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Effects of Foliar Application of Citric Acid on Morphological and Phytochemical Traits of *Calendula officinalis* L. under Drought Stress Conditions

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ABSTRACT

Drought is a commonly-known limiting factor of plant growth and productivity, while citric acid (CA) is one of the natural antioxidants in many plant species. CA plays a role in controlling the growth and development of the plant under stress conditions. The current experim ent was performed to study the effects of foliar application of citric acid on mitigating the impacts of drought stress on *Calendula officinalis* L. The effects of citric acid (0, 50, and 100 mg l^{-1}) and different levels of drought (no stress (control), 25, 50, and 75% field capacity (FC)) were studied in a factorial experiment, based on a completely randomized design with three replicates in greenhouses of north Iran during 2018. The foliar application was performed in three stages with an interval of about 20 days, including the six-leaf stage, full tillering, and first bud emergence. The results showed that drought stress and foliar CA application significantly affected the dependent variables. The highest fresh and dry weights of shoots and roots, as well as the contents of carotenoids, phenols, and flavonoids, were obtained in response to 75% FC + CA 50 mg l^{-1}. The maximum plant height, root length, and flower count were observed in response to the control group of irrigation + CA 50 mg l^{-1}, whereas the highest longevity of the flower on the plant, total carbohydrate, rutin, and total antioxidant activity were observed in response to 75% FC + CA 100 m g l^{-1}. In addition, the control group of irrigation +CA 100 mg l^{-1} resulted in the highest total chlorophyll and vitamin C contents, where as the 25% FC treatment led to the highest proline content. In general, irrigation of pot marigolds with 75% FC and spraying with CA 100 mg l^{-1} had positive effects on plant growth and biochemical parameters

Introduction

Pot marigold (*Calendula officinalis* L.) is an annual plant of the Asteraceae family. Although it originates in the Mediterranean region (Gazim et al., 2008), pot marigold usually grows in western and southwestern parts of Iran as well, with the potential to cover many other parts of the country. The flower of this plant has edible uses, is nutritious, and can function as a food coloring agent. It has active ingredients and compounds for industrial use (e.g. nylon painting and industrial paint) and pharmaceuticals (e.g. cream and lotions) (Seghatoleslami and Moosavi, 2008). The economic importance of this plant emanates from its seed oil which contains 2% of calendolic acid (Baghaeefar et al., 2018). Also, this plant contains compounds such as *sesquiterpenes*, glycosides, saponins, xanthophylls, triterpenes, flavonoids, carotenoids, vitamin E, essential oils, and calendulin. From a medicinal perspective, it is

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antiseptic, anti-inflammatory, wound-healing, and antitumor (Gazim et al., 2008).

Drought is one of the most adverse environmental stressors, which reduces plant growth and yield, thereby causing disruptions in plant physiological processes and inducing oxidative stress (Pal et al., 2015). On the other hand, while plants produce and accumulate soluble substances such as soluble sugars, amino acids, and organic acids, the osmotic potential of cells is regulated and protected against drought stress. Thus, mild and controlled drought stress can enhance the quality of medicinal plants (Farhoudi et al., 2014).

Numerous cases of research have revealed the benefits of using various compounds for reducing the effects of drought. One of these compounds is citric acid, a weak organic acid with antioxidant and pH-regulating properties (Soroori et al., 2021a). This compound plays a vital role in the Krebs cycle as a source of carbon and energy, in membrane stability and activation of transport enzymes, as well as in the metabolism and transport of carbohydrates. In addition, it positively affects chelating free radicals and stimulates plant growth (Da Silva, 2003). Foliar application of CA in Thymus vulgaris L. reportedly increased the fresh and dry weight of shoots, chlorophyll, carotenoids, and essential oil content (Miri et al., 2015). Also, CA application in *Helianthus annuus* increased antioxidant enzyme activity and protein content (Mujahid et al., 2017). In Gazania rigens L., the application of CA improved the fresh and dry weight of roots and shoots and increased plant height (Talebi et al., 2014). The present study aimed to evaluate the effects of citric acid as a foliar application on some morphological and biochemical traits of *Calendula officinalis* L.

Material and Methods

Plant materials and treatments

This study was performed in a greenhouse located in northern Iran (Behshar city) (latitude 53.44°N, 36.45°E, and 15 m above sea level) in April 2018. The seeds of *C. officinalis* were purchased from Pakan Bazr, Isfahan, Iran. The seeds were planted in coco peat and perlite (1:1) in a culture tray. Approximately three weeks after planting the seeds, the seedlings were transferred to pots (28 cm in diameter and 30 cm in height). The pots contained soil, sand, and leaf composts (1:1:1). The cultivation conditions in a greenhouse were 14/10 hours day/night photoperiod, 25/15°C day/night temperature, and relative humidity of 50-60 %.

Drought stress treatments (control, 25, 50, and 75% field capacity) were applied in six visible leaf stages. To determine field capacity, first, some of the pots were randomly selected, saturated with water, and left for the water to drain out from the bottom. The weight of each pot was measured regularly until a stable value was reached. Then, the soil samples were removed to record their fresh weight (WW) and dry weight (DW) after placing them in an oven at 72°C for 24 hours. Thus, the FC value was obtained:

FC= (WW-DW/DW) × 100.

The spray solutions of citric acid ($C_6H_8O_7$) were purchased from Sigma Company. They were made ready for application by dilution in distilled water. The foliar application of CA (0, 50, and 100 mg l^{-1}) was applied at three stages with intervals of about 20 days, i.e. six-leaf stage, complete tillering, and the emergence of the first bud. Then, sampling was done at the flowering stage.

Morphological characteristics

Fresh weight (FW) of shoots and roots was measured immediately after harvest, while dry weight (DW) was determined after 72 hours at 60°C in the oven. Subsequently, the samples were weighted using a digital scale (0.01 g accuracy). Plant height and root length were measured using a metal ruler. The number of flowers was counted and their average was recorded (Soroori et al., 2021b). The longevity of each flower on the plant was calculated from the day a flower opened to the time it wilted (Danaee and Abdossi, 2016).

Total chlorophyll

According to a method by Soroori et al. (2021b), total chlorophyll content was determined by a spectrophotometer at wavelengths of 663 and 645 nm, and was expressed as mg g-1 FW leaves, according to the following equation:

Total chlorophyll (mg g^{-1} FW) = 20.2 (A645) + 8.02 (A663) × V/1000 × W

A: wavelength, V: volume of the solution, W: sample weight

Carotenoids

To measure carotenoid content, the absorbance was read at wavelengths of 480 and 510 nm by a spectrophotometer and was expressed as mg g-1 FW petals (Soroori et al., 2021b).

Carotenoid content (mg g-1 FW) = 7.6 (A480) - 1.49 (A510) × V/1000 × W

Proline

The proline content was read by a spectrophotometer at 520 nm and the value was expressed as mg g^{-1} DW leaf (Danaee and Abdossi, 2021).

Total phenol
Total phenol content was measured according to Malekshahi and Valizadeh Kaji (2021), and the absorbance values of samples were measured at 765 nm using a spectrophotometer. Ultimately, the phenol content was expressed as mg (Gallic acid) g^{-1} FW petal.

Flavonoids
To measure flavonoid content, the samples were read for absorbance at a wavelength of 415 nm and the results were expressed as mg (Quercetin) g^{-1} FW petal (Parvaneh et al., 2022).

Vitamin C
The vitamin C content was measured by titrimetric analysis and was expressed as mg (ascorbic acid) $100g^{-1}$ FW petal (Soroori et al., 2021a).

Total carbohydrates
Carbohydrates in the petals were measured at a wavelength of 630 nm using a spectrophotometer and were expressed as mg (glucose) g^{-1} DW (Irigoyen et al., 1992).

Total antioxidant (DPPH)
For measuring total antioxidants (DPPH), the absorption was read at 515 nm and expressed as a percentage (Mahdavian et al., 2022).
DPPH % = [(A control – A sample) / A control)] × 100.

Rutin
To measure the amount of rutin, the extract was prepared according to a method by Samee and Vorarat (2007). An HPLC device was used (Merck Hitachi) with a Lactrom Pump 7100. Its diode-type detector was set at a wavelength of 356 nm. The column was C18 in length (4.6 × 250 mm) and 5 mm in diameter. The mobile phase comprised water and acetonitrile (70:30), along with 0.5% phosphoric acid (pH= 2.8), which was initially purified in an ultrasonic bath using an HPLC filter. The flow rate was 0.8 ml min^{-1} (Boligon et al., 2015).

Statistical analysis
The experiment was performed as a factorial in a completely randomized design with three replications. The data were analyzed using SAS (ver 9.1) software and the comparison of means was done by Duncan's Multiple Range test at 1 and 5% levels.

Results
The analysis of variance showed that drought stress and foliar application of CA significantly affected the fresh and dry weights of shoots and roots, root length, the longevity of the flower on the plant, total chlorophyll, carotenoids, proline, total phenol, total carbohydrates, total antioxidant activity, and rutin ($P \leq 0.01$), as well as plant height, number of flowers, flavonoids, and vitamin C ($P \leq 0.05$) (Table 1).

Morphological traits
The results of this experiment showed that applying drought stress (25% FC) caused a decrease in all morphological traits. The application of CA significantly mitigated the negative effects of drought stress. The highest fresh and dry weights of shoots, 390.67 and 35.43 g, respectively, were recorded in response to 75% FC + CA 50 mg l^{-1}, whereas the lowest values, 155.17 and 20.69 g, respectively, occurred in response to 25% FC. The treatment group of 75% FC + CA 50 mg l^{-1} achieved the highest fresh and dry weights of roots, 81.06 and 12.71 g, respectively. In contrast, the treatment group of 25% FC showed the lowest values for these parameters, 36.23 and 4.18 g, respectively. Also, the control group of irrigation + CA 50 mg l^{-1} and, separately, the treatment group of severe drought stress (25% FC) caused the highest and lowest plant height, 35.33 and 22.16 cm, as well as root length, 44 and 26.66 cm, and the number of flowers, 11.16 and 3.83, respectively (Table 2).

Total chlorophyll
As shown in Figure 1, the highest and lowest total chlorophyll content (0.53 and 0.31 mg g^{-1} FW) were related to the control irrigation + CA 100 mg l^{-1} and the 25% FC, respectively.

Carotenoids
The highest and lowest carotenoid content (1.06 and 0.46 mg g^{-1} FW) occurred in response to the 75% FC + CA 50 mg l^{-1} and the 25% FC, respectively (Fig. 2)

Proline
Data showed that the 25% FC led to the highest proline content (39.41 mg g^{-1} DW), whereas the lowest (21.52 mg g^{-1} DW) occurred in response to the control irrigation + CA 50 mg l^{-1} (Fig. 3).

Table 1. Analysis of variance of citric acid foliar application on *Calendula officinalis* L. under drought stress

		Mean Squares															
	DF	**Fresh weight of plant**	**Dry weight of plant**	**Fresh weight of roots**	**Dry weight of roots**	**Plant height**	**Root length**	**Number of flowers**	**Longevity of the flower on the plant**	**Total chlorophyll**	**Carotenoid**	**Proline**	**Phenol**	**Flavenoied**	**Vitamin C**	**DPPH**	**Rutin**
Drought	3	159.38**	536.84**	34.13**	23.68**	17.88**	33.10**	6.971**	8.57**	0.031**	0.174**	295.92**	187.33**	120.04*	20.38**	28.30**	23.12**
Citric acid	2	117.70**	178.366**	15.02**	10.38**	7.09*	10.67**	4.419*	6.22**	0.028**	0.156**	85.59**	11.25**	30.12**	2.67**	11.66**	8.59**
Drought× Citric acid	6	28.39**	27.625**	7.35**	4.88**	5.26*	7.58**	1.319*	5.89**	0.030**	0.040**	30.07**	7.98**	12.94*	1.72*	7.33**	2.86**
Error	-	7.20	5.648	9.67	1.00	3.94	7.55	2.122	0.83	0.002	0.004	2.74	9.12	0.99	0.79	5.32	0.50
Coefficient of variation (%)	-	14.42	14.23	12.21	11.25	13.63	13.84	14.58	12. 39	7.08	8.99	10.35	8.43	9.59	8.23	8.43	10.22

**, *, ns, respectively, significant at 1%, 5%, and non-significant

Table 2. Effect of drought stress (FC) and foliar application of citric acid (CA) on morphological traits

FC (%)	CA (mg l^{-1})	Fresh Weight of Shoot (g)	Dry Weight of Shoot (g)	Fresh Weight of Root (g)	Dry Weight of Root (g)	Plant Height (cm)	Root Length (cm)	Flowers Number	Flower Longevity (day)
100	0	299.69^{e}	28.86^{d}	60.16^{de}	7.81^{d}	29.83^{d}	34.33^{de}	7.53^{d}	11.20^{d}
	50	344.93^{c}	33.87^{b}	65.21^{d}	11.37^{b}	35.33^{a}	44.00^{a}	11.16^{a}	12.50^{c}
	100	332.81^{d}	31.86^{c}	79.80^{ab}	12.71^{a}	34.50^{ab}	27.66^{c}	7.93^{cd}	13.70^{ab}
75	0	367.02^{b}	32.76^{bc}	57.38^{e}	8.81^{cd}	30.83^{cd}	35.00^{d}	8.33^{c}	11.80^{cd}
	50	390.67^{a}	35.43^{a}	81.06^{a}	12.37^{ab}	33.83^{b}	41.33^{b}	10.33^{b}	13.00^{b}
	100	379.15^{ab}	35.37^{a}	77.86^{b}	9.42^{c}	31.83^{c}	43.00^{ab}	10.70^{ab}	13.80^{a}
50	0	290.74^{f}	26.22^{ef}	52.13^{f}	6.27^{fg}	25.16^{g}	34.11^{e}	6.16^{f}	8.80^{g}
	50	333.79^{d}	28.54^{de}	74.03^{c}	6.91^{e}	28.50^{e}	32.00^{f}	7.50^{de}	11.00^{e}
	100	322.82^{de}	26.70^{e}	49.58^{fg}	7.45^{de}	27.66^{ef}	36.33^{cd}	6.50^{ef}	10.20^{f}
25	0	155.17^{h}	20.69^{h}	36.23^{h}	4.18^{h}	23.50^{h}	29.33^{g}	5.16^{g}	8.30^{h}
	50	171.22^{gh}	25.71^{f}	48.46^{g}	5.83^{g}	22.16^{i}	26.66^{h}	3.83^{h}	8.70^{gh}
	100	175.85^{g}	23.81^{g}	37.80^{h}	6.49^{f}	26.50^{f}	30.66^{fg}	7.03^{e}	10.60^{ef}

Values marked by different letters are significantly different (P<0.05).

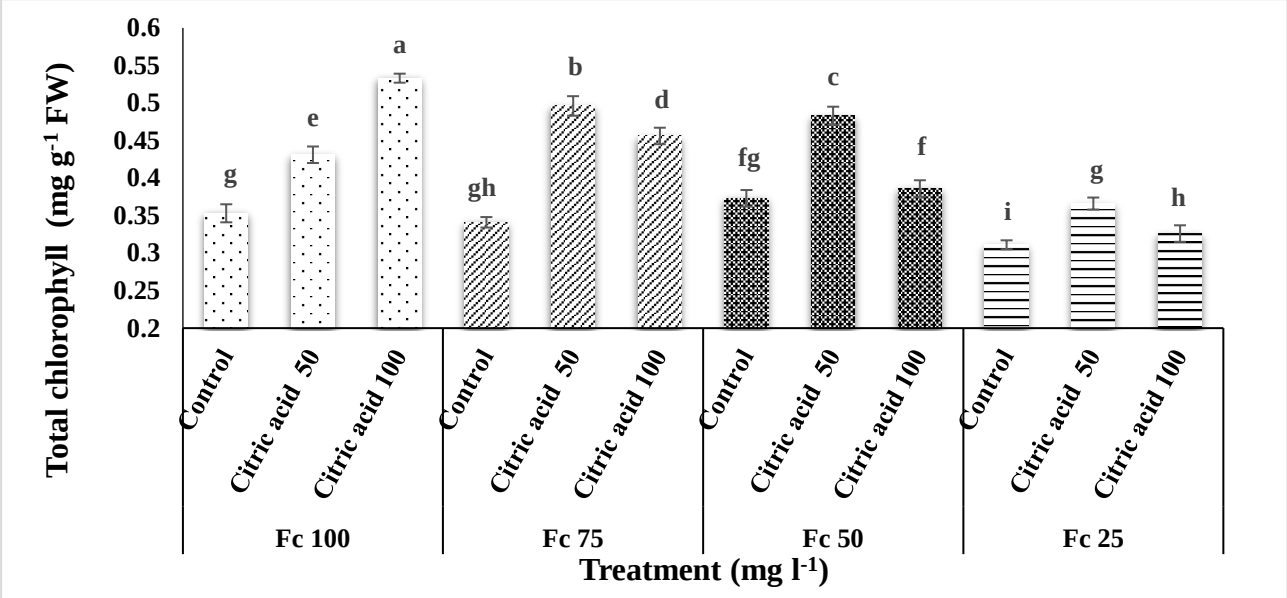

Fig. 1. Effect of drought stress (FC) and foliar application of citric acid (CA) on total chlorophyll. Values marked by different letters are significantly different (P<0.05).

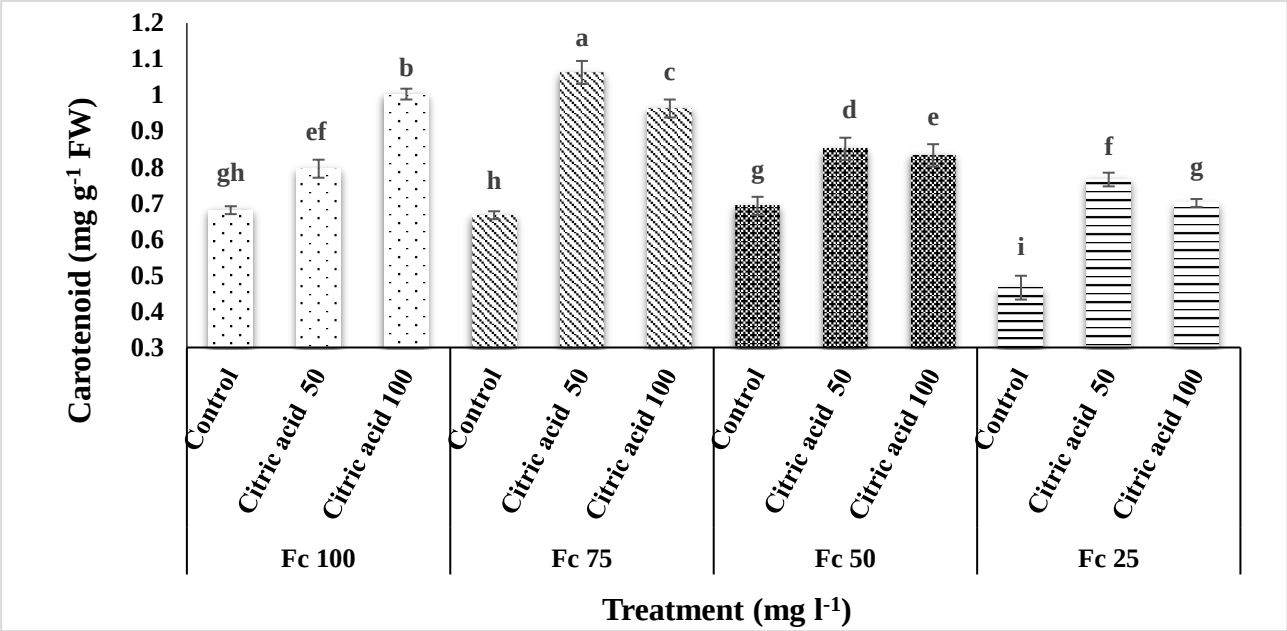

Fig. 2. Effect of drought stress (FC) and foliar application of citric acid (CA) on carotenoid content. Values marked by different letters are significantly different (P<0.05).

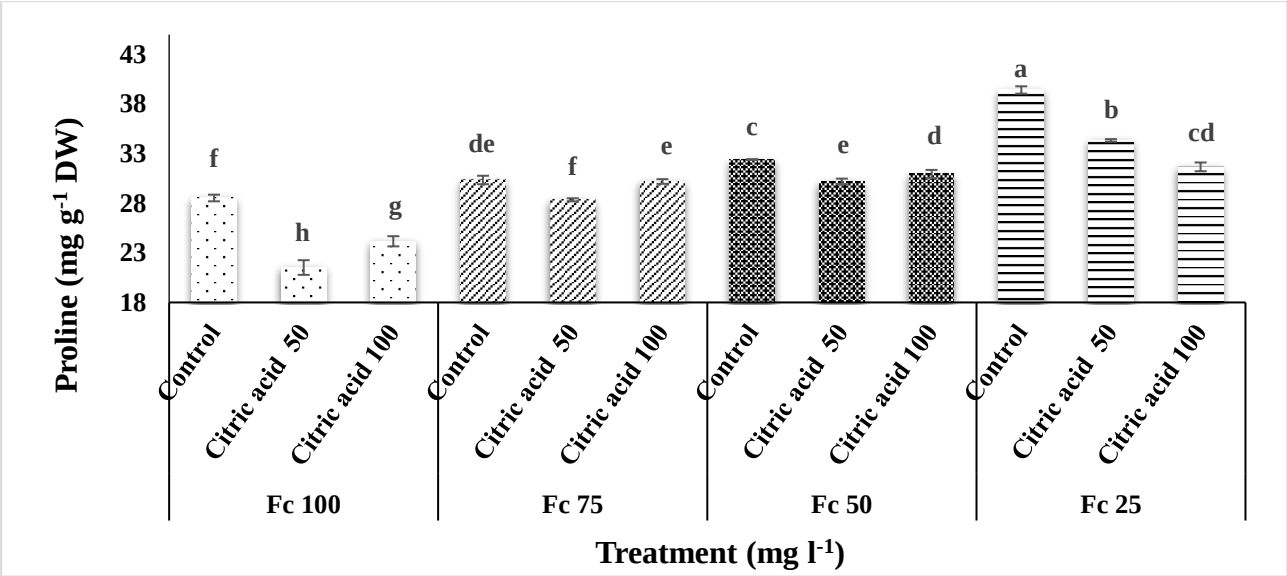

Fig. 3. Effect of drought stress (FC) and foliar application of citric acid (CA) on proline content. Values marked by different letters are significantly different (P<0.05).

Total phenol

The results showed that the highest and lowest phenol contents (130.83 and 49.75 mg g^{-1} DW) were obtained in response to the 75% FC + CA 50 mg l^{-1} and the 25% FC (Fig. 4).

Flavonoids

Data showed that the highest and lowest flavonoid contents (22.35 and 17.26 mg g^{-1} DW) were found in response to the 75% FC + CA 50 mg l^{-1} and the 25% FC (Fig. 5).

Vitamin C

As seen in Figure 6, the amount of vitamin C was highest (6.81 mg.100 g^{-1} FW) in the control irrigation + CA 100 mg l^{-1}, whereas the lowest value (2.38 mg.100 g^{-1} FW) was observed as a result of the 25% FC.

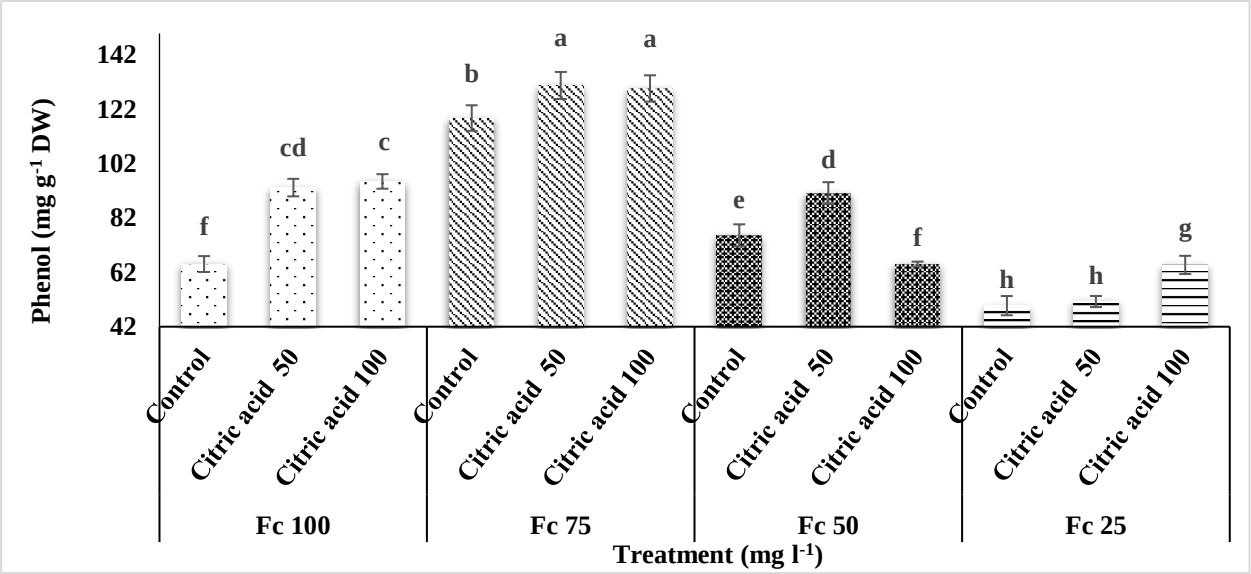

Fig. 4. Effect of drought stress (FC) and foliar application of citric acid (CA) on phenol content. Values marked by different letters are significantly different (P<0.05).

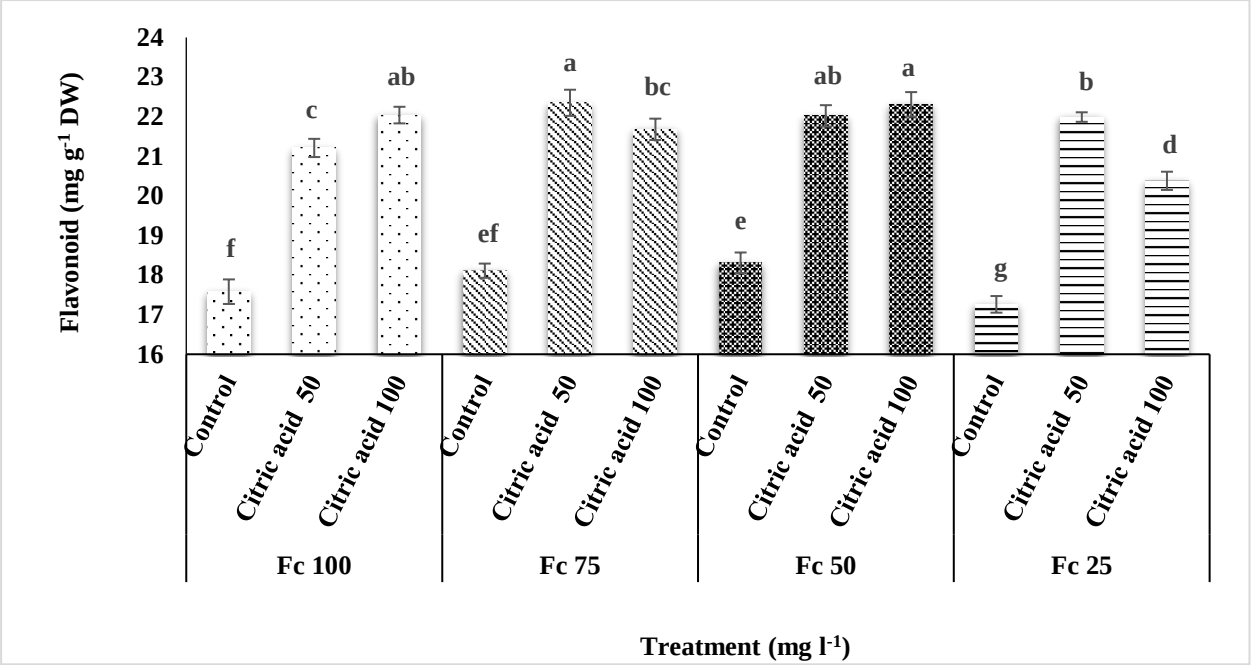

Fig. 5. Effect of drought stress (FC) and foliar application of citric acid (CA) on flavonoid content. Values marked by different letters are significantly different (P<0.05).

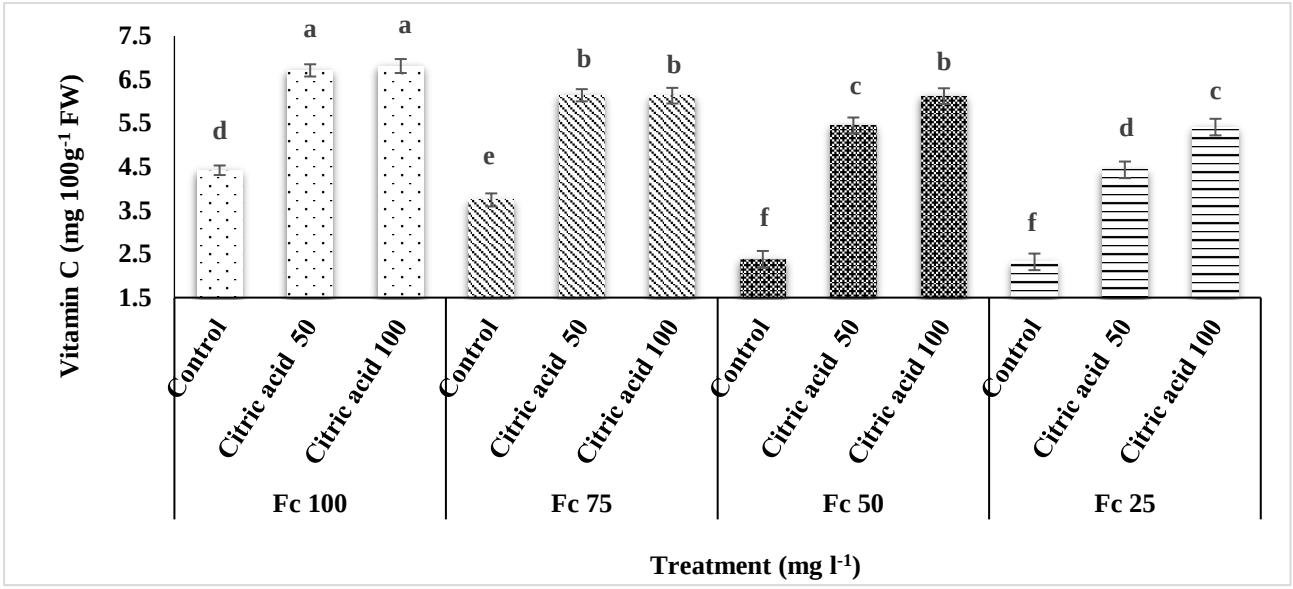

Fig. 6. Effect of drought stress (FC) and foliar application of citric acid (CA) on vitamin C content. Values marked by different letters are significantly different (P<0.05).

Total carbohydrates

Data showed that the highest and lowest total carbohydrate contents (14.21 and 9.23 mg g^{-1} DW) were observed in response to 75% FC + CA 100 mg l^{-1} and 25% FC, respectively (Fig. 7).

Antioxidant activity

As presented in Figure 8, the highest antioxidant activity (84.11%) was related to the 75% FC+ CA 100 mg l^{-1}, whereas the lowest (27.99%) was related to the 25% FC treatment.

Rutin

The present study showed that the highest and lowest amounts of rutin (9.47 and 2.04 mg l^{-1}) were obtained in the treatment groups of 75% FC + CA 100 mg l^{-1} and 25% FC, respectively (Fig. 9).

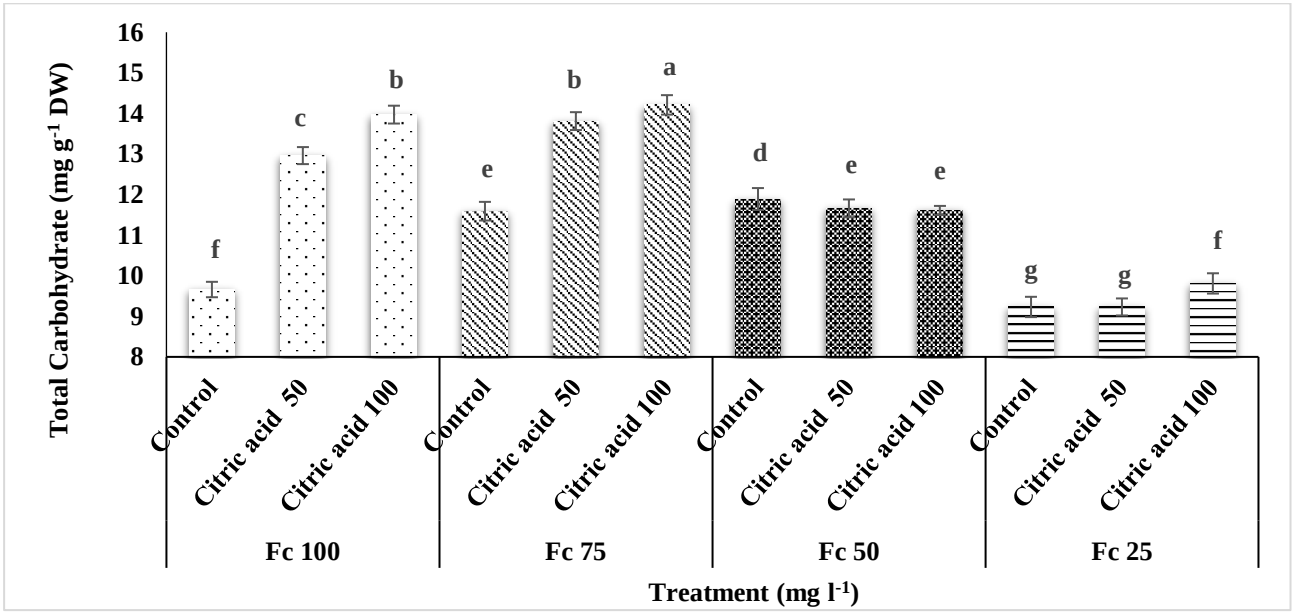

Fig. 7. Effect of drought stress (FC) and foliar application of citric acid (CA) on the total carbohydrate content. Values marked by different letters are significantly different (P<0.05).

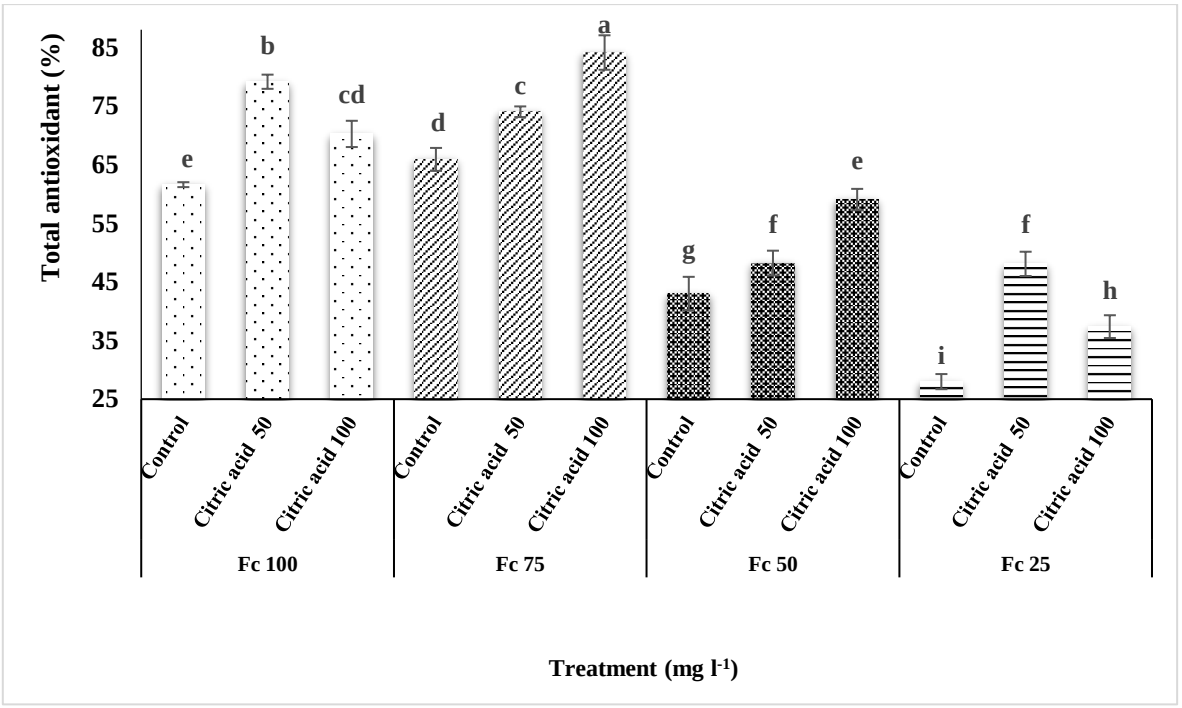

Fig. 8. Effect of drought stress (FC) and foliar application of citric acid (CA) on total antioxidant activity. Values marked by different letters are significantly different (P<0.05).

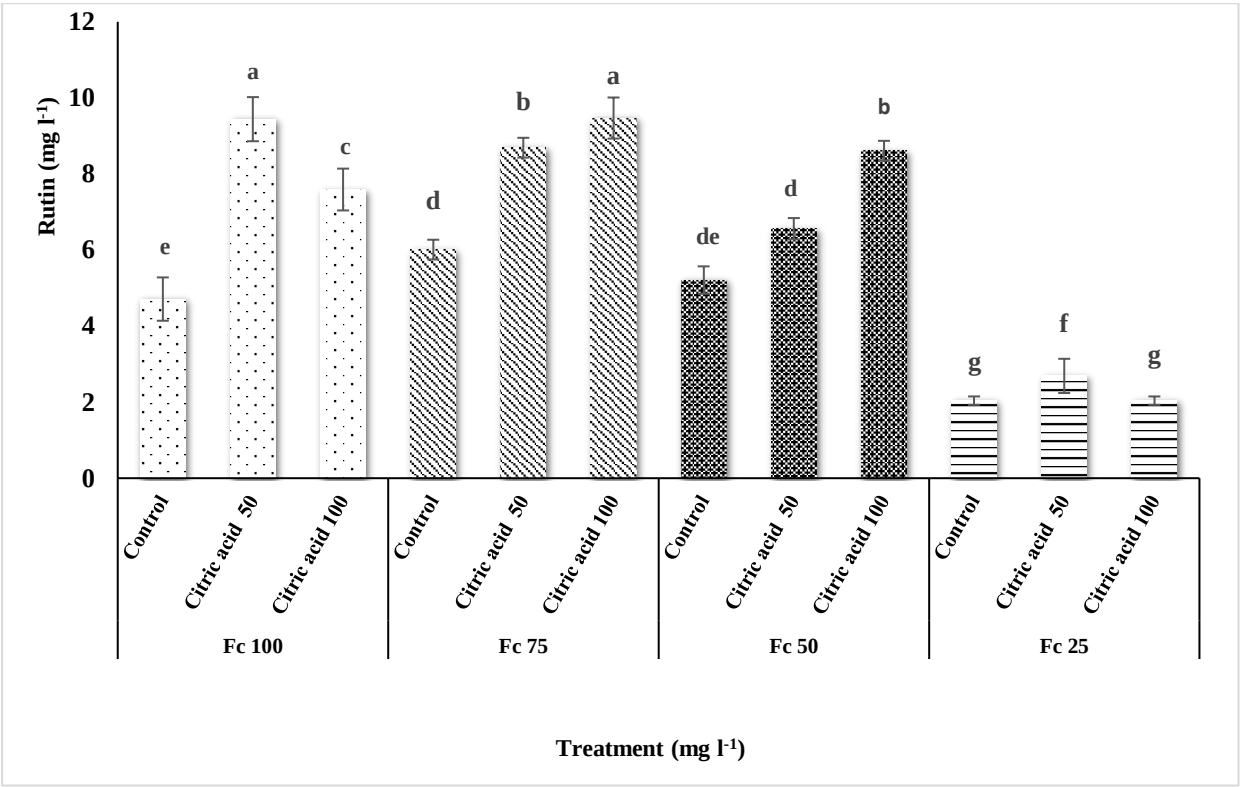

Fig 9. Effect of drought stress (FC) and foliar application of citric acid (CA) on rutin. Values marked by different letters are significantly different (P<0.05).

Discussion

The results showed that drought stress can reduce plant growth and yield by disrupting the regular functions of the photosynthetic system, thereby reducing the stability of carbon and leaf area, impeding cell division, closing stomata, and limiting water and nutrient absorption (Farzamisepehr et al., 2021). In addition, plants under severe stress are likely to produce more free oxygen radicals, which will destroy phospholipids, release fatty acids, increase membrane permeability, and result in a shortened flower longevity because of ethylene synthesis (Hossaina et al., 2005). Foliar application of CA may improve plant growth and yield under drought stress due to its role in regulating osmotic potential and the Krebs cycle. Moreover, as a natural chelating agent, it assists in increasing water and nutrient uptake and enhances phytohormone synthesis under stress (Miri et al., 2015). On the other hand, CA inhibits the activity of the synthetase enzyme by lowering the pH, acidifying the environment, preventing the formation of ethylene, and eventually increasing flower longevity (Eidyan et al., 2014). The results of this research are in line with previous findings by Hong and Sun (2011) on *Leymus chinensis*, as well as research by Naseri-Moghadam et al. (2019) on *Narsicuss tazetta* L., and Eidyan et al. (2014) on *Polianthes tuberosa* L. According to the results, total chlorophyll and carotenoid contents decreased with the increasing intensity of drought stress. The decrease in chlorophyll under drought stress can be explained by the closure or disruption of stomata, along with damage to photosystem structural proteins and chlorophyllase activity, as well as a decline in carotenoid content which may result from the decline in photosynthesis. These are usually associated with a higher production rate of oxygen radicals, leading to a faster peroxidation rate of these pigments and chemical degradation of genes related to their biosynthetic pathway (Idrees et al., 2010). The decrease in total chlorophyll and carotenoid contents corresponded to a similar pattern observed in other plants like *Origanum vulgare* L. (Minaei et al., 2019) and *Tagetes erecta* L. (Tian et al., 2012). Moreover, foliar application of CA decreased the pH of leaf extract, which activated leaf iron and increased chlorophyll synthesis. In addition, citric acid prevented the reduction of pigments under stress by reducing optical oxidation and maintaining the integrity of photosynthetic membranes. According to a previous study, CA application on *Thymus vulgaris* L. positively affected total chlorophyll and carotenoid contents (Miri et al., 2015).

Proline is considered an indicator of stress sensitivity in plants. An increase in the amount of proline in plant tissues indicates the activation of the osmotic regulation mechanism, which provides the conditions for more water and salts to be absorbed from the root environment (Chavoushi et al., 2019). This study showed that the highest proline content was observed in response to the 25% FC treatment, which confirms similar findings by Chavoushi et al. (2019) on *Carthamus tinctorius* L.

Phenols protect plants under stress by increasing osmotic potential or by controlling redox potential and eliminating active oxygen from the cells. The decrease in phenol content during severe stress conditions illustrates that these conditions are detrimental to antioxidant properties in plants (Toberman et al., 2008). In this study, the amount of phenol decreased with the increase in drought stress, which was noted in earlier research by Bettaieb et al. (2011) on *Salvia officinalis* L., in which the application of CA significantly increased phenol levels under drought stress. Several phenolic compounds accumulate in cellular vacuoles, and their hydrolysis by CA releases these inaccessible substances. The increase in phenol, following the foliar application of CA, may be attributed to the release of phenolic acids after the breakdown of cellular components and walls (Preciado-Rangel et al., 2018). Similar results were noted in the case of lentil sprout (*Lens culinaris*) (Salas-Pérez et al., 2018). The results showed that the flavonoid content decreased with increasing stress intensity. As the intensity of drought stress increased by stimulating other antioxidant mechanisms of the plant, flavonoids appeared to play a subordinate role in removing oxidative compounds. Consequently, their content decreased under stress and more reactive oxygen species were produced. Enzyme systems were activated to scavenge these radicals and flavonoids were activated in advance, although the effect of stress ultimately activated the enzyme system and resulted in a decrease in flavonoid content (Jubany-Mari et al., 2010). Foliar application of CA increased the flavonoid content in plants because CA possibly activated the signaling pathways of secondary metabolism in plants (Salas-Pérez et al., 2018), thereby having an effective role in increasing the amounts of these compounds. The results of this experiment are consistent with previous findings by Habibi (2018) on Aloe vera, and by Salas-Pérez et al. (2018) on lentil sprouts.

In horticultural produce, vitamin C plays an important role in consumer health. Since these

compounds are very susceptible to oxidation, drought stress resulted in a reduction of vitamin C due to the degradation of ascorbate by oxygen-free radicals. This was associated with the consumption of ascorbate to produce zeaxanthin and alpha-tocopherol. CA also protected cell membranes and cellular contents, including vitamin C, due to its antioxidant properties, by reducing pH and acidifying the medium. Thus, it inhibited ACC synthetase activity and prevented ethylene production and vitamin C reduction (Soroori et al., 2021a).

The results showed that severe drought stress (25% FC) decreased the total carbohydrate content. The decrease in these soluble compounds under severe stress could be related to the use of sugars in the synthesis of metabolites such as proline in the aerial parts of plants (Sodaizadeh et al., 2016). Farhoudi et al. (2014) reported similar results on *Matricaria recutita* L, whereby the increase in carbohydrates under CA spraying was explained by the effect of CA on increasing photosynthetic pigments which, in turn, increased photosynthesis and carbohydrate production (Fayed, 2010).

While the total antioxidant activity decreased with the increase in stress intensity, drought stress reduced plant growth and photosynthesis and decreased the availability of raw materials for synthesizing secondary compounds. Thus, these events reduced the antioxidant capacity of the plant (Hamidipour, 2016). Foliar application of CA reduced the levels of MDA, H_2O_2, and O_2 while protecting cells from stress damage. Generally, free radicals are generated during plant metabolism and lead to lipid oxidation, permeability loss, and cell death, whereas CA application reduces this damage by removing free radicals (Hu et al., 2016). This is in agreement with previous findings by El-Tohamy et al. (2013) on *Phaseolus vulgaris* L.

Severe drought stress reduced rutin in pot marigold petals. Rutin is a valuable flavonoid compound that is anti-inflammatory, with antioxidant activity, and can remove free radicals as the drought intensifies and more stomata are closed. The amount of carbon available to cells and the concentration of unstructured carbohydrates in specific plant tissues reduced sharply. As the stress continued to increase, the plant was forced to use its full potential to prevent cell death and use the flavonoid deposits in its vacuoles to detoxify more free radicals (Turtola et al., 2005). According to the results of this experiment, CA increased the rutin content to take advantage of its high antioxidant properties in the synthesis of phenolic and flavonoid compounds (Salas-Pérez et al., 2018).

Conclusion

Due to the prevalence of drought stress in Iran, the use of some organic substances such as citric acid in stress conditions can increase the quality and growth indicators of plants. According to the results, foliar application of citric acid increased the fresh and dry weights of aerial parts and roots, plant height, root length, number of flowers, longevity of flowers on plants, carotenoids, total chlorophyll, phenol, flavonoids, total antioxidant activity (DPPH), vitamin C, proline, and rutin. Severe drought stress reduced values in all traits except proline. In general, foliar application of different concentrations of CA on pot marigold reduced the adverse effects of drought stress due to its high antioxidant properties. CA increased the values of several growth indices and biochemical traits in pot marigold. Accordingly, the application of 75% FC and CA (100 mg l^{-1}) may be recommended for the increase in some morphological and biochemical traits of *Calendula officinalis* L.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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