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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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[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 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

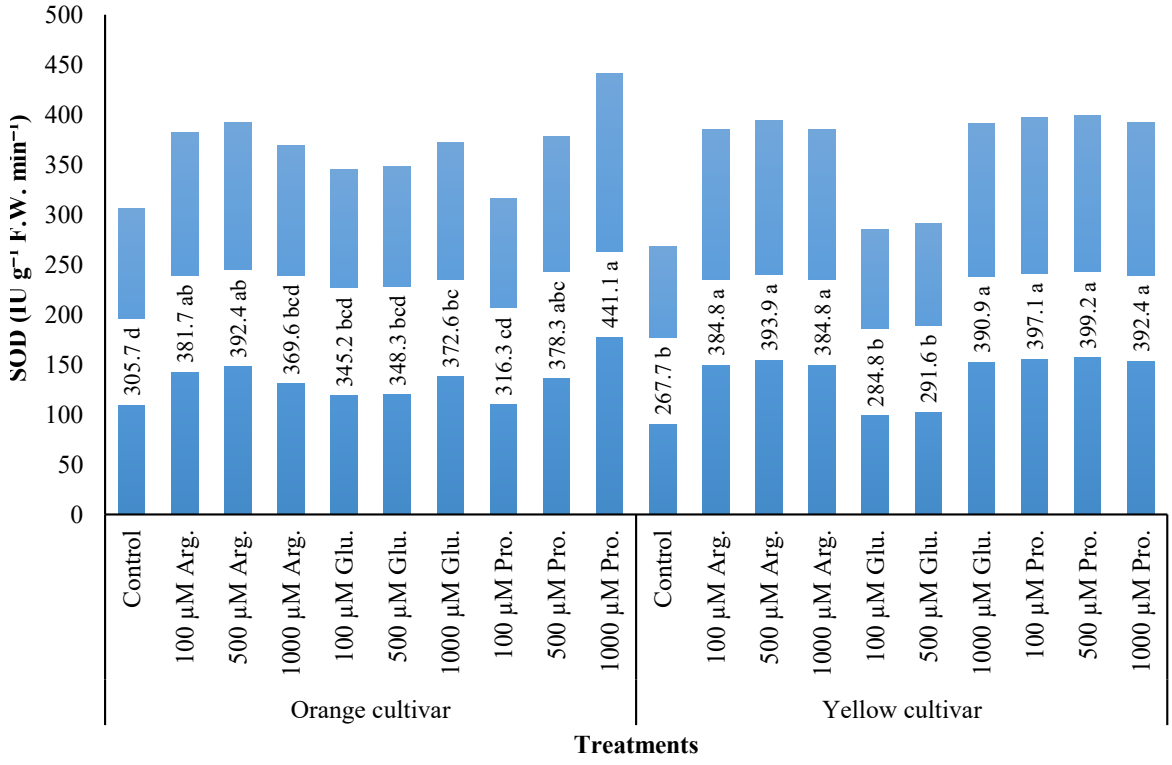

Fig. 1. Effect of different treatments on SOD activity

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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