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Research article

Improvement of physiological and phytochemical parameters of pot marigold (*Calendula officinalis*) using foliar application of zinc

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Abstract

The foliar application of micronutrients directly affects quality and yield. A pot experiment was used to investigate the effect of different concentrations of zinc (Zn) on the physiological and phytochemical traits of marigold (*Calendula officinalis*). Treatments consisted of five different concentrations of zinc nitrate (0 mg/L as the control; 0.5 mg/L; 1 mg/L; 1.5 mg/L; 2 mg/L) in four replications. The foliar application of the different concentrations of Zn was performed in two stages, with the first stage at 2–4 leaves and the second stage at the time of budding. The results indicated that different concentrations of Zn significantly affected the physiological and phytochemical parameters of marigold. The best Zn concentration for fresh weight traits, chlorophyll index, chlorophyll a, chlorophyll b, total carotenoid and β-carotene, was the fourth treatment (1.5 mg/L) of Zn. However, for the lycopene, anthocyanin, total phenol and total flavonoid traits, the highest measured value was related to the fifth treatment (2 mg/L). Finally, the best antioxidant activity in both assays applied was observed in the third treatment (1 mg/L). Therefore, application of appropriate concentrations of Zn can improve the physiological and phytochemical traits of pot marigold flowers.

Introduction

Calendula officinalis L., commonly known as marigold, is an ancient medicinal herb belonging to the Compositae family and in folk and aromatherapy medicine it has been used for fever, skin disorders, conjunctivitis (pink eye) and poor vision, irregular menstrual cycle, varicose veins, mouth and throat soreness, hemorrhoids and muscle spasms (Bisset and Wichtl, 1994). *Calendula officinalis* grows as a native and naturalized plant across Europe and North America (Verma et al., 2018). The yellow-orange-red flowers of *Calendula* are used as a herbal medicine and spice with dried and fresh flowers of marigold being used in lotions, tinctures, liniments, ointments masks and creams (Leach, 2008). The biological activities of *Calendula* are related to the existence of several major classes of natural compounds such as phenolics (flavonoids, anthocyanins, tannins), terpenoids (carotenoids, triterpene alcohols, essential oils), triterpenyl alcohols, steroids, triterpenoid aglycone, polysaccharides and mucilage (Vidal-Ollivier et al., 1989). Previous researchers reported that *Calendula* flowers contained quercetin-3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin, isorhamnetin-3-*O*-rutinosylrhamnoside and isorhamnetin-3-*O*-glucosylglucoside (Bilia et al., 2000).

Zinc (Zn) is considered as a multipurpose trace mineral that plays a critical role in many important metabolic pathways (Aravind and Prasad, 2005a; Song et al., 2015). Zn is a vital co-factor for various enzymes such as carbonic anhydrase, dehydrogenases, peroxidases and oxidases (Aravind and Prasad, 2003; 2005b). In addition, Zn contributes to the formation of chlorophyll by involvement in the regulation of cytoplasmic levels of elements. Zn can also improve the synthesis of chloroplast pigments such as carotenoids and chlorophyll, eventually proving useful for the photosynthetic system of plants (Aravind and Prasad, 2004; Song et al., 2015). In many plant species, Zn plays an essential role as a regulatory cofactor of several enzymes or structural components that catalyze an extensive range of phytochemical pathways. These roles include structural stability to cell membranes, antioxidant defense system, protein metabolism, mediator of cellular signaling, pollen formation, regulator of gene expression and the resistance to infection by certain pathogens (Foster and Chu, 2014; Fung and Gildengorin, 2015). Therefore, the presence of sufficient concentrations of

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Zn in plants is essential for the proper functioning of plant cell systems.

The Zn assimilation network comprises the harmonized activities of Zn absorption, translocation, trafficking, chelation and sequestration, supplying a sufficient amount of Zn to different types of plant cells, at all stages of growth and under various environmental conditions (Clemens, 2001; Assunção et al., 2010). However, Zn is an essential nutrient, it can be toxic to plants when present in excessive amounts. Thus, plants control Zn homeostasis using a firmly regulated network in which the coordinated expression of Zn transporters plays the main role in Zn inception from the soil, in mobilization between organs and tissues and in intracellular sequestration (Clemens, 2001; Clemens et al., 2002; Assunção et al., 2010). Zn deficiency in plants is mostly adjusted by the application of Zn to soils. Sulphate-containing fertilizers such as $ZnSO_4$ is used widely as a source of elemental Zn, due to it being very soluble in water and existing in both granular and crystalline structures (Mortvedt and Gilkes, 1993). Zn deficiency is a global and well-documented problem in plants and can lead to decreases in yield and nutritional quality (Chaudhry and Loneragan, 1970; Henriques et al., 2012; Kabir et al., 2014). When facing a shortage in Zn supply, plants adapt by enhancing Zn acquisition. Fewer studies have investigated the effect of Zn fertilizer on medicinal herbs. *Calendula officinalis* as a medicinal herb have many applications in the pharmaceutical and food industry. The aim of this study was to investigate the effect of different concentrations of Zn fertilizer on the physiological and phytochemical properties of pot marigold.

Material and Methods

Plant materials

An orange marigold cultivar (*Calendula officinalis*) was used, as it is one of the leading commercial cultivars in the world. The seeds were first cultivated in transplant culture trays (with a mixture of pit moss and perlite at a ratio of 70:30). The seedlings were then transferred to the main pots (size 16) 1 mt later (at the four-leaf stage), with one plant to a pot. The plants were grown under natural light (800 μmol/m^2/s) in the greenhouse (26/21°C day/night temperature and 55% relative humidity). During the experiment, each pot was fertigated by hand using a standard nutrient solution (a complete fertilizer containing N:P:K at 20:20:20 + trace element (TE) + amino acid) for 90 d. Each pot was fertigated every other day with 200 mL of nutrient solution. Urea fertilizer was also applied (0.5 g/L) every 2 wk.

Experimental site and design

The pot experiment was conducted in 2018 in a north-south direction. The experimental site was located in the research greenhouses of Urmia University, Urmia, Iran (44.97°E, 37.65°N at 1,365 m above mean sea level). The treatments consisted of five different concentrations of zinc nitrate (0 mg/L as the control; 0.5 mg/L; 1 mg/L; 1.5 mg/L; 2 mg/L) in four replications. Foliar application of different concentrations of Zn was performed in two stages, with the first at 2–4 leaves and the second at the time of budding.

Physiological parameters

Chlorophyll index

Three leaves (1, 2 and 2 leaflets) were selected and measured using a SPAD instrument (Minolta; Japan) and their mean was recorded as the chlorophyll index.

Fresh and dry weight of flowers

After harvesting the flowers, their fresh and dry weights were weighed to an accuracy of 0.001 g.

Diameter of flower and peduncle

The diameter of flowers was measured using a ruler and the diameter of peduncle was measured using a pair of digital calipers.

Phytochemical parameters

Extract preparation from plant samples

Dried plant samples were used for extraction. A sample of 0.2 g of the petals was powdered and poured with 5mL methanol 80% into a *conical centrifuge tube* (Falcon). The specimens were then placed in an ultrasonic apparatus at 30°C for 30 min for extraction. Samples were used for phytochemical measurements after filtration (Alirezalu et al., 2018).

Measurement of total phenol content

The phenolic contents were measured using Folin-Ciocalteu reagent. A sample of 100 μL of the extract was diluted to 1 mL (diluted 10-fold). Then, 1.6 mL of deionized water was added to 200 mL of diluted sample. Next, 200 μL of the Folin-Ciocalteu reagent was added to the mixture and after 5 min, 2 mL of 7% sodium carbonate was added and the mixture was finally made up to 5 mL with deionized water. Then, the samples were incubated at laboratory temperature for 35–45 min. Finally, the absorbance of each sample was read at 765 nm using a spectrophotometer. The standard curve was plotted based on gallic acid (GAE) and the results were reported as milligrams of gallic acid per gram on a dry weight (DW) basis (Shameh et al., 2019).

Measurement of total flavonoid content

$AlCl_3$ reagent was used to evaluate the total flavonoid content. Initially, 500 μL of each extract was added with 1.5 mL of 80% methanol, 100 μL of 10% $AlCl_3$ solution, 100 μL of 1 M CH_3CO_2K solution and 3.8 mL of distilled water. The absorbance of the mixture was read at 380 nm after 40 min. Quercetin was used to draw the standard curve. The total flavonoid content of each extract was reported based on milligrams quercetin per gram DW of plant (Moshari-Nasirkandi et al., 2020).

Measurement of total anthocyanin content

A sample of 0.1 g was crushed in Chinese mortar with 10 mL of acidic methanol (pure methanol and pure hydrochloric acid at a volume ratio of 99:1, respectively) and the extract was poured into a tube and placed in the dark for 24 hr at 25°C. Then, the extract was centrifuged for 10 min at 4,000 revolutions per minute (rpm) and the absorbance of the supernatant was read using a spectrophotometer at a wavelength of 550 nm. The extinction coefficient equation of $\varepsilon = 33{,}000/mol/cm$ was used to calculate the anthocyanin concentration (Gholizadeh-Moghadam et al., 2019). Finally, the concentration of anthocyanin was calculated according to the relation of ($A= \varepsilon bc$), where A is the sample absorption, b is the cell width and c is the concentration of the desired solution measured in micromoles per gram of petal fresh weight (FW).

Measurement of lycopene content

Lycopene was measured using a spectrophotometer based on the method of Shameh et al. (2019). A sample of 2.5 g was weighed into an Erlenmeyer flask, 4 mL of deionized water was added and stirred for 1 min using a magnetic stirrer. Then, 50 mL of mixture (absolute ethanol:hexane:acetone in the ratio 2:1:1, respectively) was added and was stirred for 10 min on a magnetic stirrer. Then, after adding 7.5 mL of deionized water, the mixture was stirred for another 5 min until the two separate layers had formed. Next, the upper yellow layer containing lycopene was carefully separated and its absorbance was read at 520 nm using a spectrophotometer.

Measurement of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl assay

An amount of 5 µL of the sample methanol extract (5 times diluted) was poured into a tube and 2,000 µL of pre-prepared 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) solution was added. The resulting solution was shaken and kept at room temperature for 35 min and the absorbance was read at 516 nm using a spectrophotometer. To prepare the blank, 50 µL of 80% ethanol was used instead of the extract (Shaghaghi et al., 2019) The calculation is shown in Equation (1):

$$\text{DPPHsc\%} = \frac{(\text{Abe control})_{t=35\ min} - (\text{Abe sample})_{t=35\ min}}{(\text{Abe control})_{t=35\ min}} \times 100 \quad (1)$$

where Abs control = the absorption rate of the blank and Abs sample = the absorption rate of the sample.

Measurement of antioxidant activity using ferric reducing antioxidant power assay

The diluted extracts of the samples were mixed with 3 mL of fresh ferric reducing antioxidant power (FRAP) reagent (300 mM sodium acetate buffer with 3.6 acidity, ferric-tripyridyl-S-triazine 2 and ferric acid). The resulting mixture was placed in a water bath for 30 min at 37°C and its absorbance was read at 593 nm using a spectrophotometer. Iron sulfate was used to draw the standard curve and the results were expressed in micromoles of Fe per gram DW (Alirezalu et al., 2020).

Measurement of chlorophyll a, b, carotenoid and β-carotene contents

Measurement was done according to Licententhaler (1987). A sample of 0.1 g of leaf tissue (fully developed leaves) was crushed and lysed in a Chinese mortar with 5 mL of 100% acetone to form a uniform mass, with the grinding and crushing of the leaf tissue being done in liquid nitrogen and at a low light intensity. A sample of 0.5 mL of the mixture was removed and 2.5 mL of distilled water was added. The samples were centrifuged at 2,500 rpm for 10 min. After centrifugation, the supernatant was separated and its absorbance was read at 663, 645 and 470 nm (A_{663}, A_{645}, A_{470}) using a spectrophotometer. Finally, using the following Equation 2–5, the contents of chlorophyll a, b, total carotenoid and β-carotene of samples were obtained (Lichtenthaler, 1987):

$$Ca = 12.7\ (A_{663}) - 2.69\ (A_{645}) \quad (2)$$

$$Cb = 22.9\ (A_{645}) - 4.68\ (A_{663}) \quad (3)$$

$$\text{Total carotenoids} = 1{,}000\ A_{470} - 2.270\ C_a - 81.4\ C_b / 227 \quad (4)$$

$$\beta\text{-Carotene} = 0.854\ A_{479} - 0.312\ A_{645} + 0.039\ A_{663} - 0.005 \quad (5)$$

Statistical analysis

Data were analyzed using analysis of variance facilitated by the SAS 9.13 software (SAS Institute, 2008). Comparisons of means was done using Duncan's new multiple range test.

Results

The analysis of variance of different morphological, physiological and phytochemical parameters in marigold grown under different concentrations of Zn demonstrated that the treatment effects were highly significant ($p < 0.01$) in all traits except flower diameter.

Fresh and dry weight of flowers

The results showed that the effect of different concentrations of Zn on flower fresh weight and dry weight were highly significant ($p < 0.01$), as shown in Table 1. The highest fresh weight was observed in the fourth treatment (9.66 g) and the lowest fresh weight (5.43 g) in the fifth treatment (Fig. 1A). The different Zn concentrations had no significant effect on flower fresh weight though with increasing Zn concentration, the flower fresh weight of marigold decreased. The highest flower dry weight (0.86 g) was observed in the control treatment and the lowest dry weight (0.55 g) in the fifth treatment. However, there was no significant difference between the control and the fourth treatment (Fig. 1B).

Diameter of flower and peduncle

The data analysis (Table 1) showed that there were no significant differences among the different concentrations of Zn in flower diameter, but a highly significant ($p < 0.01$) difference was observed in the peduncle diameter. There were no significant differences among the first to fourth treatments in peduncle diameter. The lowest peduncle diameter (3.81 mm) was obtained in the fifth treatment (Fig. 1C).

Chlorophyll index

Data analysis (Table 1) revealed that the effect of different concentrations of Zn on chlorophyll index was significant ($p < 0.01$). Different concentrations of Zn, up to the fourth treatment had an increasing effect on the chlorophyll index and this decreased with increasing Zn concentration in the fifth treatment. The highest chlorophyll index (53.03) was observed in the fourth treatment and the lowest chlorophyll index (48.23) was in the control treatment (Fig. 1D).

Table 1 Results of analysis of variance (F-value) of morphological parameters of marigold

Source of Variation	df	Fresh weight	Dry weight	Flower diameter	Peduncle diameter	Chlorophyll index
Zinc	4	9.003**	0.045**	0.321^{ns}	0.248**	11.559**
Error	10	0.074	0.001	0.092	0.038	0.32
CV		3.626	4.335	3.512	4.541	1.108

** = significant at $p < 0.01$; ^{ns} = not significant; CV = Coefficient of variation

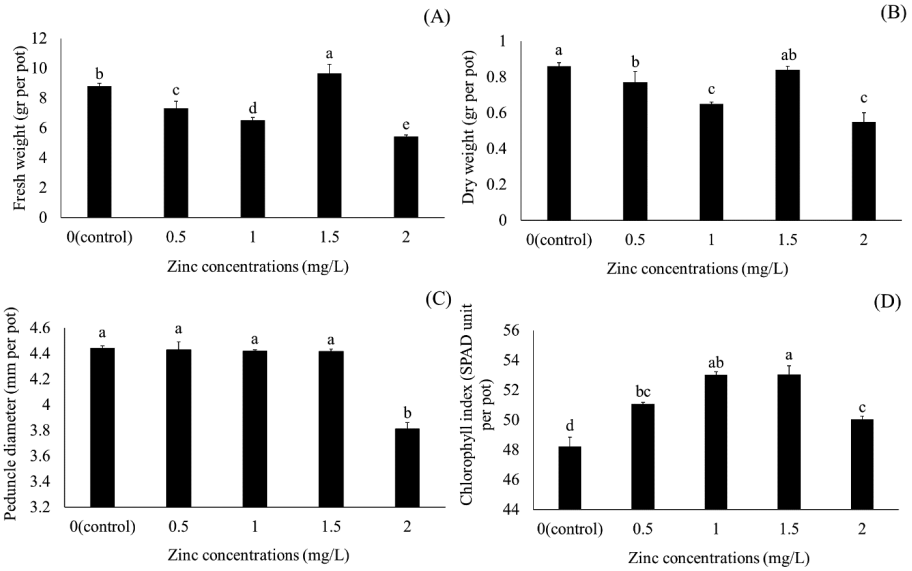

Fig. 1 Effect of different concentrations of zinc on marigold: (A) fresh weight; (B) dry weight; (C) peduncle diameter; (D) chlorophyll index, where different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD.

Chlorophyll a and b contents

Data analysis (Table 2) showed that there was a significant ($p < 0.01$) difference between different concentrations of Zn in terms of the chlorophyll a, b and carotenoids contents. Different concentrations of Zn, from the first to the fourth treatment, had an additive effect on the chlorophyll a and b contents, but in the fifth treatment the amount of chlorophyll a decreased. The highest chlorophyll a content (16 mg/g FW) was observed in the fourth treatment and the lowest chlorophyll a content (6.55 mg/g FW) was in the control treatment (Fig. 2A). In addition, the highest chlorophyll b content (6.283 mg/g FW) was observed in the fourth treatment and the lowest chlorophyll b content (3.34 mg/g FW) was in the control treatment (Fig. 2B).

Carotenoid contents

Data analysis (Table 2) showed that there was a significant ($p < 0.01$) difference between the different concentrations of Zn in terms of the carotenoids content. The highest carotenoid content (15.44 mg/g FW) was observed in the fourth treatment and the lowest (7.17 mg/g FW) was in the fifth treatment (Fig. 2C).

β-carotene content

Data analysis (Table 2) showed that there was a significant ($p < 0.01$) difference between the different concentrations of Zn for the beta-carotene content. The highest beta-carotene content (13.12 µg/g FW) was observed in the fourth treatment and the lowest (6.05 µg/g FW) was in the fifth treatment (Fig. 2D).

Lycopene content

The results of the analysis of variance in Table 2 showed that there was a significant ($p < 0.01$) difference between the different concentrations of Zn in terms of the lycopene content at 0.01 level. The highest amount of lycopene was observed in the fifth treatment (6.44 µg/g FW) and the lowest (2.98 µg/g FW) was in the third treatment (Fig. 3A).

Anthocyanin content

The results of the analysis of variance in Table 3 showed that there was a significant ($p < 0.01$) difference between the different concentrations of Zn in terms of the anthocyanin content. The highest amount of anthocyanin (144.97 µmol/g FW) was observed in the fifth treatment and the lowest amount (129.19 µmol/g FW) was in the fourth treatment (Fig. 3B).

Total phenol content

The effect of different concentrations of Zn on phenol content was significant ($p < 0.01$), as shown in Table 3. The control, second, third and fourth treatments were not significantly different. The highest amount of phenol (46.78 mg GAE/g DW) was recorded in the fifth treatment (Fig. 3C).

Total flavonoid content

The results of data analysis (Table 3) showed that there was a significant ($p < 0.01$) difference between the different concentrations of Zn in the flavonoid content. The highest flavonoid content (8.84 mg quercetin/g DW) was observed in the third treatment and the lowest (6.07 mg quercetin/g DW) was in the second treatment. However, there were no significant differences among the third, fourth and fifth treatments (Fig. 3D).

Table 2 Results of analysis of variance (F-value) of physiological and phytochemical parameters of marigold

Source of Variation	df	Chlorophyll a	Chlorophyll b	Carotenoids	Beta-carotene	Lycopene
Zinc	4	43.695**	3.946**	33.879**	24.646**	6.072**
Error	10	0.047	0.012	0.003	0.002	0.005
CV		1.983	2.386	0.517	0.522	1.555

** = significant at $p < 0.01$; df = degree of freedom; CV = Coefficient of variation

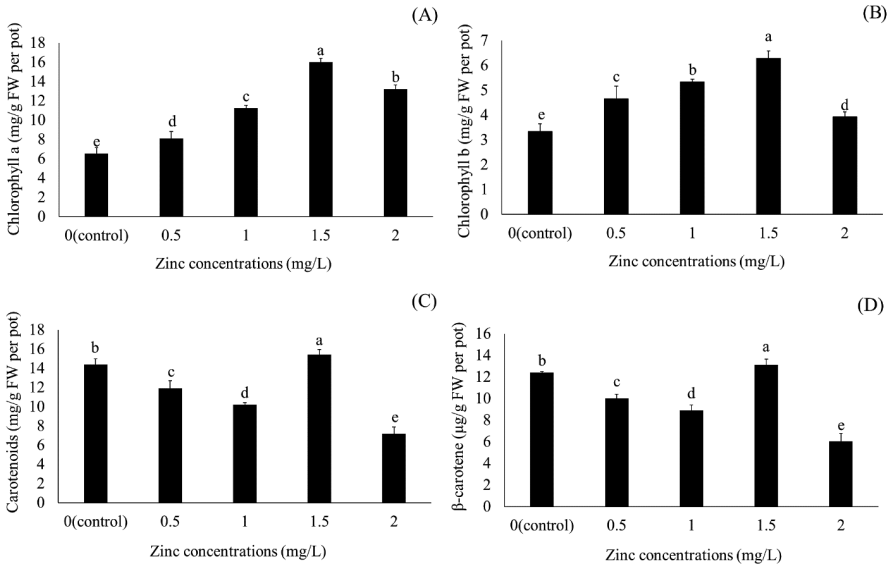

Fig. 2 Effect of different concentrations of zinc on marigold: (A) chlorophyll a content; (B) chlorophyll b content; (C) carotenoid content; (D) β-carotene content, where different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD.

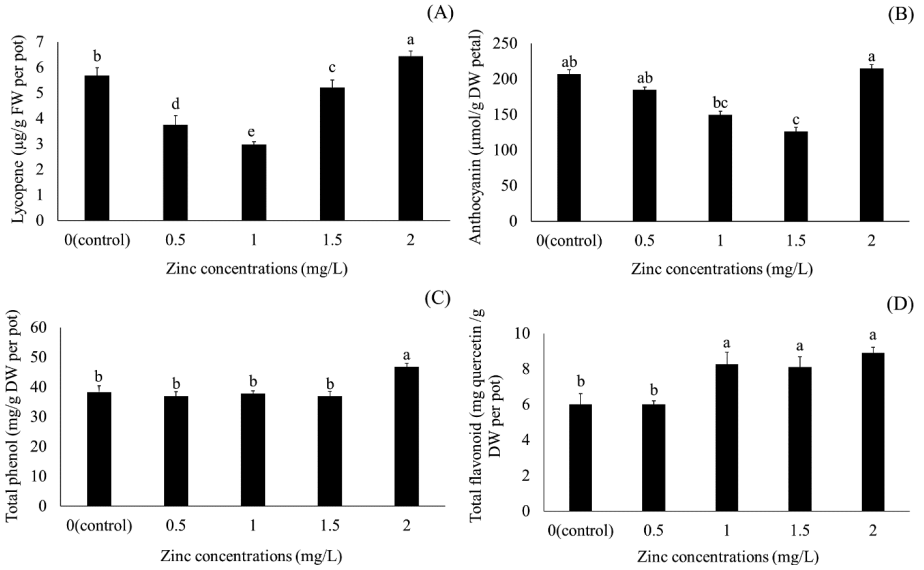

Fig. 3 Effect of different concentrations of zinc on marigold: (A) lycopene content; (B) anthocyanin content; (C) total phenol content and (D) total flavonoid content, where different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD.

Antioxidant activity using ferric ion reducing antioxidant power assay

The effect of different concentrations of Zn treatment on antioxidant activity was significant ($p < 0.01$), as shown in Table 3. The highest activity of antioxidant (309.56 µmol Fe^{++}/g DW) was observed in the third treatment and the lowest (184.81 µmol Fe^{++}/g DW) in the control treatment. There was no significant difference between the third and fifth treatments (Fig. 4A).

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl assay

The results of data analysis in Table 3 showed that there were significant ($p < 0.01$) differences among the different concentrations of Zn in terms of the DPPH antioxidant activity. The highest antioxidant activity (49.06%) was observed in the third treatment and the lowest (9.74%) was in the control treatment (Fig. 4B).

Discussion

Researchers have noted that Zn is an important element in plant development and can affect flowering (Butnariu et al., 2008; Srivastava and Singh, 2009; Hada et al. 2014; Nasiri and Najafi, 2015), which was confirmed by the current results. Srivastava and Singh (2009) indicated that a foliar application of Zn at various levels (100 g, 200 g, 300 g) significantly induced flowering intensity. As mentioned above, Zn caused an increase in the fresh and dry weights and similar results were reported by Younis et al. (2013), who argued that using essential micronutrients such as Zn resulted in improved dry and fresh weights in flowers. The current results also indicated that Zn could increase the length of the flower and peduncle in agreement with Nahed and Balbaa (2007) who reported similar results in their study on *Salvia*. In addition, Bashir et al. (2013) reported that the application of microelements on plants enhanced the stalk length.

The current word indicated that a foliar application of Zn fertilizer improved growth and photosynthetic characteristics. The biosynthesis of photosynthetic pigments such as chlorophyll is enhanced by Zn as a micronutrient acting as a co-factor with and as a catalytic agent of various enzymes for the normal development of pigment synthesis (Balashouri, 1995). Furthermore, Zn is known to have a stabilizing and protective effect on cell membranes; improving the integrity of cell membranes may be useful for the photosynthetic system of plants (Cakmak, 2010). In the current study, the chlorophyll index increased following Zn applications. In contrast, Hu and Sparks (1991) reported that Zn deficiency reduced the content of photosynthetic pigments in the leaves of *Carya illinoensis*, while, on the other hand, the use of exogenous Zn on the leaves of *Lycopersicone sculentum* increased the photosynthetic pigments in the leaves (Kaya and Higgs, 2002). Derakhshani et al. (2011) reported that an exogenous spray of Zn improved the chlorophyll index but their result was not significant. The contents of photosynthetic pigments in *Triticum aestivum* leaves revealed that these were enhanced as the levels of Zn in the soil were raised (Hemantaranjan and Garg, 1988) and the current results also reflected this. Radić et al. (2010) showed that there were significant decreases (30%) in photosynthetic pigments in plants exposed to Zn treatments compared with the control. The current data showed there was a significant ($p < 0.01$) difference between different concentrations of Zn in terms of the carotenoids content. Zhou et al. (2018) indicated that the contents of photosynthetic pigment (chlorophyll a, b and carotenoids) were increased following the application of various initial Zn concentrations. Prasad and Subbarayappa (2018) indicated that the lycopene content of the fruits increased with the increasing Zn levels, while Salam et al. (2010) clearly showed that Zn played an important role in increasing the lycopene content in fruit. Thus, the current results were in broad agreement with many other studies.

However, very few studies has been conducted on the effect of Zn fertilizer on the improvement of phenolic compounds. As sucrose has

Table 3 Results of analysis of variance (F-value)of phytochemical parameters of marigold

Source of Variation	df	Total anthocyanin	Total phenol	Total flavonoids	Antioxidant Activity (FRAP)	Antioxidant activity (DPPH)
Zinc	4	4294.7**	51.225**	6.278**	11506.7**	607.68**
Error	10	454.11	1.919	1.919	116.432	26.936
CV		12.07	3.504	3.504	4.3	16.31

FRAP = ferric ion reducing antioxidant power; DPPH = 2, 2-diphenyl-1-picrylhydrazyl
** = significant at $p < 0.01$; df = degree of freedom; CV = Coefficient of variation

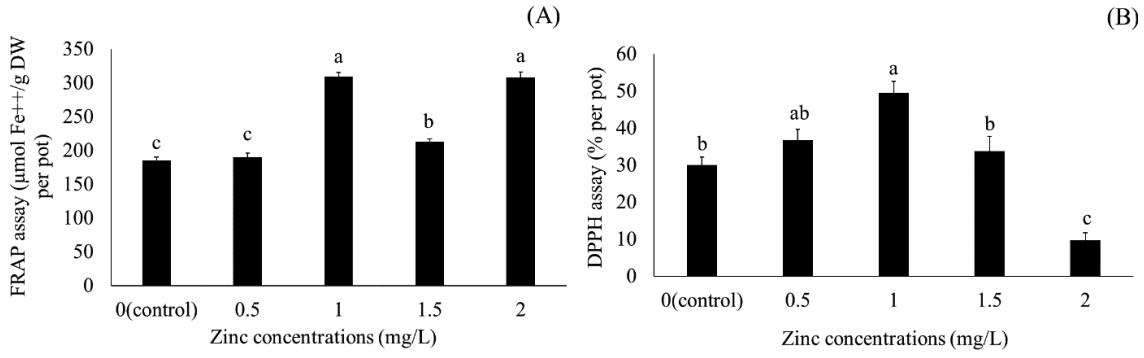

Fig. 4 Effect of different concentrations of zinc on antioxidant activity of marigold: (A) ferric reducing antioxidant power (FRAP); (B) 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, where different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD.

a positive effect on the synthesis of phenolic compounds, Zn fertilizer increases the synthesis of phenolic compounds by optimally regulating photosynthesis and sugar accumulation (Solfanelli et al., 2006; Song et al., 2015). Chalcone synthase and chalcone isomerase are key enzymes involved in the biosynthetic pathway of phytochemical compounds such as flavonoids; the overexpression of the *VvCHI* and *VvF3H* genes increased flavonoid biosynthesis and production (Pelletier and Shirley, 1996; Verhoeyen, 2001). Foliar application of Zn fertilizer to plants increases the expression of these genes and enzymes and has a positive effect on the concentration of flavonoids (Song et al., 2015). Phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are key enzymes in the initial stages of the phenylpropanoid and flavonoid pathways and it has been reported that the application of Zn fertilizer significantly enhanced the transcript abundance of PAL and CHS in some plant species (Ma et al., 2017). The current findings revealed that a foliar application of Zn could improve the transcription level of genes related to the biosynthesis of phenolic compounds as has been reported elsewhere (Ma et al., 2017). Furthermore, Marichali et al. (2014) showed that exposure to elevated Zn concentrations increased the total phenol content in all plant parts.

Zn can increase the antioxidant contents such as total phenol and total flavonoid; an exogenous spraying of Zn improved the antioxidant capacity (phenolic compounds, ascorbate, reduced glutathione) of wheat flag leaves (Ma et al., 2017). Based on the current results, the highest amount of anthocyanin (144.97 µmol/g FW) was observed in the fifth treatment. There was a high accumulation of phenolic compound such as anthocyanins in the petioles of *Gossypium* species; however, the plant anthocyanin content varied with different concentrations of Zn (Brown and Wilson, 1952). Superoxide dismutase and catalase are important enzymes as first-line defense antioxidants against reactive oxygen species (ROS) in plant tissues (Alscher et al., 2002). Zn application significantly enhanced the transcript abundance of both superoxide dismutase gene expression and the activity of superoxide dismutase in some plant species (Gao et al., 2009; Ma et al., 2017). Hence, Zn application could improve the activity of scavenging ROS by inducing gene expressions of superoxide dismutase followed by improved antioxidant activity.

Few studies have investigated the effect of Zn fertilizer on medicinal herbs. The current results indicated that different concentrations of Zn had a significant effect on the morpho-physiological and phytochemical parameters of marigold. The best Zn concentration for fresh weight traits, chlorophyll index, chlorophyll a, chlorophyll b, carotenoid and β-carotene was the fourth treatment (1.5 mg/L) of Zn. However, the lycopene, anthocyanin, total phenol and total flavonoid traits had their highest measured values following the fifth treatment (2 mg/L). Finally, the best antioxidant activity using both methods was observed in the third treatment (1 mg/L). These combined results provide new data for the agri-food industry, but further research is necessary to gain more detail and a better understanding of the effects of Zn fertilizer.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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