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Effects of Some Amino Acids and Organic Acids on Enzymatic Activity and Longevity of *Dianthus caryophyllus* cv. Tessino at Pre-Harvest Stage

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Abstract

Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers in the world. This experiment was carried out to evaluate the effects of pre-harvest application of some amino acids and organic acids on enzymatic traits and longevity of carnation flowers (*Dianthus caryophyllus* cv. Tessino) based on completely randomized design with 13 treatments and three replications. The treatments included ascorbic acid (AS), citric acid (CA), malic acid (MA), arginine (Arg), phenylalanine (Phe) and glutamine (Gln), each at two levels of 50 and 100 mg/l. The unsprayed pots constituted the control. The foliar application was carried out three times at 10-day intervals and it was so scheduled that the last stage of spraying was in the green pea stage. Sampling and evaluation of plant traits such as fresh and dry weight, petals anthocyanin, total leaf chlorophyll, phenylalanine ammonia-lyase (PAL), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) activities and flower longevity were measured on plants at the stage that at least two florets have been opened in the cluster. The results showed that 100 mg/l malic acid had the greatest effect on the improvement in fresh and dry weight, petals anthocyanin, and total leaf chlorophyll. Citric acid, ascorbic acid, and phenylalanine, all at the rate of 100 mg/l, had the greatest effect on improving the activity of catalase, superoxide dismutase, peroxidase, and phenylalanine ammonia-lyase enzymes. Also, 100 mg/l glutamine could improve the longevity of flowers on the plants. Therefore, according to the results of this study, it can be concluded that pre-harvest foliar application of amino acids and organic acids improved enzymatic traits and the longevity of flowers on the plants in carnation (*Dianthus caryophyllus*) cv. Tessino.

Keywords: Amino acids, *Dianthus caryophyllus*, Enzymatic activity, Longevity, Organic acids.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.), from the family Caryophyllaceae, can be found in a wide range of colors. In addition, it is one of the most important flowers in the global floriculture industry. Carnation flowers are often used as cut flowers although they can be used as pot plants and even sometimes in rock gardens (Kafi and Ghasemi Ghahsareh, 2007).

Endogenous organic acids and amino acids are a source of both carbon skeleton and energy for cells and are used in the respiratory cycle and other biochemical pathways (Teixeira da Silva, 2003).

One of the main components dictating plant growth is protein. Protein is composed of a sequence of amino acids. The amino acids are essential components which contribute to the growth and quality of crops. In the foliar application of amino acids, plants absorb these compounds through stomata. Nutrition of plant through biologically active amino acids could provide a source of building blocks for protein synthesis in plants. Several attempts have been made to indicate that whether bio-fertilizers containing amino acids are effective in enhancing yield and physiological properties (Glinicki *et al.,* 2010). Physiologically, amino acids account for inducing growth and protection against ammonia toxicity and act as a source of carbon and energy (Abdel Aziz *et al.,* 2010). Tajik and Danaee (2016) studied the effect of glotamine, arginine, and phenylalanine (50 or 100 ppm) pre-harvest spray on some physicochemical and enzymatic traits and longevity of *Gerbera jamesonii* cv. Sorbet flowers. They showed that the glutamine treatment at 100 ppm had the greatest effect on improving fresh weight, dry weight, relative water content, the number of flowers, the diameter of flower, flowering stem length, bent neck, leaf area, cell membrane stability index, anthocyanin content, total chlorophylls of leaves, proline, protein, superoxide dismutase, and phenylalanine ammonia-lyase activity. Flower longevity on the plant was 20 days in plants treated with 100 ppm glutamine whereas it was as short as 14 days in control plants.

Ascorbic acid (AS) is an organic compound required in trace quantities to maintain normal growth in higher plants. AS influences mitosis and cell growth in plants and affects phytohormone-mediated signaling processes during the transition from the vegetative to the reproductive phase as well as the final stage of development and senescence. Furthermore, AS affects nutritional cycle activity in higher plants and plays an important role in the electron transport system. It is also important as a cofactor for a large number of key enzymes in plants (Barth *et al*., 2006). Citric acid is a six-carbon organic acid, having a central role in CA cycle in mitochondria that creates cellular energy by phsophorylating oxidation reactions. It is created by the addition of acetyl-CoA to oxaloacetic acid that is converted to succinate and malate in the next steps. It has been documented that the exudation of citrate and malate from the roots of calcicolous plants (plants growing in alkaline soils) enables them to extract P and Fe from such soils (Lopez-Bucio *et al*., 2000). Malic acid is metabolized in plant mitochondria by the reaction of malic enzyme. Malate is a common reserve anion playing a role in the plant vacuole as a counter ion for K and Ca, especially in nitrate-dependent plants. Bedour and Rawia (2011) showed that 200 ppm ascorbic acid improved growth, delayed the flower opening of vase life and stimulated accumulation of carbohydrate in gladiolus. Eidyan *et al*. (2014) reported that the foliar application of citric acid (0.1%, w/v) increased the vase life of cut tuberose plants and the size of bulblets in a synergism with foliar Fe. Preharvest spray of citric acid (0.15%, w/v) extended the mean vase life of cut lilium flowers from 11.8 days in control treatment to 14 days (Darandeh and Hadavi, 2012).

So, the aim of the present study was to compare the potential of different concentrations of ascorbic acid, citric acid, malic acid, arginine, phenylalanine, and glutamine to improve some quantitative, qualitative, and enzymatic traits and longevity of *Dianthus caryophyllus* cv. Tessino on plants.

MATERIALS AND METHODS

This research was conducted in a commercial greenhouse in Pakdasht County in the winter of 2017, that is located 20 kilometers east of Tehran at the latitude of 51°44' N and the longitude of 28°33' E with an average elevation of 960 meters. The average temperature of the greenhouse was about 20-22°C, its relative humidity was about 60-70%, and its light intensity was about 15-20 µmol m^{-2} s^{-1}. In order to study the effects of the pre-harvest spray of amino acids and acids on quantitative, qualitative, and enzymatic traits and longevity of *Dianthus caryophyllus* cv. Tessino on plants, the experiment was based on a completely randomized design with 13 treatments and three replicates in that each replicate contained five plants amount to a total of 195 pots. The treatments included ascorbic acid, citric acid, malic acid, arginine, phenylalanine, and glutamine, each at two levels of 50 and 100 mg/l. The pot that was not treated was considered as the control. The plants were treated three times in ten days at the same base and it was so scheduled that the last stage of spraying was concurrent with the green pea stage. Also, the plants were sampled to record the traits at a stage that at least two florets have been opened in the cluster. The traits were measured as described as following.

Fresh weight and dry weight

Fresh weight and dry weight were recorded by a digital scale with the accuracy of 0.01 g (Celikel and Reid, 2002).

Anthocyanins of petals

One gram of petal was extracted using a methanol extraction solution and HCl 1N, and it was extracted using a spectrophotometer at 530 and 657 nm, and the anthocyanins in the petals were calculated by the following formula (Meng, 2004).

$$\text{Petal anthocyanin} = A_{530nm} - 0.25A_{657nm}$$

in which A is light absorption.

Total leaf chlorophyll

One gram of leaf with acetone 80% was used for leaf chlorophyll extraction, and the absorbance of the extracts was read with a spectrophotometer (Lambada 25, Perkin Elmer, USA) at 645 and 663 nm for total chlorophyll (mg/g FW) (Arnon, 1949).

$$\text{Total chlorophyll} = 20.2\,(A645) + 8.02\,(A663) \times V/\,(1000 \times W)$$

in which A represents light absorption, and V denotes final acetone volume.

Protein

Petals protein content was measured by the absorption of 1 ml Bradford reagent along with 100 µl enzymatic extract mixed completely and registered at 595 nm. Protein content was estimated using the calibration curve of cow albumin serum (BSA) (Bradford, 1976).

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase activity was measured by Redman *et al*. (1999)'s procedure at 290 nm and eventually, the PAL activity per gram of fresh petal weight was measured and expressed.

Peroxidase (POD) enzyme

Enzyme activity was assayed as per the procedure described by Putter (1974) in petals with slight modifications. Peroxidase enzyme activity was expressed as a change in absorbance at 436 nm per min/g fresh tissue.

Catalase (CAT) enzyme

Catalase activity in cytosolic extracts was measured according to the method of Aebi (1984) in petals. Catalase enzyme activity was expressed as a change in absorbance at 240 nm per min/g fresh tissue.

Superoxide dismutase (SOD) enzyme

Enzyme activity was measured in petals according to the method of Bayer and Fridovich (1987). Enzyme activity was expressed as a change in absorbance at 560 nm per min/g fresh tissue. Reaction mix absorption was measured by a spectrophotometer.

Longevity

This was calculated as the time from the coloration of the buds and the opening of the flowers to the wilting of the petals and the jaundice of the leaves and was expressed in days (Ezhilmathi *et al*., 2007).

Statistical analysis

Information was entered into MS-Excel software after measurements, and the analysis of variance was carried out using SPSS version 24 software. Then, means comparison was performed with Duncan's multiple range test at $P < 0.05$.

RESULTS

The results of the application pre-harvest spray with some amino acids and organic acids on enzymatic traits and longevity of *Dianthus caryophyllus* cv. Tessino on plants are presented in Tables 1-2.

Table 1. Analysis of variance the effect of treatments on growth factor and longevity.

SoV	df	MS				
		Fresh weight	Dry weight	Petal anthocyanin	Total leaf chlorophyll	Longevity
Treatment	12	28.443**	1.823**	3.268**	32.429**	19.410**
Error	---	0.145	0.025	0.034	0.269	0.046
CV (%)	---	13.24	12.13	10.47	13.68	12.14

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

Table 2. Analysis of variance the effect of treatments on protein content and enzymatic activity.

SoV	df	MS				
		Protein	PAL	POD	CAT	SOD
Treatment	12	1.251*	1.939**	1.184**	1.444**	2.354**
Error	---	0.014	0.021	0.013	0.016	0.021
CV (%)	---	12.55	13.91	13.43	10.46	12.86

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

Fresh weight

The highest and the lowest of fresh weight of *Dianthus caryophyllus* cv. Tessino flower with 27.78 g and 21.69 g are related to 100 mg/L malic acid and control treatments, respectively (Fig. 1). Also, in all treatments, fresh weight was higher when higher levels of amino acids and organic acids were applied.

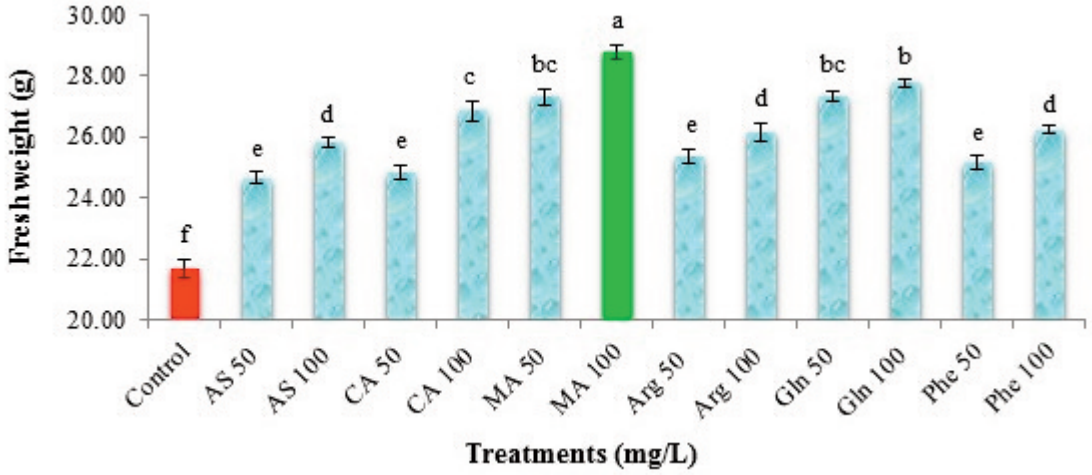

Fig. 1. The effect of the applied amino acids and organic acids on fresh weight of *Dianthus caryophyllus* cv. Tessino on plants.

Dry weight

The highest and the lowest of dry weight of *Dianthus caryophyllus* cv. Tessino flower with 6.05 g and 4.61 g are related to 100 mg/L malic acid and control treatments, respectively (Fig. 2). Also, it was observed that dry weight was increased as higher levels of amino acids and organic acids were applied.

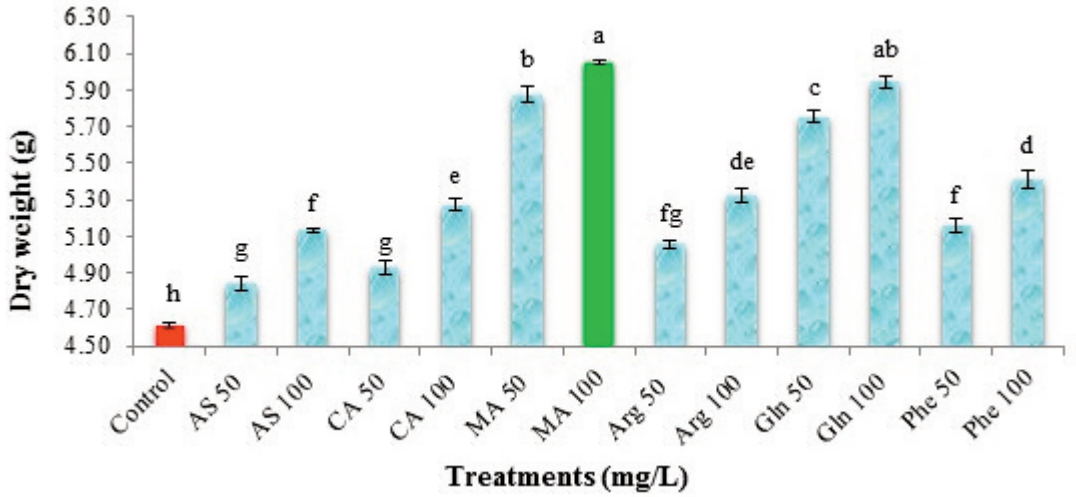

Fig. 2. The effect of the applied amino acids and organic acids on dry weight of *Dianthus caryophyllus* cv. Tessino on plants

Anthocyanin of petals

Malic acid at the rate of 100 mg/l exhibited the highest anthocyanin content in petals (10.8308 mg/g FW) whilst the lowest was 8.6308 mg/g FW observed in control plants (Fig. 3). Also, the application of glutamine at the rates of 50 or 100 mg/l and malic acid at the rate of 50 mg/l showed the next better results after malic acid at the rate of 100 mg/l.

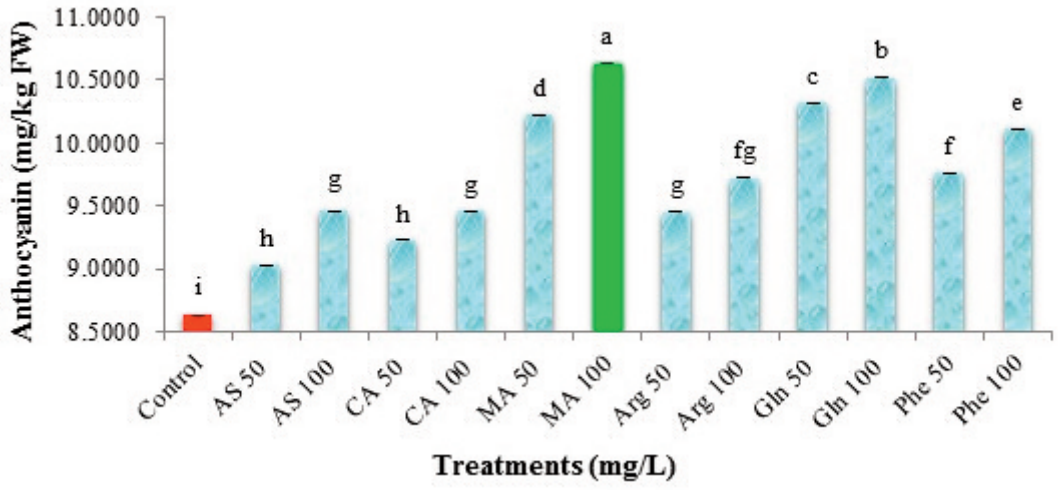

Fig. 3. The effect of the applied amino acids and organic acids on the anthocyanin content of petals of *Dianthus caryophyllus* cv. Tessino on plants.

Total leaf chlorophyll

Malic acid at the rate of 100 mg/l had the highest total leaf chlorophyll content of 47.781 mg/g FW and control had the lowest one of 41.269 mg/g FW (Fig. 4). Also, after 100 mg/l malic acid, application of 50 or 100 mg/l glutamine showed better results than other treatments.

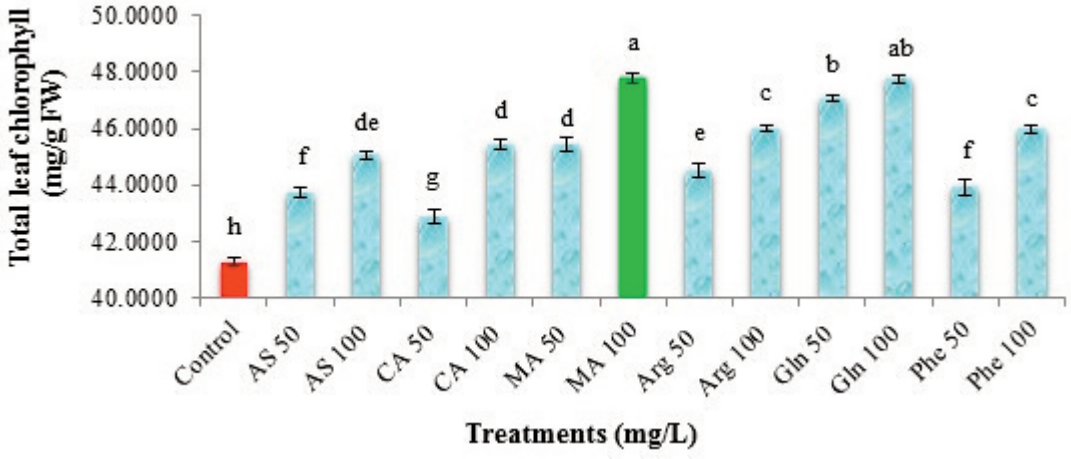

Fig. 4. The effect of the applied amino acids and organic acids on total leaf chlorophyll of *Dianthus caryophyllus* cv. Tessino on plants.

Protein

The highest and the lowest of protein content of *Dianthus caryophyllus* cv. Tessino with 3.45 mg/g FW and 2.12 mg /g FW are related to 100 mg/L glutamine and control treatments, respectively (Fig. 5). Also, among all treatments, protein content was increased with the increase in amino acids and organic acids.

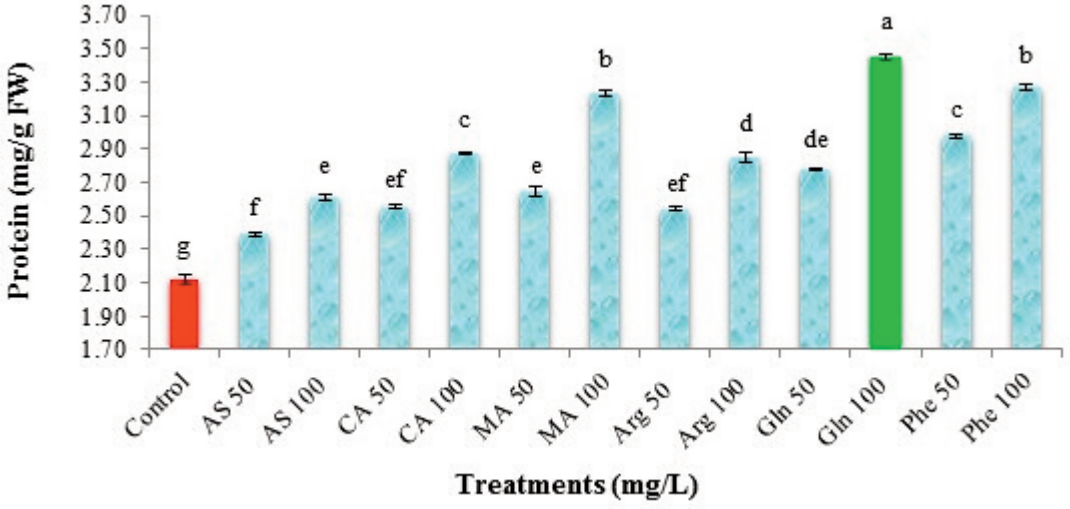

Fig. 5. The effect of the applied amino acids and organic acids on protein of *Dianthus caryophyllus* cv. Tessino on plants.

Phenylalanine ammonia-lyase (PAL) enzyme

Phenylalanine at the rate of 100 mg/l showed the highest PAL of 4.22 µg cinnamate/g FW per min and the lowest one of 2.54 µg cinnamate/g FW per min was observed in control plants (Fig. 6). Also after 100 mg/l phenylalanine, the application of 100 mg/l glutamine and 100 mg/l malic acid showed better PAL activity than other treatments.

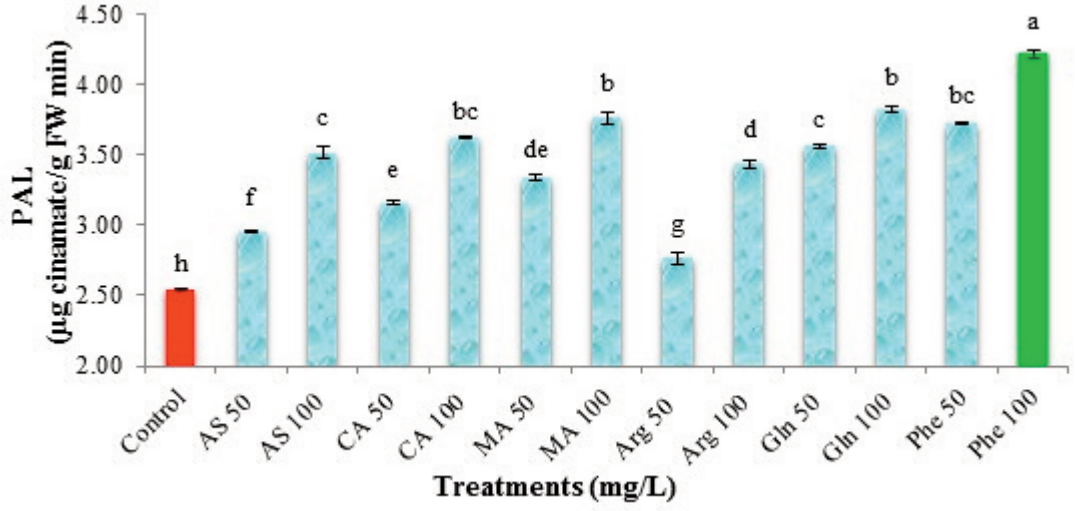

Fig. 6. The effect of the applied amino acids and organic acids on phenylalanine ammonia-lyase activity of *Dianthus caryophyllus* cv. Tessino on plants.

Peroxidase (POD) enzyme

Ascorbic acid at the rate of 100 mg/l was related to the highest POD enzyme activity of 2.95 unit E/g FW and control was related to the lowest of 1.63 unit E/g FW (Fig. 7). Also, after 100 mg/l ascorbic acid, the application of malic acid at the rate of 100 mg/l and citric acid at the rate of 100 mg/l showed better POD activity than other treatments.

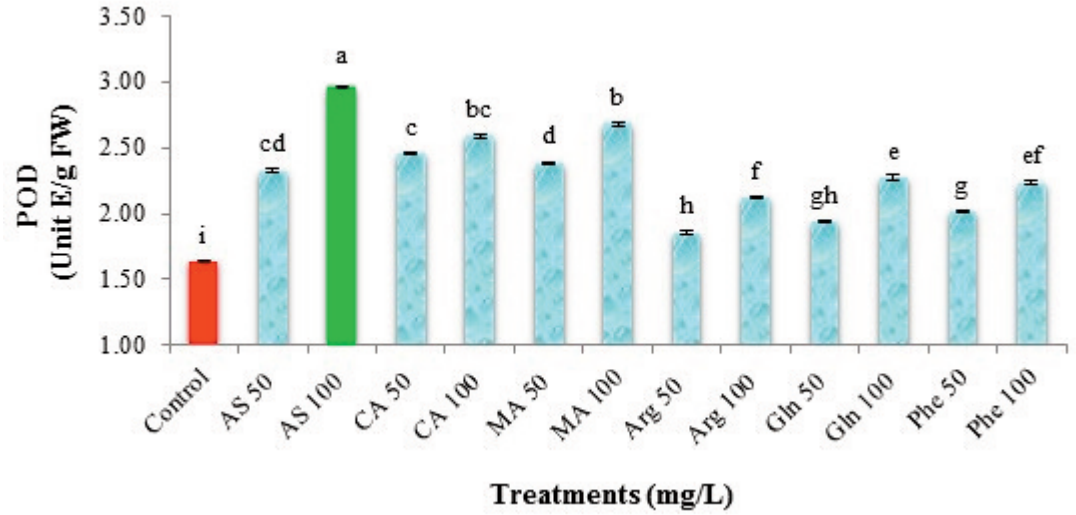

Fig. 7. The effect of the applied amino acids and organic acids on peroxidase activity of *Dianthus caryophyllus* cv. Tessino on plants.

Catalase (CAT) enzyme

Citric acid at the rate of 100 mg/l had the highest CAT enzyme activity of 5.24 unit E/g FW and control had the lowest one of 3.98 unit E/g FW (Fig. 8). Also, after 100 mg/l citric acid, the application of 100 mg/l malic acid and 100 mg/l ascorbic acid yielded better CAT activity than other treatments.

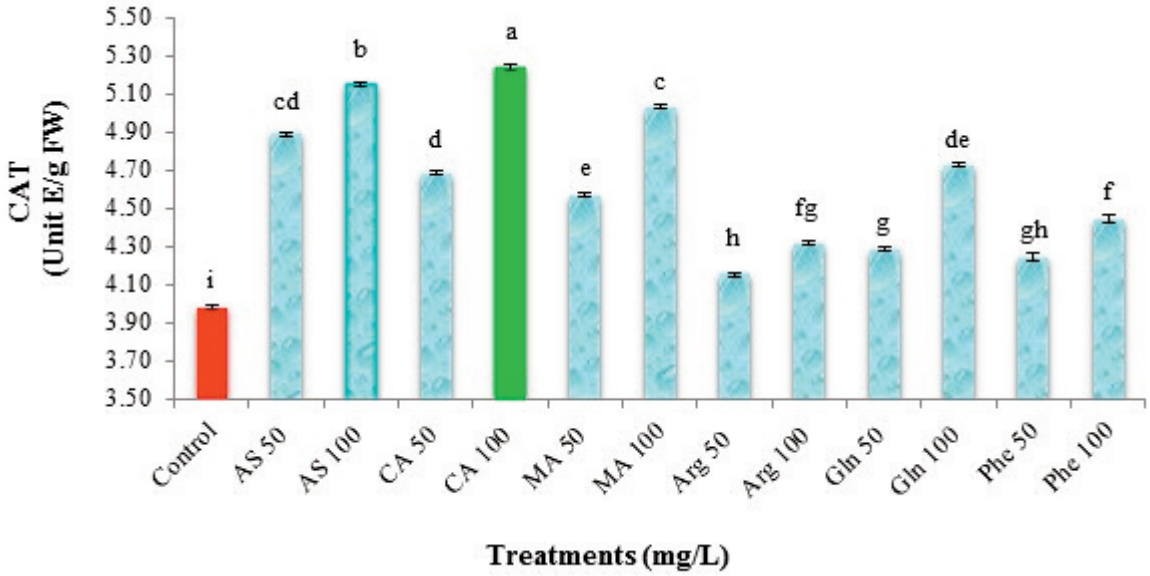

Fig. 8. The effect of the applied amino acids and organic acids on catalase activity of *Dianthus caryophyllus* cv. Tessino on plants.

Superoxide dismutase (SOD) enzyme

Citric acid at the rate of 100 mg/l was associated with the highest SOD enzyme activity of 4.92 unit E/g FW and control had the lowest one of 3.24 unit E/g FW (Fig. 9). Also, after 100 mg/l citric acid, the application of ascorbic acid at the rate of 100 mg/l showed the second best SOD activity among all treatments.

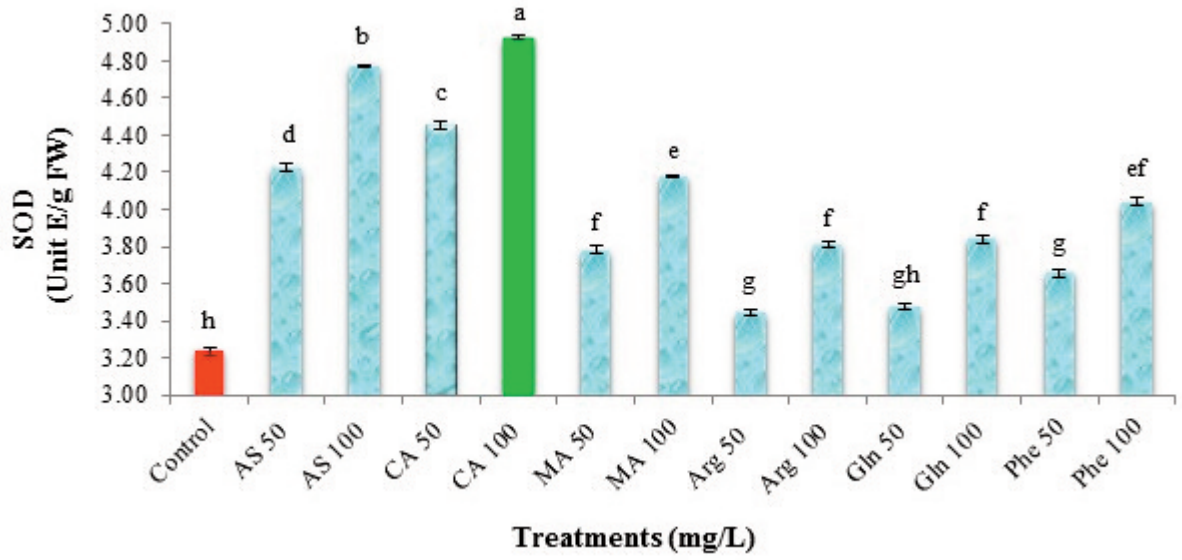

Fig. 9. The effect of the applied amino acids and organic acids on superoxide dismutase activity of *Dianthus caryophyllus* cv. Tessino on plants.

Longevity

The highest and the lowest of flowers longevity of *Dianthus caryophyllus* cv. Tessino with 11.9 days and 7.3 days are related to 100 mg/L glutamine and control treatments, respectively (Fig. 10). Also, among all treatments, longevity of flowers on the plants was extended when higher levels of amino acids and organic acids were applied.

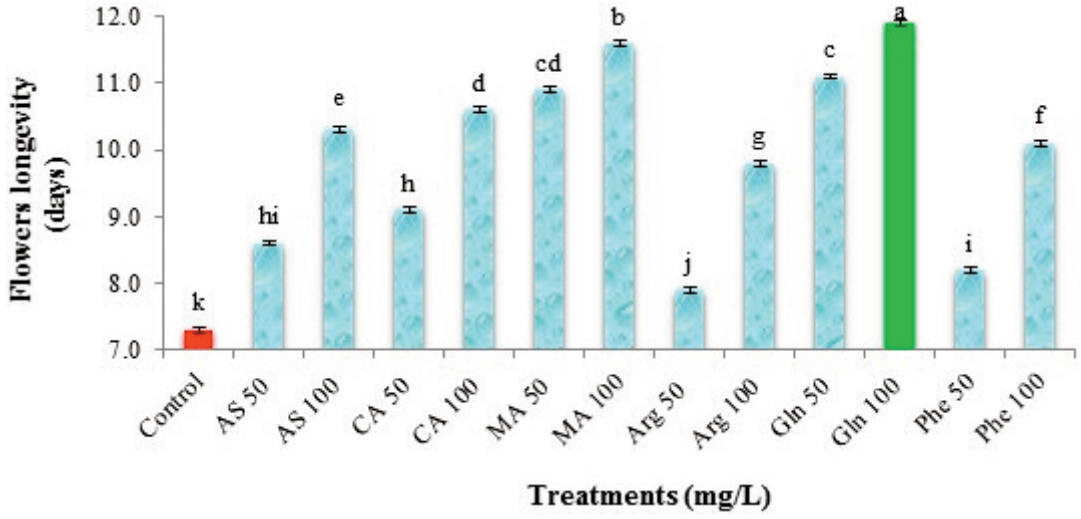

Fig. 10. The effect of the applied amino acids and organic acids on the longevity of *Dianthus caryophyllus* cv. Tessino.

DISCUSSION

Initial metabolites like amino acids, carbohydrates, fatty acids, and organic acids are involved in growth, respiration and photosynthesis, hormone synthesis, and protein synthesis (Mirzapour *et al*., 2013).

Amino acids used in this study, could increase protein content and enzymatic activity due to their roles in the synthesis of proteins, plant hormones, and growth (Tajik and Danaee, 2016). These compounds stimulate cell growth and act as a buffer and a carbon source and energy, protecting plants against ammonium toxicity (with amide formation) (Talebi *et al*., 2014). Also, they play a significant role in the synthesis of other organic compounds such as proteins, amines, purines, pyrimidines, alkaloids, vitamins, enzymes, and terpenes (Tajik and Danaee, 2016). Organic acids used in this study could extend the longevity of carnation flowers on the plants (*Dianthus caryophyllus* cv. Tessino) by playing an important role in the physiological activity of the plants. Some of them such as citric, malic, succinic, and oxalic acids are part of the Krebs cycle, and important metabolic reactions in carbohydrates, lipids, and proteins are the intermediate products (Zandieh *et al*., 2015; Emami *et al*., 2015). The quality and, therefore, marketability can be affected by the factors at pre-harvest, harvest, and postharvest stages (Danaee and Abdossi, 2016). Foliar application of amino acids and organic acids during plant growth can be effective in enhancing plant resistance when exposed to environmental stresses, opening and closing stomata, increasing the quantity and quality of the product, strengthening the plant's defense system, increasing the post-harvest longevity of the plants, inducing the pollination process, increasing the product ripening, contributing to the soil microflora balance, increasing the absorption of micronutrients, accelerating the formation of aerial parts and enhancing photosynthesis, increasing photosynthetic pigments, and affecting plant growth. In this experiment the rate of 100 mg/l amino acids and organic acids could be improved morphological, physiological, enzymatic activity and flowers longevity and our findings are consistent with Eidyan *et al*. (2014)'s study on pre-harvest foliar application of iron sulfate and citric acid combined with urea fertigation influencing the growth and vase life of tuberose (*Polianthes tuberosa* L.) and Kazemi *et al*. (2012)'s study on the effect of salicylic acid, malic acid, citric acid, and sucrose on antioxidant activity, membrane stability and ACC-oxidase activity in relation to the vase life of carnation cut flowers. In fact, foliar application of amino acids and organic acids could improve growth, flowering, and quality and longevity of flowers on plants by metabolism instigation and metabolical processes (Starck, 2007). This experiment reveals the positive effects of amino acids and organic acids on morphology, physiology, enzyme, and longevity of carnation flowers on plants (*Dianthus caryophyllus* cv. Tessino).

CONCLUSION

Overall, two rates (50 or 100 m/l) of ascorbic acid, citric acid, malic acid, arginine, phenylalanine, and glutamine were applied to *Dianthus caryophyllus* cv. Tessino on plants before their harvest. Then, sampling and evaluation of traits were done at a stage that at least two florets were open in the cluster. The results showed that malic acid at the rate of 100 mg/l had the highest effect on such traits as fresh and dry weight, anthocyanin of petals, and total chlorophyll of leaf. Ascorbic acid at the rate of 100 mg/l had the highest effect on improving the peroxidase enzymes activity. Citric acid at the rate of 100 mg/l improved catalase and superoxide dismutase enzymes activity. Phenylalanine at the rate of 100 mg/l had the strongest impact on improving the activity of phenylalanine ammonia-lyase enzymes. Also, the treatment of 100 mg/l glutamine improved the longevity of the flowers on plants.

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