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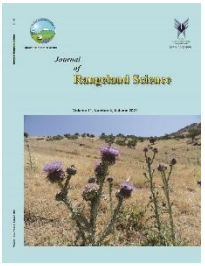

Research and Full Length Article:

Effect of Drought Stress on Morphophysiological Traits of *Thymus vulgaris* L. in a Field Experiment, Borujerd, Iran

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Abstract. The plants can absorb nutrients and reduce water in face to drought stress. These conditions reduce plant growth rate and change physiological traits. In this study, the effect of drought stress on morphological and physiological characteristics of *Thymus vulgaris* L. was investigated under water deficit condition. A field experiment was conducted using three irrigation levels as 90% Field Capacity (90%FC), 50% FC and 25% FC based on a Randomized Complete Block Design (RCBD) in four replications. The seedlings of *T. vulgaris* were moved to the field and planted in early spring. The field was irrigated for each treatment for 3 months (June to August 2019). The soil moisture for each treatment was determined by the pressure plate method. At the end of the experiment, three plants in each plot were taken with their roots. The results of ANOVA showed significant differences between treatments for all the traits ($P<0.05$). For shoot length, the highest and lowest values of 43.2 and 19.9 cm were obtained in 90% FC and 25% FC, respectively. The highest shoot weight (13.66 g) and the lowest root weight (7.33 g) were obtained in 90% FC treatment. The highest and lowest values of both (total nitrogen and potassium) were obtained in 90% FC and 25% FC, respectively. The highest and lowest proline contents with values of 4.2 and 2.3 µmol/g FW were obtained in 25% FC and 90% FC, respectively. The highest values of soluble carbohydrates, Catalase (CAT) and Peroxidase (POD) activity were obtained in 25% FC compared to other treatment. Based on the results of present study, *T. vulgaris* L. is a suitable plant species for cultivation under drought stress conditions.

Key words: Catalase, Thyme, Proline, Morphological, Field capacity

Introduction

Thymus as a main native rangeland plant belongs to the Lamiaceae family. In general, Thyme species are all aromatic and medical with antioxidant and antibacterial effects that are used in the production of medicines as well as using in the food industry (Lorenzo *et al*., 2018). The plants of this genus are highly diverse in terms of morphology and chemical composition. The essential oil of thyme could be used for reducing the bacterial and fungal infections or anti-inflammatory in the traditional pharmacopeia (Boukhatem *et al*., 2020).

Drought stress has negative effects on plant growth and physiological traits variation of plants (Akhzari and Ghasemi Aghbash, 2013). Based on literature review, *Thymus* spp. as a perennial shrub could be survived under drought stress in arid environments. Babaee *et al*. (2010) studying *T. vulgaris* L. showed that the plant height, number of lateral shoots, shoot dry weight, root volume, fresh and dry root weight and root length were decreased under drought stress condition. They also showed that with increasing drought stress, the dry matter of the plant decreased, but the essential oil content, composition and amount of active ingredients were increasd (Babaee *et al*., 2010). According to investigation of Pazoki *et al*. (2012), drought stress reduced shoot length, root and shoot dry weight and relative water content of *T. vulgaris* L. Ghaderi *et al*. (2018) also reported that dry weight of root and shoot of *T. vulgaris* decreased as compared to control while root length increased.

Potassium content of *Solanum lycopersicum. L.* was significantly decreased under drought stress (Ali and Rab, 2017). Another investigation (Akhzari and Shayganfar, 2019) indicated a negative effect of drought stress on total nitrogen (TNC) in *Ferula haussknechtii* H.
Ghaderi *et al*. (2018) found a significant increase of proline under drought stress condition.Similarly, Catalase and peroxidase enzymes significantly increased under drought stress conditions (Jabeen *et al*., 2019).

Many studies indicated that the effect of drought stress on morphological and physiological traits of plants varies depending on plant species and growing conditions. Many of drought stress studies in thyme species have been performed mostly in greenhouse conditions and there is less report under field experiment. Field experiments generally give yield results with higher ecological validity than laboratory experiments.

This study aimed to investigate the effects of water-deficit stress on plant growth, morphological and physiological traits of *T. vulgaris* in field condition.

Materials and Methods

Study Area

This study was conducted in a crop farm in the Sarband Region (33° 52' 12" N, 48° 58 10" E) located in 20th km Borujerd, Lorestan Province, Iran. Meteorology data indicated that average annual precipitation and temperature in the study area are 456.2 mm and 14.8°C, respectively. The amberotermic curve was drawn for 12-year period (2004-2016) of precipitation and temperature (Fig. 1). The altitude of station is 2246 m above sea. Based on the results of hydrometric method adopted by Farid Giglo *et al.* (2014), the soil texture of the station is silty clay loam. The general direction of the slope in this area, which is considered a mountainous area, is to east direction.

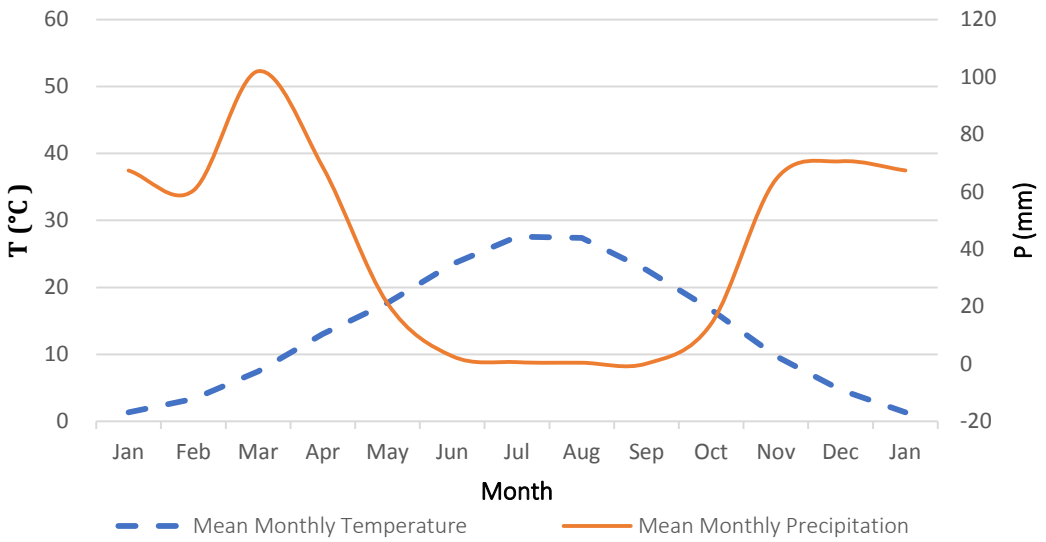

Fig. 1. Amberotermic curve of study area (2004-2016) (References: weather station of Borujerd)

Experiment layout

A field experiment was conducted using three irrigation levels as 90% Field Capacity (90%FC), 50% FC and 25% FC based on a Randomized Complete Block Design (RCBD) in four replications. Each plot was 2x5 m. The seedlings of *T. vulgaris* were moved to the field and planted at a distance of 20 cm from each other (50 plants in each plot). For each treatment, plots were irrigated for 3 months (June to August 2019). In the first treatment, plots were fully irrigated (90%FC). The second treatment was irrigated with 50% FC and the third was irrigated with 25% FC. The soil moisture for each treatment was determined by the pressure plate method

Data collection

At the end of the experiment, three plants in each plot were taken with their roots. The soil around the roots was rinsed with water; then, plant roots and shoots were separated in all treatments. Plants were dried at 70°C for 48 hours in the oven, dry weights of the roots and shoots were determined in g, separately.

Total nitrogen (TNC) and potassium (K) content was determined in the leaves by the Kjeldhal (AOAC, 1984) and flame photometer methods, respectively. The proline content was determined in leaves (µmol/g FW) based on Bates *et al*. (1973) method. The soluble carbohydrates (mg/g FW) were determined by absorbance at 485 nm spectrophotometer (Kochert, 1978).

The catalase (CAT) and peroxidase (POD) activity were determined based on the description of Sariri *et al*. (2006).

The collected data were analyzed of variance (ANOVA) and the means comparisons between treatments were made using the LSD method. The SAS9 software was used for data analysis

Results

The results of ANOVA showed significant differences between treatments for all the traits in *T. vulgaris* ($P<0.05$). For shoot length, the highest and lowest values of 43.2 and 19.9 cm were obtained in 90% FC and 25% FC, respectively. The highest shoot weight (13.66 g/plant) and the lowest root weight (7.33 g/plant) were also obtained in 90% FC treatment (Table 1 and Fig. 1).

The highest and lowest values of both (total nitrogen and potassium content) were obtained in 90% FC and 25% FC, respectively. For nitrogen, the highest and the lowest concentration values of 2.16% and 0.52% were obtained for 90%FC and 25% FC, respectively. A similar trend was observed for potassium concentration. The highest and lowest potassium contents with

values of 1.43% and 0.42% were obtained in 90%FC and 25% FC, respectively (Table 2 and Fig. 2)

The highest and lowest proline contents with values of 4.2 and 2.3 µmol/g FW were obtained in 25% FC and 90% FC, respectively. The amount of soluble carbohydrates increased significantly at 25% FC compared to 90% FC treatment ($P<0.05$).

Drought stress considerably increased the Catalase and Peroxidase enzymes activities in the study plants at varying water stress levels ($P<0.05$). Both enzymes activities significantly increased in *T. vulgaris* as water stress progressed from FC to 50% and 25% FC (Table2, Fig. 2).

Table 1. Effect of different levels of drought stress on shoot and root length and weight in *T. vulgaris*

Treatment	Shoot height (cm)	Root length (cm)	Shoot weight (g/plant)	Root weight (g/plant)
90% FC	43.50 a	6.66 c	13.66 a	7.33 c
50% FC	31.63 b	14.86 b	9.00 b	11.00 b
25% FC	19.96 c	20.53 a	4.33 c	14.67 a

Mean of column followed by similar letters has no significant differences based on LSD 5%

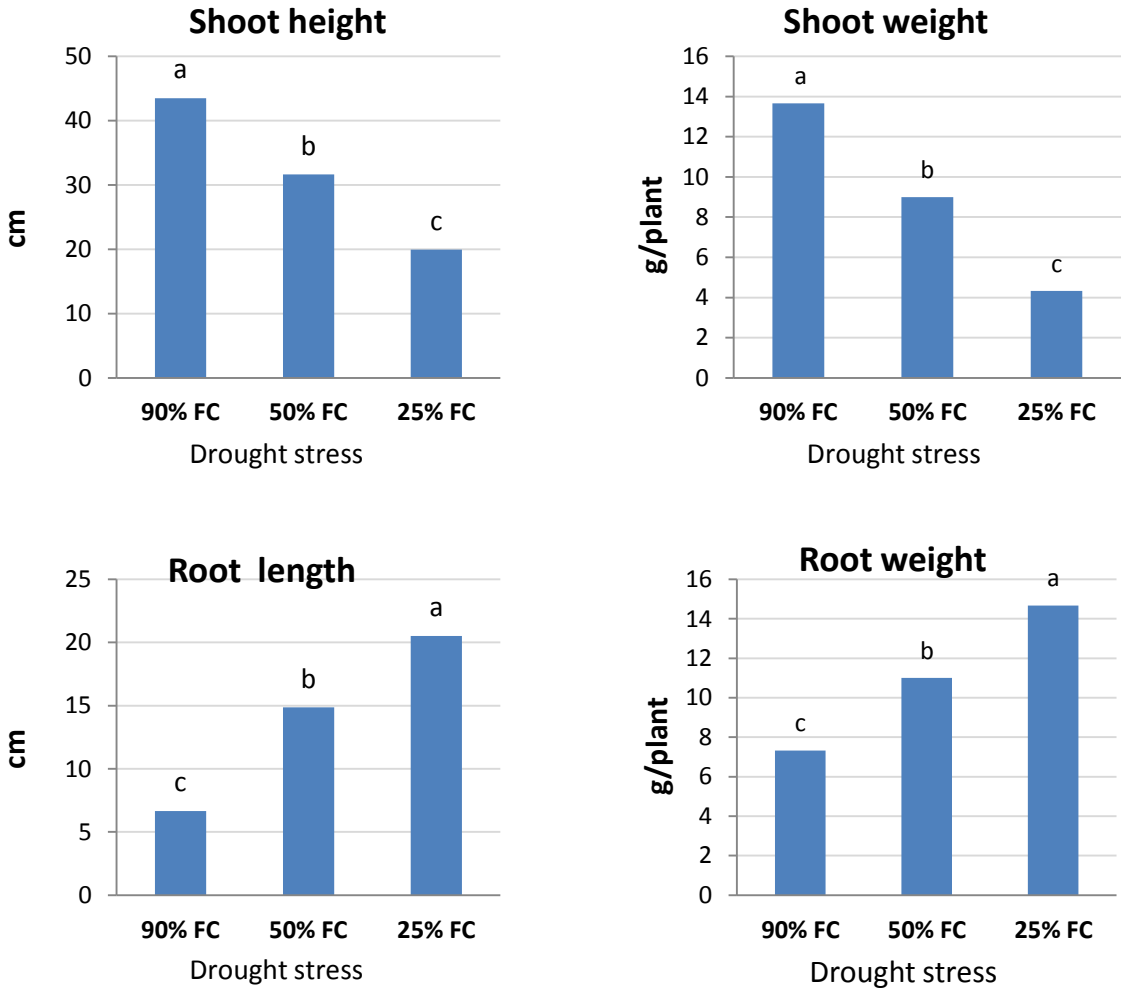

Fig. 1. Effect of different levels of drought stress on shoot and root length and weight in *T. vulgaris*

Table 2. Effect of different levels of drought stress on physiological traits in the *T. vulgaris*

Treatment	Nitrogen %	Potassium %	Proline (µmol/g FW)	Carbohydrates (mg/g FW)	Catalase (Unit/mg protein)	Peroxidase (Unit/mg protein)
90% FC	2.16 a	1.43 a	2.3 c	1.7 c	0.63 c	0.14 c
50% FC	1.34 b	0.84 b	3.6 b	2.9 b	0.84 b	0.23 b
25% FC	0.52 c	0.42 c	4.2 a	3.8 a	1.21 a	0.32 a

Mean of column followed by similar letters has no significant differences based on LSD 5%

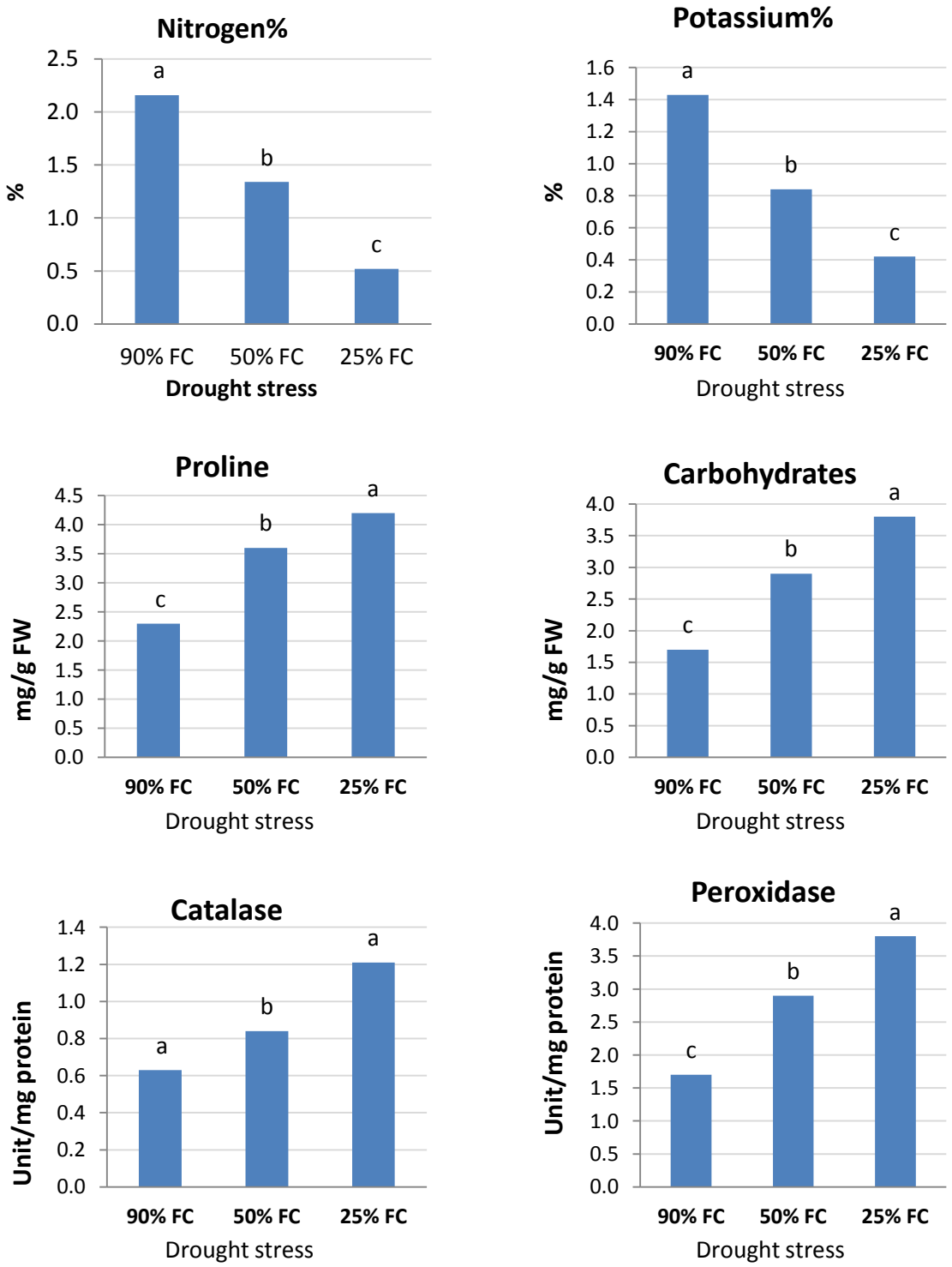

Fig. 2. Effect of different levels of drought stress on physiological traits in the *T. vulgaris*

Discussion

Many aspects of plant metabolism and growth could be affected by moisture stress. Drought stress causes physiological changes in plants and the physiological changes eventually cause morphological changes in plants. In this study, the effects of drought stress on morphological and physiological characteristics of thyme were studied. Successful cultivation of *T. vulgaris* plants in dry land farming requires investigation of morphological and physiological responses of this plant to drought stress conditions. *T. vulgaris* as an important medicinal herb in natural resources was mostly used in the traditional medicine and pharmaceutical industry.

According to the results of this research, the shoot length significantly decreased as the drought level increased ($P<0.05$). In contrast, the plants grow in 90%FC had lower root length than the 50% and 25% FC treatments ($P<0.05$). This is in contrast to Pazoki *et al*. (2012) results that stated that drought stress reduces both root length and root weight in *T. vulgaris*.

Potassium content decreased significantly by increasing drought stress. This result is in agreement with the results of Pratima *et al.* (2018) research on various cultivars of kiwifruit. On the other hand, the amount of nitrogen also decreased significantly with increasing drought stress (Fig. 2). This finding coincides with the results of Yue *et al*. (2019) who stated that nitrogen content were reduced significantly in *Leymus chinensis* under drought stress. Similar reports also were stated by Khan *et al.* (2017), which indicated negative effect water-deficit stress on nitrogen in two contrasting rice varieties.

The amount of proline significantly increased with increasing drought stress (Fig. 2). Bayat and Moghadam (2019) found similar results in a study on *Salvia nemorosa* which showed proline content under water stress was significantly higher than control. In face to water-deficit stress, some plant species had the ability to produce a type of protein (i.e., dehydrins), which increases their resistance to drought stress. The result of the present research maybe reflects the ability of *T. vulgaris* to produce proline as a dehydrins protein to alleviate drought stress impacts.

Whereas proline and soluble carbohydrate accumulations are physiological products which have effective use for alleviation of water-deficit stress in plants. The concentration of soluble carbohydrates increases significantly with increasing drought stress (Fig. 2). Yu *et al*. (2019) showed a significant increase in soluble carbohydrates due to drought stress. In contrast to our study, Razavizadeh *et al.* (2019) stated that the amount of soluble carbohydrates and proline of *T. vulgaris* decreased significantly under polyethylene glycol (PEG)-induced osmotic stress.

Biotic and abiotic stressors lead to the formation of reactive oxygen species (ROS) in plant cells. Plants have different mechanisms to reduce the harmful effects of ROS. One of these mechanisms is the antioxidant defense system. Antioxidant enzymes such as catalase and peroxidase play the role in the removal of oxygen free radicals in the cell (Agarwal and Pandey, 2004). The results of the present study indicated that Catalase and Peroxidase activities increased significantly under drought stress condition (Fig. 2). This result was in agreement with the results of Hosseini *et al*. (2015) researches.

Conclusion

The results of our study indicate that by increasing the length and weight of the roots, the plant responds well to drought stress, thereby continuing to absorb water and nutrients with minimal energy consumption. The accumulation of proline, soluble carbohydrates and Catalase and peroxidase enzymes in *T vulgaris* aerial parts in the face of drought stress are other physiological mechanisms of drought resistance of this plant. The accumulation of these organic substances, which regulate

osmotic pressure, increases the osmotic potential of plant cells and thereby absorbs water from the soil. So, *Thymus vulgaris* is a suitable plant species for cultivation under dry land farming system. In addition, it was able to reduce irrigation at drought areas and could save soil from erosion.

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اثر تنش خشکی بر خصوصیات مرفوفیزیولوژیکی گونه گیاهی آویشن باغی (*Thymus vulgaris L.*): در شرایط مزرعه‌ای (بروجرد، ایران)

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چکیده. توانایی گیاهان در جذب مواد غذایی و آب در شرایط تنش خشکی کاهش می‌یابد. این شرایط باعث کاهش سرعت رشد و تغییرات فیزیولوژیکی گیاهان می‌شود. در این مطالعه، تأثیر تنش خشکی بر خصوصیات مورفولوژیکی و فیزیولوژیکی گیاه .*Thymus vulgaris* L تحت شرایط تنش خشکی بررسی شد. یک آزمایش میدانی با سه سطح آبیاری شامل ظرفیت زراعی90٪ FC ،50٪ FC و 25٪ FC در قالب طرح بلوک‌های کامل تصادفی (RCBD) در ۴ تکرار به مدت ۳ ماه آبیاری (تیر تا شهریور ۱۳۹۸) انجام گرفت. در پایان آزمایش، ۳ بوته از هر کرت با ریشه‌های آن به طور کامل برداشت و مورد ارزیابی قرار گرفتند. نتایج نشان داد که با افزایش سطح خشکی، ارتفاع اندام هوایی کاهش یافت به طوری که بیشترین و کمترین ارتفاع ساقه با ۴۳/۲ و ۱۹/۹ سانتی‌متر به ترتیب در تیمار 90% FC و 25%FC بدست آمد (P<0.05). بیشترین وزن ساقه با ۱۳/۶۶ گرم در بوته در تیمار90 ٪ FC بدست آمد. در عین حال، کمترین وزن ریشه با ۷/۳۳ گرم در بوته نیز در تیمار90 ٪ FC مشاهده شد (P<0.05). تفاوت بین تیمارها از لحاظ غلظت نیتروژن کل و پتاسیم معنی داری بود. بیشترین و کمترین غلظت نیتروژن کل و پتاسیم به ترتیب در تیمارهای90 ٪ FC و 25٪ FC گزارش شدند. بیشترین و کمترین میزان پرولین با ۴/۲ و ۲/۳ میکرومول بر گرم وزن تر در تیمارهای FC 25٪ و 90٪ FC گزارش شد. مقدار قندهای محلول در 25٪ FC نسبت به تیمار90 ٪ FC به طور قابل توجهی افزایش یافت. فعالیت کاتالاز(CAT) و پراکسیداز (POD) با افزایش تنش از 50٪ FC به 25٪ FC به طور معنی داری افزایش یافت (P<0.05). بر اساس نتایج مطالعه حاضر گونه آویشن باغی گیاهی مناسبی برای کشت در شرایط تنش خشکی است.

کلمات کلیدی: کاتالاز، آویشن، پرولین، مرفولوژیکی، ظرفیت زراعی