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Effect of Arginine, Proline and Glutamine Amino Acids on Morphological and Physiological Traits of Two African Marigold (*Tagetes erecta* L.) Cultivars

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Abstract

In addition to its ornamental and medicinal applications, marigold is considered an edible flower, too. To produce marigold with safe and non-chemical methods, a factorial experiment was conducted based on a completely randomized design with 3 replications and 20 treatments. The experimental treatments included 2 cultivars of marigold ('Yellow' and 'Orange') and 3 amino acids including arginine, glutamine and proline at 3 levels (100, 500 and 1000 µM), as well as distilled water as the control treatment. The results showed that amino acids had positive effects on the recorded yield and growth characteristics. The treatment of 100 µM arginine outperformed other treatments in increasing leaf number, flower diameter, shoot fresh weight and shoot dry matter, reducing electrolyte leakage and improving catalase activity in cv. 'Orange'. In cv. 'Yellow', the highest leaf number, shoot fresh weight and root dry matter, the lowest polyphenol oxidase activity, the lowest electrolyte leakage and the highest catalase activity were related to the treatment of 1000 µM proline. The highest total phenol was obtained from 100 µM arginine in two cultivar. With the application of amino acids, flavonoids were increased in both cultivars versus the control. Therefore, it is recommended to apply amino acids, especially arginine (100 µM) and proline (1000 µM), to produce marigolds 'Orange' and 'Yellow' in an organic way, respectively.

Keywords: Edible flower, Growth stimulator, Organic nitrogen, Phenol compounds.

INTRODUCTION

African marigold (*Tagetes erecta* L.) is an ornamental, medicinal, and edible species from the family Asteraceae (Kaisoon *et al*., 2012; Petrova *et al*., 2016). Nowadays, due to the importance of crop production by sustainable agricultural systems, growing attention is paid to organic and biological matters, e.g., the application of amino acids-containing organic fertilizers to improve soil properties and crop yields (Faten *et al*., 2010; Ali and Hassan, 2013; Raeisi *et al*., 2014). Amino acids are the organic form of nitrogen and among the most important sources of organic nitrogen in soils (Keutgen and Pawelzik, 2008; Cerdana *et al*., 2009; Faten *et al*., 2010). Research shows that amino acids can complex metals and increase nutrient availability, so they are used by themselves as chelating factors to escalate plant growth and development (Liu *et al*., 2008; Ali and Hassan, 2013). The positive effects of both foliar and soil application of amino acids have been reported on the morphological and physiological traits of the strawberry (Shehata *et al*., 2011; Bidaki *et al*., 2018) and African marigold (Ali and Hassan, 2013).

Proline is one of the most important constituents of plant proteins and is involved in preserving plant resistance to stresses. It is also regarded as a source of carbon, nitrogen, and energy in plants (El-Din and El-Wahed, 2005; de Sousa *et al*., 2020). Proline improves plant growth by contributing to increasing the uptake of specific ions (e.g., Mg^{2+}), increasing photosynthesizing pigments (Khattab and Afifi, 2009), preserving stomatal conductance, enhancing photosynthesis rate (Ali *et al*., 2007) and maintaining the structure and activity of enzymes (de Sousa *et al*., 2020).

Glutamine is one of the most available amino acids and a source of energy. Glutamine is a precursor for the synthesis of chlorophyll and other amino acids. This amino acid plays a key role in the metabolic cycles of carbon and nitrogen and the accumulation of sugars and proteins in plants. It is also essential for germination and plant growth and development (Taiz and Zeiger, 2010; Bidaki *et al*., 2018).

Arginine is one of the most important storage and nitrogenous compounds in plants. Arginine is an essential amino acid, a major component of proteins and a precursor for the biosynthesis of polyamines, proline, nitric oxide, and agmatine. This amino acid at a ratio of 6C/4N plays important physiological roles in plants (Jubault *et al*., 2008). Arginine is involved in the transportation and storage of nitrogen, the activity of antioxidant enzymes and the synthesis of plant enzymes related to flowering and fruit-bearing (Bidaki *et al*., 2018). In addition to the positive effects of arginine in plants, the metabolic products of this amino acid (such as polyamines, nitric oxide, and proline) have key roles to play in plant growth and development and plant resistance to stresses and adverse environmental conditions (Groppa and Benavides, 2008; Trovato *et al*., 2008).

Given the positive effect of amino acids on the growth and development of different plants, the present research aimed to shed light on the effect of applying arginine, glutamine and proline as safe and plant growth-promoting compounds on the morphological and physiological traits of two types of African marigold including ‘Yellow’ and ‘Orange’ marigolds.

MATERIALS AND METHODS

This research investigated the effect of amino acids on morphological and physiological traits of two African marigold cultivars in a factorial experiment laid on a completely randomized design with 20 treatments, 3 replications and 60 plots, each containing 8 plants. The experimental treatments included two African marigold cultivars (‘Yellow’ and ‘Orange’) and three amino acids (glutamine, arginine, and proline) at three levels (100, 500 and 1000 µM), as well as distilled water as the control.

The F1 seeds of the two cultivars (Taishan™ Orange and Yellow) were purchased at the PanAmerican Seed Institute with a vigor of >95%. After the germination test, the seeds were cultured on seedling trays containing perlite + garden soil + river sand at equal proportions. After the seedlings reached the 2-leaf stage, they were transferred to pots with a diameter of 14 cm containing perlite + garden soil + river sand at equal proportions. The amino acids were sprayed for the first time 10 days after the transfer of the seedlings (6-leaf stage). The second and third steps of foliar application were performed at 10-day intervals. During the experimental period, the plants were fertilized with Kristalon^{TM} NPK fertilizer (20-20-20) and irrigated. Also, all measures were taken to control weeds and pests. All experimental steps were conducted in a greenhouse with standard conditions (Humidity 70 to 75% and temperature 22 to 25 ºC). 55 days after the last foliar application, traits evaluation performed.

Assessment of traits

The number of leaves per plant was measured by counting the leaves of three plants from each replication at the end of the experiment (flower withering) and the average was reported. After the flowers opened, the flower diameter of three plants was measured from each replication with a caliper and the average was reported. To measure root and shoot fresh and dry weight, the shoots were cut at crown from the root at the end of the experiment. After the mud and dirt were cleaned from the roots, they were weighed with a 0.01 g digital scale to record their fresh weight. Then, the shoots and roots were separately oven-dried at 105°C for 24 hours to find out their dry weight with the 0.01-g digital scale. It was reported in g. The root dry matter and shoot dry matter was calculated by dividing their dry weight by their fresh weight multiplied by 100. To measure electrolyte leakage, the leaves were sampled. Also, to estimate total phenol, flavonoids and the enzymes catalase (CAT) and polyphenol oxidase (PPO), the petals were sampled after the first flower was opened on the plants. The electrolyte leakage was measured by Kaya *et al*.'s (2001) method, flavonoid was measured by Du *et al.*'s (2009) method and total phenol was estimated by Singleton *et al*.'s (1999) method. CAT and PPO activities were also determined by Dhindsa *et al.*'s (1981) and Nicoli *et al*. (1991) procedures, respectively.

Data analysis

Data were analyzed by the SPSS 19.0 statistical software package and the means were compared by the LSD statistical test.

RESULTS

Leaf number

The interaction of 'cultivar × amino acid' was significant ($P < 0.05$) for the leaf number of the African marigold (Table 1). The marigolds 'Orange' produced more leaves when they were treated with proline and arginine than when they were treated with glutamine and the control. Increasing the rate of the amino acids resulted in increasing the leaf number in the marigold 'Yellow'. This cultivar produced the highest number of leaves (28 leaves) in the treatment of 1000 µM proline. Both cultivars exhibited the lowest number of leaves in the control (Table 2).

Flower diameter

Flower diameter was significantly ($P < 0.05$) affected by the interaction of 'cultivar × amino acid' (Table 1). The lowest flower diameter in both cultivars was observed in the control. The highest flower diameter of cv. 'Orange' belonged to the treatments of 100 µM arginine (4.61 cm), 100 µM glutamine (3.97 cm), 500 µM proline (3.64 cm) and 100 µM proline (3.62 cm), but these four treatments did not differ significantly. Cv. 'Yellow' showed the highest flower diameter in the treatments of 500 µM arginine (4.11 cm) and 100 µM proline (4.03 cm) with no statistically significant difference between them (Table 2).

Shoot fresh weight

The interactive effect of 'cultivar × amino acid' was found to be significant ($P < 0.01$) on shoot fresh weight (Table 1). The lowest shoot fresh weight of cv. 'Orange' was observed in the treatment of 100 µM glutamine (5.14 g) and the control (5.30 g) and the highest in the treatments of 100 µM arginine (10.80 g), 500 µM proline (9.73 g) and 1000 µM proline (9.37 g). Regarding cv. 'Yellow', the treatments of 1000 µM proline, 500 µM glutamine and 100 µM arginine, which did not differ significantly, were related to the highest shoot fresh weight of 9.30, 8.18 and 7.66 g, respectively. The control showed the lowest shoot fresh weight (4.27 g) of cv. 'Yellow', which had no significant difference from the treatments of 500 and 1000 µM arginine, 100 and 1000 µM glutamine (Table 2).

Table 1. Analysis of variance for the effect of different treatments on the measured traits.

S.o.V	**df**	**Lear no.**	**Flower diameter**	**Shoot fresh weight**	**Shoot dry matter**	**Root fresh weight**	**Root dry matter**	**Electrolyte leakage**
Cultivar (C)	1	41.3^{ns}	0.261^{ns}	18.07**	0.196^{ns}	6.81**	3.64^{ns}	0.782^{ns}
Amino acid (A)	9	64.83**	1.409**	6.53**	23.3**	0.676^{ns}	14.4**	10.37**
C × A	9	45.9*	0.893*	13.3**	20.4**	0.997*	2.752^{ns}	6.44*
Error	38	12.25	0.308	1.77	4.81	0.35	3.83	2.245
CV (%)		16..81	17.26	19.67	10.02	20.84	14.53	10.16

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 1. Continued.

S.o.V	**df**	**Total Phenol**	**Flavonoid 270 nm**	**Flavonoid 300 nm**	**Flavonoid 330 nm**	**Catalase activity**	**Polyphenol oxidase**
Cultivar (C)	1	0.0395^{ns}	0.8283^{ns}	4.24**	0.177^{ns}	9805**	0.669**
Amino acid (A)	9	0.1038*	2.628**	1.88**	1.70**	11308**	0.724**
C × A	9	0.1180*	0.913*	1.196**	2.98**	21864**	0.291**
Error	38	0.04	0.351	0.176	0.52	857.63	0.019
CV (%)		14.14	6.04	5.14	10.59	8.10	15.03

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 2. Means comparison for the effect of 'cultivar × amino acid' on the measured traits.

	Treatments	**Leaf no.**	**Flower diameter (cm)**	**Shoot fresh weight (g)**	**Shoot dry matter (%)**	**Root fresh weight (g)**	**Root dry matter (%)**
Orange	Control	15.08 ^{e}	2.56 ^{c}	5.30 ^{d}	17.55 ^{c}	2.49 ^{d}	11.74 ^{a}
	100 µM Arg.	29.10 ^{a}	4.61 ^{a}	10.80 ^{a}	27.29 ^{a}	3.68 ^{abc}	15.69 ^{a}
	500 µM Arg.	23.20 $^{a\text{-}d}$	3.00 ^{bc}	5.82 ^{cd}	21.34 ^{bc}	2.716 ^{cd}	13.32 ^{a}
	1000 µM Arg.	20.13 $^{b\text{-}e}$	3.10 ^{bc}	6.30 ^{cd}	21.61 ^{bc}	3.92 ^{ab}	13.70 ^{a}
	100 µM Glu.	15.26 ^{de}	3.16 ^{bc}	5.14 ^{d}	18.08 ^{c}	2.60 ^{cd}	12.30 ^{a}
	500 µM Glu.	17.65 ^{cde}	2.82 ^{bc}	6.04 ^{cd}	20.64 ^{bc}	2.96 ^{bcd}	13.12 ^{a}
	1000 µM Glu.	19.25 $^{b\text{-}e}$	2.87 ^{bc}	6.70 ^{cd}	21.59 ^{bc}	2.32 ^{d}	12.82 ^{a}
	100 µM Pro.	25.26 ^{abc}	3.62 ^{abc}	7.90 ^{bc}	22.76 ^{b}	3.16 $^{a\text{-}d}$	13.30 ^{a}
	500 µM Pro.	26.93 ^{ab}	3.64 ^{abc}	9.73 ^{ab}	23.74 ^{ab}	3.94 ^{ab}	16.21 ^{a}
	1000 µM Pro.	24.73 ^{abc}	3.97 ^{ab}	9.37 ^{ab}	23.49 ^{ab}	4.24 ^{a}	16.75 ^{a}
Yellow	Control	16.37 ^{e}	2.18 ^{d}	4.27 ^{bc}	17.38 ^{d}	2.05 ^{b}	8.44 ^{a}
	100 µM Arg.	18.76 ^{cde}	4.11 ^{a}	7.66 ^{a}	25.32 ^{a}	2.51 ^{ab}	14.61 ^{a}
	500 µM Arg.	19.86 ^{cd}	3.18 ^{bc}	5.50 ^{b}	23.80 ^{ab}	2.63 ^{ab}	14.57 ^{a}
	1000 µM Arg.	20.58 ^{c}	3.38 ^{b}	5.62 ^{b}	22.47 ^{ab}	2.20 ^{ab}	13.65 ^{a}
	100 µM Glu.	17.22 ^{de}	2.72 ^{bc}	5.30 ^{b}	18.50 ^{cd}	2.92 ^{a}	11.65 ^{a}
	500 µM Glu.	18.52 ^{cde}	2.64 ^{cd}	8.18 ^{a}	21.33 ^{bc}	2.72 ^{ab}	12.00 ^{a}
	1000 µM Glu.	24.33 ^{b}	2.60 ^{cd}	5.79 ^{b}	21.26 ^{bc}	2.77 ^{ab}	12.37 ^{a}
	100 µM Pro.	17.26 ^{de}	3.06 ^{bc}	5.44 ^{b}	21.48 ^{bc}	2.48 ^{ab}	13.08 ^{a}
	500 µM Pro.	19.06 ^{cde}	3.02 ^{bc}	5.05 ^{b}	22.43 ^{ab}	2.72 ^{ab}	14.93 ^{a}
	1000 µM Pro.	28.00 ^{a}	4.03 ^{a}	9.30 ^{a}	25.26 ^{a}	2.27 ^{ab}	15.09 ^{a}

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

Shoot dry matter

The interactive effect of 'cultivar × amino acid' was significant (P < 0.01) on shoot dry matter (Table 1). Both 'Yellow' and 'Orange' recorded their minimum shoot dry matter in the control. The highest shoot dry matter of 'Orange' was observed in the treatments of 100 µM arginine (27.29%), 500 and 1000 µM proline (23.74% and 23.49%, respectively). There was not a significant difference among these three treatments. The highest shoot dry matter of 'Yellow' was related to the treatments of 100 µM arginine (25.23%), which had no statistically significant difference with the treatments of 500 and 1000 µM proline and arginine (Table 2).

Roof fresh weight

Shoot fresh weight was significantly (P < 0.05) affected by the interaction of 'cultivar × amino acid' (Table 1). The shoot fresh weight of cv. 'Orange' was increased versus the control (2.49 g) when it was treated with either level of proline or arginine or 100 or 500 µM glutamine. This cultivar recorded its lowest shoot fresh weight (2.32 g) in the treatment of 1000 µM glutamine, which did not differ from the control (2.49 g) significantly. Regarding cv. 'Yellow', no significant difference was observed in this trait among different levels of amino acids. However, the application of amino acids was effective in increasing their root fresh weight versus the control whose root fresh weight was 2.05 g (Table 2).

Root dry matter

This trait was not influenced by the interaction of the experimental treatments significantly (Table 1). Nonetheless, the application of the amino acids increased the root dry matter of both cultivars versus the control (Table 2).

Total phenol

Total phenol was significantly (P < 0.05) influenced by the interaction of 'cultivar × amino acid' (Table 1). In both cultivars, the control had the lowest total phenol content. The highest total phenol content of 'Orange' was related to the application of 100 µM arginine (1.90 mg GAE g^{-1} F.W.), which had no statistically significant difference with the treatments of 500 µM arginine, 1000 and 500 µM proline. cv. 'Yellow' exhibited the highest total phenol content when it was treated with 100 µM arginine (1.71 mg GAE g^{-1} F.W.), which was in the same statistical group with the other levels of amino acids but differed from the control (1.12 mg GAE g^{-1} F.W.) significantly (Fig. 1).

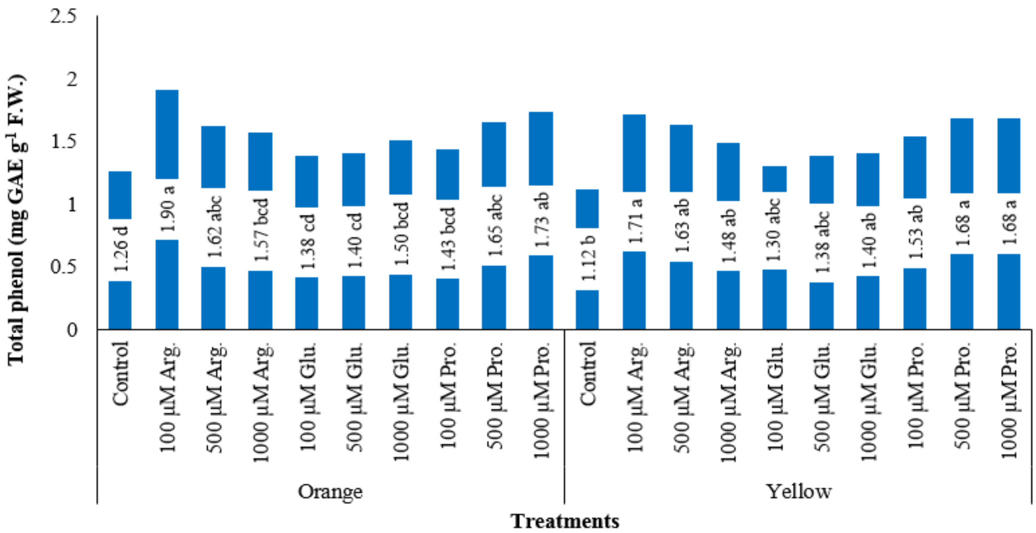

Fig. 1. Means comparison of different levels of amino acids on total phenol of *Tagetes erecta* 'Orange' and 'Yellow'. Arg: Arginine, Glu: Glutamine and Pro: Proline.

Flavonoid

The flavonoid content of the African marigolds ‘Yellow’ and ‘Orange’ was assessed at three wavelengths of 270, 300 and 330 nm. Based on the analysis of variance, the interaction of the experimental treatments was significant for flavonoid 270 nm ($P < 0.05$) and flavonoid at 300 and 330 nm ($P < 0.01$) (Table 1).

In ‘Orange’, the lowest flavonoid content at 270, 300 and 330 nm was related to the control. The highest flavonoid content at 270 nm was obtained from the application of 100 µM arginine (10.53 µmol g^{-1} F.W.), which did not differ from 500 µM arginine and glutamine and 100, 500 and 1000 µM proline significantly. The highest flavonoid content at 300 nm in cv. ‘Orange’ was recorded by the treatments of 500, 1000 µM proline and 100 µM arginine, but there was not a significant difference among them. As is evident in Fig. 2, all amino acids increased flavonoid content of ‘Orange’ at 330 nm versus the control, although the control and the treatment of 1000 µM glutamine did not differ significantly (Fig. 2).

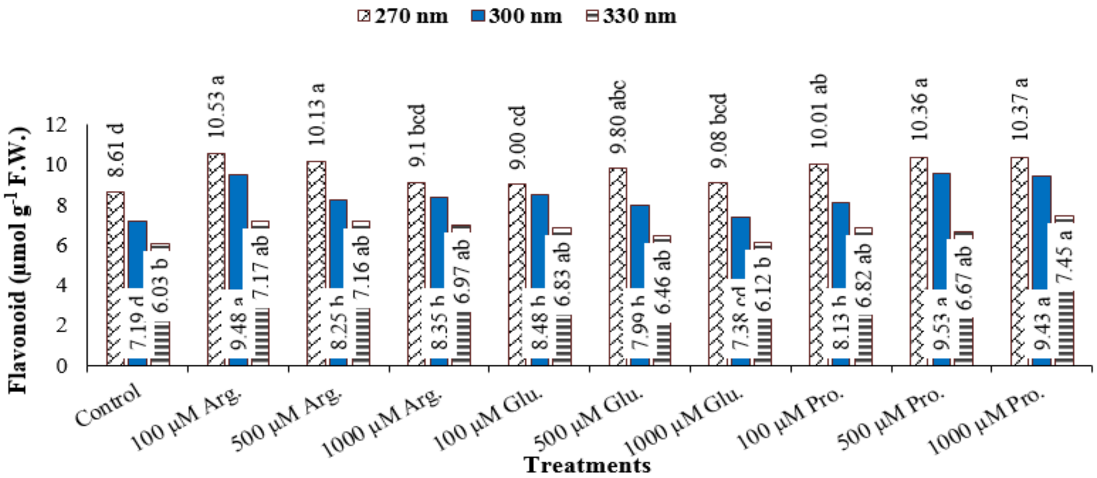

Fig. 2. Means comparison of different levels of amino acids on flavonoid 270, 300 and 330 nm of *Tagetes erecta* ‘Orange’. Arg: Arginine, Glu: Glutamine and Pro: Proline.

In cv. ‘Yellow’, the treatments of 1000 µM proline, 100 µM arginine and 100 µM proline were related to the highest flavonoid content at 270 nm. The highest flavonoid content of ‘Yellow’ at 300 nm was obtained from the treatments of 1000 µM proline and 100 µM arginine, but they had no significant difference. The maximum flavonoid content at 300 nm was recorded by the plants treated with 1000, 500 µM proline and 100 µM arginine, which did not differ from one another significantly (Fig. 3).

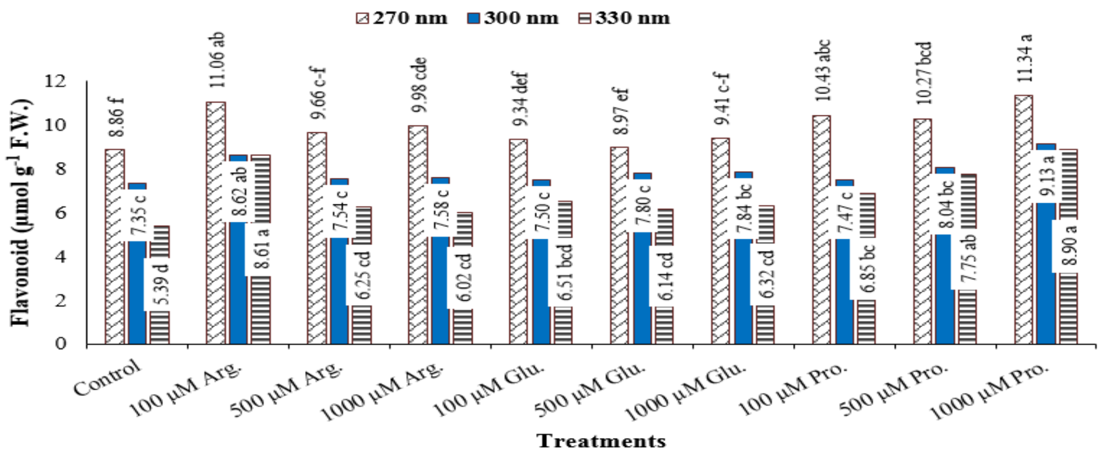

Fig. 3. Means comparison of different levels of amino acids on flavonoid 270, 300 and 330 nm of *Tagetes erecta* ‘Yellow’. Arg: Arginine, Glu: Glutamine and Pro: Proline.

Electrolyte leakage

The interaction of 'cultivar × amino acid' was significant ($P < 0.05$) for electrolyte leakage (Table 1). In 'Orange', the control had the highest electrolyte leakage (17.67%), which did not differ from that of the treatment of 100 µM glutamine (16.16%) significantly. The lowest was 12.62% observed in the treatment of 100 µM arginine. In 'Yellow', the lowest and highest electrolyte leakage was recorded by 1000 µM proline (11.50%) and the control (16.80%), respectively. There were no statistically significant differences among the control and the treatments of 100 and 500 µM proline, 100, 500 and 1000 µM glutamine and arginine. Indeed, it can be said that the application of amino acids was more effective in reducing electrolyte leakage of 'Orange' than that of 'Yellow' (Fig. 4).

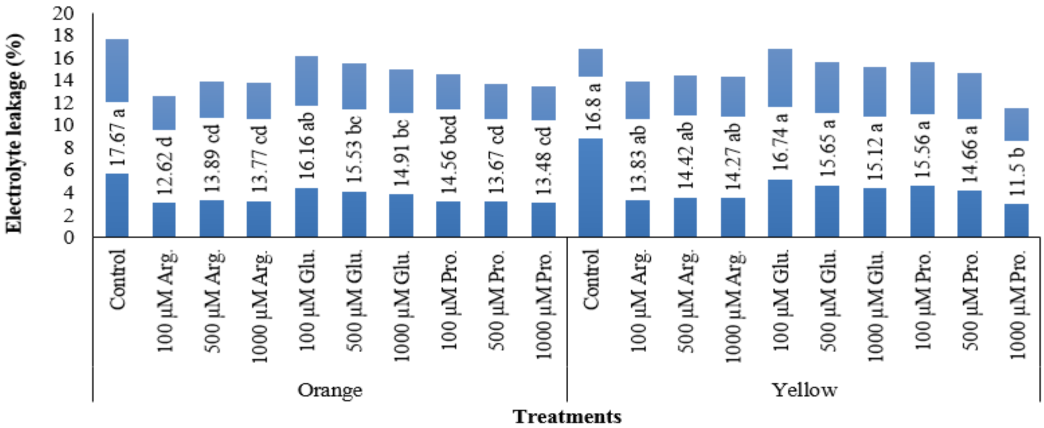

Fig. 4. Means comparison of different levels of amino acids on electrolyte leakage of *Tagetes erecta* 'Orange' and 'Yellow'. Arg: Arginine, Glu: Glutamine and Pro: Proline.

Catalase (CAT) activity

The interaction of the experimental treatments was significant ($P < 0.01$) for the CAT activity (Table 1). In both cultivars, the application of the amino acids increased the CAT activity versus the control. In 'Orange', the control (143.3 IU g^{-1} F.W. min^{-1}) and 100 µM glutamine (188.5 IU g^{-1} F.W. min^{-1}) had the lowest CAT activity with no statistically significant difference with one another. The highest CAT activity of 'Orange' was recorded at the treatment of 100 µM arginine (666.01 IU g^{-1} F.W. min^{-1}) followed by 1000 µM proline (470.2 IU g^{-1} F.W. min^{-1}). In 'Yellow', the control exhibited the lowest CAT activity (166.8 IU g^{-1} F.W. min^{-1}), whereas the highest was observed in the treatments of 1000 µM proline (610.3 IU g^{-1} F.W. min^{-1}) and 1000 µM arginine (567.5 IU g^{-1} F.W. min^{-1}) (Fig. 5).

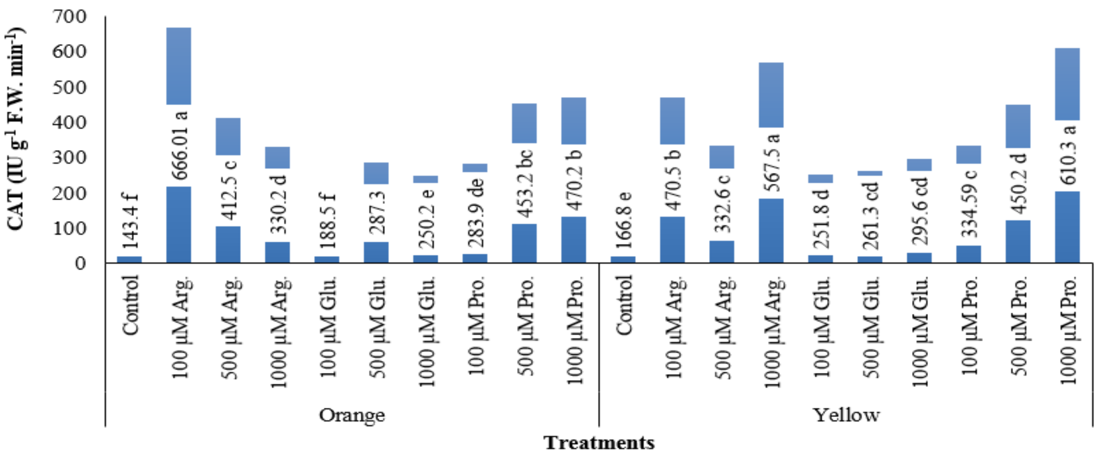

Fig. 5. Means comparison of different levels of amino acids on catalase (CAT) activity of *Tagetes erecta* 'Orange' and 'Yellow'. Arg: Arginine, Glu: Glutamine and Pro: Proline.

Polyphenol oxidase (PPO) activity

The effect of 'cultivar × amino acid' was significant ($P < 0.01$) on the PPO activity (Table 1). The control and the treatment of 100 µM glutamine were related to the highest PPO activity (1.7 and 1.40 IU g^{-1} F.W. min^{-1}, respectively). The best treatments in reducing the PPO activity in 'Orange' were 1000 µM proline (0.43 IU g^{-1} F.W. min^{-1}) and 100 µM arginine (0.57 IU g^{-1} F.W. min^{-1}). In 'Yellow', the control (1.71 IU g^{-1} F.W. min^{-1}) and 1000 µM proline (0.26 IU g^{-1} F.W. min^{-1}) exhibited the highest and lowest PPO activity, respectively (Fig. 6).

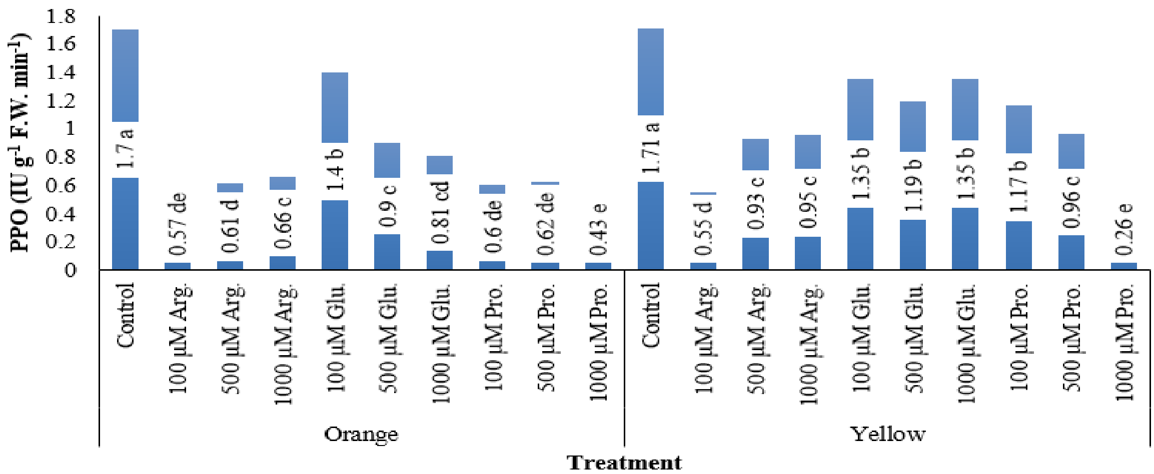

Fig. 6. Means comparison of different levels of amino acids on polyphenol oxidase (PPO) activity of *Tagetes erecta* 'Orange' and 'Yellow'. Arg: Arginine, Glu: Glutamine and Pro: Proline.

DISCUSSION

Amino acids are the organic form of nitrogen and play a specific role as the main constituent of proteins in plant growth and development (Cerdana *et al.*, 2009; Faten *et al.*, 2010). In the present work, the foliar application of amino acids to African marigold cvs. 'Orange' and 'Yellow' increased leaf number and flower diameter. The positive effect of the amino acids on these traits was stronger in 'Orange' than in 'Yellow'. Amino acids are growth stimulators and facilitate nutrient uptake, improve metabolic activities and enhance the vegetative and reproductive growth of plants (de Sousa *et al.*, 2020). Amino acids are a source of organic nitrogen, carbon, and energy (de Sousa *et al.*, 2020). Nitrogen is also an essential element for plant growth and development (Chaudhary *et al.*, 2018). It can, therefore, be said that the increase in leaf number and flower diameter has resulted from the increased availability of nitrogen and nutrients for the marigold plants. There are reports as to the increased number of leaves in green mint (Azarpira *et al.*, 2020), pepper (Dinoo *et al.*, 2009) and the increased growth and yield of Chinese cabbage (Cao *et al.*, 2010) with the application of amino acids, which is consistent with our findings.

As well, it has been reported that the application of amino acids increases vegetative and reproductive growth by increasing the uptake of water and nutrients, reducing stress imposed on the plants, increasing leaf area and number, increasing photosynthetic pigments and enhancing photosynthesis efficiency, thereby expanding carbohydrate reserve and increasing the fresh and dry weight of the plants (Ameri *et al.*, 2007; Faten *et al.*, 2010). Similarly, the application of amino acids in the present study increased the fresh and dry weight of the marigolds' shoots and roots. Similar results have been reported about the positive effect of amino acids on the fresh and dry weight of Chinese cabbage (Hua-Jing *et al.*, 2007), *Thuja orientalis* L. (Nahed *et al.*, 2010), and chamomile (El-Din and Abd El-Wahed, 2005), which agrees with our findings. Researchers argue that higher photosynthesis efficiency increases plant weight and yield (Simkin *et al.*, 2019).

So, it can be claimed that amino acids increased the fresh and dry weight of the marigolds by influencing chlorophyll pigments and increasing the photosynthesis process (Faten *et al*., 2010).

Phenol compounds are the most important secondary metabolites in plants that have antioxidant properties. The phenol compounds in foods are divided into three groups – phenols and simple phenol acids, the derivatives of cinnamic hydroxyl acid, and flavonoids. Flavonoids are the most important group of phenol compounds, which are beneficial to human health due to their antioxidant effect (de la Rosa *et al*., 2019). African marigolds, which are consumed as ornamental and edible flowers, are an invaluable natural source of phenol and antioxidant compounds (Youssef *et al*., 2020). In the present research, the foliar application of the amino acids increased total phenols and flavonoids in both cultivars. Among the environmental factors, nutrients play a more highlighted role in increasing secondary metabolites in medicinal and edible plants. In fact, nutrients impact the quantity and quality of active ingredients by influencing the vegetative and reproductive growth of the plants (de la Rosa *et al*., 2019; Omidbigi, 2007). Therefore, the role of amino acids in improving nutrient uptake can be regarded as a reason for the positive effect of amino acids on improving total phenols and flavonoids in the African marigold versus the control. Previous studies have revealed the effect of the foliar application of amino acids on increasing phenol compounds and flavonoids in fenugreek (Abd-EL Hamid *et al*., 2016), mint (Taraseviciene *et al*., 2021) and *Aloe vera* (Oraghi Ardebili *et al*., 2012).

Reactive oxygen species (ROS) is a toxic byproduct of aerobic metabolism that is produced in response to biotic and abiotic stresses, as well as apoptosis. The increased level of ROS and the disruption of the balance in ROS synthesis and elimination in plant tissues are harmful to plant cells and lipid peroxidation. Therefore, to cope with these compounds, plants use antioxidant systems to overcome ROS toxicity (Bailey-Serres and Mittler, 2006).

Catalase (CAT) is an antioxidant enzyme and the most important H_2O_2 scavenger and decomposer in the peroxisome. This enzyme in plant cells decomposes H_2O_2 into water and oxygen. The increased activity of CAT signals that the antioxidant system is active against environmental stresses (Bahari *et al*., 2013). We observed that the application of amino acids increased CAT activity and decreased electrolyte leakage. This implies that amino acids create optimal conditions in marigolds so that the increased activity of antioxidants contributes to alleviating stresses imposed on the plant, preserving macromolecules, and inhibiting electrolyte leakage. Oraghi Ardebili *et al*. (2012) report that the activity of antioxidant enzymes enhances plant tolerance of stresses by suppressing ROS, thereby postponing senescence. They found that the foliar application of amino acids to aloe vera plants increased the activity of their antioxidant enzymes. Bahari *et al*. (2013) reported similar results, which is consistent with our findings. According to Ahmadi and Ceiocemardeh (2004), the application of amino acids protected membranes against adverse environmental factors by increasing the activity of antioxidant enzymes and reducing free oxygen radicals. In Ghaffari *et al*.'s (2018) study, the foliar application to sugar beet plants reduced electrolyte leakage – a result similar to our findings.

Polyphenol oxidase (PPO) is a defensive enzyme that forms brown pigments on the damaged tissues of plants to protect them against damages, pests, and diseases. However, these brown pigments have an adverse impact during storage and in postharvest fruits and vegetables, and in general, in the food industry (Constable and Barbehenn, 2008). Therefore, reducing the activity of this enzyme plays an important role in improving the quality and shelf-life of agricultural products. The extent of PPO activity depends on respiration, nutrition or nutrient availability, the presence of antioxidants, and the resistance of the plant tissue (Taranto *et al*., 2017). Therefore, given the positive effect of amino acids on marigold growth and development and the preservation of antioxidant activity, the decreased activity of PPO was expected, which was recorded in the results, too.

In general, especially in cv. 'Orange', arginine and proline outperformed glutamine in improving the studied traits. The positive effect of proline can be attributed to this antioxidant and anti-stress role, and the positive effect of arginine can be attributed to its metabolic compounds, including polyamines, which were effective in enhancing the morphological and physiological traits of the marigold.

CONCLUSION

The foliar application of amino acids to the African marigolds cv. 'Orange' and 'Yellow' improved morphological and physiological traits. Arginine and proline outperformed glutamine in improving the assessed traits. Overall, the application of 100 µM arginine was the best and 1000 µM proline was the second-best treatment in improving the quantitative and qualitative traits of the marigolds 'Orange' and 'Yellow'.

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