

Effect of Different Concentrations and Application Methods of Polyamines (Putrescine, Spermine, Spermidine) on Some Morphological, Physiological, and Enzymatic Characteristics and Vase Life of *Rosa hybrida* cv. 'Dolce Vita' Cut Flower

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In order to study the effect of different application methods of polyamines (putrescine, spermine, spermidine) on some morphological, physiological and enzymatic traits and vase life of *Rosa hybrida* cv. 'Dolce Vita', as a factorial experiment was conducted in a completely randomized design with 32 treatments, 3 replicates and each replication containing 5 vases, amounting to a total of 480 vases. The first factor was assigned to putrescine, spermine, spermidine (50 or 100 mg/l) and the second factor was devoted to four application methods (pre-harvest spray, postharvest spray, 24 hrs pulse, and continuous). We applied two blanks in the experiment: distilled water and 3% sucrose + 3 mg/l nanosilver. Morphological, physiological and enzymatic traits, such as relative fresh weight, cell membrane stability index, anthocyanin of petals, total chlorophyll of leaves, protein, phenylalanine ammonia- lyase, superoxide dismutase enzyme activity, and vase life, were measured. The results showed that 100 mg/l Spm in pre-harvest spray method was related to the highest rates of protein, activity of phenylalanine ammonia-lyase, and superoxide dismutase enzymes. Therefore, pre-harvest spraying of polyamines can be recommended as the best way to improve morphological, physiological and enzymatic traits as well as the vase life of *Rosa hybrida* cv. 'Dolce Vita'.

Abstract

Keywords: Phenylalanine ammonia- lyase, Polyamines, Rose, Superoxide dismutase.

INTRODUCTION

Rose with the scientific name of *Rosa hybrid* is one of the plant of Rosaceae family. There are about 107 genera and 3100 species in this family. About 70 genera of the rose family are known as ornamental, oral and medicinal plants (Rashidi, 2008). Flowering is different in roses. The greenhouse roses are day neutral and can be flowering throughout the year. The flower initiation does not depend on environmental factors. The garden roses consist of permanent flower varieties and any other non-flowered cultivars. Effective environmental factors in rose cultivation include temperature, light, relative humidity, media, nutrition, irrigation, and so on (Kafi and Ghasemi Ghahsareh, 2007).

Polyamines are aliphatic amines that are found extensively in living organisms. In plant cells, diamine putrescine, triamine spermidine and tetramine spermine are the main polyamines (Bhattacharjee and De, 2005). In plant cells, like other chemical compounds, the lower limit of concentration of polyamines is determined by the minimum requirements for cell growth and its upper limit due to their potential toxicity for the cell. Therefore, the intracellular concentration of these compounds is highly controlled and regulated. The concentration of polyamines in the cells is regulated by a series of fully controlled biosynthesis pathways. Polyamines as plant growth regulators involved in a wide range of growth and development processes, including cell division, embryogenesis, morphogenesis, flowering, fruit emergence, root development, aging delay, membrane stability, active radical's scavenging and tolerance of various stresses (Esna Ashari and Zokaee Khosroshahi, 2008). Also, the total activity of polyamines have been applied as antiseptic and anti-fungal agents in the plant, especially in the harvested crop, and, on the other hand, they have been competitively produced with ethylene, and these compounds are considered as important compounds for increasing the life of the

The production of active oxygen species activates the immune system, resulting in antioxidants such as superoxide dismutase enzymes (SOD), peroxidase (POD) and catalase (CAT) to protect the effects of active oxygen species.

After water, carbohydrates are the most abundant compounds in vegetable tissues. Sucrose is the most common carbohydrate used in preservative solutions, and the dominant form of carbohydrates is transferable to the flower bud, which in some cases also uses glucose and fructose (Esna Ashari and Zokaee Khosroshahi, 2008). Using sugars to partially prevents the production of ethylene in flowers and reduces their sensitivity to ethylene.

In addition, many studies have reported on the application of polyamines in extending the vase life of cut flowers.

Polyamins are recommended to increase the longevity of flowers and delaying their senescence in which has been showed in the finding of Farjadi Shakib *et al.* (2013) on the *Cyclamen persicum*. Their results showed that a exogenesis application of spermidin (5, 10 and 20 Mm) significantly extended the longevity of flowers. It has been also showed that spermidin 10 Mm improved protein, CAT and SOD anzyms activity compared to control.

Ismaeilkhan Zandi and Danaee (2016) study about the effect of polyamines spraying with 2 concentration (50 and 100 ppm) on rose flower (*Rosa hybrida*) cv. Black Baccara on the plant. Traits assessed include fresh weight, dry weight, relative water content, number of flowers, leaf surface, superoxide dismutase enzymes activity and flower longevity. Analysis of variance results showed that 100 ppm spermine greatest effects on improving the morphological, physiological and enzymes traits and the results were significant at 1%.

So, the aim of this study was to comparison of the different concentrations and methods of application of putrescine, spermine and spermidine to improve some quantitative and qualitative traits and postharvest life of rose cut flower cv. Dolce Vita.

MATERIALS AND METHODS

This research was conducted in commercial greenhouses in Pakdasht County in the spring of 2017. The city of Pakdasht is located 20 kilometers east of Tehran (Lat. 51°44' N., Long 28°33' E., Elevation 960 m.). The average temperature of the greenhouse was about 20 to 22°C, the relative humidity was about 60 to 70 percent, and the light intensity was about 15 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The present work focused on the effect of different methods of application of polyamines (putrescine, spermine, spermidine) on some morphological, physiological and enzymatic traits and the vase life of Dolce Vita in a factorial experiment based on a completely randomized design with 32 treatments, 3 replicates, and each replicate containing 5 vases. A total of 480 vases were made from the same flower and age. In the first method, two weeks before harvesting, flowers were sprayed with different levels of putrescine, spermine, and spermidine (0, 50, or 100 mg/l), and after two weeks, the flowers were harvested and stored in a laboratory in a nanosilver preservative solution (3 mg/l) with 3% sucrose until the end of their vase life. In the second method, the harvested flowers were sprayed with different levels of putrescine, spermine, and spermidine (0, 50, or 100 mg/l), and then they were transferred into vases containing a silver nanoparticles solution (3 mg/l) with 3% sucrose until the end of their vase life. In the third method, short-term treatment (24 hours) of flowers after harvest was performed with different levels of putrescine, spermine, and spermidine (0, 50, or 100 mg/l), and then the flowers were stored in a silver nanoparticles solution (3 mg/l) with 3% sucrose until the end of their vase life. In the fourth method, long-term treatment of flowers after harvest was performed with different levels of putrescine, spermine, and spermidine (0, 50, or 100 mg/l) until the end of vase life. The following traits were evaluated as described below.

Relative fresh weight

In order to calculate the relative fresh weight, the fresh weight was recorded on days 0, 2, 4 and 6 by a digital scale with an accuracy of 0.01 g. Finally, the changes in the fresh weight of flowers were expressed as percentages of the original weight on the days indicated (Celickel and Ried, 2002).

$$\text{Relative fresh weight} = (\text{The fresh weight on a certain day} / \text{Fresh weight on the first day}) \times 100$$

Cell membrane stability index

To determine cell membrane stability, two samples of petals each including 200 mg of each replication were weighted and dipped in 10 ml of double-distilled water. One of them was placed at 40°C Bain-marie for 30 min (C1) and the second one at 100°C Bain-marie for 15 min. After reaching the room temperature (C2), the electrical conductivity of the solutions was measured with an EC-meter and the stability percent of the membrane was determined according to Singh *et al.* (2008)'s procedure as follow:

$$\text{Membrane stability index (\%)} = [1 - (C1/C2)] \times 100$$

Petal's anthocyanin

1.5 g of petal was extracted using a methanol extraction solution and 1 normal HCl and extracted using spectrophotometer in two wavelengths of 530 and 657 nm, and the anthocyanins in the petals were calculated by the following formula (Meng, 2004):

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

In which *A* denotes light absorption.

Total leaf chlorophyll

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

$$\text{Chl} = 20.2 (A_{645}) + 8.02 (A_{663}) \times V / (1000 \times W)$$

In which A represents light absorption and V shows final acetone volume.

Petal's protein

Petal protein was measured using Bradford (1976)'s method. Absorption of 1 ml of Bradford reagent along with 100 μ l of the enzymatic extract was mixed completely and registered at 595 nm. Protein content was estimated using the calibration curve of cow albumin serum (BSA).

Phenylalanine ammonia- lyase (PAL)

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

Superoxide dismutase (SOD) enzyme

200 mg of plant tissues were extracted in 50 mM HEPES-KOH buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was transferred to centrifuge tubes and was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to an enzyme extract. For assay, a mixture consisting of 50 mM HEPES-KOH (pH 7.8) containing 0.1 mM NaEDTA, 50 mM Na₂CO₃ (pH 10.2), 12 mM L-methionine, 75 μ M nitro blue tetrazolium (NBT), 1 μ M riboflavin and crude extract was prepared and enzymatic extract as a unit of SOD activity was considered as enzymatic amount which resulted in 50% inhibition of NBT in 560 nm (Chance and Maehly, 1955). Reaction mix absorption was measured by a spectrophotometer.

Flower vase life

Since the opening of the three external petals of the flowers and the curvature of the sepals from the bottom to the wilting or dying of flowers and the loss of petals and stems, it was calculated in the day (Ezhilmathi *et al.*, 2007).

Statistical analysis

The information was entered into MS-Excel software after measurement. Then, analysis of variance was carried out using SPSS version 24 software package. Means separation was performed with Duncan's Multiple Range Test at the $P < 0.05$ level.

RESULTS

The results are presented below on the effect of the application of various methods and levels of polyamines (putrescine, spermine, spermidine) on some morphological, physiological and enzymatic traits and the vase life of Dolce Vita. The interaction of different treatments were significant for all traits at the $P < 0.01$ level and for cell membrane stability index at the $P < 0.05$ level (Table 1).

Table 1. Analysis of variance the experiment.

S.o.V	df	S M							
		Relative fresh weight	Cell membrane stability index	PetalS anthocyanin	Total leaf chlorophyll	Petal protein	PAL	SOD	Vase life
Treatment (T)	7	3041.15**	881.83**	0.188**	128.82**	0.045**	51.275**	469.15**	52.901**
Method (M)	3	401.36**	250.14**	0.011*	3.370*	0.011*	8.135**	202.58**	2.893**
T×M	21	2.89**	0.89*	0.235*	0.401*	8.809**	0.240**	11.06**	0.143**
Error	---	0.446	0.423	0.005	0.081	0.001	0.062	2.47	0.028
CV (%)	---	13.73	11.67	12.88	10.63	9.29	11.71	13.52	11.78

**, * show significance at the 1% and 5% levels, respectively.

Relative fresh weight

In pre-harvest spraying method, Spm 100 ppm treatment had the highest relative fresh weight of 82.74% and control had the lowest relative fresh weight of 69.05%. In the pulse method, Spm100 ppm treatment showed the highest relative fresh weight of 83.70% and control showed the lowest one, i.e. 68.08%. In the short-term preservative solution method, the highest and lowest relative fresh weight were 81.74 and 67.09% observed in plants treated with Spm100 ppm and in control plants, respectively. In post-harvest spraying method, Spm100 ppm treatment showed the highest relative fresh weight of 80.76% and control treatment showed the lowest one of 66.36% (Fig. 1).

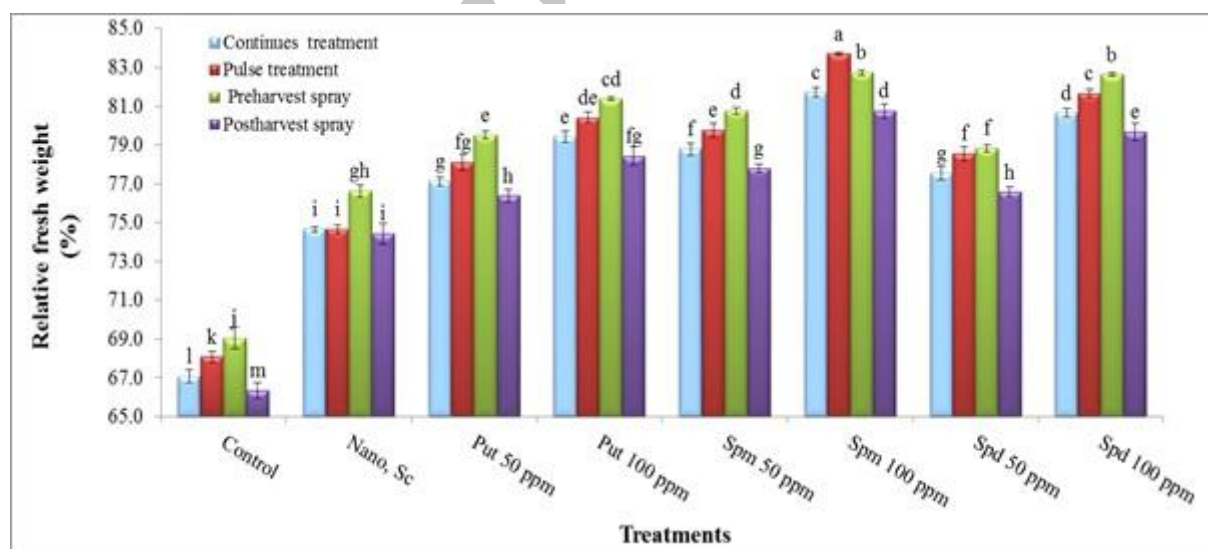


Fig. 1. The variations relative fresh weight in *Rosa* 'Dolce Vita' cut flower.

Cell membrane stability index

In pre-harvest spraying method, Spd 100 ppm treatment had the highest and control treatment had the lowest cell membrane stability index of 75.82 and 64.43%, respectively. In the long-term preservative solution method, Spm100 ppm treatment had the highest and control treatment

had the lowest cell membrane stability index of 78.57 and 62.68%, respectively. In the short-term preservative solution method, Spm100 ppm treatment had the highest and control treatment had the lowest cell membrane stability index of 75.12 and 62.05%, respectively. In post-harvest spraying method, Spm100ppm treatment had the highest and control treatment had the lowest cell membrane stability index of 75.34 and 61.43%, respectively (Fig. 2).

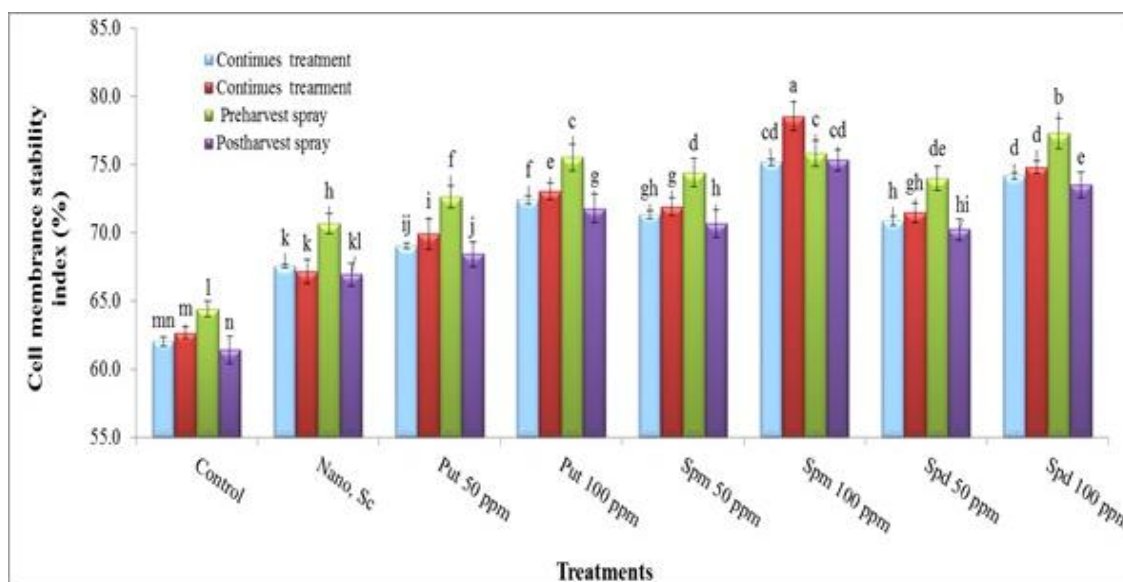


Fig. 2. The variations of cell membrane stability index in *Rosa* 'Dolce Vita' cut flower.

Petal's anthocyanin

In pre-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest petal anthocyanin content of 0.8304 and 0.6241 mg/g FW, respectively. In the long-term preservative solution method, Spm100 ppm treatment had the highest and control treatment had the lowest petal anthocyanin content of 0.6286 and 0.8174 mg/g FW, respectively. In the short-term preservative solution method, Spm100 ppm treatment had the highest and control treatment had the lowest petal anthocyanin content of 0.864 and 0.6149 mg/g FW, respectively. In postharvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest petal anthocyanin content of 0.81.06 and 0.6302 mg/g FW, respectively (Fig. 3).

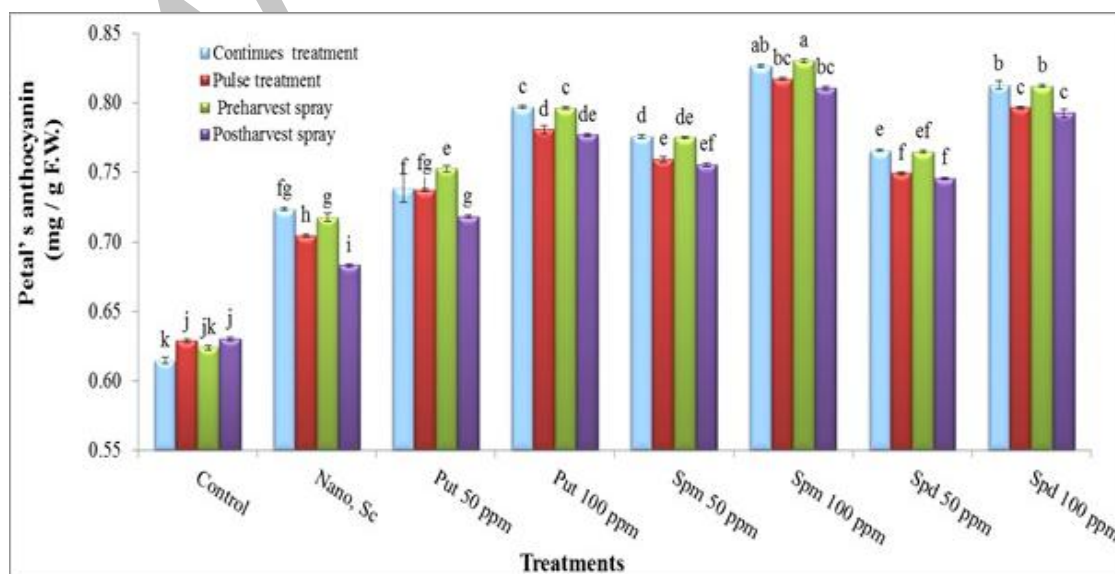


Fig. 3. The variations of petal's anthocyanin content in *Rosa* 'Dolce Vita' cut flower.

Total leaf chlorophyll

In pre-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest total leaf chlorophyll content of 15.6995 and 10.4300 mg/g FW, respectively. In the long-term preservative solution method, Spm 100 ppm treatment had the highest and control treatment had the lowest leaf chlorophyll content of 15.2185 and 10.4158 mg/g FW, respectively. In the short-term preservative solution, Spm100 ppm treatment had the highest and control treatment had the lowest leaf chlorophyll content of 15.1604 and 10.4109 mg/g FW, respectively. In post-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest total leaf chlorophyll content of 15.0714 and 10.2729 mg/g FW, respectively (Fig. 4).

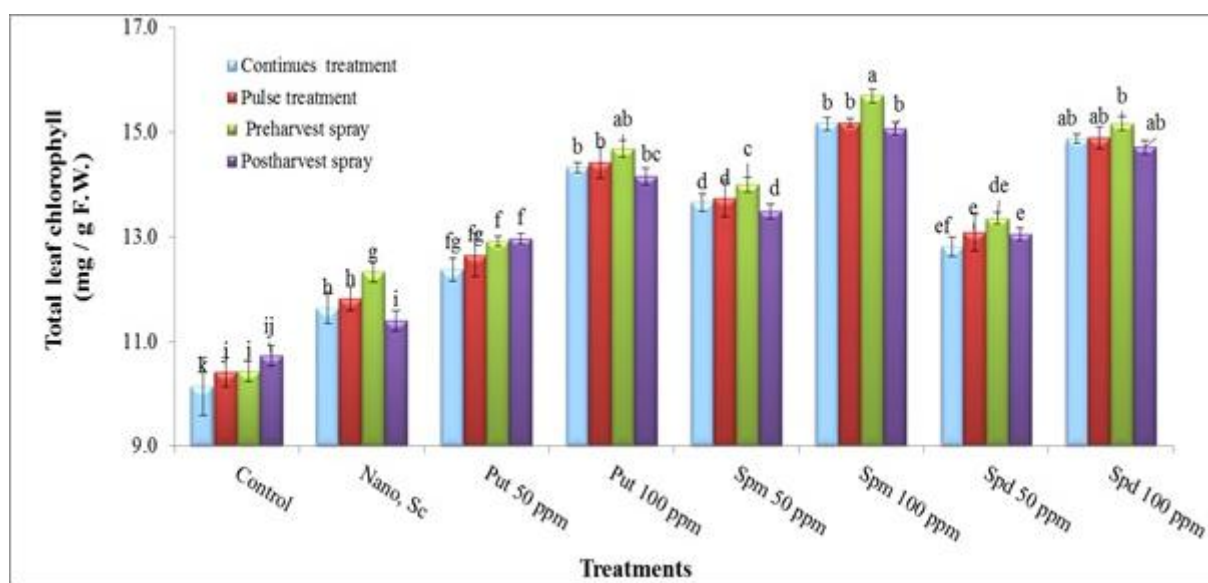


Fig. 4. The variations of total leaf chlorophyll in *Rosa* 'Dolce Vita' cut flower.

Petal's protein

In pre-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest protein content of 0.162 and 0.107 $\mu\text{g} / \text{ml F.W.}$, respectively. In the long-term preservative solution method, Spm100 ppm treatment had the highest and control treatment had the lowest protein content of 0.158 and 0.106 $\mu\text{g} / \text{ml F.W.}$, respectively. In the short-term preservative solution, Spm100 ppm treatment had the highest and control treatment had the lowest protein content of 0.160 and 0.099 $\mu\text{g} / \text{ml F.W.}$, respectively. In post-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest protein content of 0.161 and 0.098 $\mu\text{g} / \text{ml F.W.}$, respectively (Fig. 5).

Phenylalanine ammonia-lyase enzyme (PAL)

In pre-harvest spraying method, Spm 100 ppm treatment had the highest and control treatment had the lowest PAL activity of 14.28 and 11.27 $\mu\text{g cinnamate/g FW/min}$, respectively. In the long-term preservative solution method, Spm 100 ppm treatment had the highest and control treatment had the lowest PAL activity of 14.13 and 10.27 $\mu\text{g cinnamate/g FW/min}$, respectively. In the short-term preservative solution method, Spm 100 ppm treatment had the highest and control treatment had the lowest PAL activity of 14.17 and 10.67 $\mu\text{g cinnamate/g FW/min}$, respectively. In postharvest spraying method, Spm 100 ppm treatment had the highest and control treatment had the lowest PAL activity of 13.87 and 10.53 $\mu\text{g cinnamate/g FW/min}$, respectively (Fig. 6).

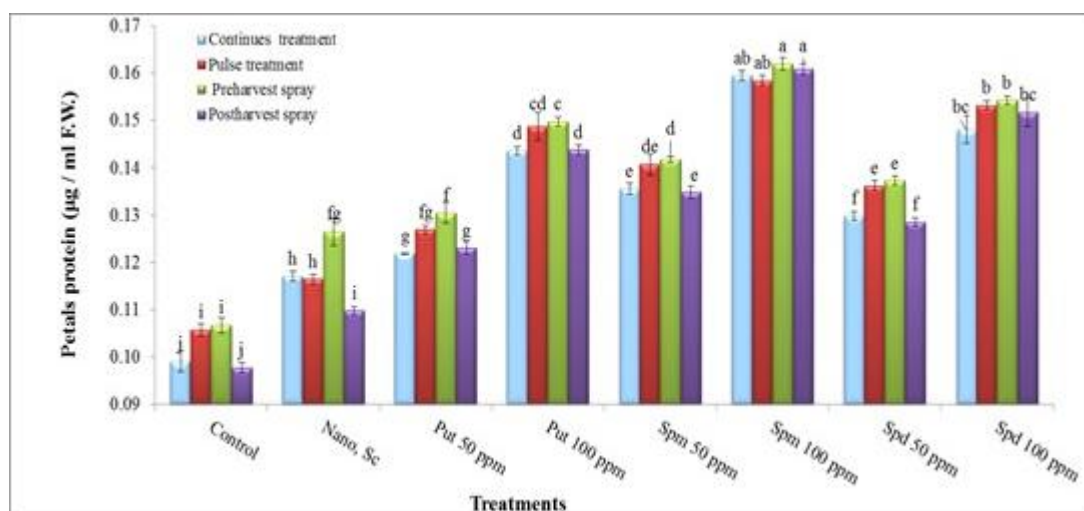


Fig. 5. The variations of petal's protein in *Rosa* 'Dolce Vita' cut flower.

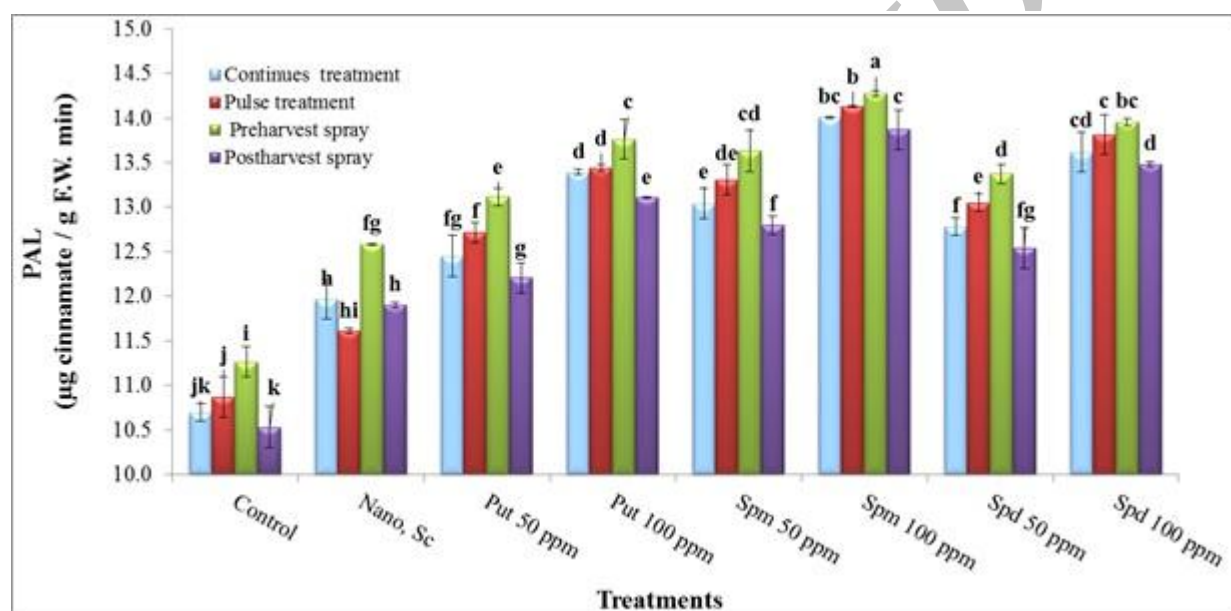


Fig. 6. The variations PAL activity in *Rosa* 'Dolce Vita' cut flower.

Superoxide dismutase enzyme (SOD)

In pre-harvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest SOD activity of 467.02 and 374.39 units/g FW, respectively. In the long-term preservative solution method, Spm100 ppm treatment had the highest and control treatment had the lowest SOD activity of 461.11 and 364.89 units/g FW, respectively. In the short-term preservative solution method, Spm 100 ppm treatment had the highest and control treatment had the lowest SOD activity of 458.98 and 364.84 units/g FW, respectively. In postharvest spraying method, Spm100 ppm treatment had the highest and control treatment had the lowest SOD activity of 461.53 and 365.47 units/g FW, respectively (Fig. 7).

Flower vase life

In pre-harvest spraying method, Spm100 ppm treatment had the longest and control treatment had the shortest postharvest vase life of 8.5 and 4.6 days, respectively. In the long-term preservative solution method, Spm100 ppm treatment had the longest and control treatment had the shortest postharvest vase life of 8.1 and 4.5 days, respectively. In the short-term preservative so-

lution method, Spm100 ppm treatment had the longest and control treatment had the lowest post-harvest vase life. In postharvest spraying method, Spm100 ppm treatment had the longest and control treatment had the shortest postharvest vase life of 7.6 and 4.5 days, respectively (Fig. 8).

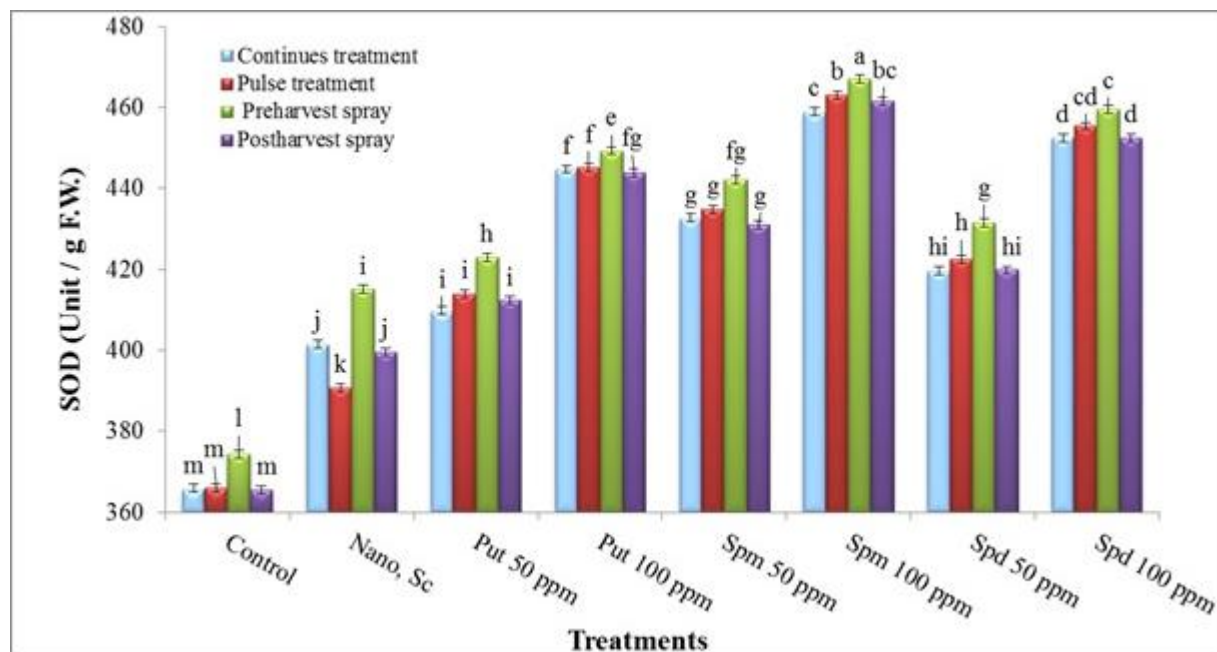


Fig. 7. The variations of SOD activity in *Rosa* 'Dolce Vita' cut flower.

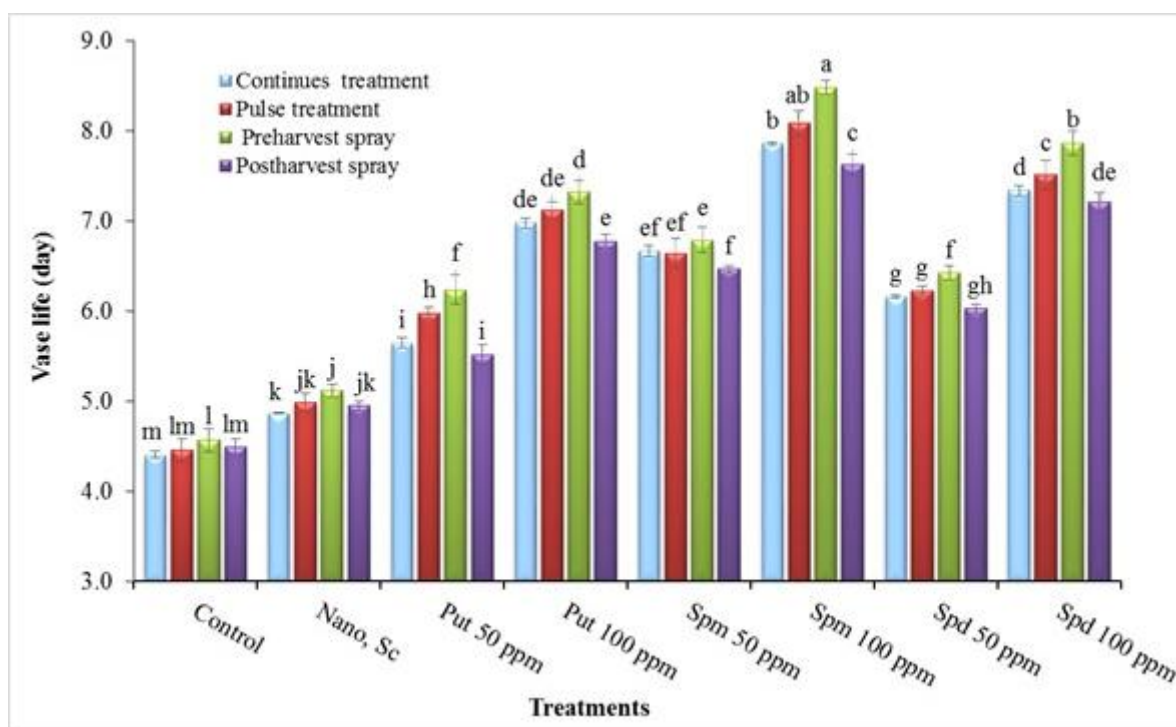


Fig. 8. Vase life of *Rosa* 'Dolce Vita' cut flower.

DISCUSSION

Vase life of cut flowers is very limited. Several factors accelerate the aging and disappearance of marketable appearance and reduce the postharvest vase life of cut flowers. Loss of quality and, consequently, marketability can result from the factors of the breeding period, that is, before harvest, at harvest and after harvest. In general, the most important indicators of quality loss in all flowers are petal fall, petal wilting, leaf loss, leaf yellowing and stem bending. Improvement of pre-harvest, harvest and postharvest conditions can effectively improve the quality and vase life of cut flowers (Bosma and Dole, 2002). Also, lower access of cut flowers to the required carbohydrates can be another reason for the loss of quality and vase life of cut flowers. The reduction in available carbohydrates for respiration results in the consumption of soluble proteins and amino acids in flowers, which, in turn, results in changes in the pH of the vacuoles and, consequently, changes in the color of the flowers. Therefore, the carbohydrates contained in the preservative solution of the cut flowers cause it to grow inside the plant, preserving the soluble proteins and the color of petals by increasing their synthesis. As a result, aging in petals is delayed (Figuerola *et al.*, 2005).

Polyamines can also be effective in improving the quality, aging and prolonging postharvest life, with the effect on cell membrane stability, the accumulation of active radicals, anti-aging, anti-stress and anti-fungal effects, and the competitive ability of ethylene production (Esna Ashari and Zokaee Khosroshahi, 2008).

The postharvest application of polyamines, silver nanoparticles and sucrose with acidic pH controls and inhibits the growth of microorganisms in vase solution and prevents vascular obstruction, inhibits ethylene synthesis, increases osmotic pressure of petals, increases the synthesis of plant colors, and ultimately enhances relative fresh weight, cell membrane stability index, petal anthocyanin and total chlorophyll content of leaves. The PAL activity as the key enzyme involved in the biosynthetic pathway of phenylpropanoid with the effect on the phenylalanine amino acid and the production of cinnamic acid as a precursor of phenolic substances such as lignin escalates lignin and, thus, increases the strength of the stem (Hatfield and Vermerris, 2001). Also, postharvest vase life of cut flowers can be improved by the effects on ROS or cause defects in the mechanism of their synthesis. Also, the activity pattern of antioxidant enzymes is a very important factor in controlling the process of aging processes. In fact, the compatibility and overcoming of oxidative stresses and the prevention of the production of free radicals require regulating the synthesis and reinstatement of proteins and enhancing the antioxidant capacity of the cells. Also, increased activity of antioxidant enzymes improves plant protection against oxidative stresses and aging and death of cells. Thus, antioxidants oxidize reactive oxygen species by releasing electrons to free radicals and eliminating the aging process (Ananieva *et al.*, 2004).

Despite the fact that the plants are equipped with antioxidant enzymatic systems, such as superoxide dismutase and catalase, these systems may have much less power in cut flowers and have no antioxidant capacity to reduce the negative effects of oxidation by free radicals. Compounds such as polyamines and silver nanoparticles that are able to modulate the resistance to stress and reduce the function of free radicals through the strengthening of antioxidant systems are used for this purpose (Senaranta *et al.*, 2000). The results of this research are in agreement with the findings of Nemeth *et al.* (2002) about the effect of Put on vegetative and flowering growth of *Gladiolus grandiflorum* L., Basiri *et al.* (2010) on the effect of five concentrations of silver nanoparticles with 6% sucrose in preservative solution of cut *Dianthus*, Hoseini Farahi *et al.* (2013) about the effect of spermidine and calcium sulfate on the qualitative, qualitative and post-harvest characteristics of Dolce Vita varieties in hydroponic system, Mittler (2002) on the use of Put for two consecutive seasons on cut chrysanthemum, Zobideh *et al.* (2014) about the effect of non-chemical treatments in various time application on quality and vase life of cut *Alstroemeria*, and Farjadi Shakib *et al.* (2013) on external spermidine application on the quantity and quality of n cyclamen.

CONCLUSION

Among the four methods used in evaluating the traits, pre-harvest spraying was found to be the best method for increasing the vase life of the cut rose 'Dolce Vita'. In different forms of polyamines, spermine has the best results in improving the measured quantitative, qualitative and enzymatic traits. Among the concentrations used, 100 ppm gave better results than the other concentrations. Finally, pre-harvest spraying with 100 ppm of spermine can be recommended for prolonging post-harvest life and quantitative and qualitative traits of *Rosa hybrida* cv. Dolce Vita cut flowers.

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