: aajafai@rifr-ac.ir

ج استادیار، دانشکده کشاورزی و صنایع غذایی، دانشگاه آزاد اسلامی -واحد علوم و تحقیقات، تهران

چکیده. خشکی یکی از عوامل محدود کننده رشد، تولید و زنده‌مانی گیاهان زراعی و گیاهان داروئی در مناطق مختلف ایران می‌باشد. به منظور بررسی اثرات تنش رطوبتی بر رشد گیاهچه و صفات فیزیولوژیکی در چهار گونه آویشن (*Thymus spp.*) بومی ایران در شرایط گلخانه، بذر چهار گونه *Thymus pubescens*، *T. daenensis*، *T. lacnifolius* و *T. kotschyanus* در آزمایش گلدانی با استفاده در سه سطح تنش رطوبتی شامل: شاهد بدون تنش (ظرفیت زراعی ٪۹۰FC)، تنش متوسط (٪۷۰FC) و تنش شدید (٪۴۰FC) با استفاده از آزمایش فاکتوریل در قالب طرح کاملا تصادفی در سه تکرار در مرکز تحقیقات کشاورزی استان لرستان در شهرستان خرم آباد در سال ۱۳۹۷ به اجرا درآمد و صفات مورفوفیزیولوژیکی گیاه‌چه‌ها اندازه‌گیری شد. نتایج تجزیه واریانس نشان داد که تفاوت بین گونه‌ها و بین سطوح تنش خشکی برای کلیه صفات معنی‌دار بود. اثرات متقابل گونه در خشکی برای کلیه صفات بجز کربوهیدرات‌های محلول و آنزیم پراکسیداز معنی‌دار شد که نشان‌دهنده عکس العمل متفاوت گونه‌های آویشن نسبت به تنش رطوبتی بود. در مقایسه میانگین سطوح تنش رطوبتی، نتایج نشان داد که با افزایش تنش خشکی، میانگین صفات طول ریشه، وزن ریشه، حجم ریشه، نشت الکترولیت، محتوی قندها و پرولین و فعالیت آنزیم‌های پراکسیداز و کاتالاز در همه گونه‌ها افزایش یافت و مقدار محتوی آب نسبی و رنگدانه‌های برگ کاهش یافت. در مقایسه میانگین اثرات متقابل، بیشترین رشد طولی ریشه در تنش شدید ٪۴۰FC و بیشترین وزن ماده خشک ریشه در تنش متوسط ٪۷۰FC در گونه‌های *T. lacnifolius* و *T. daenensis* به‌دست آمد. به همین ترتیب بیشترین محتوی نسبی آب برگ، نشت الکترولیت برگ، کربوهیدرات های محلول و رنگدانه‌های برگ در گونه‌های *T. lancifolius* و *T. daenensis* به‌دست آمد که نشان دهنده مقاومت بیشتر این گونه‌ها به تنش خشکی نسبت به سایر گونه‌ها می‌باشد. با این وجود گونه *T. daenensis* با تولید سرشاخه فراوان و عملکرد اسانس بیشتر در شرایط مزرعه برای اهلی کردن و کشت در دیمزارها و مرتع نیمه استپی ایران توصیه شد.

کلمات کلیدی: خشکی، *Thymus pubescens, T. lacnifolius,T. daenenesis,T. kotschyanus*