Journal of Ornamental Plants
www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Research Paper
DOR: https://dorl.net/dor/20.1001.1.22516433.2020.11.1.2.5

Effect of Foliar Application of Proline on Morphological and Physiological Traits of *Calendula officinalis* L. under Drought Stress

Sophia Soroori[1], Elham Danaee[2*], Khodayar Hemmati[3] and Alireza Ladan Moghadam[4]

[1] PhD Student, Medicinal Plant, Department of Horticultural Sciences, Aliabad Katoul Branch, Islamic Azad University, Aliabad Katoul, Iran
[2] Assistant Professor, Department of Horticultural Sciences, Garmsar Branch, Islamic Azad University, Garmsar, Iran
[3] Associate Professor, Department of Horticulture Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[4] Associate Professor, Department of Horticultural Sciences, Garmsar Branch, Islamic Azad University, Garmsar, Iran

Received: 25 June 2020 Accepted: 21 December 2020

*Corresponding author's email: dr.edanaee@yahoo.com

Abstract

Drought stress is one of the most important abiotic stresses that seriously reduces the production of ornamental plants such as pot marigold. Proline is one of the active amino acids in the osmotic regulation phenomenon and it has an effective role in reducing cell damage and improving drought tolerance. A pot experiment as factorial arrangement in a completely randomized design with three replications was performed in order to study the effect of foliar application of proline (0, 50 and 100 mg L^{-1}) on some morpho- physiological characteristics of pot marigold under drought stress (normal irrigation, 25, 50 and 75% field capacity). The results showed that the highest fresh and dry weight of aerial organ, longevity of the flower on the plant, carotenoid and total chlorophyll were observed in normal irrigation+ proline 100 mg L^{-1}. The highest fresh and dry root weight, root length and total antioxidant activity (DPPH) were for 75 % FC + proline 100 mg L^{-1}. The highest plant height and number of flowers were in normal irrigation+ proline 50 mg L^{-1}. The highest proline content was belonged to 25 % FC+ proline 100 mg L^{-1}. In general, the results of this experiment showed that application of proline can partially compensate for the adverse effects of dehydration and on the other hand, despite the fact that the pot marigold plant is always able to survive in drought stress, it is recommended to irrigate 75% FC in order to increase pot marigold performance.

Keywords: Antioxidant activity, Carotenoid, Chlorophyll, Field capacity, Plant longevity.

INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is considered as a bushy and annual plant of the Asteraceae family, originating in the Mediterranean and West Asian regions and grown as an ornamental, medicinal and industrial plant (Jalali and Naderi, 2019). Terpenoids, flavonoids, coumarins, essential oils, carotenoids and amino acids are among the most important compounds in this plant (Buntariu and Zepa Cradini, 2012). Plants face a variety of environmental stresses during the growing season, and these stresses can limit the chances of plants growing and surviving. Water deficit has been recognized as a limiting factor in plant growth in most parts of the world (Pei *et al.,* 2013). In addition to reducing growth and yield, drought stress disrupts plant physiological processes, increases the production of reactive oxygen species, resulting in oxidative stress in plants (Pal *et al*., 2015). Different plants use different strategies to deal with drought stress which one of them is the use of osmotic substances such as proline, glycine betaine, trehalose, etc. Through foliar application, which reducing significantly the destructive effects of stress on plants. Among these, the use of proline (exogenous) has been an effective way to reduce the adverse effects of stress (Dawood *et al*., 2014).

Proline is an amino-acid existing in protein structures. Proline is osmolite abductor of reactive oxygen species (non-enzymatic antioxidant) and stabilization of protein structure and it is able to protect cells against stress damage (Szabados and Savoure, 2009). Also, the effect of proline spraying on plants depends on plant species, plant growth stage and time of use and proline concentration (Dawood *et al*., 2014). The use of proline amino acid in the form of foliar application at low concentrations greatly improves the plant's tolerance to environmental stresses (Deivanai *et al*., 2011). At the same time, high concentrations of proline in many cases have negative and irreversible effects on vital plant metabolism and inhibit growth (Dawood *et al*., 2014). Numerous studies have been conducted on application of proline in plants to control the effects of stress. It was shown that foliar application of proline, under salinity stress, significantly increased physiological characteristics such as plant height, number and leaf area, stem diameter, number of sub-stems, root volume, number and diameter of flowers, fresh and dry weight of plant, relative water content, total chlorophyll, carotenoids, catalase, peroxidase, ascorbate peroxidase enzymes and endogenous proline in henna flowers (*Impatiens walleriana*) (Roozbehani *et al*., 2018). In another study, proline foliar application increased the number of flowers, fresh and dry weight of the flowers, total chlorophyll and the activity of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) of chamomile (*Matricaria chamomila* L.) under drought stress (Darvizheh *et al*., 2017). Also, in a study on *Rosa hybrid* L. Grand Gala, the results showed that foliar application of proline, reduced oxidative stress and delayed leaf aging (Kumar *et al*., 2010). Alipour *et al.* (2016), showed that foliar application of proline on cut flowers of tuberose (*Polianthes tuberosa* L.) decreased water loss and ion leakage and increased activity of guaiacol peroxidase, catalase, ascorbate peroxidase and phenylalanine ammonia-lase enzymes but decreased the activity of polyphenol oxidase enzyme.

Thus, the present paper assesses the effect of foliar application of proline on some morphological and physiological characteristics of *Calendula officinalis* L. under drought stress.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse in northern Iran (latitude 53.44°N, 36.45 °E, and 15 m above sea level) in 2018. Factorial experiment was performed in a completely randomized design with 3 replications. The factors of the experiment consisted of foliar application of proline (0, 50 and 100 mg L^{-1}) and drought stress (normal irrigation, 25, 50, and 75% field capacity). Pot marigold was grown in the greenhouse at 25/15°C day/night temperature and 14/10 hours day/night photoperiod. Approximately, three weeks after planting the seeds in the appropriate bed, the seedlings were transferred to a pot (containing a mixture of soil, sand and leaf composts in a volume ratio of 1:1:1). The field capacity of the soil and permanent wilting point (PWP) were measured by a pressure–plate apparatus (Shakarami *et al*., 2019) and the drought stress treatments were applied in six visible leaves stages. To determine FC, some pots containing the tested beds were saturated with water so that water drains out from the bottom of the pots. To prevent evaporation, the pot surface was covered with aluminum foil. The weight of pots was evaluated each day until it reached a stable level. Thereafter, the soil of each pot was mixed to make a uniform mixture and then some soil was removed to record its wet weight. To evaluate the soil dry weight, it was placed in the oven for 24 hours at 72 °C. Then the FC was calculated. According to the following equation: FC= (WW-DW/DW) × 100 Where, FC is field capacity, WW is the soil wet weight and DW is the soli dry weight.

The spray solutions of proline ($C_5H_9NO_2$) (Sigma Aldrich, Germany) were prepared based on distilled water. Foliar application was applied in three stages with intervals of about 20 days including six visible leaves, complete tillering and emergence of the first bud. Control pots were sprayed with distilled water. Then, at the appropriate stage of harvesting sampling was done. The traits were measured as described as following.

Fresh weight of the aerial and root organs

The fresh weight of the aerial and root organs was calculated by a digital scale with 0.01 g accuracy (Dolatkhahi *et al*., 2013).

Dry weight of the aerial and root organs

To measure the dry weight of the aerial and root organs, the samples were kept in an oven at 40 °C and the weight was measured by a digital scale with 0.01 g accuracy (Hassanzadeh *et al*., 2018).

Plant height and root length

Plant height and root length were measured by metal meters (Mirzaee Esgandian *et al*., 2020).

Number of flowers

The number of flowers was calculated by counting and their average was recorded (Mostofi and Najafi, 2005).

Longevity of the flower on the plant

Longevity of the flower on the plant was calculated from the flower opening to wilting or pale flowering and reported in days (Ezhilmathi *et al*., 2007).

Total chlorophyll

For measuring total chlorophyll, the pigments were extracted with dimethyl sulfoxide (DMSO(, then the absorbance of the solution obtained was read using an optical spectrophotometer at wavelengths of 663 nm for chlorophyll a and 645 nm for chlorophyll b, the total chlorophyll of leaves were expressed in mg g^{-1} fresh weight by using the following formulas (Barnes *et al*., 1992).

$$\text{Total Chl. (mg g}^{-1}\text{ FW)} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times V/1000 \times W$$

A: Wavelength, V: Final volume of solution, W: Sample weight.

Carotenoid

Carotenoid was determined using Barnes *et al*., (1992) method, the absorbance was read with spectrophotometer at 480 and 510 nm and expressed in mg g^{-1} FW petal.

$$\text{Carotenoid (mg g}^{-1}\text{ FW)} = 7.6\,(A480) - 1.49\,(A510) \times V/1000 \times W$$

A: Wavelength, V: Final volume of solution, W: Sample weight.

Proline

Proline was determined following Bates *et al*. (1973). The absorbance of the organic phase was recorded at 520 nm. The results were compared with a standard curve of proline and the concentration was expressed in mg g^{-1} dry weight.

Total antioxidants (DPPH)

Total antioxidants (DPPH), was measured by (Lama *et al*., 2013). Finally their absorption was read at 517 nm and it was expressed as a percentage.

$$\text{DPPH radical-scavenging activity \%} = [(A_{control} - A_{sample}) / A_{control})] \times 100$$

Statistical analysis

The data were analyzed using SAS software. The comparison of the means was done by Duncan's multiple range at 1 and 5% levels, and the graphs were drawn using the Excel software.

RESULTS

Fresh weight of plant

The results of the present study showed that foliar application of proline, drought stress and their interaction were significant on fresh weight of aerial organs at 1 % level (Table 1), drought stress 75% FC showed the highest fresh weight of the aerial organs compared to the control and proline application was able to reduce the effects of stress to some extent. Generally, the highest fresh weight of organs with 459.68 g was related to the normal irrigation + 100 mg L^{-1} of proline and also the lowest with 155.17 g was for 25 % FC (Fig. 1).

Table 1. Analysis of variance of proline foliar application in *Calendula officinalis* L. under drought stress.

S.o.V	df	MS					
		Fresh weight of plant	Dry weight of plant	Fresh weight of roots	Dry weight of roots	Plant height	Root length
Drought (D)	3	159.383**	6.841**	34.138**	3.685*	17.888**	23.104**
Proline (P)	2	117.706**	1.366ns	23.026*	1.384ns	5.093ns	10.679*
D×P	6	218.392**	7.625**	47.350**	4.884**	7.262*	32.583**
Error	-	17.208	1.648	9.678	1.009	3.949	4.554
CV (%)	-	11.42	9.23	12.21	10.25	8.63	11.84

*, ** and ns: Significant at P<0.05, P<0.01 and insignificant, respectively.

Table 1. Continued

S.o.V	df	MS					
		Number of flowers	Longevity of the flower on the plant	Carotenoid	Total chlorophyll	Proline	DPPH
Drought (D)	3	4.971**	2.575**	0.174**	0.031**	15.927**	28.303**
Proline (P)	2	1.419ns	1.220ns	0.056**	0.028**	8.590*	11.668ns
D×P	6	6.319*	4.892**	0.040**	0.030**	21.070**	37.338**
Error	-	1.122	0.830	0.004	0.002	2.747	5.325
CV (%)	-	10.58	9. 39	8.99	7.08	10.35	8.43

*, ** and ns: Significant at P<0.05, P<0.01 and insignificant, respectively.

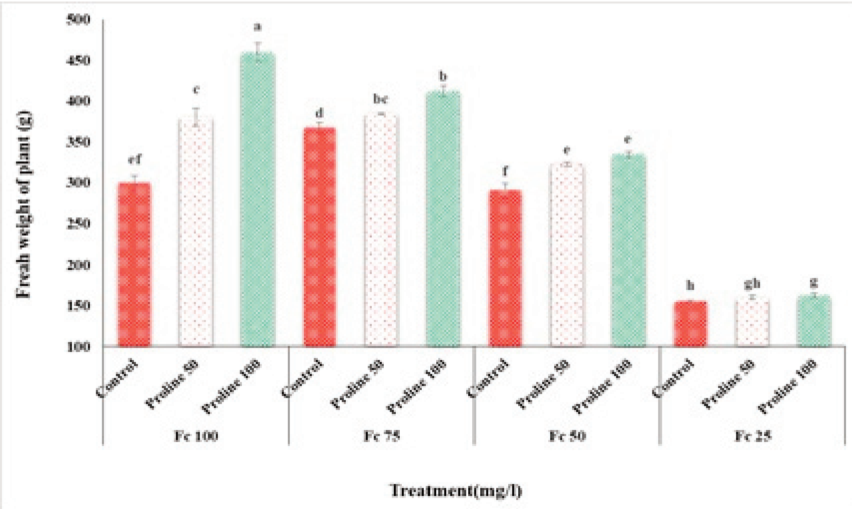

Fig. 1. Mean comparison of the effect of proline foliar application on fresh weight of plant in *Calendula officinalis* L. under drought stress.

Dry weight of plant

Data showed that foliar application of proline, drought stress and their interaction were significant on dry weight of aerial organs at 1 % level (Tabel 1). In general, the highest dry weight of aerial organs (42.45 g) was for the normal irrigation+ 100 mg L^{-1} of proline and the lowest (20.69 g) was belonged to 25 % FC (Fig. 2).

Fresh weight of roots

Data analysis showed that foliar application of proline, drought stress and their interaction were significant on fresh weight of roots at 1 % level (Tabel 1). Drought stress 75% FC showed the highest fresh weight of roots compared to the control and proline application was able to reduce the effects of drought stress (Fig. 3). The highest and the lowest fresh weight of roots with 86.4 g and 36.23 g are related to 75 % FC+ 100 mg L^{-1} of proline and 25 % FC, respectively.

Dry weight of roots

The results of the present study showed that foliar application of proline, drought stress

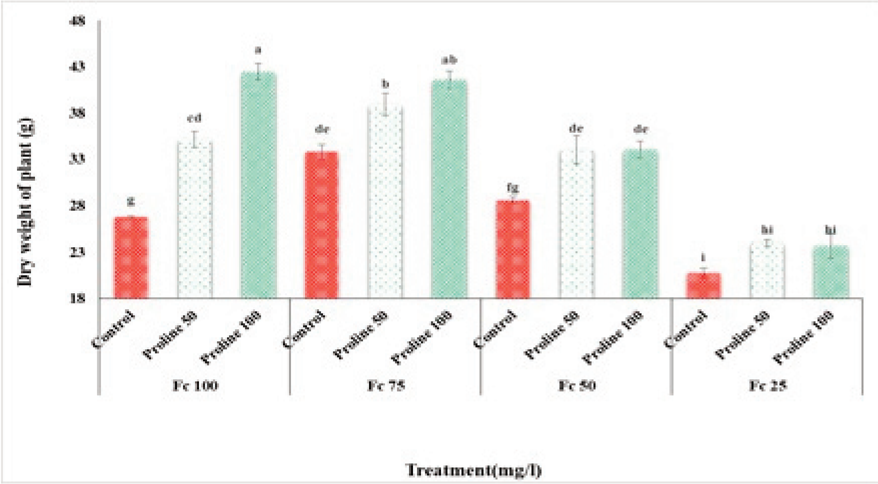

Fig. 2. Mean comparison of the effect of proline foliar application on dry weight of plant in *Calendula officinalis* L. under drought stress.

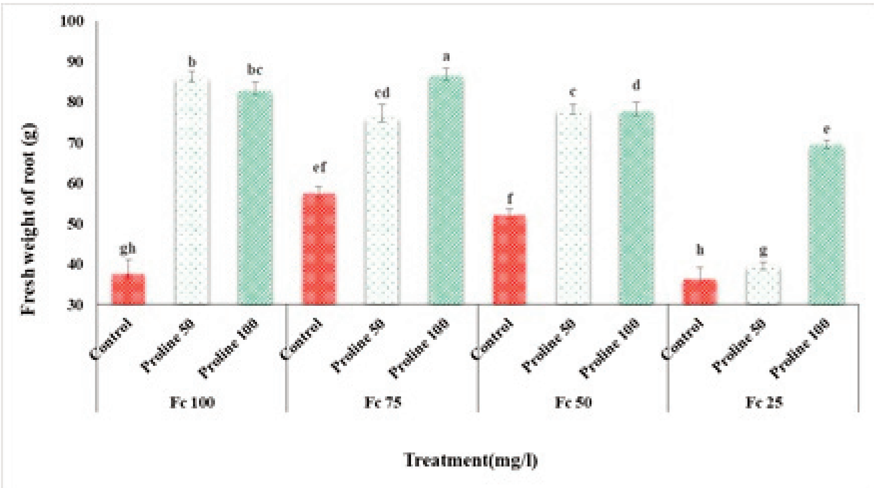

Fig. 3. Mean comparison of the effect of proline foliar application on fresh weight of roots in *Calendula officinalis* L. under drought stress.

and their interaction were significant on dry weight of roots at 1 % level (Tabel 1). Generally, 75% FC+ proline 100 mg L^{-1} exhibited the highest dry weight of roots (18.1 g) and the lowest (5.88 g) observed in 25 % FC (Fig. 4).

Plant height

The results showed that drought stress was significant on plant height at 1 % level, while foliar application of proline and their interaction were significant at 5% level (Tabel 1). Drought stress 75% FC increased plant height compared to the control, which was not significantly different from 50% FC. Moreover, proline application was able to reduce the severity of drought stress to some extent (Fig. 5). In general, the highest plant height (35.01 cm) was for the normal irrigation+ 50 mg L^{-1} of proline and the lowest (22.66 cm) was for 25 % FC and 50 mg L^{-1} of proline.

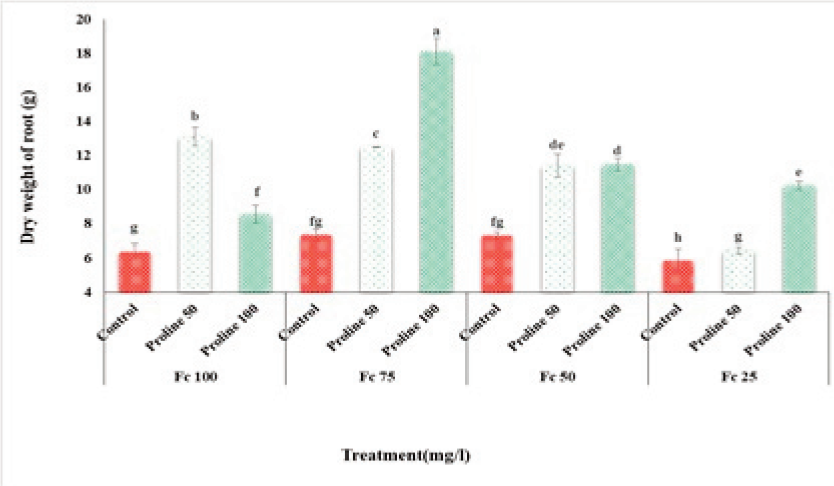

Fig. 4. Mean comparison of the effect of proline foliar application on dry weight of roots in *Calendula officinalis* L. under drought stress.

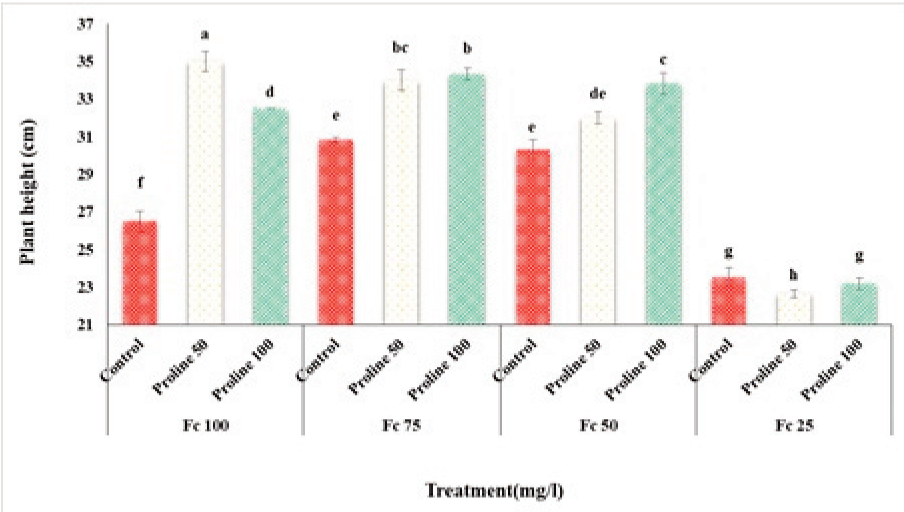

Fig. 5. Mean comparison of the effect of proline foliar application on plant height in *Calendula officinalis* L. under drought stress.

Root length

Examination of the data showed that foliar application of proline, drought stress and their interaction were significant on root length at 1 % level (Tabel 1). Drought stress 75% FC increased root length compared to control and proline application was able to reduce the stress effect (Fig. 6). The highest and the lowest root length (47.33 cm and 28.33 cm) were related to 75 % FC + 100 mg L^{-1} of proline and 25 % FC, respectively.

Number of flowers

The results showed that drought stress was significant on number of flowers at 1 % level, while foliar application of proline and their interaction were significant at 5 % level (Tabel 1). Drought stress 75 % FC increased number of flowers compared to control but proline application reduced the effect of drought stress on the number of flowers. In general, normal irrigation+ 50 mg L^{-1} of proline exhibited the highest number of flowers (12) whilst the lowest was (5.16) observed in 25 % FC (Fig. 7).

Longevity of the flower on the plant

The results of the present study showed that foliar application of proline, drought stress and their interaction were significant on longevity of the flower on the plant at 1 % level (Tabel 1). Drought stress 75 % FC increased longevity of the flower on the plant compared to control and proline application also played an effective role in increasing longevity of the flower on the plant (Fig. 8). In general, the highest longevity of the flower on the plant (15.16 days) was related to normal irrigation+ 100 mg L^{-1} of proline and the lowest (8.33 days) was for 25 % FC.

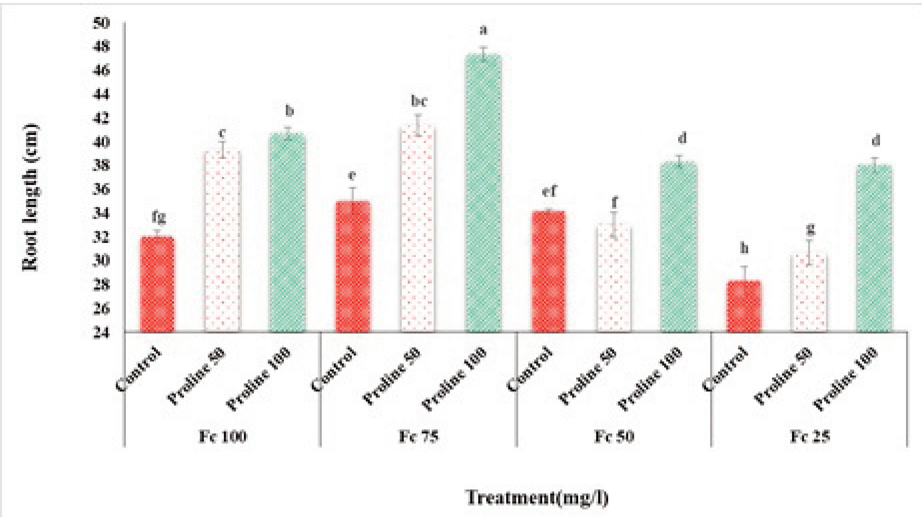

Fig. 6. Mean comparison of the effect of proline foliar application on root length in *Calendula officinalis* L. under drought stress.

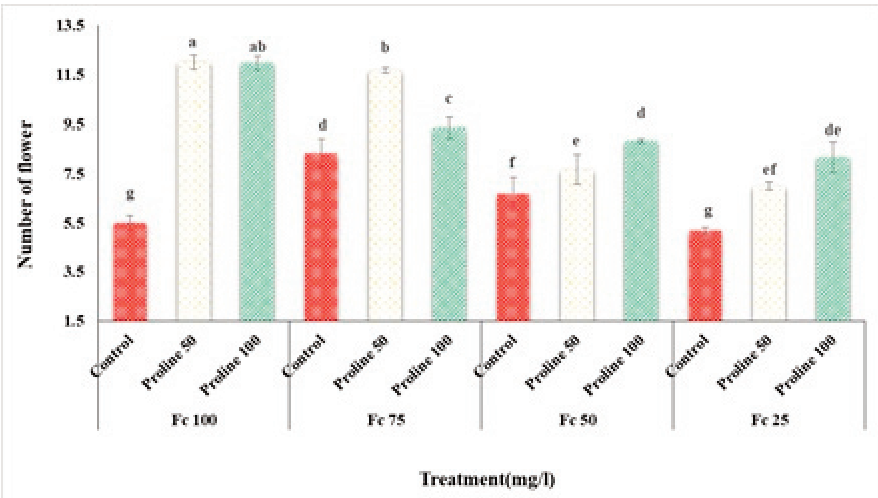

Fig. 7. Mean comparison of the effect of proline foliar application on number of flowers in *Calendula officinalis* L. under drought stress.

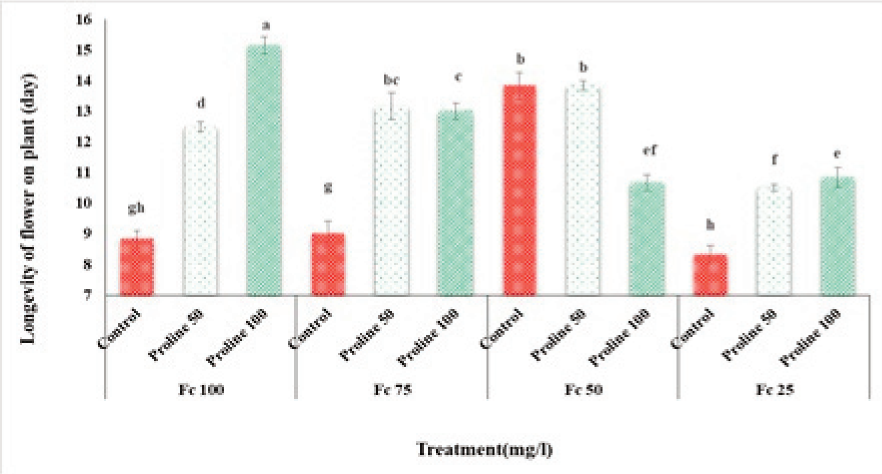

Fig. 8. Mean comparison of the effect of proline foliar application on longevity of the flower on the plant in *Calendula officinalis* L. under drought stress.

Carotenoid

The results showed that foliar application of proline, drought stress and their interaction were significant on carotenoid at 1 % level (Tabel 1). Drought stress 75% FC increased the carotenoid compared to the control, which was not significantly different from the 50% FC. Proline application was able to compensate the carotenoid reduction in stress condition. In general, proline at the rate of 100 mg L^{-1}+ normal irrigation had the highest carotenoid content (1.20 mg g^{-1} FW) and 25 % FC had the lowest (0.46 mg g^{-1} FW) (Fig. 9).

Total chlorophyll

The results showed that foliar application of proline, drought stress and their interaction were significant on total chlorophyll at 1 % level (Tabel 1). Drought stress 75 % FC increased total chlorophyll compared to control and proline application was able to compensate for the reduction in stress (Fig. 10). The highest and the lowest of total chlorophyll of pot marigold leaves with 0.64 mg g^{-1} FW and 0.32 mg g^{-1} FW were related to normal irrigation+ 100 mg L^{-1} of proline and 25 % FC, respectively.

Proline

Studies have shown that foliar application of proline, drought stress and their interaction was significant on proline at 1 % level (Tabal 1). In general, with increasing drought stress, the amount of proline content in the plant increased and the foliar application of proline also increased the amount of endogenous proline. In general, proline at the rate of 100 mg L^{-1} exhibited the highest proline content in pot marigold leaves (42.02 mg g^{-1} DW) whilst the lowest was 21.52 mg g^{-1} DW observed in control FC (Fig. 11).

Total antioxidants (DPPH)

Data analysis showed that foliar application of proline, drought stress and their interaction were significant on proline at 1 % level (Table 1). Drought stress 75% FC increased DPPH compared to control and proline application also increased the antioxidant activity of the plant. The highest DPPH (77.58 %) was for the 75 % FC+ 100 mg L^{-1} of proline and the lowest (27.99 %) was for 25 % FC (Fig. 12).

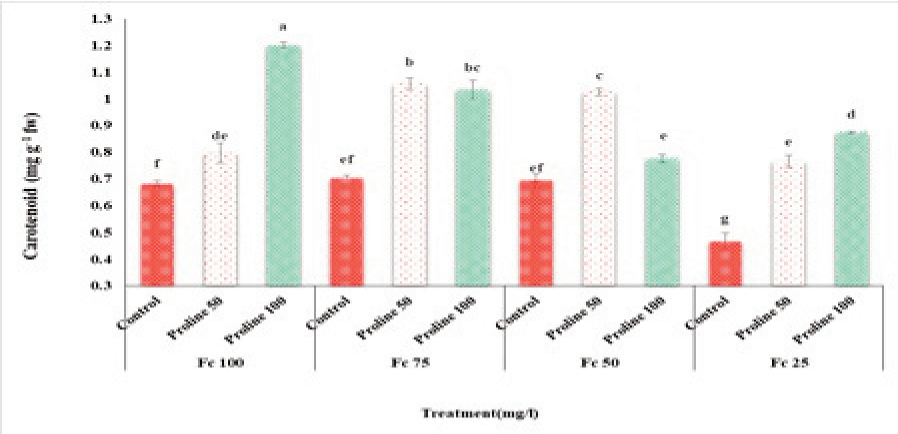

Fig. 9. Mean comparison of the effect of proline foliar application on carotenoid of petals in *Calendula officinalis* L. under drought stress.

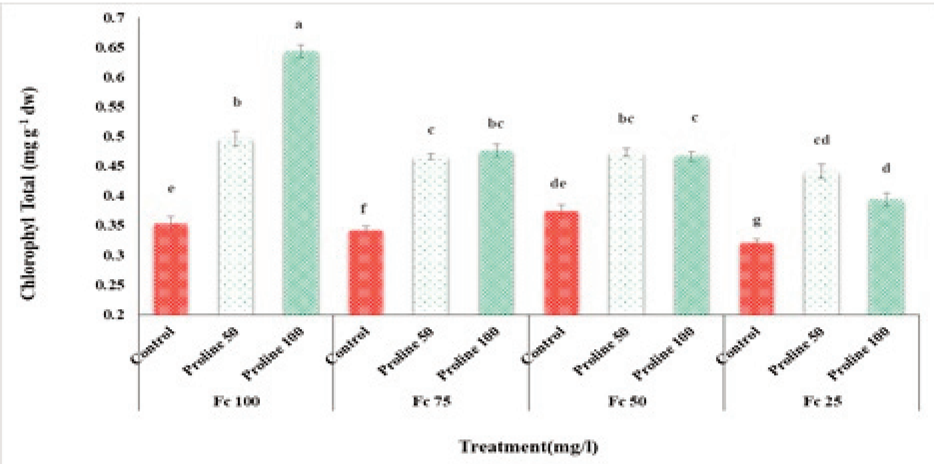

Fig. 10. Mean comparison of the effect of proline foliar application on total chlorophyll of leaves in *Calendula officinalis* L. under drought stress.

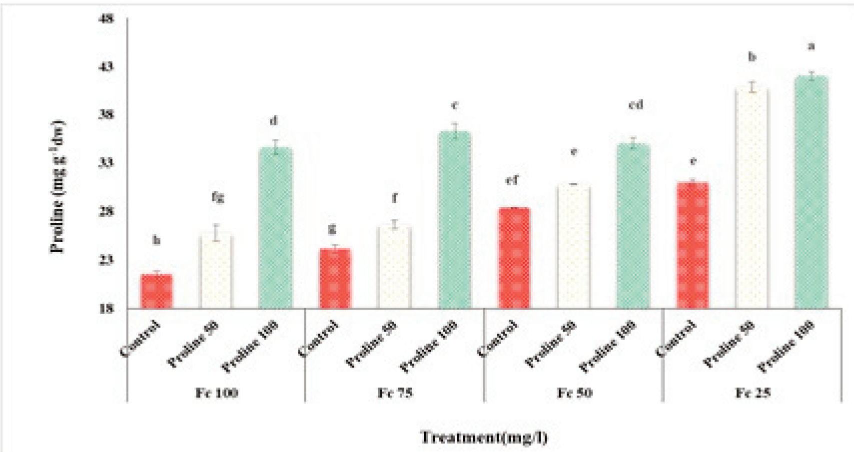

Fig. 11. Mean comparison of the effect of proline foliar application on proline content of leaves in *Calendula officinalis* L. under drought stress.

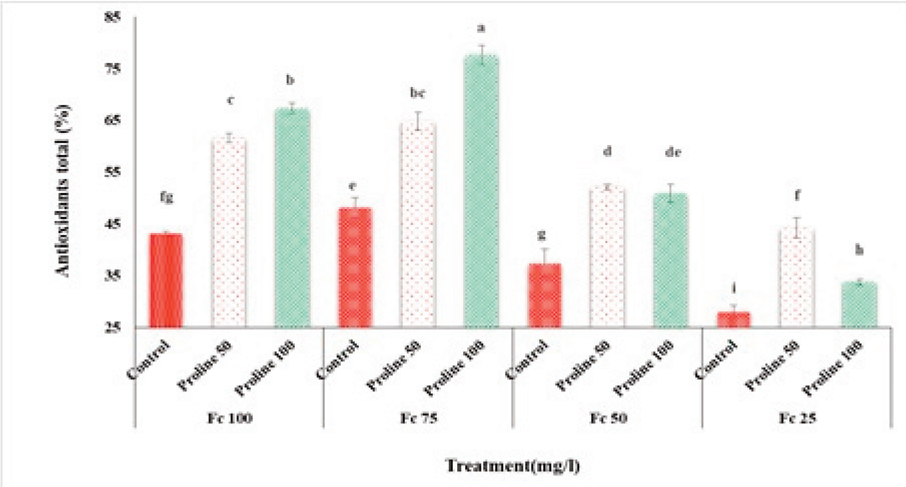

Fig. 12. Mean comparison of the effect of proline foliar application on DPPH of petals in *Calendula officinalis* L. under drought stress.

DISCUSSION

In the present study, the results showed that severe drought stress (25 % FC) reduced the fresh and dry weight of the plant. Drought stress significantly changes the content of chlorophyll, damaging the photosynthetic system, inhibiting photochemical activity and enzymes in the Calvin cycle (Dulai *et al*., 2006). On the other hand, reducing the stomatal conductance (Hasanuzzaman *et al*., 2013), reducing carbon stabilization, limiting water absorption and nutrients (Ashraf and Foolad, 2007) leads to lower leaf area and accelerates the aging process of leaves and limit the production and export of photosynthetic materials. In plants, the fresh and dry weights of plant organs decrease under drought stress compared to control (Baghalian *et al*., 2011). Proline spraying solution increased the fresh and dry aerial weight. According to research, it has been proven that exogenous application of proline has adverse effects of drought stress on vegetative parameters in some plants (Ali *et al*., 2007; Khalil Soha and El- Noemani, 2012). Various mechanisms have been identified for the effect of proline on plant growth. Increased growth by proline in drought stress is likely due to increased proline accumulation, which not only protects the enzymes and structures of proteins and organ membranes, but also provides a source of energy for growth and helps plants survive through resistance to stress (Ashraf and Foolad, 2007). The study results were consistent with the results of Hatamifar and Babadaei Samani) 2017) on *Petunia* × *grandiflora* 'Bravo F1 Blue and Darvizheh and Zavareh (2018) on *Matricaria chamomilla* L.

Fresh and dry weight of root decreased with increasing intensity of stress. It has been discussed that the reduction of fresh and dry weight of roots during stress may due to the fact that drought stress reduces leaf area, closes stomata and reduces the absorption and transfer of water and nutrients in the root region (Ganjeali and Bagheri, 2010). The results also showed that proline increased the fresh and dry weight of roots because it increased the amount of potassium in the plant, which in turn increased leaf area and photosynthetic production (Foyer and Spancer, 1986). The results of this study are in agreement with the results of Zekavati *et al*., (2019) on *Polianthes tuberose*, Mohammadi Torkashvand *et al*. (2015) on *Araucaria excelsa* L. and Zarinkolah *et al*. (2013) on *Parrotia persica* L.

The results of this study showed that with increasing stress, plant height decreased. The most important reasons for the decrease in plant height under dehydration conditions are probably due to reduced cell division, photosynthesis disorder, reduced production of photosynthetic materials to provide growing areas, clogging of wooden vessels and phloem, and ultimately lack of genetic potential in plant height (Kaya *et al.*, 2007). Proline solution spraying increased plant height. Proline, as a defense mechanism, reduced the osmotic potential of the cell leading to increased water uptake by the cell (Ali *et al*., 2007). In general, the availability of amino acids and nitrogen in the early stages of growth under water stress causes extension of the aerial part (Darvizheh and Zavareh, 2018). The study results are consistent with the study results of Hashemabadi *et al*., (2019(on *Catharanthus roseus* L. and Darvizheh and Zavareh (2018 (on *Matricaria chamomilla* L.

The results showed that root length increased by drought stress 75 and 50% FC compared to control and decreased in 25 % FC. The root behavior of the plant is affected by soil moisture stress, and with increasing moisture stress, the roots have sought moisture and become more developed (Sodaiezadeh *et al*., 2016). In this study, with increasing intensity of drought stress, root length decreased, which may be due to the fact that drought stress significantly reduces the rate of carbon dioxide gas exchange, the total level of assimilation and the wet and dry matter of the plant (Misra and Srivastava, 2000). The results also showed that proline spraying increased root length. The reasons for this are that proline acts as an osmotic regulator, reducing the cell's osmotic potential and thus increasing water absorption. In addition, proline increases phosphorus levels, which are essential for cell division and the development of meristematic tissues (Maiato *et al*., 2004). The results of this study implied the results by Naseri Moghadam *et al.,* (2019) on *Narcissus tazetta* and Mansouri Motehaver *et al*. (2019) on *Catharanthus roseus*.

Drought stress at 75 and 50 % FC increased the number of flowers compared to the control. However, the 25% FC reduced the number of flowers. Decreased number of flowers under drought stress, due to reduced photosynthesis (closing the stomata), which further disrupts cell metabolism and reduces the rate of yield (Baghalian *et al*., 2011). In this experiment, the use of proline increased the number of flowers. The use of amino acids significantly improved morphological characteristics (Karima *et al.,* 2005). In addition, amino acids play an important role in protein biosynthesis and improve plant growth. Exogenous proline affects the flowering time and thus increases the flowering period and the number of flowers per plant (Saxena *et al*., 2008). The study results agree with Mansouri Motehaver *et al*. (2019) on *Catharanthus roseus* and Oraee *et al*. (2019) on *Alcea rosea*.

With increasing drought stress, the longevity of the flower on the plant decreased. Increased oxygen free radicals (ROS) in severe stresses destroys phospholipids and releases fatty acids and peroxidation, which increases membrane permeability, resulting in membrane degradation leading to ethylene synthesis and ultimately reducing flower shelf life (Hossaina *et al*., 2005). In addition to the role of proline in reducing the damage caused by ROS (Kumar *et al.,*2010) and its positive effects on enzymes and membrane strength (Hassanzadeh Fard *et al*., 2013), studies have shown that amino acids delay ACC oxidase activity and affect enzymatic and non-enzymatic systems. It produces ethylene and ultimately reduces the production of ethylene or prevents its effect on flowers and delays aging and ultimately increases the longevity of flowers on the plant (Zamani *et al*., 2011). The study results of Naseri Moghadam *et al*. (2019) on *Narsicuss tazetta* L.; Abdossi and Danaee (2018) on *Dianthus caryophyllus* cv. Tessino were consistent with these results.

The results of the present study showed that under severe drought stress, petal carotenoid content was reduced and could not play its protective role. The reduction in the content of carotenoids can be due to stimulation of ionic stress, a reduction in photosynthetic level, an increase in the production of oxygen radicals, peroxidation of these pigments and chemical degradation of genes related to their biosynthetic path (Idrees *et al*., 2010). Foliar application of solutions greatly increased the amount of carotenoid of pot marigold but in fact in this study, proline foliar application enhanced the biosynthesis and protection of photosynthetic pigments further that is probably due to the effect of proline stimulation on photosynthetic pigments due to the stability of the active site of the enzymes (Ali *et al*., 2013) The results agree with the study results of Tian *et al*. (2012) on *Tagetes erecta* L. and Gholami Zali and Ehsanzadeh (2018) on *Foeniculum vulgare* Mill.

Chlorophyll levels were also reduced in severe drought stress which can be due to the closure of the stomata or the disruption and damage to the structural proteins used in photosystems (Lawlor, 2002). Moreover, chlorophyll reduction is another reason for the stress activity of chlorophyll enzyme activity, in which the expression of the gene for this enzyme is induced (Ranjan *et al*., 2001). In this study, proline foliar application increased total chlorophyll, which may be due to the role of proline in increasing the stomatal conductance and subsequently increasing the amount of carbon dioxide in the stomata space, thus increasing the rate of photosynthesis in the plant (Sharkey *et al*., 2007). Moreover, proline protects the chloroplast structure by using the additional energy of photosystem □ and inhibiting free radicals (Moustakas *et al*., 2011). The results of this study implied the results by Hatamifar and Babadaei Samani (2017) on *Petunia × grandiflora* 'Bravo F1 Blue', Roozbehani *et al.* (2018) *Impatiens walleriana* and Shakarami *et al.* (2019) *Polianthes tuberosa*.

The findings showed that proline levels increased with increasing intensity of stress. Increased proline during drought stress may be due to protein breakdown (Habibi *et al.,* 2010). Under conditions of dehydration stress and the consequent accumulation of soluble substances such as proline, the osmotic potential of cells decreases, which absorbs water into the cell and aids in turgor (Farooq *et al*., 2009). In this study, exogenous proline increased the amount of proline in the plant. Under drought stress, proline helps the plant grow more efficiently by regulating osmosis (Chen and Dickman, 2005), preventing the destruction of enzymes and proteins, and maintaining the stability of the cell membrane (Kocheva and Georgive, 2003). Proline application improves energy production and storage by affecting nitrogen metabolism in plants (Hashem *et al*., 2015). The study results were consistent with the study results of Kashefi and Bahri (2019) on *Malva sylvestris* and Roozbehani *et al.* (2018) on *Impatiens walleriana,*

The results showed that the total antioxidant capacity increased by 75% FC, but decreased with increasing stress intensity. Plant cells produce free radicals when exposed to any oxidative stress. The antioxidant capacity of plants includes enzymatic and non-enzymatic compounds (Spinardi, 2005). Antioxidants oxidize reactive oxygen species by releasing electrons to free radicals (Danaee and Abdossi, 2018). Severe drought stress limits growth and photosynthesis, leading to reduced access to raw materials for the synthesis of secondary compounds, and ultimately it has a negative effect on the antioxidant capacity of the plant (Hamidipour *et al.,* 2017). Among the various adaptive substances, proline is one of the most effective inhibitors of active oxygen varieties and free radicals, which can reduce their harmful effects under stress (Okuma *et al*., 2004). Islam *et al*., 2009 also reported that the use of proline improves plant tolerance to stress and prevents cell death by reducing the accumulation of active oxygen species and increasing the activity of antioxidant enzymes. The study results were consistent with the study results of Hamidipour *et al*. (2017) on *Mentha piperita*.

CONCLUSION

According to the results, foliar application of proline increased the wet and dry weight of aerial and root organs, plant height, root length, number of flowers, longevity of the flower on plant, petal carotenoids, total chlorophyll and proline of leaves and total antioxidant activity (DPPH). It had a positive effect on the growth and flowering of *Calendula officinalis* L. plants. Severe drought stress also reduced all traits except proline and irrigation at 75 % FC had the highest morphological and physiological characteristics in *Calendula officinalis* L. In general, it can be suggested that foliar application of proline (100 mg L^{-1}) as a defense agent in plants used by foliar application under stress can be effective in saving water consumption due to the water shortage crisis in agriculture and increase growth and yield by reducing the amount of damage caused by drought stress.

ACKNOWLEDGEMENTS

Thanks to Gorgan University of Agricultural Sciences and Natural Resources for providing the necessary facilities for this research.

Literature Cited

Abdossi, V. and Danaee, E. 2018. Effects of some amino acids and organic acids on enzymatic activity and longevity of *Dianthus caryophyllus* cv. Tessino at pre-harvest stage. Journal of Ornamental Plants, 9 (2): 93-104.

Ali, Q., Anwar, F., Ashraf, M., Saari, N. and Perveen, R. 2013. Ameliorating effects of exogenously applied proline on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. International Journal of Molecular Sciences, 14 (1): 818-835.

Ali, Q., Ashraf, M. and Athar, H.R. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pakistan Journal of Botany, 39 (4): 1133-1144.

Alipour, S., Farahmand, F. and Nasibi, F. 2016. Influence of proline treatment on some physiological morphological characteristics and postharvest life of cut tuberose (*Polianthes tuberosa* L.). Plant Process and Function, 4 (14): 114-106.

Ashraf, M. and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress tolerance. Environmental and Experimental Botany, 59 (2): 206- 216.

Baghalian, K., Abdoshah, S.H., Khalighi-Sigaroodi, F. and Paknejad F. 2011. Physiological and phytochemical response to drought stress of German chamomile (*Matricaria recutita* L.). Plant Physiology and Biochemistry, 49 (20): 201-207.

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S. and Davison, A.A. 1992. A reappraisal of the use of DMSO for extraction and determination of chlorophyll a and b in lichens and higher plants. Environmental and Experimental Botany, 32: 85-100.

Bates, L.S., Waldran, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water studies. Plant Soil, 39: 205-207.

Buntariu, M. and Zepa Cradini, C. 2012. Evaluation of biologically active compounds from *Calendula officinalis* flowers using spectrophotometry. Butnariu and Coracini Chemistry Central Journal, 6 (35): 1-7.

Chen, C. and Dickman, M. B. 2005. Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Proceedings of the National Academy of Sciences. United States of America, 102 (9): 3459-3464.

Danaee, E. and Abdossi, V. 2018. Effect of different concentrations and application methods of polyamines (putrescine, spermine, spermidine) on some morphological, physiological, and enzymatic characteristics and vase life of *Rosa hybrida* cv. 'Dolce Vita' cut flower. Journal of Ornamental Plants, 8 (3): 171-182.

Darvizheh, H. and Zavareh. M. 2018. The effect of proline foliar application on reducing the effects of dehydration stress on German chamomile (*Matricaria chamomilla* L). Ecological Quarterly Journal of Crop Plants, 14 (1): 43-33. (In Persian)

Darvizheh, H., Zavareh, M. and Ghasmanjad, M. 2017. Effect of proline spraying on biochemical properties of German chamomile in water stress conditions (*Matricaria chamomilla* L). Journal of Applied Research in Plant Ecophysiology, 4 (1): 35-60. (In Persian)

Dawood, M.G., Taie, H.A.A., Nassar, R.M.A., Abdelhamid, M.T. and Schmidhalter, U. 2014. The changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under seawater stress. South African Journal of Botany, 93: 54–63.

Deivanai, S., Xavier, R., Vinod, V., Timalata, K. and Lim, O.F. 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. Journal of Stress Physiology and Biochemistry, 7 (4): 157-174.

Dolatkhahi, A., Matloobi, M., Motallebiazar, A. and Vahdati, N. 2013. Shading impact on qualitative characteristics and chlorophyll content of cut rose (*Rosa hybrida* cv. Avalanche). Journal of Ornamental Plants, 3 (4): 215-220.

Dulai, S., Molnar, I., Pronay, J., Csernak, A., Tarnai, R. and Molnar-Lang, M. 2006. Effects of drought on photosynthetic parameters and heat stability of PSII in wheat and in *Aegilops* species originating from dry habitats. Acta Biologica Szegediensis, 50 (1-2): 11-17.

Ezhilmathi, K., Singh, V., Arora, P. and Sairam, R.K. 2007. Effect of 5-sulfocalicylic acid on antioxidant in relation to vase life of gladiolus cut flower. Plant Growth Regulation, 51 (2): 99-108.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. 2009. Plant drought stress effects, mechanisms and management. Agronomy for Sustainable Development, 29 (1): 185-212.

Foyer, C. and Spancer, C. 1986. The relationship between phosphate status and photosynthesis in leaves effects on intracellular orthophosphate distribution photosynthesis and assimilate partitioning. Planta, 167 (3): 369-375.

Ganjeali, A. and Bagheri, A. 2010. Evaluation of morphological characteristics of root chickpea (*Cicer arietinum* L.) in response to drought stress. Iranian Journal of Pulses Research, 1 (2):101-110.

Gholami Zali, A. and Ehsanzadeh, P. 2018. Exogenous proline improves osmoregulation, physiological functions, essential oil, and seed yield of fennel. Industrial Crops and Products, 111: 133–140.

Habibi, D., Ardakani, M.R., Mahmoudi, A. and Asgharzadeh, A. 2010. Effects of super absorbent polymer and plant growth promoting rhizobacteria (PGPR) on yield and oxidative damage of maize under drought stress. International Conference on Chemistry and Chemical Engineering. Kyoto, Japan. 1-3 August, 253-257.

Hamidipour, P., Amoaghaei, R. and Bahreininejad, B. 2017. Effect of aminolevulinic acid on growth, essential oil content and antioxidant responses in *Mentha piperita* under drought stress. M.Sc. Thesis. Shahrekord University. (In Persian)

Hasanuzzaman, M., Nahar, K., Gill, S.S. and Fujita, M. 2013. Drought stress responses in plants, oxidative stress, and antioxidant defense. Climate Change and Plant Abiotic Stress Tolerance, First Edition. Wiley-VCH Verlag GmbH and Co. KGaA. Part 9, pp. 209- 249.

Hashem, A., Abd Allah, E.F., Alqarawi, A.A. and Egamberdieva, D. 2015. Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill. by arbuscular mycorrhizal fungi. Saudi Journal of Biological Sciences, 23 (1): 39-47.

Hashemabadi, D., Asadi Beigzadeh-Mahaleh, Z. and Pourzarnegar, F. 2019. Morphological and physiological traits of *Catharanthus roseus* L. at different irrigation intervals as affected by salicylic acid application. Journal of Ornamental Plants, 9 (3): 165-178.

Hassanzadeh, K., Hemmati, Kh. and Mehdipour, M. 2018. The effect of different drying methods (natural and aven) on the drying time and some secondary metabolites of the medicinal plant lemongrass (*Melissa officinalis* L.). Journal of Plant Production Research, 25 (1): 137-143. (In Persian)

Hassanzadeh Fard., Sh. and Arvin, M.J. 2013. The role of glycine betaine and proline in increasing drought resistance with emphasis on its functional aspects. The First National Conference on Agricultural Sciences with an Emphasis on Non-biological Stresses. Payame Noor Naghadeh University. 18-19 September. https://www.civilica.com/Paper-NCASPNU01-NCASPNU01_079.html.

Hatamifar, N. and Babadaei Samani, R. 2017. Effect of paclobutrazol on some morphological and physiological characteristics of petunia under drought stress. Journal of Ornamental Plants, 7 (2): 125-136.

Hossaina, Z., Kalam, A., Mandala, A., Kumar Dattaa, S. and Krishna Biswasb, A. 2005. Decline in ascorbate peroxidase activity –A prerequisite factor for tepal senescence in *Gladiolus*. Journal of Plant Physiology, 163 (2): 186-194.

Idrees, M., Khan, M. M. A., Aftab, T., Naeem, M. and Hashmi, N. 2010. Salicylic acid induced physiological and biochemical changes in lemongrass varieties under water stress. Journal of Plant Interactions, 5 (4): 293-303.

Islam, M.M., Hoque, M.A., Okuma, E., Banu, V., Shimoishi, Y. and Nakamura, Y., 2009. Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. Journal of Plant Physiology, 166 (15):1587-97.

Jalali, F. and Naderi, D. 2019. Study of morphological and biochemical traits of marigold as influenced by phosphorous biofertilizer and zinc. Journal of Ornamental Plants, 9 (4): 275-290.

Karima, M., Gamal, E.D. and Abdel-Wahed, M.S.A. 2005. Effect of some amino acids on growth and essential oil content of chamomile plant. International of Journal of Agriculture and Biology, 7 (3): 376-380.

Kashefi, B. and Bahri, F. 2019. The effect of drought stress and hexaconazole on physio-morphological characteristics of *Malva sylvestris* L. Environmental Stress in Crop Sciences, 12 (3): 877-887. (In Persian)

Kaya, C., Levent Tuna, A., Ashraf, M. and Altunlu, H. 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. Environmental and Experimental Botany, 60 (3): 397–403.

Khalil Soha, E. and El-Noemani, A.A. 2012. Effect of irrigation intervals and exogenous proline application in improving tolerance of garden cress plant (*Lepidium sativum* L.) to water stress. Journal of Applied Sciences Research, 8 (1):157-167.

Kocheva, K. and Georgive, G .2003. Evaluation of the reaction of two contrasting barley (*Hordeum vulgare* L.) cultivars in response to osmotic stress with PEG6000. Bulgarian Journal of Plant Physiology, 1 (1): 290-294.

Kumar, N., Pal, M., Singh, A., Sairam R.K. and Srivastava, G.C. 2010. Exogenous proline alleviates oxidative stress and increase vase life in rose (*Rosa hybrida* L. 'Grand Gala'). Scientia Horticulture, 127 (1): 79–85.

Lama, B., Ghosal, M., Gupta, S. and Mandal, P. 2013. Assessment of different preservative solutions on vase life of cut roses. Journal of Ornamental Plants, 3 (3): 171-181.

Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: Stomata versus metabolism and the role of ATP. Annals of Botany, 89 (7): 871-85.

Maiato, H., DeLuca, J., Salmon, E. and Earnshaw, W.C. 2004. The dynamic kinetochore-microtubule interface. Journal of Cell Science, 117 (23): 5461-5477.

Mansouri Motehaver, M., Mosavi Fard, S. and Rezaeinejad, A. 2019. The effect of proline application on the morphophysiological characteristics of *Catharanthus roseus* salinity stress. M.Sc. Thesis, Faculty of Agriculture, Physiology and Flower and Ornamental Plant Breeding, Lorestan University. (In Persian)

Mirzaee Esgandian, N., Jabbarzadeh, Z. and Rasouli Sadaghiani, M.H. 2020. Investigation on some morphological and physiological characteristics of *Gerbera jamesonii* as affected by humic acid and nano-calcium chelate in hydroponic culture conditions. Journal of Ornamental Plants, 10 (1): 1-13.

Misra, A. and Srivastava, N.K. 2000. Influence of water stress on Japanese mint. Journal of Herbs, Spices and Medicinal Plants, 7 (1): 51-58.

Mohammadi Torkashvand, A., Misaghi, M. and Ramezanzadeh Bisheh Gahi, F. 2015. The effect of growth bed containing organic substrates and irrigation period on the growth of *Araucaria excelsa* L. Journal of Ornamental Plants, 5 (4): 231-238.

Mostofi, Y. and Najafi, F. 2005. Analytical laboratory methods in horticultural sciences. Translation of Tehran University Press. (In Persian)

Moustakas, M., Sperdouli, I., Kouna, T., Antonopoulou, C. I. and Therios, I. 2011. Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. Journal of Plant Growth Regulation, 65 (2): 315–325.

Naseri Moghadam, A., Bayat, H., Aminifard, M. and Moradi Nezhad, F. 2019. Effect of drought and salinity stress on growth, flowering and biochemical characteristics of *Narsicuss tazetta* L. Journal of Horticultural Sciences, 33 (3): 466-451. (In Persian)

Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M. and Murata, Y. 2004. Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil Science and Plant Nutrition, 50 (8): 1301-1305.

Oraee, T., Shoor, M., Tehranifar, A. and Nemati, H, 2019. Alleviation of drought stress effects by exogenous application of spermidine and salicylic acid on hollyhock (*Alcea rosea*). Journal of Ornamental Plants, 9 (4): 303-318.

Pal, M., Szalai, G. and Janda, T. 2015. Polyamines are important in abiotic stress signaling. Plant Science, 237: 16-23.

Pei, F., Li, X., Liu, X. and Lao, C. 2013. Assessing the impacts of droughts on net primary productivity in China. Journal of Environmental Management, 114: 362–371.

Ranjan, R., Bohra, S.P. and Jeel, A.M. 2001. Book of plant senescence. Jodhpur, Agrobios New York. pp 18- 42.

Roozbehani, F., Mosavi Fard, S. and Rezaeinejad, A. 2018. The effect of proline on some morphophysiological, physiological and biochemical properties of two varieties of *Impatiens walleriana* under salinity stress. M.Sc. thesis. University of Lorestan. (In Persian)

Saxena, S., Kaushik, N. and Sharma, R. 2008. Effect of abscisic acid and proline on *in vitro* flowering in *Vigna aconitifolia*. Biologia Plantarum, 52 (1): 181-190.

Shakarami, Kh., Zahedi, B., Rezaei Nejad, A. and Mousavi Fard, S. 2019. Evaluation of physiological responses of tuberose (*Polianthes tuberosa* L.) to water deficit stress. Journal of Ornamental Plants, 9 (4): 259-273.

Sharkey, T.D., Carl, J.B., Graham, D.F. and Singsaas, E.L. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant Cell Environment, 30 (9): 1035-1040.

Sodaiezadeh, H., Shamsayi, M., Tajamolian, M., Mirmohammadi Meybodi, A. and Hakimzadeh, M. 2016. The effect of drought stress on some morphological and physiological traits of *Satureja hortensis*. Plant Process and Function, 5 (15): 1-11.

Spinardi, A.M. 2005. Effect of harvest date and storage on antioxidant systems in pears. Acta Horticulture, 682: 655-662.

Szabados, L. and Savoure, A. 2009. Proline: A multifunctional amino acid. Trends in Plant Sciences, 15 (2): 89-97.

Tian, Z., Wang, F., Zhang, W., Liu, CH. and Zhao, X. 2012. Antioxidant mechanism and lipid peroxidation patterns in leaves and petals of marigold in response to drought stress. Horticulture, Environment and Biotechnology, 53:183-193.

Zamani, S., Kazemi, M. and Aran, M. 2011. Postharvest life of cut rose flowers as affected by salicylic acid and glutamine. World Applied Science Journal, 12 (9): 1621- 1624.

Zarinkolah, M., Shamshiri, M., Karimi, H. and Esmailizadeh, M. 2013. Effect of irrigation levels and exogenous proline application in improving tolerance of ironwood seedlings (*Parrotia persica*) to drought stress. M.Sc. Thesis, Vali-e-Asr University of Rafsanjan. (In Persian)

Zekavati, H., Shoor, M., Rohani, H., Fazeli Kakhki, F. and Ganji Moghadam, A. 2019. Effect of *Trichoderma* fungus (Separation 65) on morphological and biochemical characteristics of sage under drought stress. Crops Improvement, 21 (1): 62-72. (In Persian)

How to cite this article:

Soroori, S., Danaee, E., Hemmati, K., and Ladan Moghadam, A. 2021. Effect of foliar application of proline on morphological and physiological traits of *Calendula officinalis* L. under drought stress. *Journal of Ornamental Plants,* 11(1), 13-30.

URL: http://jornamental.iaurasht.ac.ir/article_680553_14bd61764e1b056d9a7619342b2a5c78.pdf