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THE EFFECT OF FOLIAR APPLICATION OF AMINO ACIDS ON SOME NUTRITIONAL PROPERTIES, ANTIOXIDANT CAPACITY AND SOME OTHER PHYSIOLOGIC PARAMETERS OF AFRICAN MARIGOLD (*Tagetes erecta* L.), TAISHAN 'YELLOW' AND 'ORANGE'

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ABSTRACT

African marigold (*Tagetes erecta* L.) is one of the most well-known ornamental, medicinal and edible flowers in the world. A factorial experiment based on completely randomized design with 20 treatments in 3 replications, 60 plots and 5 plants per plot were conducted in order to investigate the effect of amino acids on growth, nutritional parameters and antioxidant capacity in African marigold. Experimental treatments included two cultivars of African marigold ('Yellow' and 'Orange') and foliar application of three amino acids (arginine, glutamine and proline) each one at three levels (100, 500 and 1000 µM). Distilled water was used as control. The results showed that the application of amino acids increased plant height, display life, fresh weight and dry matter of flower, leaf total chlorophyll and petal carotenoid compared to the control in both African marigold cultivars. Petal protein content and malondialdehyde (MDA) were not affected by the above treatments, but the use of amino acids, especially arginine and proline, increased proline and calcium, zinc and iron in the petals of both African marigold cultivars. Treatments of 100 µM arginine and 1000 µM proline induced the highest vitamin C in both African marigold cultivars. In 'Orange' cultivar, the highest enzyme activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) belonged to 1000 µM proline and the highest peroxidase (POD) activity belonged to two treatments of 100 µM arginine and 1000 µM proline. In 'Yellow' cultivar, all three levels of arginine and proline together with 1000 µM glutamine significantly increased SOD and POD activity compared to other treatments. Also, the highest APX enzyme activity was recorded for 100 µM arginine. The results of the present study showed that external application of amino acids, especially arginine and proline, improved the nutritional properties of both African marigold cultivars by increasing the activity of antioxidants.

Key words: spray, nitrogen, bio-stimulators, vitamin C, oxygen free radicle

INTRODUCTION

In addition to being an ornamental plant, African marigold (*Tagetes erecta* L.) has been considered for many years due to its antioxidant capacity and nutritional and nutritional value. Today, the use of ornamental flowers, which have been proven to be edible, has increased in the human diet. As the demand for fresh edible flowers increases, research into the organic and healthy production of these flowers and increasing their nutritional value should also be considered. African marigold is also one of the most popular and

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the most widely used edible flowers in the world, its petals are used fresh in the preparation of desserts and salads [Moliner et al. 2018, Singh et al. 2020].

Nitrogen is the most essential element needed by the plant, which is involved in all the vital processes of plants. Therefore, the use of nitrogen fertilizers for plant production with optimal yield and quantitative and qualitative characteristics is essential [Leghari et al. 2016]. Different nitrogen fertilizers from various chemical and organic sources are available in the market, which today are more important for healthy organic production and environmental protection. One of the organic sources of nitrogen are amino acids that are commercially available in the market in the form of a mixture of amino acids or amino chelates and their positive effect on the growth and quantitative and qualitative yield of various plants has been reported [Faten et al. 2010, Raeisi et al. 2014].

Amino acids are a source of organic nitrogen in plants. These compounds are also precursors of growth hormones as well as anti-stress agents. Am no acids are able to form complexes with metals and accelerate the absorption and transport of nutrients in plants [Teixeira et al. 2018]. The positive effect of consumption of amino acids in the form of foliar spray application and soil drench on the morphological and physiological characteristics of strawberries [Bidaki et al. 2018], broccoli [Shekari and Javanmardi 2017] and soybeans [Teixeira et al. 2018] has been reported.

Proline is a source of carbon, nitrogen and energy. This amino acid is involved in increasing plant resistance to environmental stresses and protecting proteins and membrane structures against oxygen free radical damage [El-Din and El-Wahed 2005, Soroori et al. 2021]. The role of proline in improving vegetative and generative parameters, nutrient uptake, content of proline, chlorophyll and carotenoid in leaves, flower permanency [Soroori et al. 2021], increasing photosynthetic pigments [Khattab and Afifi 2009], and maintaining the structure and activity of antioxidant enzymes [Yaqoob et al. 2019] have been reported.

Glutamine is one of the most readily available amino acids and an energy source. Glutamine is a precursor to the synthesis of proteins, polypeptides, chlorophyll and other amino acids. This amino acid plays an important role in the metabolic cycles of carbon and nitrogen and causes the accumulation of sugars and proteins in the plant. This amino acid also plays an important role in seed germination, root formation and resistance to pathogens and stresses [Bidaki et al. 2018, Qiu et al. 2020].

Arginine is an essential amino acid and a precursor to the biosynthesis of polyamines, nitric oxide, proline and agmatine [Jubault et al. 2008]. This amino acid plays an important role in the transport and storage of nutrients, the synthesis of growth hormones and the improvement of the activity of antioxidant enzymes [Bidaki et al. 2018]. In addition to the positive effects of arginine in plants, the metabolic production of this amino acid (polyamines, nitric oxide and proline) also plays a decisive role in growth and development as well as plant resistance to biotic and abiotic stresses [Neill et al. 2003, Trovato et al. 2008].

Foliar feeding is an effective and efficient way to provide the nutrients needed by the plant through the growing shoots [Zulaikha 2013]. The positive effect of amino acid foliar application on the quantitative and qualitative properties of carnation [Abdossi and Danaee 2019] has been reported. Considering the positive effect of foliar application of amino acids on quantitative and qualitative yield of different plants, the present study aimed to investigate the effect of foliar spray application of amino acids arginine, glutamine and proline on morphological and physiological characteristics of African marigold (*Tagetes erecta* L.), cultivars of 'Yellow' and 'Orange'.

MATERIALS AND METHODS

Experimental design and treatments. In this study, the effect of amino acids on ornamental, edible and antioxidant parameters of African marigold (*Tagetes erecta* L.) was investigated. This experiment was performed as factorial in a completely randomized design with 20 treatments, 3 replications, 60 plots and 5 pots or plants in each plot. Experimental treatments included two cultivars of African marigold ('Yellow' and 'Orange') and foliar application with three types of amino acids (arginine, glutamine and proline), each at three levels of 100, 500 and 1000 µM. Distilled water was used as a control.

Plant materials. F1 seeds of two cultivars of African marigold (Taishan™ Orange and Yellow) were obtained from the Pan American Seed Institute.

In mid-March 2020, seeds were planted in seedling tray and in greenhouses. Forty days after sowing the seeds, the two-leaf seedlings were transplanted to 14 cm in diameter pots. The substrate used in both seed sowing and seedling transfer was a mixture of river sand, garden soil and perlite with an equal volume ratio. 10 days after transplanting, the first foliar spray application of amino acids was performed and the second and third stages were performed 10 days apart. Plant needs (light, water, nutrients and pest and disease control) were assessed continuously during the experiment period.

Evaluation of traits. At the end of the experiment, plant height was measured from the crown (soil surface) with a ruler. Display life was calculated by counting the days from the time of seedling transfer until the wilting the flowers. Time until flowering was measured by counting the days from the transfer the seedling until emergence of first visible bud. At the time of full flowering, two-plant flowers were separated from each plant without a peduncle, and the fresh weight of the flowers was measured with a digital scale to the nearest 0.01 g. After measurement of fresh weight, the flowers were dried in an oven at 105°C for 24 h and their dry weight was measured with a digital scale to the nearest 0.01 g. Then, by dividing the fresh weight by the dry weight and multiplied by 100, the percentage of flower dry matter was obtained.

Chlorophyll a, b, and total of the leaves were measured by Mazumdar and Majumder [2003] procedure. So, the leaves were sampled at the time of full flowering and they were extracted in 80% acetone. The absorption of the filtered samples was read at 643 and 660 nm with an APEL PD-330UV spectrophotometer, and chlorophyll a, b, and total were calculated by the following equations:

$$\text{Chlorophyll a} = 9.93\ (A_{660}) - 0.777\ (A_{643})$$

$$\text{Chlorophyll b} = 17.6\ (A_{643}) - 2.81\ (A_{660})$$

$$\text{Total chlorophyll} = 7.12\ (A_{660}) + 16.8\ (A_{643})$$

To measure petal carotenoids, extraction was performed with 80% acetone. The absorbance of the extract was read with a spectrophotometer (Shimadzu UV-120-02, Japan) at 440, 645, and 663 nm and then, the following equation was used to calculate carotenoid content in μg g^{-1} F.W. [Mazumdar and Majumder 2003]:

$$\text{Petal carotenoids} =$$

$$= (4.69 \times A_{440}) - (0.286 \times 20.2 \times A_{645}) + (8.02 \times A_{663})$$

The vitamin C content was measured using the titration method with 2,6-dichlorophenol-indophenol (DCIP) for which 2 g of the petals was extracted with 20 mL of 3% metaphosphoric acid and was mixed in a homogenizer for 30 minutes. The extract was filtered through a Whatman paper. At the next step, 15 mL of the filtered extract was titrated with DCIP until the emergence of pink color at which step the amount of DCIP applied for titration was recorded. Finally, the following equation was applied to determine the vitamin C content of the petals in mg 100 g^{-1} F.W. [Mazumdar and Majumder 2003]:

$$\text{Vitamin C} = \frac{e \times d \times b}{c \times a} \times 100$$

in which; a – is the sample weight, b – is the volume of metaphosphoric acid used for extraction, c – is the volume of the solution taken for titration, d – is the color factor, and e – is the volume of the colored solution consumed.

The petals of marigold were sampled at the time of full flowering to determine their total protein content by Kjeldahl indirect method. So, first nitrogen percentage and then protein percentage were calculated by the following equations:

$$\text{Nitrogen (\%)} = 0.56 \times t \times (a - b) \times \frac{V}{W} \times \frac{100}{DM}$$

in which; t – is the concentration of acid used for titration in mol l^{-1}, a – is the amount of acid used for sample in mol l^{-1}, b – is the amount of acid used as control in ml, V – is the volume of extract taken from digestion in ml, W – is the plant sample weight for digestion in g, and DM – is the plant dry matter.

$$\text{Total protein (\%)} = \text{nitrogen} \times 6.25$$

Proline content was determined spectrophotometric by adopting the ninhydrin method of Bates et al.

[1973]. Fresh leaf samples weighing 300 mg were homogenized in 3 ml of 3% sulfosalicylic acid. The homogenate filtrate was reacted with 1 ml each of acid ninhydrin and glacial acetic acid for 1 h in a test tube placed in a water bath at 100°C. The mixture was extracted with toluene and the absorbance was measured on a spectrophotometer (JASCO Model V-530) at 520 nm using L-proline as a standard.

The petals of marigold were sampled at the time of full flowering to determine their Iron, calcium and zinc in petals by Rengel and Romheld [2000] method. For this purpose, the petals were placed in an oven at 550° C for 2 h and turned into ash. Then using hydrochloric acid 2 N the extract was prepared. Finally, the three elements iron, zinc, and calcium were obtained from the atomic absorption.

Malondialdehyde (MDA) was calculated by Heath and Parker [1968] method. So, 0.5 g of petal tissue at the time of full flowering was sampled by liquefied nitrogen and potassium phosphate buffer. The extract was centrifuged at 4°C at a speed of 14 000 and 10 500 rpm and the supernatant was separated with a sampler. Then, 200 µl of the supernatant was mixed with 1000 µl of TCA and TBAS and was heated in a hot water bath for 30 minutes. Immediately after that, the samples were placed in an ice-filled container for 30 minutes. The cooled mixture was centrifuged at 4°C at 10500 rpm for 10 minutes. Then, it was read with a spectrophotometer at 532 and 600 nm and the readings were placed in the following equation to yield MDA content in nmol g^{-1} F.W.

Peroxidase (POD) activity was measured by In et al. [2007] method for which some petal samples were detached from the time of full flowering and was extracted with 50 mM of potassium phosphate buffer. The extract was centrifuged at 10 500 rpm at 4°C for 20 minutes. The supernatant was used as the enzymatic extract. So, 100 µl of the extract was added with 450 µl of H_2O_2 and 450 µl of guaiacol solution. Then, it was read at 470 nm with a JASCO Model V-530 spectrophotometer and POD activity was reported in nmol g^{-1} F.W. min^{-1}.

SOD enzyme activity was measured by the method described in Giannopolitis and Ries [1997]. The reaction solution to measure SOD activity was composed of 0.1 mL of the enzymatic extract, 25 mM of nitro blue tetrazolium chloride, 13 mM of methionine, 0.1 mM of EDTA, 50 mM of carbonate sodium, and 50 mM of potassium phosphate buffer. It was slowly shaken in specific tubes for 15 minutes under florescent light exposure at 22°C. Then, the samples were placed in a dark room. After that, their absorption was read at 560 nm, and the SOD activity was expressed in the IU g^{-1} F.W. min^{-1}.

Data analysis. Data were analyzed using SPSS statistical software and mean data were compared using LSD test.

RESULTS

Plant height. The interaction effect of cultivar × amino acid on plant height was significant at the level of 1% probability (Tab. 1). Table 2 revealed that the plant height in 'Orange' cultivar was taller than 'Yellow' cultivar. In both African marigold cultivars, application of amino acid increased plant height compared to the control. The tallest plant (29.16 cm) was recorded in the cultivar 'Orange' for 100 µM arginine (Tab. 3). In 'Yellow' cultivar, the tallest plant was obtained using 1000 µM arginine (26.74 cm), 1000 µM proline (26.63 cm), and 500 µM proline (23.86 cm) which were not statistically significant (Tab. 4).

Display life. The interaction effect of cultivar × amino acid on the display life of African marigold was not significant, but the effect of cultivar ($P < 0.05$) and amino acid ($P < 0.01$) on the display life was significant (Tab. 1). Table 2 showed the display life was longer in the 'Orange' cultivar. Also 100 µM Arg. (26.66 days) was the most appropriate treatment to increase the display life. The results of table 3 and 4 showed that display life in 'Orange' cultivar varies between 58 to 69 days and in 'Yellow' cultivar between 57.30 to 64.70 days.

Time until flowering. The interaction effect of cultivar × amino acid on time until flowering was significant at the level of 1% probability (Tab. 1). Mean comparison showed that in both 'Orange' and 'Yellow' cultivars, the shortest time until flowering belonged to the control. In 'Orange' cultivar, the highest time to flowering (23 days) belonged to plants treated with 100 µM arginine, which was not statistically significant with treatments of 100, 500 and 1000 µM proline and 500 and 1000 µM arginine (Tab. 3). In 'Yellow' cultivar, the highest time until flowering (23.33 days)

Table 1. Analysis of variance (ANOVA) of the effect of different treatments on traits

S.o.V	df	Plant height	Display life	Time until flowering	Flower fresh weight	Flower dry matter	Chlorophyll a	Chlorophyll b	Total chlorophyll	Petals carotenoids	Petals protein
Cultivar (C)	1	76.4**	240.0**	8.06*	0.339 ^{ns}	39.33**	0.106^{ns}	0.493**	0.995**	0.00864*	0.00073^{ns}
Amino acid (A)	9	15.25**	81.2*	16.67**	0.707^{ns}	31.8**	0.437**	0.238**	1.199**	0.0385**	0.0146**
C × A	9	25.4**	62.7^{ns}	9.25**	0.896*	13.13**	0.277**	0.162**	0.596*	0.0117**	0.0086^{ns}
Error	38	4.28	20.13	1.83	0.348	3.776	0.039	0.045	0.158	0.0012	0.0029
CV (%)	–	9.01	7.16	6.62	31.41	17.28	13.12	20.67	15.78	6.78	4.86

* and ** significant difference at 5% and 1% probability level, respectively, ns – not significant

Table 1. Continued

S.o.V	df	Vitamin C	Calcium	Zinc	Iron	Proline	Malondialdehyde (MDA)	Superoxide dismutase activity (SOD)	Peroxidase activity (POD)	Ascorbate Peroxidase activity (APX)
Cultivar (C)	1	14.32**	0.0000268^{ns}	0.0992**	0.211**	0.363^{ns}	0.00504^{ns}	614^{ns}	0.245**	99.92**
Amino acid (A)	9	28.52**	0.004915**	0.075**	0.206**	33.54**	0.197**	6442**	0.497**	303.9**
C × A	9	27.5**	0.00272*	0.0407**	0.1561**	12.09**	0.157^{ns}	6814**	0.164**	96.3**
Error	38	3.830	0.00093	0.00148	0.0276	1.255	0.0465	1156.84	0.0256	6.51
CV (%)		9.882	13.21	10.89	13.50	8.43	10.730	9.397	19.39	11.10

* and ** significant difference at 5% and 1% probability level, respectively, ns – not significant

Table 2. The effects of cultivar and amino acid treatments on traits

Treatments	Plant height (cm)	Display life (day)	Time until flowering (day)	Flower fresh weight (g)	Flower dry matter (%)	Chlorophyll a (mg/g F.W.)	Chlorophyll b (mg/g F.W.)	Total chlorophyll (mg/g F.W.)
'Orange' cultivar	24.08^{a}	20.80^{a}	64.60^{a}	1.95	12.05^{a}	1.549	1.126^{a}	2.64^{a}
'Yellow' cultivar	21.82^{b}	20.06^{b}	60.60^{b}	1.80	10.43^{b}	1.464	0.944^{b}	2.391^{b}
Control	21.89^{de}	17.83^{e}	57.66^{d}	1.41^{c}	7.87^{e}	1.098^{d}	0.708^{c}	1.69^{c}
100 µM Arg.	24.62^{ab}	22.66^{a}	65.16^{ab}	2.50^{a}	13.30^{b}	1.863^{a}	1.335^{a}	2.98^{a}
500 µM Arg.	21.12^{e}	21.16^{abc}	64.33^{ab}	2.09^{abc}	11.86^{bc}	1.765^{a}	1.025^{b}	2.78^{ab}
1000 µM Arg.	24.3^{abc}	20.83^{bc}	60.00^{bcd}	1.95^{abc}	11.07^{bcd}	1.641^{ab}	0.870^{bc}	2.36^{b}
100 µM Glu.	21.31^{e}	18.33^{de}	58.33^{cd}	1.52^{bc}	8.82^{de}	1.128^{d}	0.971^{b}	1.89^{c}
500 µM Glu.	22.06^{cde}	19.66^{cd}	63.66^{ab}	1.69^{bc}	10.26^{cd}	1.451^{bc}	0.900^{b}	2.36^{b}
1000 µM Glu.	24.16^{a-d}	19.00^{de}	63.50^{abc}	1.55^{bc}	9.97^{cde}	1.275^{cd}	1.056^{b}	2.52^{ab}
100 µM Pro.	21.89^{de}	20.66^{bc}	66.33^{a}	1.80^{bc}	11.14^{bc}	1.698^{a}	1.018^{b}	2.70^{ab}
500 µM Pro.	22.45^{b-e}	22.00^{ab}	63.00^{abc}	2.07^{abc}	12.07^{bc}	1.446^{bc}	1.110^{ab}	2.91^{a}
1000 µM Pro.	25.65^{a}	22.16^{ab}	64.00^{ab}	2.17^{ab}	16.00^{a}	1.700^{a}	1.358^{a}	2.97^{a}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 2. Continued

Treatments	Petals carotenoids (µg/ g F.W).	Petals protein (%)	Vitamin C (mg/100 g F.W.)	Calcium (mg/g F.W.)	Zinc (mg/100 g F.W.)	Iron (mg/100 g F.W.)	Proline (µM/g F.W.)	Malondialdehyde (µM/g F.W.)
'Orange' cultivar	0.511^{b}	1.116	19.31	0.071	0.313^{b}	1.292^{a}	13.201	2.00
'Yellow' cultivar	0.535^{a}	1.103	20.29	0.075	0.3941^{a}	1.173^{b}	13.356	2.02
Control	0.450^{e}	1.021^{c}	16.66^{d}	0.0603^{c}	0.266^{e}	0.955^{b}	9.74^{d}	2.58^{a}
100 µM Arg.	0.731^{a}	1.155^{a}	21.06^{ab}	0.0868^{a}	0.600^{a}	1.368^{a}	16.44^{a}	1.72^{cd}
500 µM Arg.	0.506^{cd}	1.135^{a}	20.35^{bc}	0.0720^{b}	0.415^{c}	1.428^{a}	13.54^{bc}	1.90^{c}
1000 µM Arg.	0.493^{d}	1.133^{a}	19.86^{bc}	0.0713^{bc}	0.303^{de}	1.343^{a}	12.52^{c}	1.88^{c}
100 µM Glu.	0.476^{de}	1.031^{c}	16.71^{d}	0.0663^{bc}	0.2583^{e}	1.083^{b}	11.03^{d}	2.42^{ab}
500 µM Glu.	0.483^{de}	1.115^{ab}	18.71^{cd}	0.0703^{bc}	0.298^{de}	1.040^{b}	10.49^{d}	2.24^{b}
1000 µM Glu.	0.493^{de}	1.068^{bc}	19.05^{bc}	0.0690^{bc}	0.290^{de}	1.015^{b}	14.03^{b}	1.95^{c}
100 µM Pro.	0.490 ^{de}	1.121^{ab}	19.72^{bc}	0.0751^{b}	0.300^{de}	1.403^{a}	13.58^{bc}	1.90^{c}
500 µM Pro.	0.538^{bc}	1.128^{ab}	22.91^{a}	0.071^{bc}	0.316^{d}	1.385^{a}	14.69^{b}	1.88^{c}
1000 µM Pro.	0.566^{b}	1.161^{a}	22.98^{a}	0.090^{a}	0.488^{b}	1.305^{a}	16.70^{a}	1.54^{d}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 3. Mean comparisons of the effect of different treatments on traits measurement in 'Orange' cultivar

Cultivar	Treatments	Plant height (cm)	Display life (day)	Time until flowering (day)	Flower fresh weight (g)	Flower dry matter (%)	Chlorophyll a (mg/g F.W)	Chlorophyll b (mg/g F.W)	Total chlorophyll (mg/g F.W)
'Orange'	Control	20.94^{c}	69.0	18.33^{d}	1.28^{b}	6.25^{e}	1.053^{d}	0.643^{d}	1.696^{de}
	100 µM Arg.	29.16^{a}	58.0	23.00^{a}	2.12^{ab}	15.02^{a}	2.170^{a}	1.600^{a}	3.770^{a}
	500 µM Arg.	24.45^{bc}	58.0	21.66^{ab}	2.00^{ab}	11.48^{abc}	1.973^{ab}	1.163^{bc}	3.136^{a}
	1000 µM Arg.	23.73^{bc}	68.0	21.33^{ab}	2.10 ^{ab}	10.37^{bcd}	1.900^{b}	0.943^{b-d}	2.843^{b}
	100 µM Glu.	22.03^{bc}	68.0	18.66^{cd}	1.81^{ab}	6.72^{de}	1.223^{d}	0.763^{cd}	2.036^{bcd}
	500 µM Glu.	23.10^{bc}	68.0	20.33^{bcd}	1.71^{ab}	9.92^{bcd}	1.173^{cd}	0.840^{cd}	1.963^{d}
	1000 µM Glu.	23.66^{bc}	63.0	19.66^{bcd}	1.69 ^{ab}	8.31^{cde}	1.143^{d}	1.153^{bc}	2.296^{bc}
	100 µM Pro.	24.32^{bc}	68.0	21.00 ^{abc}	1.92^{ab}	11.46^{abc}	1.523^{c}	1.100^{bc}	2.623^{b}
	500 µM Pro.	24.66^{bc}	68.0	22.00^{ab}	2.22^{ab}	11.57^{abc}	1.410^{c}	1.370^{ab}	2.780^{b}
	1000 µM Pro.	24.76^{c}	58.0	22.00^{ab}	2.49^{a}	13.23^{ab}	1.920^{ab}	1.683^{a}	3.603^{a}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 3. Continued

Cultivar	Treatments	Petals carotenoids (µg/ g F.W).	Petals protein (%)	Vitamin C (mg/100 g F.W.)	Calcium (mg/g F.W.)	Zinc (mg/100 g F.W.)	Iron (mg/100 g F.W.)	Proline (µM/ g F.W.)	Malondialdehyde (µM/ g F.W.)
'Orange'	Control	0.430^{e}	0.990	16.33^{d}	0.0513^{e}	0.233^{f}	1.050^{e}	9.00^{e}	2.59
	100 µM Arg.	0.810^{a}	1.143	24.96^{a}	0.088^{ab}	0.460^{a}	1.713^{a}	15.33^{ab}	1.90
	500 µM Arg.	0.533^{b}	1.123	21.75^{b}	0.071^{cd}	0.330^{c}	1.460^{abc}	15.14^{b}	1.88
	1000 µM Arg.	0.453^{de}	1.140	18.53^{cd}	0.070^{cd}	0.310^{cd}	1.410^{bcd}	14.73^{bc}	1.88
	100 µM Glu.	0.431^{e}	1.033	16.35^{d}	0.060^{de}	0.253^{ef}	1.083^{e}	9.55^{e}	2.42
	500 µM Glu.	0.456^{de}	1.053	16.87^{d}	0.065^{cde}	0.263^{def}	1.063^{e}	12.50^{d}	2.24
	1000 µM Glu.	0.460^{cde}	1.100	17.03^{d}	0.069^{cd}	0.283^{c-f}	1.163^{de}	12.43^{d}	1.95
	100 µM Pro.	0.503^{bcd}	1.110	17.41^{d}	0.076^{bc}	0.286^{cde}	1.206^{cde}	12.66^{cd}	1.90
	500 µM Pro.	0.520^{bc}	1.200	20.96^{bc}	0.068^{cd}	0.313^{cd}	1.263^{cde}	13.25^{bcd}	1.72
	1000 µM Pro.	0.513^{bcd}	1.213	22.93^{ab}	0.0910^{a}	0.396^{b}	1.506^{ab}	17.40^{a}	1.54

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 4. Mean comparisons of the effect of different treatments on traits measurement in 'Yellow' cultivar

Cultivar	Treatments	Plant height (cm)	Display life (day)	Time until flowering (day)	Flower fresh weight (g)	Flower dry matter (%)	Chlorophyll a (mg/g F.W)	Chlorophyll b (mg/g F.W)	Total chlorophyll (mg/g F.W)
'Yellow'	Control	18.51^{c}	61.3	14.66^{e}	0.91^{d}	8.92^{d}	1.113^{c}	0.780^{b}	1.893^{c}
	100 µM Arg.	20.07^{c}	57.3	22.66^{ab}	2.13 ^{b}	14.23^{abc}	1.753^{ab}	0.960^{ab}	2.713^{a}
	500 µM Arg.	21.69^{bc}	60.7	20.00^{cd}	1.90^{bc}	12.25^{bc}	1.780^{ab}	1.036^{ab}	2.816^{a}
	1000 µM Arg.	26.74^{a}	58.7	21.00^{bc}	1.81^{bc}	11.94^{bcd}	1.360^{bc}	0.900^{ab}	2.260^{abc}
	100 µM Glu.	19.02^{c}	62.0	18.33^{d}	1.69^{bcd}	9.02^{d}	1.143^{c}	0.773^{b}	1.916^{c}
	500 µM Glu.	19.46^{c}	60.0	19.66^{cd}	1.54^{bcd}	9.06^{d}	1.276^{c}	0.850^{ab}	2.126^{abc}
	1000 µM Glu.	21.24^{bc}	64.0	19.33^{cd}	1.17^{cd}	11.40^{cd}	1.383^{bc}	0.936^{ab}	2.319^{ab}
	100 µM Pro.	21.02^{bc}	64.7	19.33^{cd}	1.57^{bcd}	11.64^{cd}	1.873^{a}	1.070^{ab}	2.943^{a}
	500 µM Pro.	23.86^{ab}	58.0	23.333^{a}	1.98^{bc}	15.03^{ab}	1.483^{abc}	1.106^{a}	2.589^{ab}
	1000 µM Pro.	26.63^{a}	59.3	22.33^{ab}	3.31^{a}	16.99^{a}	1.480^{abc}	1.033^{ab}	2.513^{ab}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 4. Continued

Cultivar	Treatments	Petals carotenoids (µg/g F.W.)	Petals protein (%)	Vitamin C (mg/100 g F.W.)	Calcium (mg/g F.W.)	Zinc (mg/100 g F.W.)	Iron (mg/100 g F.W.)	Proline (µM/g F.W.)	Malondialdehyde (µM/ g F.W.)
'Yellow'	Control	0.440^{g}	1.010	16.30^{d}	0.060^{c}	0.266^{e}	0.826^{d}	9.57^{d}	2.21
	100 µM Arg.	0.573^{bc}	1.147	23.36^{ab}	0.086^{a}	0.663^{b}	1.396^{ab}	17.55^{a}	1.83
	500 µM Arg.	0.533^{cde}	1.113	20.37^{bc}	0.075^{abc}	0.370^{c}	1.230^{bc}	14.50^{b}	1.88
	1000 µM Arg.	0.550^{cd}	1.127	20.99^{abc}	0.080^{ab}	0.316^{cde}	1.276^{bc}	11.94^{c}	1.93
	100 µM Glu.	0.470^{fg}	1.010	16.56^{d}	0.061^{c}	0.280^{de}	1.003^{cd}	10.48^{cd}	2.19
	500 µM Glu.	0.480^{d-g}	1.107	16.46^{d}	0.066^{bc}	0.293^{de}	0.980^{cd}	11.43^{c}	2.06
	1000 µM Glu.	0.506^{d-f}	1.133	19.56^{cd}	0.073^{abc}	0.283^{de}	0.896^{d}	10.31^{cd}	2.11
	100 µM Pro.	0.530^{c-f}	1.110	22.03^{abc}	0.082^{ab}	0.323^{cde}	1.609^{a}	15.63^{b}	2.03
	500 µM Pro.	0.613^{ab}	1.110	22.90^{abc}	0.075^{abc}	0.343^{cd}	1.016^{cd}	16.0^{ab}	2.03
	1000 µM Pro.	0.653^{a}	1.170	24.36^{a}	0.092^{a}	0.803^{a}	1.506^{ab}	16.13^{ab}	1.93

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

was recorded for 500 µM proline treatment, which was not significantly different from 100 µM arginine and 1000 µM proline treatments, and were the most suitable treatments to increase the time until flowering of African marigold cultivar 'Yellow' (Tab. 4).

Fresh weight and dry matter percentage. The interaction effect of cultivar × amino acid on fresh weight ($P < 0.05$) and dry matter percentage ($P < 0.01$) of flowers was significant (Tab. 1). The results of mean comparison of interaction effect showed that the lowest amount of fresh weight and dry matter of flowers in both cultivars of African marigold belonged to the control. In 'Orange' cultivar, the highest fresh weight of flowers (2.49 g) was recorded for 1000 µM proline and the highest percentage of dry matter (15.02%) was recorded for 100 µM arginine, which was not statistically significant with the treatments of 100, 500, 1000 µM proline and 500 µM arginine (Tab. 3). In 'Yellow' cultivar, the highest fresh weight of flower (3.31 g) belonged to 1000 µM proline. The highest flower dry matter (16.99, 15.03 and 14.23%) in 'Yellow' cultivar was recorded for three treatments of 1000 µM proline, 500 µM proline and 100 µM arginine, respectively, which were not statistically significant with each other (Tab. 4).

Chlorophyll a, b and total. The interaction effect of experimental treatments on chlorophyll a and b ($P < 0.01$), and total ($P < 0.05$) was significant (Tab. 1). In the 'Orange' cultivar, the lowest amount of chlorophyll a, b and total was extracted from control. The highest amount of chlorophyll a and total in 'Orange' cultivar belonged to 100 and 500 µM arginine and 1000 µM proline. Treatments of 1000 µM proline, 100 µM arginine and 500 µM proline were the most successful treatments for chlorophyll b retention in 'Orange' cultivar, respectively (Tab. 3). In 'Yellow' cultivar, the lowest chlorophyll a (1.113 mg/g F.W.) was obtained in the control. The highest amount of chlorophyll a in 'Yellow' cultivar was obtained in treatments of 100 µM proline, 500 and 100 µM arginine and 500 and 1000 µM proline, respectively. In 'Yellow' cultivar, control and 100 µM glutamine had lower chlorophyll b and total content than other treatments and the rest of the treatments caused chlorophyll b and total retention and were not statistically significant (Tab. 4).

Petal carotenoid. Evaluation of mean comparison (Tabs 3 and 4) showed that in both 'Orange' and 'Yellow' cultivars, control and 100 µM glutamine had the lowest amount of petal carotenoid. While the highest petal carotenoid in the 'Orange' cultivar (0.810 µg/g F.W.) belonged to 100 µM arginine treatment and the highest amount of petal carotenoid in the 'Yellow' cultivar belonged to the 1000 and 500 µM proline treatments, respectively. The interaction effect of experimental treatments on petal carotenoid was significant at the level of 1% probability (Tab. 1).

Petal protein. Table 1 showed that the interaction effect of cultivar × amino acid on petal protein was not significant, but the effects of amino acid on petal protein was significant ($P < 0.01$). Table 2 showed that application of amino acid increased petals protein compared to the control (1.021%). Also, the best treatments in maintaining petals protein were 1000 µM Pro. (1.611%) and 100 µM Arg. (1.155%). Table 3 revealed that in 'Orange' cultivar, the use of all 9 levels of amino acids increased the percentage of petal protein compared to the control. In 'Yellow' cultivar (Tab. 4), except for 100 µM glutamine treatment, the percentage of protein in other amino acid levels increased compared to the control, but the differences were not statistically significant.

Vitamin C. The interaction effect of cultivar × amino acid on vitamin C was significant at the level of 1% probability (Tab. 1). According to the comparison of means, in the 'Orange' cultivar, the highest amount of vitamin C (24.96 and 22.93 mg/100 g F.W.) was extracted from treatments of 100 µM arginine and 1000 µM proline, respectively, which were not statistically significant with each other. The lowest vitamin C in the 'Orange' cultivar belonged to the control, 100, 500 and 1000 µM glutamine, respectively, which were not statistically significant (Tab. 3). In 'Yellow' cultivar, using all three levels of proline and arginine, and 1000 µM glutamine, the amount of vitamin C increased significantly compared to the control and the highest amount of vitamin C (24.36 mg/100 g F.W.) belonged to 1000 µM proline. The lowest amount of vitamin C (16.30 mg/100 g F.W.) in the 'Yellow' cultivar belonged to the control, which was not statistically significant with the 100 and 500 µM glutamine treatments (Tab. 4).

Mineral elements. The effect of experimental treatments on calcium ($P < 0.05$), zinc ($P < 0.01$), and iron ($P < 0.01$) concentrations was significant (Tab. 1).

The lowest amount of calcium, zinc and iron in the 'Orange' cultivar was calculated in the control. The highest amount of calcium in 'Orange' cultivar was calculated in 1000 µM proline and 100 µM arginine treatments, respectively. Treatments of 100 µM arginine and 1000 µM proline had the highest amounts of zinc and iron among the treatments, respectively (Tab. 3). Glutamine had a weak effect on increasing mineral elements in 'Orange' and 'Yellow' cultivars compared to arginine and proline. In 'Yellow' cultivar, the highest amount of calcium (0.092 mg/g F.W.) was recorded for 1000 µM proline, which was not statistically significant with all three levels of arginine, 100 and 500 µM proline and 1000 µM glutamine. The lowest amount of calcium in 'Yellow' cultivar was allocated to control and 100 µM glutamine. The highest (0.803 mg/100 g F.W.) and lowest (0.266 mg/100 g F.W.) content of zinc in 'Yellow' cultivar was obtained in 1000 µM proline and control treatments, respectively. The highest amount of iron in the petals of 'Yellow' African marigold was obtained by using 100 and 1000 µM proline and 100 µM arginine. The lowest amount of iron in the 'Yellow' cultivar was obtained in the control (Tab. 4).

Proline. The effect of experimental treatments on petal proline content was significant at the level of 1% probability (Tab. 1). In the 'Orange' cultivar, application of 1000 µM proline and 100 µM arginine increased proline accumulation (17.40 and 15.33 µmol/g F.W, respectively). The lowest amount of petal proline in the 'Orange' cultivar belonged to the control and 100 µM glutamine (Tab. 3). In the 'Yellow' cultivar, the control had the lowest and the treatments of 100 µM arginine and 1000 and 500 µM proline had the highest amount of proline storage in the petals (Tab. 4).

MDA accumulation. The effects of amino acid on MDA accumulation was significant at the level of 1% probability, but the interaction effect of cultivar × amino acid on MDA accumulation was not significant (Tab. 1). Table 2 showed that the use of amino

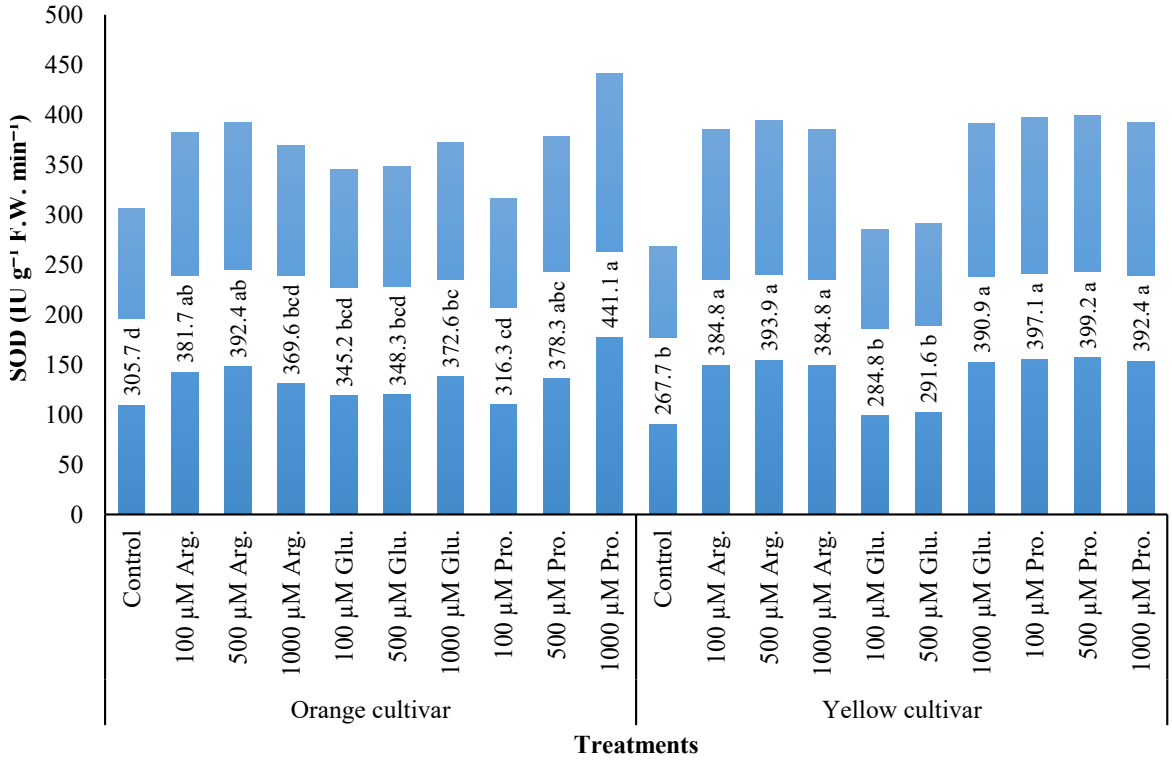

Fig. 1. Effect of different treatments on SOD activity

acids can prevent lipid peroxidation and accumulation of MDA in plant tissues compared to the control. The best treatments for controlling and reducing MDA, were 1000 µM Pro. (1.54 µM/ g F.W.) and 100 µM Arg. (1.72 µM/ g F.W.).

Antioxidant enzymes activity. Analysis of variance of the data showed that the interaction effect of amino acid × cultivar on the activity of enzymes SOD, POD and APX was significant at the level of 1% probability (Tab. 1). Mean comparison of the interaction effect of cultivar × amino acid on the activity of antioxidant enzymes is shown in Figures 1, 2 and 3. As can be seen, in both African marigold cultivars, with the use of amino acids, the activity of enzymes SOD, POD and APX has increased compared to the control. The lowest activity of these enzymes was recorded in both African marigold cultivars for the control. In 'Orange' cultivar, the highest SOD activity was recorded for 1000 µM proline treatment, which was not statistically significant with 100 and 500 µM arginine and 500 µM proline treatments. Treatments of 1000 µM proline and 100 µM arginine had the highest POD activity (1.24 IU/g F.W./min) and were not statistically significant different from 500 µM proline. APX activity was highest in 'Orange' cultivar with 1000 µM proline application. In the 'Yellow' cultivar, treatments of 100 and 500 µM glutamine were not successful in altering the function of SOD and POD enzymes. The highest SOD activity in the 'Yellow' cultivar belonged to the treatments of 100, 500 and 1000 µM arginine and proline together with 1000 µM glutamine, which were not statistically significant. The best treatments in maintaining POD enzyme activity were all three levels of arginine and proline amino acids that were not statistically significant. In 'Yellow' cultivar, arginine and proline were more successful in increasing APX activity than glutamine, and the highest APX activity was recorded for 100 µM arginine, which was the most successful treatment in maintaining APX enzyme activity (Fig. 1, 2 and 3).

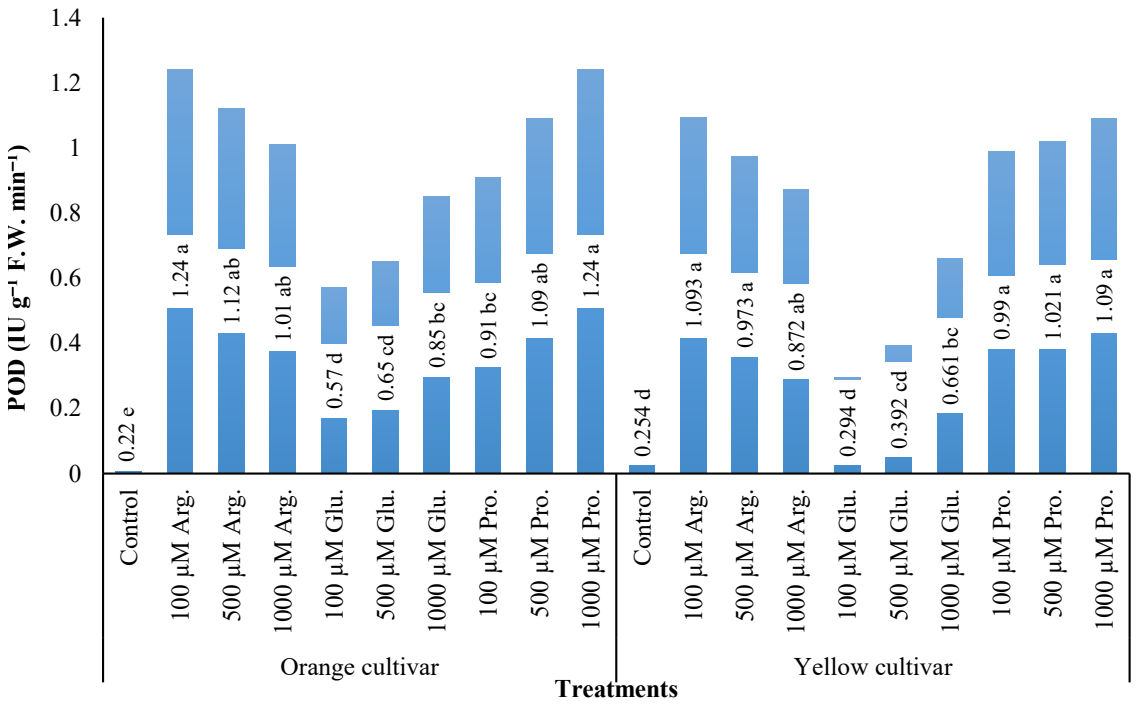

Fig. 2. Effect of different treatments on POD activity

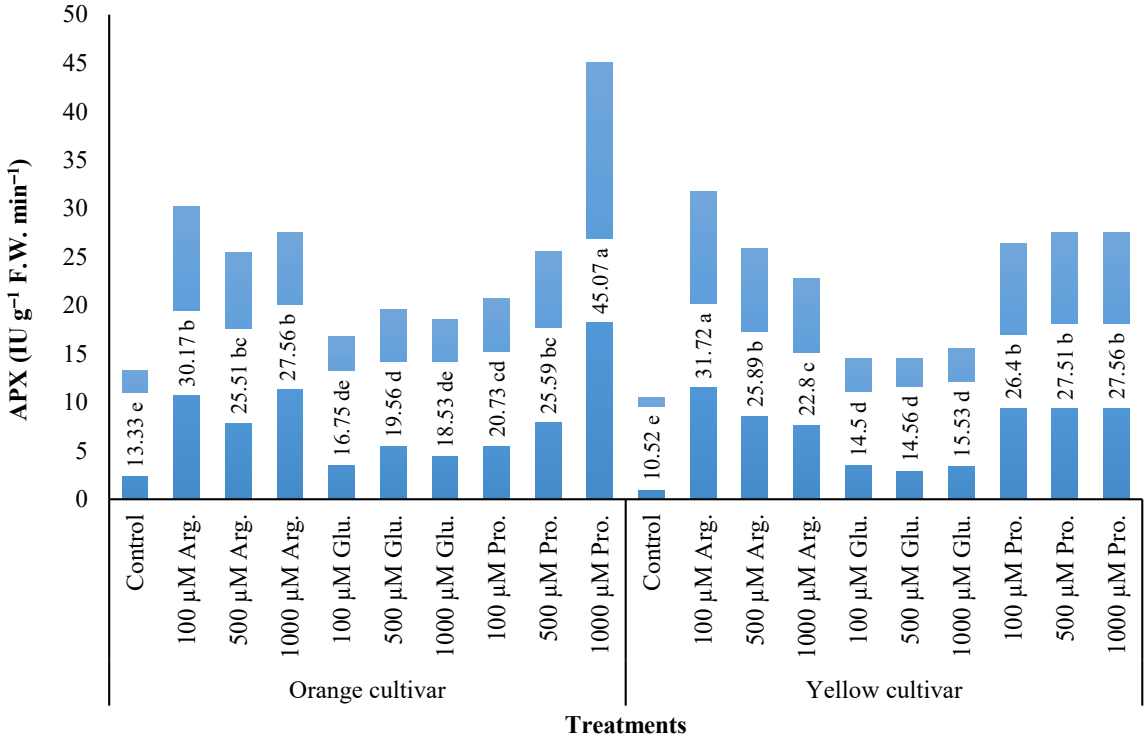

Fig. 3. Effect of different treatments on APX activity

DISCUSSION

In the present study, foliar application of African marigold cultivars 'Orange' and 'Yellow' with amino acids increased plant height, display life and fresh and dry weight of flowers. Nitrogen is essential for plant growth and development. Amino acids are a source of organic nitrogen, carbon and energy [Winter et al. 2015]. It has also been reported that consumption of amino acids increases vegetative and reproductive growth by increasing water and nutrient uptake, reducing plant stress, increasing surface area and number of leaves, increasing photosynthetic pigments and increasing photosynthetic efficiency that resulted in storing more carbohydrates and increasing fresh and dry weight [Porcel and Ruiz-Lozano 2004, Faten et al. 2010]. Therefore, increasing plant height, display life and fresh and dry weight of flowers has been the result of more nitrogen and nutrients supply in African marigold plants. Similar results have been reported on the fresh and dry weight of carnation [Abdossi and Danaee 2019] and chamomile [El-Din and Abd El-Wahed 2005], which agrees with the results of the present study. Researchers believe that increasing photosynthetic efficiency increases plant weight and yield [Hirose et al. 1997]. Ghafari et al. [2018] reported that the amount of chlorophyll in the leaves is one of the most important signs of maintaining photosynthetic capacity in the plant, improving growth and dry matter production. In fact, the amount of leaf chlorophyll indicates the strength and ability of the plant to photosynthesize. Therefore, another reason for the positive effect of amino acids on increasing vegetative and reproductive growth, as well as fresh and dry weight of the plant can be attributed to the effect of amino acids in improving photosynthetic pigments and increasing photosynthetic efficiency, which is consistent with the present results.

Chlorophyll is the most important pigment in the photosynthesis process [Croce and van Amerongen

2014]. During the growing season, chlorophylls are constantly degraded and replaced in the presence of favorable conditions such as light and oxygen. However, the onset of aging and the occurrence of stresses are associated with the destruction of chlorophyll and a decrease in its amount in the leaves [Croft and Chen 2017]. In the present study, the control plants had the lowest amount of chlorophyll a, b and total. One of the reasons for the lower amount of leaf chlorophyll in the control plants than those treated with amino acids can be attributed to the occurrence of stress or premature aging in this treatment, which has caused the destruction of chlorophyll or reduced its synthesis. Leaf chlorophyll contains most of the leaf nitrogen, and estimating the amount of chlorophyll in the leaves indirectly indicates the nutritional status of the plant [Croce and van Amerongen 2014, Croft and Chen 2017]. Amino acids are growth stimulants that facilitate plant access to water and nutrients. Therefore, the effective role of amino acids in the maintenance of chlorophylls can be related to the nutritional effect of these compounds. Nitrogen is a part of the structure of chlorophyll and protein. Therefore, nitrogen deficiency in plants is associated with a decrease in chlorophyll and protein [Madadkar Haghjou 2013]. Plants can use amino acids as a source of nitrogen. Therefore, external application of amino acids with the provision of nitrogen required by the plant have prevented the degradation of chlorophyll in the leaves of African marigold. The positive effect of amino acids on chlorophyll retention in calendula [Soroori et al. 2021], leaves has been reported, which agrees with the results of the present study. In the present study, arginine and proline were more successful in maintaining chlorophyll than glutamine. Due to the presence of glutamic acid helps the production of chlorophyll and is the main factor for the formation and synthesis of chlorophyll [Fischer et al. 1998].

Carotenoids are the most important plant pigments after chlorophylls and are effective in absorbing light, especially at wavelengths that chlorophyll pigments are unable to absorb. In addition to the role of carotenoids in the process of photosynthesis, their effect on the synthesis of abscisic acid, opening and closing of stomata and defense of the plant against stresses has also been reported [Croft and Chen 2017]. Carotenoids are precursors of vitamin A and have antioxidant effects. These pigments are used in food, pharmaceutical and cosmetic industries. African marigold is also rich in carotenoids and lutein [Jothi 2018]. Therefore, maintaining and increasing carotenoids in the petals of African marigold can increase its nutritional value. In the present study, the use of amino acids, especially arginine and proline, increased petal carotenoids in both types of African marigold compared to the control. Researchers believe that amino acids play an important role in the synthesis of photosynthetic pigments and increase the intensity of color in plant tissues [Shafie et al. 2020]. The amount of chlorophyll a and b and carotenoids in sunflower under drought stress was increased with the use of proline [Sadak and Mostafa 2015]. An increase in plant pigments has been reported with the use of amino acids in various plants [Hasanuzzaman et al. 2014, Soroori et al. 2021], which is consistent with the results of the present study.

Amino acids have an osmotic role and in addition to participating in the synthesis of proteins, they play an important role in numerous processes such as the transfer of ions and nutrients in plants [Souri and Hatamian 2018]. Amino acids facilitate the absorption of nutrients in plants through root development and increased nitrogen fixation [Khan et al. 2019]. A group of researchers believe that the use of amino acids acts as a signaling compound and by increasing the activity of antioxidants, they facilitate the absorption and transfer of water and nutrients in various plants [Calvo et al. 2014, Khan et al. 2019]. Abo Sedera et al. [2010] reported that the use of amino acids increases photosynthesis and the production of more metabolites in plants by increasing the absorption of water and nutrients. In the present research, the application of amino acids in both African marigold cultivars increased the concentrations of zinc, iron and calcium compared to the control. The use of amino acids increased the concentration of zinc in lettuce leaves, but their effect on the concentration of iron and calcium was not increased [Khan et al. 2019]. The secretion of amino acids from the roots is one of the factors involved in the uptake and transport of zinc in plants [Mirzapour et al. 2020]. Increased nutrient uptake in strawberries [Abo Sedera et al. 2010], bean [Sadak et al. 2015], garlic [Fawzy et al. 2012] and carnation [Abdossi and Danaee 2019] has been reported with the use of amino acids, which is agreement with us. The effect of amino acids varies

from plant to plant. So that foliar application of amino acids in various studies increased vitamin C in strawberries [Abo Sedera et al. 2010], while they did not have a significant effect on increasing vitamin C in lettuce [Khan et al. 2019]. The results of various studies have shown that foliar application of amino acids can create more favorable conditions for the production of plant proteins [Stijn et al. 2007]. In our study, although there was no significant difference between the interaction treatments on petal protein, but in 'Orange' and 'Yellow' cultivars, the highest petal protein was recorded for 100 µM arginine and 1000 µM proline treatments, respectively. Amino acids increase mRNA transcription and facilitate the synthesis of carbohydrates and macromolecules such as proteins in plants [Thomas et al. 2009]. The positive effect of proline and arginine on the increase of petal protein compared to the control, in addition to their role in protein synthesis, can be related to the antioxidant effect of these amino acids and their resulted metabolic compounds that have anti-stress effect, which by reducing reactive oxygen species (ROS), they preserve macromolecules and proteins in plant tissue. Increased protein in garlic by spraying amino acids has been reported [Fawzy et al. 2012]. ROS is a by-product and toxic biochemical reactions that play important roles in signaling stress, growth and development, and programmed cell death. ROS are highly reactive and cause damage to other molecules and alter their function. ROS concentration is very important in living organisms. Their presence in small amounts can be useful to strengthen the plant's defense mechanisms, but overproduction of these molecules accelerates the aging process and plant decline [Bailey Serres and Mittler 2006, Alici and Arabaci 2016]. Environmental stresses increase ROS production. Increased ROS levels are associated with the destruction of macromolecules and the peroxidation of fats, and by increasing the fluidity of the membrane, it causes electrolyte leakage [Bailey Serres and Mittler 2006].

Vitamin C has antioxidant properties and plays an important role in plant growth and plant defense mechanisms and is a cofactor of many enzymes [Khan et al. 2019]. Amino acids are involved in the production of secondary metabolites, growth hormones, alkaloids, enzymes, and vitamins [Shekari and Javanmardi 2017]. Amino acids are also involved in biological cycles such as the production of ascorbic acid and citric acid [Heldt and Piechulla 2010]. Foliar application of strawberry plants with amino acids increased sugar and vitamin C in the fruit [Abo Sedera et al. 2010]. In the present study, the amount of vitamin C was increased by the use of amino acids. The increase in vitamin C in African marigold can be attributed to the antioxidant effect of amino acids as well as their effect on improving plant nutrition, which increases secondary metabolites.

Malonedialdehyde (MDA) is also the result of lipid peroxidation, which is produced by the breakdown of unstable peroxides of unsaturated fatty acids. MDA is a small and stable molecule, that its measurement shows the extent of oxidative stress damage in plants [Yaqoob et al. 2019]. Nawaz and Ashraf [2010] demonstrates that the use of compatible osmolytes reduces the effects of oxidative stress, increases the activity of antioxidants, increases turgor and water retention in plant tissues. The use of proline reduces H_2O_2 and MDA accumulation [Hasanuzzaman et al. 2014]. A group of researchers also believe that the use of proline reduces the level and production of ROS and consequently reduces lipid peroxidation and MDA accumulation [Siddiqui et al. 2012]. As well as proline itself increased proline accumulation in both African marigold cultivars and subsequently decreased MDA accumulation. Proline abducts ROS and is effective in maintaining proline external spraying to maintain its level in plant tissue [Yaqoob et al. 2019]. In the present study, in both African marigold cultivars, treatments of 100 µM arginine and 1000 µM proline had the highest proline levels, which were also the most effective treatments in improving the activity of antioxidant enzymes. These treatments also prevented the accumulation of MDA in African marigold. Therefore, foliar application of amino acids has prevented membrane damage, which is consistent with the findings of some researchers [Yan et al. 2011].

Antioxidant enzymes protect cells from the damaging effects of oxygen by reducing the energy of oxygen free radicals, donating electrons to ROS and stabilizing these molecules, and interrupting the oxidation chain reactions [Alici and Arabaci 2016]. SOD is the first enzyme in the plant's antioxidant defense line. As stress occurs and oxygen levels rise, SOD activity first increases. SOD converts oxygen free

radicles into H_2O_2 and O_2. After that, POD and CAT start working and convert H_2O_2 to oxygen and water [Abaspour Esfaden et al. 2019]. APX is also one of the main enzymes in the glutathione ascorbate cycle, which plays an effective role in dehydration of H_2O_2 [Foyer and Noctor 2005]. In the present study, MDA accumulation decreased with the use of amino acids in both African marigold cultivars and the activity of antioxidant enzymes increased. By increasing the activity of antioxidant enzymes, the amount of stress damage to the plant is reduced [Abaspour Esfaden et al. 2019]. Therefore, increasing the activity of antioxidant enzymes has prevented membrane damage and prevented electrolyte leakage and lipid peroxidation. Antioxidant enzymes are a type of protein and the use of amino acids that stimulate protein production in the plant increases the enzymes in the plant [Guerra-Guimarães et al. 2016]. Foliar application of arginine, phenylalanine and glutamine on carnation increased the activity of POD, SOD and CAT [Abdossi and Danaee 2019]. The external application of amino acids increased the activity of POD, CAT and SOD enzymes in quinoa [Yaqoob et al. 2019]. These findings confirmed our findings. Anosheh et al. [2012] showed that in order to protect cells against the effects of ROSs, the activity of antioxidants can be stimulated through the use of growth stimulants, which is consistent with the results of the present study. Our findings showed that arginine and proline compared to glutamine in both types of African marigold were effective in reducing the accumulation of MDA as well as increasing the activity of antioxidant enzymes. Arginine, as a precursor to polyamines, nitric oxide and proline, plays an important role in increasing plant resistance to stress [Winter et al. 2015]. The effectiveness of arginine in reducing the effects of ROSs and increasing the activity of antioxidant enzymes can be related to the ability of metabolic products of this amino acid to prevent stress and deterioration. The use of arginine increased the activity of antioxidant enzymes [Rezasefat Arbani et al. 2020], which is agreement with the results of the present study.

CONCLUSIONS

In conclusion, according to the results of the present study, foliar application of amino acids had a significant effect on the evaluated traits except for the time required for flower emergence, MDA accumulation and petal protein. The two amino acids, proline and arginine, were more effective than glutamine in improving the studied traits. Concentration of 100 µM arginine in 'Orange' cultivar and 1000 µM proline in 'Yellow' cultivar showed the best results. In general, the external application of the amino acids arginine at a concentration of 100 µM and proline at a level of 1000 µM is recommended to improve the ornamental and edible parameters of two cultivars of African marigold ('Orange' and 'Yellow'). However, it is recommended to do more research to achieve the effective concentration of the above amino acids, especially glutamine.

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REFERENCES

Ansari, S., Hadavi, E., Salehi, M., Moradi, P. (2011). Application of microorganisms compared with nanoparticles of silver, humic acid and gibberellic acid on vase life of cut gerbera 'Good Timing'. J. Ornam. Hortic. Plants., 1 (1), 27–33.

Arora, A.V., Singh, S.S., Sindhu, D.N., Voleti, S.R. (2007). Oxidative stress mechanisms during flower senescence. Plant Stress Global Science Books, Japan, pp: 235.

Abaspour Esfaden, M., Kalate Jari, S., Fatehi, F. (2019). The effect of salicylic acid and L-arginine on morpho-physiological properties and leaf nutrients of *Catharanthus roseus* under drought stress. J. Hortic. Sci. (Agric. Sci. Technol.), 33, 417–432. https://doi.org/10.22067/jhorts4.v33i3.73631

Abdossi, V., Danaee, E. (2019). Effects of some amino acids and organic acids on enzymatic activity and longevity of *Dianthus caryophyllus* cv. Tessino at pre-harvest stage. J. Ornam. Plants, 9, 93–104. https://dorl.net/dor/20.1001.1.22516433.2019.9.2.2.7

Abo Sedera, F., Bader, L.A., Rezk, S.M. (2010). Effect of NPK mineral fertilizer levels and foliar application

with humic and amino acids on yield and quality of strawberry. Egypt. J. Basic Appl. Sci., 25, 54–169.

Alici, E.H., Arabaci, G. (2016). Determination of SOD, POD, PPO and CAT enzyme activities in *Rumex obtusifolius* L. Ann. Res. Rev. Biol., 11, 1–7. https://doi.org/10.9734/ARRB/2016/29809

Anosheh, H.P., Emam, Y., Ashraf, M., Foolad, M.R. (2012). Exogenous application of salicylic acid and chlormequat chloride alleviates negative effects of drought stress in wheat. Adv. Stud. Biol., 4, 501–520.

Bailey Serres, J., Mittler, R. (2006). The roles of reactive oxygen species in plant cells. Plant Physiol., 141, 311. https://doi.org/10.1104/pp.104.900191

Bates, L.S., Waldren, S.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39, 205–207. https://doi.org/10.1007/BF00018060

Bidaki, S., Tehranifar, A., Khorassani, R. (2018). Postharvest shelf life extension of fruits of two strawberry (*Fragaria× ananassa* Duch.) cultivars with amino acids application in soilless culture system. J. Sci. Technol. Greenhouse Cult., 2, 1–9. http://dx.doi.org/10.29252/ejgcst.9.2.1

Calvo, P., Nelson, L., Kloepper, J. (2014). Agricultural uses of plant biostimulants. Plant Soil, 383, 3–41. https://doi.org/10.1007/s11104-014-2131-8

Croce, R., van Amerongen, H. (2014). Natural strategies for photosynthetic light harvesting. Nature Chem. Biol., 10, 492–501. https://doi.org/10.1038/nchembio.1555

Croft, H., Chen, J.M. (2017). Leaf pigment content. Elsevier, Oxford, p. 26. http://dx.doi.org/10.1016/B978-0-12-409548-9.10547-0

El-Din, K.M.G., El-Wahed, M.S.A. (2005). Effect of some amino acids on growth and essential oil content of chamomile plant. Intl. J. Agric. Biol., 7, 376–380.

Faten, S.A., Shaheen, A.M., Ahmed, A.A., Mahmoud, A.R. (2010). Effect of foliar application of amino acids as antioxidant on growth, yield and characteristics of squash. Res. J. Agric. Biol. Sci., 6, 583–588.

Fawzy, Z., El-Shal, Z., Yunsheng, L., Zhu, O., Sawan, O. (2012). Response of garlic (*Allium sativum* L.) plants to foliar spraying of some bio-stimulants under sandy soil condition. J. Appl. Sci. Res., 8, 770–776.

Fischer, W.N., André, B., Rentsch, D., Krolkiewicz, S., Tegeder, M., Breitkreuz, K., Frommer, W.B. (1998). Amino acid transport in plants. Trends Plant Sci., 3, 188–195. https://doi.org/10.1016/S1360-1385(98)01231-X

Foyer, C.H., Noctor, G. (2005). Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ., 28, 1056–1071. https://doi.org/10.1111/j.1365-3040.2005.01327.x

Giannopolitis, C., Ries, S. (1997). Superoxide dismutase. I: Occurrence in higher plant. Plant Physiol., 59, 309–314. https://doi.org/10.1104/pp.59.2.309

Ghafari, H., Tadayon, M.R., Razmjoo, J. (2018). Effect foliar of proline on some physiological indices of sugar beet (*Beta vulgaris* L.) to water deficit condition. J. Plant Proc. Funct., 7, 13–26. http://dorl.net/dor/20.1001.1.23222727.1397.7.26.2.1

Guerra-Guimarães, L., Pinheiro, C., Chaves, I., Barros, D.R., Ricardo, C.P. (2016). Protein dynamics in the plant extracellular space. Proteomes, 4, 22. http://doi.org/10.3390/proteomes4030022

Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., Fujita, M. (2014). Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. BioMed. Res. Intl., 1–17. https://doi.org/10.1155/2014/757219

Heath, R.L., Packer, L. (1968). Photoperoxidation in isolated chloroplasts. Arch. Biochem. Biophys., 125, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Heldt, H.W., Piechulla, B. (2010). Plant biochemistry. Academic Press, 145.

Hirose, T., Ackerly, D.D., Traw, M.B., Ramseier, D., Bazzaz, F.A. (1997). CO_2 elevation, canopy photosynthesis and optimal leaf area index. Ecology, 78, 2339–2350. https://doi.org/10.1890/0012-9658(1997)078[2339:CECPAL]2.0.CO;2

In, B.C., Motomura, S., Inamoto, K., Doi, M., Mori, G. (2007). Multivariate analysis of relation between preharvest environmental factors, postharvest morphological and physiological factors and vase life of cut 'Asomi Red' roses. Jap. Soc. Hortic. Sci., 76, 66–72. https://doi.org/10.2503/jjshs.76.66

Jothi, D. (2018). Extraction of natural dyes from African marigold flower (*Tagetes erecta* L.) for textile coloration. Autex Res. J., 8, 49–53.

Jubault, M., Hamon, C., Gravot, A., Lariagon, C., Delourme, R., Bouchereau, A., Manzanares-Dauleux, M.J. (2008). Differential regulation of root arginine catabolism and polyamine metabolism in club root-susceptible and partially resistant *Arabidopsis* genotypes. Plant Physiol., 146, 2008–2019. https://doi.org/10.1104/pp.108.117432

Khan, S., Yu, H., Li, Q., Gao, Y., Noman Sallam, B., Wang, H., Liu, P., Jiang, W. (2019). Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. Agronomy, 9, 1–17. https://doi.org/10.3390/agronomy9050266

Khattab, E.A., Afifi, M.H. (2009). Effect of proline and glycine betaine on canola plants grown under salinity stress condition. Modern J. Appl. Biol. Sci., 3, 42–51.

Leghari, S.J., Wahocho, N.A., Laghari, G.M., Laghari, A.H., Bhabhan, G.M., Talpur, H.K., Bhutto, T.A., Wahocho, S.A., Lashari, A.A. (2016). Role of nitrogen for plant growth and development: a review. Adv. Environ. Biol., 10, 209–218.

Madadkar Haghjou, M. (2013). Comparative study of some physiological characteristics (chlorophyll content, photosynthetic activity) in selection of *Dunaliella* sp. strains (isolated from Iranian waters). J. Cell Tiss., 4, 85–102.

Mazumdar, B.C., Majumder, K. (2003). Methods on physicochemical analysis of fruits. Univ. College Agric., Calcutta Univ., 136–150.

Mirzapour, M., Golchin, A., Khoshgoftarmanesh, A., Tehrani, M. (2020). Effect of amino acid and zinc application on uptake and transport of zinc and iron in rapeseed in nutrient solution culture. J. Crops Improv. (J. Agric.), 21, 379–392. https://doi.org/10.22059/jci.2019.280063.2204

Moliner, C., Barros, L., Dias, M.I., López, V., Langa, E., Ferreira, I.C.F.R., Gómez-Rincón, C. (2018). Edible flowers of *Tagetes erecta* L. as functional ingredients: phenolic composition, antioxidant and protective effects on *Caenorhabditis elegans*. Nutrients, 10, 2–14. https://doi.org/10.3390/nu10122002

Nawaz, K., Ashraf, M. (2010). Exogenous application of glycine betaine modulates activities of antioxidants in maize plants subjected to salt stress. J. Agron. Crop Sci., 196, 28–37. https://doi.org/10.1111/j.1439-037X.2009.00385.x

Neill, S., Desikan, R., Hancock, J.T. (2003). Nitric oxide signaling in plants. New Phytol., 159, 11–35. https://doi.org/10.1046/j.1469-8137.2003.00804.x

Porcel, R., Ruiz-Lozano, J.M. (2004). Arbuscular mycorrhizal influence on leaf water potential, solute accumulation and oxidative stress in soybean plants subjected to drought stress. J. Exp. Bot., 55, 1743–1750. https://doi.org/10.1093/jxb/erh188

Qiu, X.M., Sun, Y.Y., Ye, X.Y., Li, Z.G. (2020). Signaling role of glutamate in plants. Front. Plant Sci., 10, 1743. https://doi.org/10.3389/fpls.2019.01743

Raeisi, M., Farahani, L., Palashi, M. (2014). Changes of qualitative and quantitative properties of radish (*Raphanus sativus* L.) under foliar spraying through amino acid. Int. J. Biosci., 4, 463–468. http://dx.doi.org/10.12692/ijb/4.1.463-468

Rengel, Z., Romheld, V. (2000). Root exudation and Fe uptake and transport in wheat genotypes differing in tolerance to Zn deficiency. Plant Soil, 222, 25–34. https://doi.org/10.1023/A:1004799027861

Rezasefat Arbani, M., Kalateh Jari, S., Fatehi, F., Khalighi, A. (2020). The effect of humi-forthi and L-arginine amino acid on growth, physiological and biochemical characteristics of marigold (*Tagetes erecta*) under drought stress. Iran. J. Hortic. Sci. (Iran. J. Agric. Sci.), 51, 365–373. https://doi.org/10.22059/ijhs.2019.262454.1483

Sadak, M.S., Mostafa, H.A. (2015). Physiological role of pre-sowing seed with proline on some growth, biochemical aspects, yield quantity and quality of two sunflower cultivars grown under seawater salinity stress. Sci. Agric., 9, 60–69.

Sadak, M.S.H., Abd El-Hamid, M.T., Schmidhalter, U. (2015). Effect of foliar application of amino acids on plant yield and physiological parameters in bean plants irrigated with seawater. Acta Biol. Colombiana, 20, 141–152. https://doi.org/10.15446/abc.v20n1.42865

Shafie, F., Bayat, H., Aminifard, M.H., Daghighi, S. (2020). Biostimulant effects of seaweed extract and amino acids on growth, antioxidants, and nutrient content of yarrow (*Achillea millefolium* L.) in the field and greenhouse conditions. Commun. Soil Sci. Plant Anal., 52, 964–975. https://doi.org/10.1080/00103624.2021.1872596

Shekari, G., Javanmardi, J.E. (2017). Effects of foliar application pure amino acid and amino acid containing fertilizer on broccoli (*Brassica oleracea* L. var. Italica) transplant. Crop Sci. Technol., 5, 280. http://dx.doi.org/10.4172/2329-8863.1000280

Siddiqui, M.H., Al-Whaibi, M.H., Sakran, A.M., Ali, H.M., Basalah, M.O., Faisal, M., Alatar, A., Al-Amri, A.A. (2012). Calcium-induced amelioration of boron toxicity in radish. J. Plant Growth Reg., 61–71. https://doi.org/10.1007/s00344-012-9276-6

Singh, Y., Gupta, A., Kannojia, P. (2020). *Tagetes erecta* (marigold): a review on its phytochemical and medicinal properties. Curr. Med. Drug Res., 4, 1–7.

Soroori, S., Danaee, E., Hemmati, K., Ladan Moghadam, A. (2021). Effect of foliar application of proline on morphological and physiological traits of *Calendula officinalis* L. under drought stress. J. Ornam. Plants, 11, 13–30. https://dorl.net/dor/20.1001.1.22516433.2021.11.1.1.8

Souri, M.K., Hatamian, M. (2018). Amino chelates in plant nutrition: A review. J. Plant Nutr., 1–13. https://doi.org/10.1080/01904167.2018.1549671

Stijn, S., Jos, V., Roseline, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. Fems Microbiol. Rev., 31, 425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Teixeira, W.F., Fagan, E.B., Soares, L.H., Soares, J.N., Reichardt, K., Neto, D.D. (2018). Seed and foliar

application of amino acids improve variables of nitrogen metabolism and productivity in soybean crop. Front. Plant Sci., 9, 396. https://doi.org/10.3389/fpls.2018.00396

Thomas, J., Mandal, A.K.A., Raj Kumar, R., Chrodia, A. (2009). Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camelia* sp.). Int. J. Agric. Res., 4, 228–236. https://dx.doi.org/10.3923/ijar.2009.228.236

Trovato, M., Matioli, R., Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. Rend. Lincei, 19, 325–346. https://doi.org/10.1007/s12210-008-0022-8

Winter, G., Todd, C.D., Trovato, M., Forlani, G., Funck, D. (2015). Physiological implications of arginine metabolism in plants. Front. Plant Sci., 6, 223–231. https://doi.org/10.3389/fpls.2015.00534

Yan, Z., Guo, S., Shu, S., Sun, J., Tezuka, T. (2011). Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. Afr. J. Biotechnol., 10, 18381–18390. https://doi.org/10.5897/AJB11.1073

Yaqoob, H., Akram, N.A., Iftikhar, S., Ashraf, M., Khalid, N., Sadiq, M., Alyemeni, M.N., Wijaya, L., Ahmad, P. (2019). Seed pretreatment and foliar application of proline regulate morphological, physio-biochemical processes and activity of antioxidant enzyme in plants of two cultivars of quinoa (*Chenopodium quinoa* Willd.). Plants, 8, 1–17. https://doi.org/10.3390/plants8120588

Zulaikha, R. (2013). Effect of foliar spray of ascorbic acid, Zn, seaweed extracts (Sea) force and biofertilizers (EM-1) on vegetative growth and root growth of olive (*Olea europaea* L.) transplants cv. Hoj Blanca. Int. J. Pure Appl. Sci. Technol., 17, 79–89.