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Effect of drought stress on some physiological and morphological characteristics of *Hymenocrater yazdianus*

Mohsen Sadeghian, Mohammad Hossein Hakimi*, Hamid Sodaeizadeh

Faculty of Natural Resources and Desert, Yazd University, Yazd, I.R. of Iran

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Key words: Drought Stress, *Hymenocrater yazdianus*, Physiological characteristics, Morphological characteristics.

Abstract

Hymenocrater yazdianus is endemic of Yazd province, Iran. A completely randomized design with six replications was used to investigate the effects of drought stress on some morphological and physiological characteristics of the plant. The results showed that the soil moisture content has a significant effect on the properties of canopy and root size, leaf thickness, average of leaf area, root to shoot ratio, chlorophyll (a, b and total), proline and soluble sugar. The minimum and maximum values of the canopy, leaf area, root volume and weight, was related to the field capacity of 25 and 75 percent, respectively, and the highest amount of root to shoot ratio with an average of 1.86 was related to the field capacity of 100%. Relating to the physiological factors, the lowest and the highest amounts of a, b and total chlorophyll as well as proline were related to the field capacity of 100% and 25%, respectively. In the case of water soluble sugars, no significant differences were observed between treatments. Drought stress had no significant impact on the characteristics such as plant height, shoot dry weight, relative water content (RWC) and water saturation deficit (WSD). The overall results of this study suggest that *Hymenocrater yazdianus* by utilizing some defense mechanisms such as reducing the size of the canopy, leaf area, volume and weight of the roots and increasing the thickness of leaves, the amount of chlorophyll and proline would be compatible with dry conditions.

***Corresponding Author:** Mohammad Hossein Hakimi ✉ mhhakimi@yazd.ac.ir

Introduction

Arid and semi-arid regions are regions where the total transpiration rate is 50% or even less than 50% of the transpiration rate in non-stress conditions. The water shortage is not unique to these areas, but even in the wet climate, irregular distribution of rainfall leads to reduce the available water and limited plant growth (Kafi *et al.*, 2005). Drought stress occurs when the plant's water intake is less than its loss. This may be due to excessive transpiration, reduction of water absorption or a combination of them (Koochaki and Alizadeh, 1995). Drought stress adversely affects different aspects of plant growth, including seed germination as well as plant growth and development. The severe drought stress causes severe disrupting in photosynthesis and physiological processes, halting growth and eventually death of the plant (Singh and Patel, 1996). To survive or to escape from drought, the plants show reaction or adapt to the conditions. To tolerate or adapt to dry conditions, Plants change their physiological, biochemical and morphological status. Morphological factors such as changes in leaf area, volume or weight of the canopy, the weight of total biomass, plant height, root volume and weight, and also physiological characteristics such as proline, soluble sugars, chlorophyll and relative water content can play a role in plant resistance to drought stress. During the occurence of drought stress, plants with storage osmotic regulator, such as amino acids, sugars, some mineral ions, hormones and proteins are trying to deal with stress. Among the organic compounds, Proline is one of the main osmotic regulators (Reddy *et al.*, 2004). Proline regulates the osmotic pressure, reduces water loss and maintains the turgidity of leaf (Azarmjoo *et al.*, 2009). So far, the impact of drought stress on morphological and physiological characteristics of some rangeland and forest species of arid and semiarid regions is examined. Lebaschi and Sharifi Ashoorabadi (2004) have investigated the effect of different levels of drought stress on *Plantago psyllium, Achillea millefolium, Salvia officinalis, Calendula officinalis and Matricaria Chamomilla*. They have reported that with increased drought stress, the shoot weight and plant height have decreased in all studied plants. Safi Khani *et al.*, (2007) reported that compared to the 60% and 100% of field capacity treatments, in the 40% of field capacity treatment, the height, leaf length and width, internode length, biomass and essential oil yield (kg^{-ha}) of *Dracocephalum moldavica* was reduced but the essential oil percentage increase compared to the nonstressed treatment. Abbaszadeh *et al.* (2007) have investigated the Effect of drought stress on physiological characteristics of *Melissa officinalis* and concluded that the maximum amount of chlorophyll a, b and total chlorophyll related to the control and 20% of field capacity treatments and the most proline related to 20% of field capacity treatment. The highest amount of soluble sugar and RWC obtained in 60% of field capacity and control treatments, respectively. Zirehzadeh *et al.* (2009) have examined the effect of salinity and drought stresses on germination of *Thymus vulgaris* and concluded that the salinity and drought significantly reduced the percentage and speed of germination, root and shoot length and dry weight. Jafarian (2014), by applying drought treatments and investigating the role of iodine in decreasing the effects of drought stress in two varieties of *Carthamus tinctorius* and *Brassica napus* concluded that with increased drought stress, all morphological factors of *Brassica napus* dropped, severe drought also led to increase the amount of sugar and chlorophyll in the shoots. So it seems that the plant uses this mechanism to deal with drought. Petropoulos *et al.* (2008) reported that the level of water stress reduced leaf fresh weight, number of leaves and the root weight of parsley. The essential oil yield increased with increasing water stress. Since most species of medicinal plants have a relative low resistance to water shortage and their planting is an appropriate way to exploit and enhance the yield in the dry climate, so it is essential oil that their reaction to drought stress were investigated. *Hymenocrater yazdianus* is an exclusive automotive plant which grows in rangelands around the Yazd. So far, no research has been done on the reaction of the plant to drought stress. So, the purpose of this study is to evaluate the effect of drought stress on the function of *Hymenocrater yazdianus*. The results of this study can be used to identify the tolerance to drought and the possibility of its large-scale cultivation in areas with limited range of water.

Materials and methods

Plant characteristics

Hymenocrater yazdianus is an exclusive plant which grows in Yazd and it belongs to Lamiaceae, woody plant with a wooden base, multiple flower stems with the height of 30-45 cm, thinning leaves with medicinal properties (Mozaffarian, 1996). Average annual rainfall and temperature in the habitat of the species is 312 mm and 15.9 - 17 ° C, respectively. Slope gradient in the habitat of the species is 5-50% with the northern and eastern slope aspects. It has been distributed in Shirkooh (Yazd), Lakheseh Valley (Mehriz), Nodoushan between Milsefid and Sadrabad, and Gloeek farm.

Experimental methods

Moisture treatments evaluated in this experiment were 100% (the control treatment), 75%, 50% and 25% of field capacity (FC), which was applied in a completely randomized design with 6 replications. In order to measure soil moisture, the pots weight was determined. Considering the reduction of the amount of water, irrigation was provided at given times. For planting, plastic pots (height 20.5 cm and diameter 33 cm) with drainage holes were used. To improve drainage, five centimeters of fine sand were poured at the bottom of the pot. The soil texture was sandy loam, the chemical and physical characteristics of the soil are shown in table. 1. For the production of seedlings, seeds of this species were collected from the highlands of Sadrabad, Nodooshan (natural habitat, with the situation 31° 49'39" N and 53 °42 '27" E). A total of 20 healthy seeds were planted in each pot and after the full deployment and the relative growth, the moisture treatments were applied. After sixty days, morphological characteristics such as plant height and diameter, the canopy volume, shoot dry weight, leaf thickness, average of leaf area, root dry weight, root to shoot dry weight and root volume were measured. The physiological characteristics, including relative water content, chlorophyll, proline and soluble sugar content were calculated. At the end of the period of drought stress, the average of plants height and diameter (North-South and East-West) was selected as a basis for evaluation. By measuring the height and diameter of plants, the plant volume was calculated according to the following equations:

$$V = \frac{4}{3}\pi a^2 b \quad (1)$$

When the height is less than the diameter.

$$V = \frac{4}{3}\pi a b^2 \quad (2)$$

When the diameter is less than the height.

Where a, b and v is the plant height, plant average diameter and the volume of the crown, respectively.

Leaf thickness was measured with a digital caliper. The average of leaf area in 3 sizes (large, medium and small) was obtained using graph paper. Proline and soluble sugar were measured using the Bates *et al.* (1973) and Kochert (1978) methods, respectively. The chlorophyll content was determined spectrophotometric ally by measuring the absorbance (optical density - OD) of the extract at 663 and 645 (nm) wave lengths and then it was used for calculating the amount of chlorophyll a and b (Starnes & Hadley 1965).

Chlorophyll a (mg^{-gr} plant tissue) = 12.25(A663) – 2.798(A645).

Chlorophyll b (mg^{-gr} plant tissue) = 21.5(A645) – 5.1(A633).

The leaf relative water content (RWC) was measured using the following equation and it was used to assess the plant water status (Alizadeh, 2005).

$$RWC (\%) = [(W - DW) / (TW - DW)] \times 100. \quad (3)$$

W: Sample fresh weight.

TW: Sample turgid weight.

DW: Sample dry weight.

Statistical analysis

After securing the normality of the data and by the assumption of equal variance, statistical analysis of the data was performed using ANOVA analysis and Duncan method was used to compare data. SPSS (version 20) software and EXCEL 2010 were used for data analysis and drawing Graphs.

Results

The results of analysis of variance showed in table. 2 and table. 3. that soil moisture content had a significant impact on the morphological and physiological properties such as leaf thickness, average of leaf area, root dry weight, root to shoot ratio, root volume, chlorophyll (a, b and total), proline, canopy volume and sugar content. Plant height, shoot dry weight, water saturation deficiency and leaf relative water content were not significantly affected by the drought treatments.

Table 1. Soil properties at experimental site.

EC (ds/m)	Soil pH	N %	Organic matter %	Soil Texture
.87	7.4	.05	1.48	Sandy loam

Effect of drought stress on morphological properties of Hymenocrater yazdianus

The maximum canopy volume of 88556.8 cubic centimeters was observed in the 75% FC that had no significant difference with 50% and 100% FC. The minimum canopy volume of 54322.2 cubic centimeters was observed in the 25% FC (Fig. 1-a).

In severe drought stress (25%FC), leaf thickness showed approximately 75 % increase when compared to 100 % FC. (Fig. 2-b). The results also indicated that highest and lowest leaf area (2.97 and 1.41cm2) was related to the 75% and 25% FC, respectively. So, in comparison with the control treatment there was an increase and a decrease of about 15.56% and 45.13%, respectively (Fig. 2-c). The maximum weight and volume of the root was related to the 75 % treatment and compared to the control treatment, 22 and 11% increased, respectively.

Table 2. Mean square of the effects of drought treatments on the morphological characteristics of *Hymenocrater yazdianus*.

Root volume	Root to shoot ratio	Root dry weight	Average of leaf area	Shoot dry weight	Leaf thickness	Canopy volume	Height	Degrees of freedom	Variable
3480.556**	.182 **	65.343**	2.656 **	2.693 ns	.026 **	1349983918	9.81 ns	3	Drought stress
74.583	.122	1.233	.142	1.233	.001	350182119.9	3.921	20	Error

ns: not significantly different. ** Statistically significant at the level of 1%, * significant at the level of 5%.

Also, the minimum weight and volume of the root was related to the 25 % treatment and compared to the control treatment, 49 and 55.5% increased, respectively (Fig. 2-d). The maximum and minimum amount of root to shoot dry weight was observed at the 100% (1.86) and 25% (0.88) of field capacity treatments, respectively, and it had no significant difference with the 50% and 75% of field capacity treatments. Compared to the control treatment (100% of the field capacity), the root to shoot dry weight (in the 25% of field capacity treatment) decreased 52.6 %. (Fig. 2 -e).

Effect of drought stress on physiological properties of Hymenocrater yazdianus

The results showed that the highest amount of chlorophyll a (17.72 mg -gr plant tissue) was related to the 25% of field capacity treatment which compared to the control treatment (100% of the field capacity), increased 69.4 % (Fig. 2-a). The lowest amount of chlorophyll a (10.46 mg -gr plant tissue) was related to the 100% of field capacity treatment and there was no significant difference between the it and 75% of field capacity treatment (Fig. 2-a). The highest amount of chlorophyll b (2.9 mg -gr plant tissue) was related to the 25% of field capacity treatment, which compared to the control treatment (100% of the field capacity), increased 85.9 % (Fig. 2-b).

Table 3. Mean square of the drought treatments on the physiological characteristics of *Hymenocrater yazdianus*.

Sugar	Proline	Water saturation deficiency (WSD)	Leaf relative water content (RWC)	Total Chlorophyll	Chlorophyll b	Chlorophyll a	Degrees of freedom	Variable
.007*	.017**	32.944 ns	32.472 ns	93.667**	2.041**	9.655**	3	Drought stress
.001	.003	40.683	41.439	9.066	.316	5.435	20	Error

ns: not significantly different. ** Statistically significant at the level of 1%, * significant at the level of 5%.

The highest amount of total chlorophyll (21.15 mg- gr plant tissue) was related to the 25% of field capacity treatment and the lowest amount of total chlorophyll (12.03 mg -gr plant tissue) was related to the 100% of field capacity treatment (Fig. 2-c). The highest (0.15 mg -gr) and the lowest (0.03 mg -gr) amount of proline was related to the 25% and 100% of field capacity treatment, respectively. Also, compared to the control treatment (100% of the field capacity), treatments of 25%, and 50% and 75% of field capacity increased 3.87, 1 and 0.96, respectively (Fig. 2-d). The highest (0.819 mg -gr) and the lowest (0.809 mg -gr) amount of sugar was related to the 50% and 75% of field capacity treatment, respectively, and there was no significant difference between the it and 25%, 50% and 75% of field capacity treatments (Fig. 2-e).

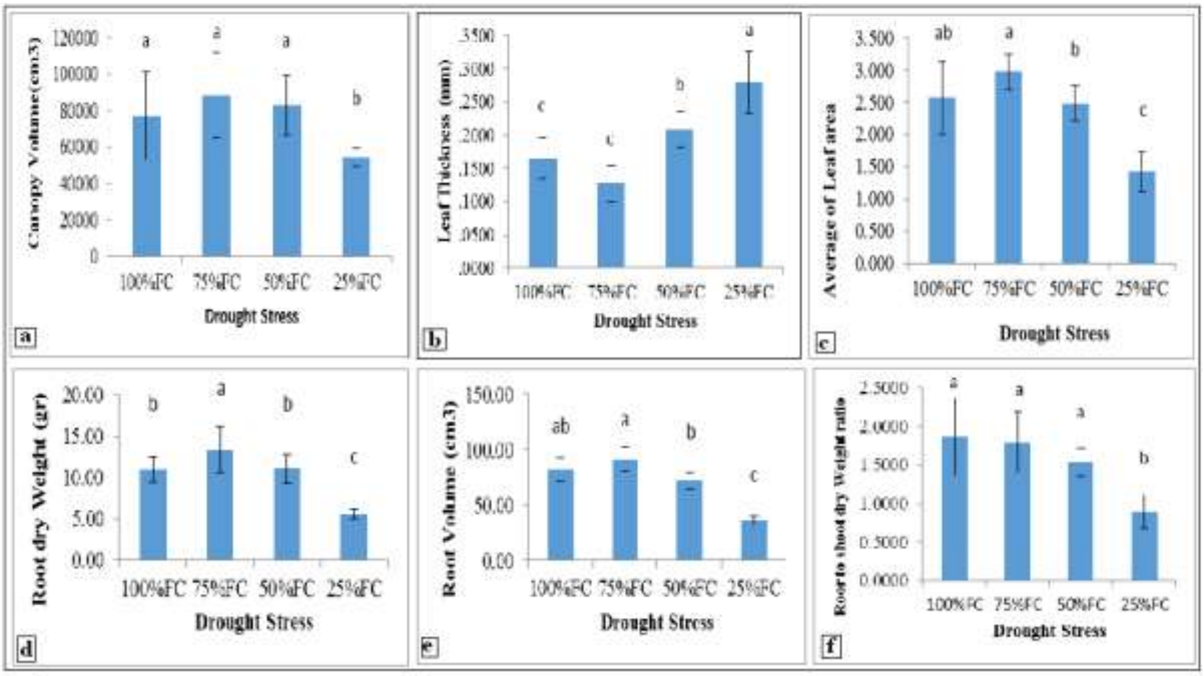

Fig. 1. The effect of different moisture treatments on average of some morphological characteristics of *Hymenocrater yazdianus*: a) the canopy volume, b) the leaf thickness, c) the average of leaf area, d) the root volume and dry weight, e) root to shoot dry weight ratio. Means that at least in each characteristics have a common letter, are not significantly different at the level of 5% in Duncan test.

Discussion

Changes in the morphological structure of plants in the face of drought stress are one way of tolerance or adaptation to drought condition. Faced with a drought stress, the plant reduces the angle of its branches towards the main stem to decrease the amount of absorbed radiation, so the canopy diameter and the volume of the plant is reduced (Raad *et al.*, 2009). In the present study, it was also found that drought stress have affected the canopy volume of *Hymenocrater yazdianus* so that the lowest volume was obtained in the 25% of field capacity treatment.

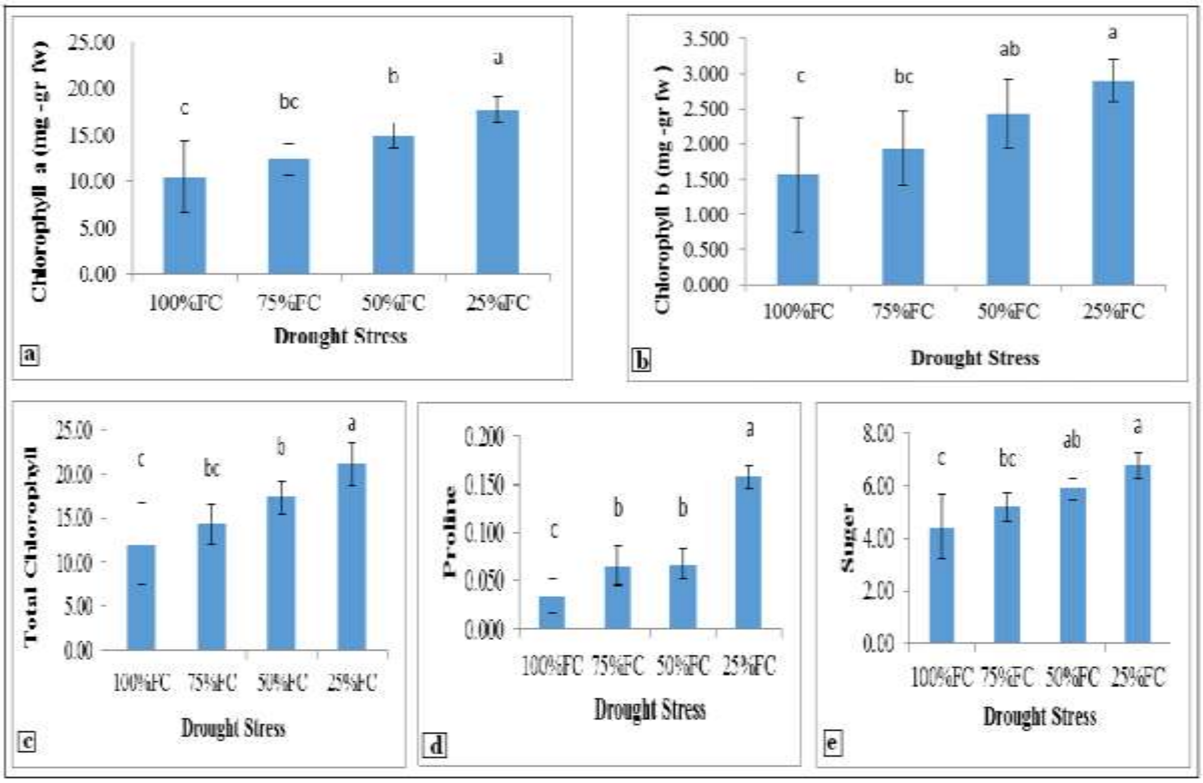

Fig. 2. The effect of different moisture treatments on average of some physiological characteristics of Hymenocrater yazdianus . A) chlorophyll a, b), chlorophyll b, c) total chlorophyll, D) proline, E) soluble sugar. Means that at least in each characteristics have a common letter, are not significantly different at the level of 5% in Duncan test.

This reaction leads to the reduction of water loss in tissues of the plant, which is one of the mechanisms of drought tolerance. Besides that, as another way to deal with the severe drought stress during the growing season, *Hymenocrater yazdianus* increases the thickness of its leaves to reduce the water loss. More moisture, leads to increase in the leaf area and decrease in the leaf thickness so that the maximum leaf area and minimum leaf thickness was observed in the 75% of field capacity treatment. The results of studies on *Carthamus tinctorius* (Baghkhany and farahbakhsh, 2008) are consistent with the results obtained in the present study. The results of this study also indicate that there is no significant reduction in the height and dry weight of shoots against water stress, indicating that in drought stress, reduction in the growth of shoots is prevented by morphological and physiological changes of *Hymenocrater yazdianus*. Root volume and weight also decreased by applying soil moisture restrictions and it shows the inefficiency of the plant root development in the use of water in terms of water stress. A significant reduction in root dry weight as a result of increased water stress, indicating the impact of the water stress on the plant's roots as an important component of this environmental phenomenon. In fact, with the advancement of drought, the photosynthesis decreases and the sugar need to adjust the osmotic pressure increases. Subsequently, the root growth inevitably stops (Lu and Neumann. 1998). The results of studies on *Fortuynia bungei* (Tajamolian *et al.*, 2012) and *Thymus vulgaris* (Shamsaee, 2014) are consistent with the results obtained in the present study. Root to shoot ratio is one of the most important indicators of the relationship between the aerial and underground parts of plants for evaluation the drought tolerance. Comparison of different treatments showed that the highest value of this attribute was observed in the 100% of field capacity and the lowest was observed in the treatment of 25% of field capacity. It indicates that *Hymenocrater yazdianus* in the lack of moisture limits (100% field capacity) has expanded its roots to absorb more water. While, in severe drought stress (25% of field capacity), it did not use this mechanism and the other mechanisms such as proline accumulation was used for increasing osmotic potential and subsequently more water absorption. Vafabakhsh *et al.* (2009) investigated the effect of drought stress on yield of *Brassica napus* and reported that shoots are increased in the plant. Their results were not consistent with the results obtained in the present study. The leaf relative water content of *Hymenocrater yazdianus* is not affected by soil moisture treatments and in all treatments RWC was greater than 50%. Ludlow (1989) represented that plants with more than 50% relative water content have a drought escape mechanism. Kaiser (1987) stated that if the amount of RWC is between 70 to 100 percent, decrease in photosynthesis due to stomatal closing will be rapidly reversible. According to our findings and based on Ludlow classification, this plant has a drought escape mechanism. High turgidity in conditions such as longtime droughts, indicating enough water storage in the tissues of the plant. *Hymenocrater yazdianus* stores water through stomatal closing and reducing the transpiration, so it can survive in drought condition. The results obtained in the present study are not consistent with the results of studies on *Melissa officinalis* L. (Abbaszadeh *et al.*, 2007) and *Eucalyptu camaaldulensis* (Raad *et al.*, 2010) but they are consistent with the results obtained from studies on *Fortuynia bungei* by Tajamolian *et al.* (2012). The results showed that the highest and the lowest amount of chlorophyll a, b and total was related to the 25% and 100% of field capacity treatments, respectively. According to the results, it seems that in severe drought stress, the plant by increasing the production of chlorophyll in leaves has increased the photosynthesis rates. It leads to proper growth and therefore, to some extent the effect of drought stress on other factors such as reducing the leaf area and increasing the leaf thickness would be corrected. Luvha *et al.* (2008) by examining the mango plant (*Mangifera indica*) reported that with an increase in drought stress the amount of chlorophyll a and total increased steadily while the amount of chlorophyll b in all treatments remained stable except the severe drought stress with a slight increase. The results were consistent with our findings. In contrast with the results of the present study, Tehranifar *et al.* (2009) investigated the native and imported *Festuca arundinacea* and *Lolium perenne* and reported the reduction in chlorophyll a, b and total because of drought stress. In the plants under drought stress or other stresses, the water inside the cytosol decreased, so the osmotic pressure must be increased to absorb water during drought stress period. Among the factors affecting the osmotic pressure, proline reduces water loss from the cell and maintains the turgidity (Kuznetsov and Shevyakova, 1999). The accumulation of proline due to drought stress is a common response which occurs because of the production of proline in tissues (Schonfeld *et al.*, 1988) and inhibition of oxidative proline to participate in proteins (Pedrol *et al.*, 2000). In this study, the maximum and the minimum amount of proline was related to the 25% and 100% of field capacity treatments, respectively. The results of studies on 4 species of Eucalyptus by Osareh and Shariat (2008) and the results obtained from studies on *Pyrus communis* by Javadi *et al.*

(2004) represents an increase of proline with an increase in drought stress. These results are consistent with the results obtained in the present research. Soluble sugars are another cell osmotic protective, but they are not only important parameter in osmotic adjustment and maintenance of osmotic pressure. The results showed that the amount of soluble sugars in severe drought stress was not significantly different with that amount in non-stressed treatment. Therefore, it seems the plant to deal with drought stress have used other methods such as proline accumulation, increase in chlorophyll content and increase in leaf thickness. Osareh and Shariat (2008), Yang *et al.* (2009) and Javadi *et al.* (2004) have reported the increase in the amount of soluble sugars with increase in drought stress. Their results are different with the results of the present research.

Conclusion

In general, the results indicate that *Hymenocrater yazdianus* using some defense mechanism (morphological and physiological) such as reducing the size of the canopy and leaf area, increasing leaf thickness, chlorophyll of the leaves and proline accumulation have the ability to adapt or to tolerate dry conditions. However, it is essential that the reaction of the plant to apply other environmental stresses such as salinity and temperature are examined.

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