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Effect of Sodium Nitroprusside on the Vase Life of Cut Rose, Lisianthus, and Sunflower

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Abstract

Long vase life is the most important factor determining the economic value of cut flowers. The effect of the pulse treatment of sodium nitroprusside (SNP) was studied at four levels of 0, 20, 40, and 60 µM on the vase life of cut roses (*Rosa hybrida* L.), lisianthus (*Eustoma grandiflorum*), and sunflowers (*Helianthus annuus* L.) in a factorial experiment based on a randomized complete design with three replications and 12 treatments. The results showed that the longest vase life of cut roses (14.33 days) and sunflowers (14.5 days) were obtained from the application of 40 µM SNP whereas cut lisianthus flowers exhibited the longest vase life (14.00 days) when they were treated with 20 µM SNP. This treatment was the most effective in conserving the dry matter of cut roses. SNP significantly reduced ethylene synthesis in cut roses and lisianthus versus the control. The lowest ethylene synthesis in cut sunflowers (0.03 nl l^{-1} h^{-1} g^{-1} FW) was, however, obtained from the treatment of 60 µM SNP. SNP had no significant effect on water uptake, vase solution and stem-end bacterial population, and chlorophyll b. But, it significantly contributed to maintaining the protein content of the studied cut flowers. Overall, it can be said that SNP improves the postharvest longevity of cut roses, lisianthus, and sunflowers by suppressing ethylene synthesis and protecting proteins.

Keywords: Ethylene, Nitric Oxide, Preservative solution, Senescence.

INTRODUCTION

Cut flowers with short vase life are economically less valuable and marketable. Various flowers are involved in the early aging of cut flowers, such as genotype, environmental conditions, oxidative stresses, microorganisms, and sensitivity to ethylene. In addition to controlling the temperature and humidity of the cut flower preservative, maintaining water uptake by controlling vase solution and stem-end microbial population and reducing ethylene synthesis and flower sensitivity to ethylene can play a key role in extending the vase life of cut flowers. Therefore, the treatment of cut flowers with antimicrobial and anti-ethylene compounds is a common way to keep the quality of these horticulturally valuable crops (Seyf *et al*., 2012; Sudaria *et al*., 2017; Shabanian *et al*., 2018; Gupta and Dubey, 2018).

Nitric oxide (NO) is an unstable environmentally-friendly gas radical that is used to protect the postharvest longevity of different horticultural crops (Tongfei *et al*., 2011; Ashouri Vajari and Nalousi, 2013; Naing *et al*., 2017). In addition to controlling harvested crop aging, NO is involved in many plant processes, e.g., germination, growth and development, photosynthesis, pigment synthesis, defensive system, and many others (Seyfe *et al*., 2012). Ethylene and NO have an antagonistic effect (Del Rio *et al*., 2004). NO inhibits early aging in higher plants by reducing ethylene synthesis and activity (Leshem and Wills, 1998; Sankhla *et al*., 2005). Sodium nitroprusside (SNP) is the most common NO-releasing compound whose positive effect has been reported on extending the postharvest longevity of cut flowers (Shabanian *et al*., 2018; Naing *et al*., 2017).

Naing *et al*. (2017) investigated the effect of SNP at six rates (0, 1, 5, 10, 15, and 20 mg L^{-1}) on improving the postharvest life of cut carnations. The results revealed that the rates of 1, 5, and 10 mg L^{-1} extended the vase life of this cut flower, but the higher rates decreased it. It was also found that the longest vase life, the highest fresh weight, and the lowest ethylene synthesis were obtained from the application of 10 mg L^{-1} SNP. The positive effect of SNP has been reported on extending the vase life of cut roses (Liao *et al*., 2013), gerbera (Shabanian *et al*., 2018), and gladiolus (Dwivedi *et al*., 2016).

Rose (*Rosa hybrida* L.) from the family Rosacea is the top cut flower in the world. Susceptibility to ethylene and water stress due to vascular blockage reduces the quality and longevity of cut roses via the suppression of bud opening, neck bending, and early withering of petals (Abri *et al*., 2014; Farazmandi *et al*., 2020).

Lisianthus (*Eustoma grandiflorum*) from the family Gentianaceae is a famous cut flower in international flower markets, which is popular among many people due to its similarity to the rose, the cluster and standard form of its flowers, color diversity, and beauty. The vase life of lisianthus varies among the cultivars from 5 to 28 days. The main factors limiting the vase life of this cut flower include ethylene sensitivity, the inability of water uptake resulting in bending, and suppression of bud opening and coloring, which should be considered at the postharvest stage (Cho *et al*., 2001; Skutnik *et al*., 2021).

Sunflower (*Helianthus annuus* L.) is a cut flower the demand for which is growing in the global markets. But, its postharvest life is shortened by neck bending and early withering and shedding of petals, which are mainly the negative consequence of its susceptibility to ethylene and water stress (Kilic *et al*., 2020).

The present research aimed to investigate the effect of sodium nitroprusside (SNP) as a nitric oxide (NO)-releasing compound on conserving the postharvest longevity and quality of cut roses, lisianthus, and sunflowers.

MATERIALS AND METHODS

The study was conducted as a factorial experiment based on a randomized complete design with three replications, 12 treatments, and 36 vases, each containing five flower branches. The cut

rose, lisianthus, and sunflower flowers were procured at the bud stage from a greenhouse with standard production conditions in Tehran province and were transported to the study site in appropriate packages and by caring for all postharvest physiological conditions. In the laboratory, the flowers were divided into 5-branch groups based on tissue health and size. To apply the treatments, the flowers were re-cut from a height of 50 cm under water at 40°C. In this study, SNP was applied at four rates of 0, 20, 40, and 60 µM in 24-hour pulse form. Then, the flowers were kept in a vase solution containing 8-HQS and 3% sucrose until the end of the experiment. The experiment was carried out in a controlled environment at a temperature of 20 ± 2 °C, relative humidity of 60-70%, and lightness duration of 12 hours at an intensity of 12 µM m^{-2} s^{-1}.

Assessment of traits

Vase life

It was calculated by counting the number of days from the initiation of the treatments until 50% wilting of florets in the lisianthus (Choe *et al*., 2001), neck bending and the wilting of two-third of petals in the roses (Seyf *et al*., 2012; Farazmandi *et al*., 2020), and neck bending and 50% shedding of petals in the sunflowers (Kilic *et al*., 2020).

Solution uptake

The amount of solution taken up by the cut flowers was obtained from the following equation:

$$\text{Solution uptake (ml/g FW)} = \frac{V_{t0}-(E_t+V_{t1})}{FW}$$

in which V_{t0} is the initial solution volume, V_{t1} is the final-day solution volume, E_t is the final amount of evaporation from the solution surface, and FW is the cut flower fresh weight on the first day.

Dry matter

At the end of the vase life, flower branches were weighed and was then oven-dried at 70 °C for 48 hours. Dry weight (%) was calculated by the following equation:

$$\text{Dry weight (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Calculation of vase solution and stem-end bacteria

It was performed by Liu *et al*.'s (2009) procedure for which 24 hours after the application of the pulse treatments, 5 mL of the vase solution and 1 cm of the stem ends of the roses, lisianthus, and sunflowers were separated. The vase solution samples were diluted with 0.9 % normal saline serum. The stem samples were extracted in a mortar using 0.9 % normal saline serum. Then, 0.1 mL of the diluted solution of the vase solution and the stem-end extract were separately cultured on nutritious agar. The culture media were kept in an incubator at 37 °C for 24 hours. The bacterial colonies were, then, counted with an optical microscope.

Total protein

The petals of the roses, lisianthus, and sunflowers were sampled on day 5, and their total protein contents were determined by the Kjeldahl indirect method and the following equation:

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

In which **t** represents the concentration of the acid used for titration (mol L^{-1}), **a** represents the quantity of the acid used for the sample (ml), **b** represents the quantity of the acid used for the control (ml), **V** represents the volume of the extract derived from digestion (ml), **W** represents the plant sample weight for digestion (g), and **DM** represents plant dry matter.

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

Chlorophyll b

The healthy leaves of the roses, lisianthus, and sunflowers were sampled on day 5. Then, the leaves were extracted with 80% acetone and their absorbance was read at 643 and 660 nm with an Apple PD-330UV spectrophotometer. The following equation was applied to determine chlorophyll b content (Mazumdar and Majumdar, 2003):

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

Ethylene synthesis

The amount of ethylene production by the cut roses, lisianthus, and sunflowers was measured by the gas chromatography method. So, 24 hours after the application of the pulse treatments, one flower branch was sampled from each plot and vacuumed in specific jars. After 12 hours, the gas produced in the jars was sampled with a venoject. The ethylene content of the samples was measured with a Shimadzu gas-chromatography device (Japan) and reported in nl l^{-1} h^{-1} g^{-1} F.W.

Data analysis

Data collected during daily readings and from the laboratory analyses were analyzed in the SPSS statistical software package. The means were compared by the LSD test.

RESULTS

Vase life

The interaction of 'species × SNP' was significant ($P < 0.05$) for the vase life of all three species (Table 1). The comparison of the means revealed that the application of SNP up to the rate of 40 µM extended the vase life of all three cut flowers. The longest vase lives of the cut roses (14.33 days) and the cut sunflowers (14.5 days) were obtained from the application of 40 µM SNP. But, the vase life of the cut lisianthus extended the most in the treatment of 20 µM SNP, but it did not differ from the treatment of 40 µM SNP (13.55 days), significantly. SNP at a rate of 60 µM significantly reduced the postharvest vase life of all three flowers versus the lower rates (20 and 40 µM). The lowest vase lives of the cut rose (10.66 days), lisianthus (11.66), and sunflower (11.00 days) were all related to the control treatment (Fig.1).

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

S.o.V	df	Vase life	Solution uptake	Dry matter	Bacteria colonies in vase solution	Bacteria coloniesin end stem	Chlorophyll b	Petals protein	Ethylene production
Species (S)	2	0.72^{ns}	0.034^{ns}	6.438^{**}	1.194^{ns}	6.083^{ns}	0.005^{ns}	3.267^{**}	0.008^{**}
Sodium Nitroprusside (SNP)	3	0.505^{ns}	0.022^{ns}	0.899^{ns}	2.509^{ns}	2.815^{ns}	0.001^{ns}	0.571^{**}	0.001^{**}
S × SNP	6	6271.5^{*}	0.048^{ns}	56.94^{**}	3.604^{ns}	4.121^{ns}	0.0034^{ns}	10.73^{**}	0.02^{**}
Error	22	1.992	0.053	1.849	7.00	2.861	1.47	0.00	0.0005
CV (%)		39.32	54.26	27.43	19.61	45.90	28.18	34.06	33.12

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

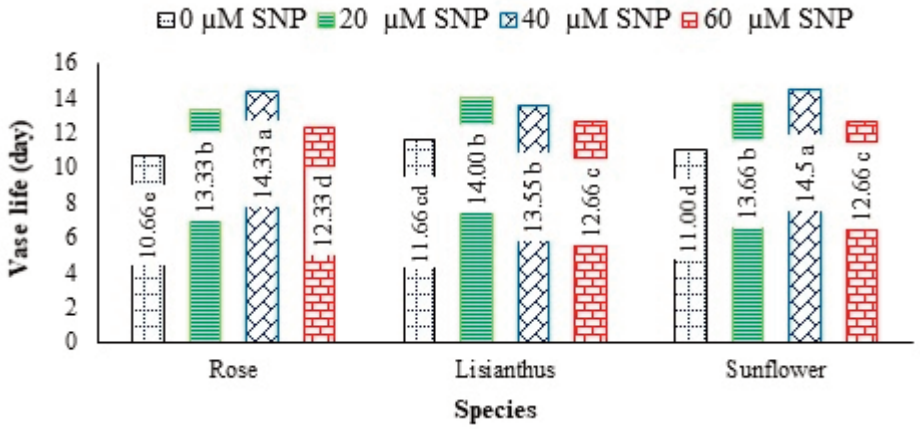

Fig. 1. The effect of different SNP rates on the vase life of the studied plant species.

Solution uptake

The effect of 'species × SNP' was statistically insignificant on solution uptake (Table 1). However, the highest solution uptake of the cut roses (1.10 ml g^{-1} F.W.) and sunflowers (0.89 ml g^{-1} F.W.) were recorded by those treated with 60 µM SNP, but the cut lisianthus flowers (0.92 ml g^{-1} F.W.) exhibited the highest solution uptake in the control treatment (Table 2).

Table 2. Means comparison for the effect of different treatments on the recorded traits.

Treatments		Solutionuptake (ml g^{-1} F.W.)	Bacteria coloniesin vase solution (Log_{10} CFU ml^{-1})	Bacteria coloniesin end stem (Log_{10} CFU ml^{-1})	Chlorophyllb (mg g^{-1} F.W.)
Species					
Rose		0.86[a]	4.66[a]	2.58[a]	0.294[a]
Lisianthus		0.85[a]	5.16[a]	3.41[a]	0.310[a]
Sunflower		0.76[a]	5.66[a]	4.00[a]	0.355[a]
SNP (µM)					
0		0.83[a]	5.00[a]	2.77[a]	0.302[a]
20		0.87[a]	5.44[a]	3.22[a]	0.318[a]
40		0.76[a]	6.11[a]	3.22[a]	0.310[a]
60		0.85[a]	5.08[a]	4.11[a]	0.323[a]
Species	**SNP(µM)**				
Rose	0	0.86[a]	6.00[a]	2.33[a]	0.350[a]
	20	0.67[a]	6.00[a]	3.66[a]	0.460[a]
	40	0.79[a]	6.00[a]	3.66[a]	0.428[a]
	60	1.10[a]	6.33[a]	4.33[a]	0.380[a]
Lisianthus	0	0.92[a]	5.00[a]	2.66[a]	0.365[a]
	20	0.91[a]	5.00[a]	2.00[a]	0.307[a]
	40	0.68[a]	3.00[a]	5.66[a]	0.391[a]
	60	0.80[a]	6.33[a]	2.66[a]	0.350[a]
Sunflower	0	0.74[a]	5.66[a]	2.33[a]	0.118[a]
	20	0.88[a]	5.33[a]	5.00[a]	0.221[a]
	40	0.68[a]	2.33[a]	3.33[a]	0.197[a]
	60	0.89[a]	5.66[a]	2.33[a]	0.190[a]

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the LSD test

Vase solution and stem-end bacterial population

The interaction of 'species × SNP' was not statistically significant for vase solution and stem-end bacterial population (Table 1). However, the lowest bacterial colonies were recorded in the vase solution of the lisianthus (3.00 Log_{10} CFU mL^{-1}) and sunflowers (2.33 Log_{10} CFU mL^{-1}), which were related to the treatment of 40 µM SNP (Table 2).

The lowest stem-end bacterial population was observed in the untreated cut roses (2.33 Log_{10} CFU mL^{-1}) and in the cut lisianthus treated with 20 µM SNP (2 Log_{10} CFU mL^{-1}). Regarding the cut sunflowers, the lowest stem-end bacterial population was recorded in the treatments of 0 and 60 µM SNP, which did not differ significantly (Table 2).

Dry matter

The dry matter contents of the cut roses, lisianthus, and sunflower were significantly ($P < 0.01$) affected by the interaction of 'species × SNP' (Table 1). As is seen in table 2, the application of SNP at all three levels to the cut roses and sunflowers increased their dry matter percentages versus the control although the increase was not significant in the cut sunflowers. The cut roses and sunflowers had the highest dry matter percentages (25.21 % and 12.20 %, respectively) when they were treated with 20 µM SNP. The cut lisianthus had the highest and lowest dry matter percentages at the SNP rates of 60 and 40 mM (18.48% and 16.58%, respectively)(Fig. 2).

Total protein content of petals

The interaction of the experimental treatment was significant ($P < 0.01$) for the protein content of the petals (Table 1). The treatment of the cut roses, lisianthus, and sunflowers with SNP increased the protein content in their petals. The lowest level of this trait in all three species was obtained when they were not applied with SNP. The highest was related to the treatments of 20 and 40 µM SNP regarding the cut roses and sunflowers (14.65 %). Regarding the cut lisianthus flowers, the highest levels were obtained from the treatments of 20 and 40 µM SNP (14.80 % and 14.67 %, respectively)(Fig. 3).

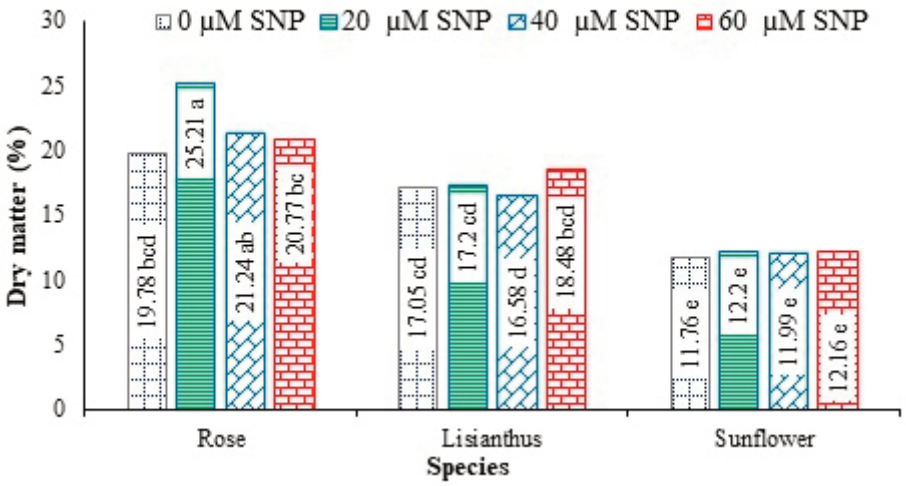

Fig. 2. The effect of SNP at different rates on dry matter percentage.

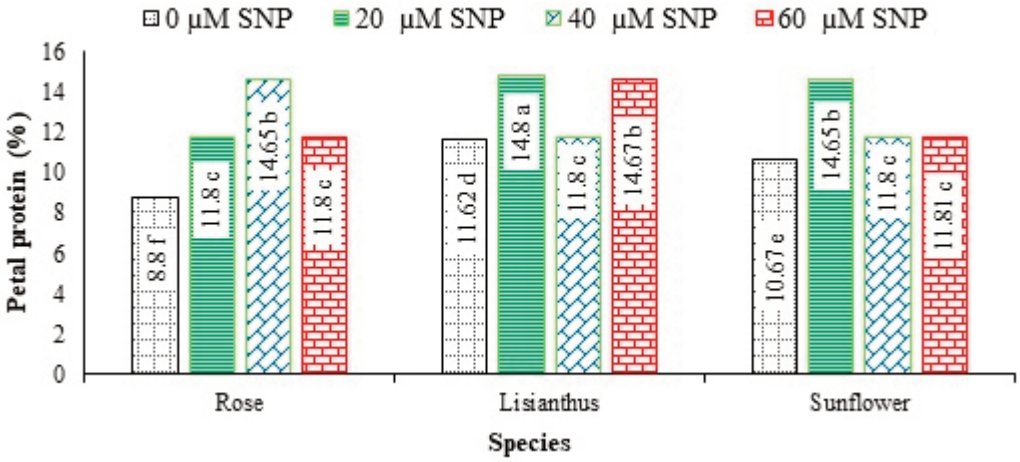

Fig. 3. The effect of SNP at different rates on the petals protein.

Chlorophyll b content

The chlorophyll b content was not significantly influenced by the interaction of the experimental treatments (Table 1). However, the treatment of the cut roses and sunflowers with SNP increased their chlorophyll b content versus the control. The leaves of the lisianthus produced the highest amount of chlorophyll b (0.39 mg g^{-1} FW) when they were treated with 40 µM SNP (Table 2).

Ethylene production

The interaction of 'species × SNP' was significant ($P < 0.01$) for ethylene production (Table 1). Fig. 4 shows that the application of SNP reduced ethylene production by the cut roses significantly although increasing SNP rate to 60 µM increased ethylene production by this species compared to its lower rates (20 and 40 µM). The cut lisianthus flowers treated with 20, 40, and 60 µM SNP had lower ethylene content than the control (0.24 nl l^{-1} h^{-1} g^{-1} F.W.). As for the cut sunflower, the highest ethylene production (0.07 nl l^{-1} h^{-1} g^{-1} F.W.) was recorded in the treatments of 20 and 40 µM SNP and the lowest (0.03 nl l^{-1} h^{-1} g^{-1} F.W.) in the treatment of 60 µM SNP (Fig. 4).

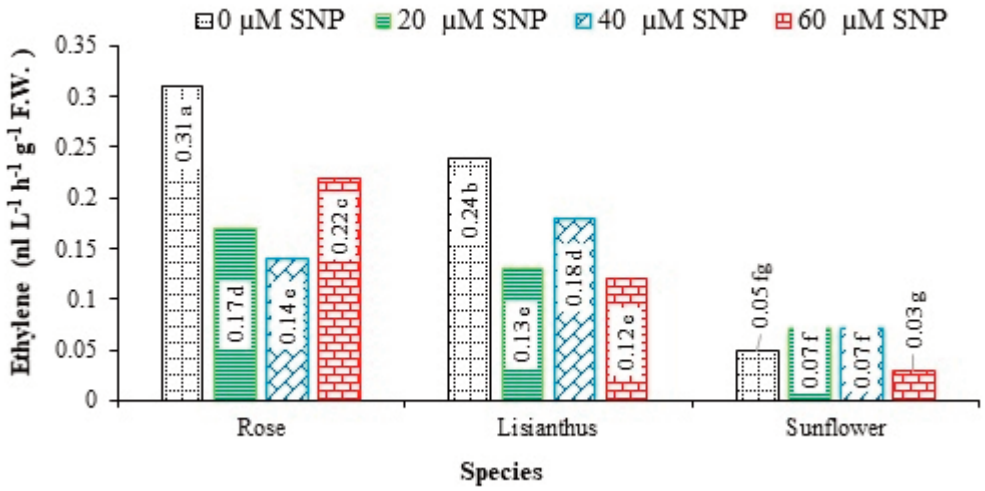

Fig. 4. The effect of SNP at different rates on ethylene production.

DISCUSSION

Sensitivity to ethylene and water stress is the main factor limiting the postharvest life of cut roses, lisianthus, and sunflowers. Our results revealed that the treatment of the cut roses, lisianthus, and sunflowers with different levels of SNP, especially 20 and 40 µM, was effective in extending their vase life. The positive effect of SNP has been reported on improving the postharvest longevity of cut carnations 'Monte' (Zeng *et al*., 2011), roses 'Kardinal' (Liao *et al*., 2013), and carnations (Naing *et al*., 2017), which supports our finding.

SNP is an NO-releasing compound. It is believed that NO protects plants against oxidative stress and early senescence by two mechanisms, i.e., the antioxidant effect and changing the expression of defensive genes in plants (Lamattina *et al*., 2003; Ashouri Vajari and Nalousi, 2013; Shi *et al*., 2016). However, NO has both protective and toxic effects. In most research studies, NO has extended the vase life of cut flowers at low rates as with cut *Eucomis* (Salachna and Byczynska, 2017), carnations 'Monte' (Zeng *et al*., 2011), and roses (Liao *et al*., 2013). Like our finding, these studies also reported that SNP at high rates reduced its effectiveness in conserving vase life. Researchers argue that NO at high rates produces peroxynitrite (ONOO) radical, and this toxic compound destroys macromolecules and advances the aging process at high concentrations. Thus, the positive effect of NO on reducing the aging rate strongly depends on its concentration (Lamattina *et al*., 2003; Badiyan and Wills, 2004).

Based on the results, SNP had no significant effect on solution uptake and bacterial population in the vase solution and at the stem end. Few studies have addressed the antimicrobial effect of SNP. However, there are reports about the positive effect of NO on water conservation in cut roses, gerberas, and tulips (Badiyan and Wills, 2004), chrysanthemums (Mansouri, 2012), and gerberas (Karamian *et al*., 2019), which is inconsistent with our findings. Nonetheless, it is suggested that the positive effect of NO on improving water uptake and retention is associated with its effect on reducing the activity of polyphenol oxidase and inhibiting the production of suberin and vascular blockage (Karamian *et al*., 2019; Shabanian *et al*., 2018), as well as its effect on stomatal closure, the protection of membrane structure and macromolecules, and its antioxidant activity (Neill *et al*., 2002). Some researchers have also reported that NO induces plant resistance to water stress by influencing stomatal closure and reducing transpiration (Jiang, 2012; Seyf *et al*., 2012). Salachna and Byczynska (2017) reported that solution uptake in cut *Eucomis* flowers was decreased with the application of SNP at the rates of 1 mM and 1 µM versus the control.

Although, SNP had no effect on water uptake by the cut roses, lisianthus, and sunflowers, it reduced ethylene production by all three species significantly. So, SNP retards aging and withering in cut roses, lisianthus, and sunflowers by controlling ethylene production and its detrimental impacts. Leshem and Wills (1998) reported that the positive effect of NO on extending vase life and delaying senescence was mainly related to its inhibitory effect on ethylene synthesis and emission. They reported that NO reduced ethylene production and biosynthesis and extended postharvest life of climacteric species by inactivating the cofactor of ACC-oxidase and ACC-synthase enzymes. In Naing *et al*.'s (2017) study, the application of SNP at rates up to 10 mg L^{-1} contributed to extending the vase life of carnations by reducing the expression of genes responsible for ethylene biosynthesis and decreasing ethylene production and emission, which corroborates our results.

SNP was influential on protecting dry matter and total protein percentage of the cut roses, lisianthus, and sunflowers. NO has antioxidant activity and hinders damages to macromolecules in stressful conditions by increasing antioxidants (Zeng *et al*., 2011; Ashouri Vajari and Nalousi, 2013). According to Mirzaie Esgandian and Jabbarzadeh (2019), the dry weight of cut roses 'Utopia' and 'Dolce Vita' was increased by the application of SNP. They reported that carbohydrates are the production of photosynthesis and NO is involved in the synthesis of photosynthesizing pigments, so it can increase dry matter production by synthesizing these pigments.

In Talebi *et al*.'s (2013) research, SNP increased the protein content of petals and leaves of cut roses 'Sensiro'. They reported that SNP improved vase life by reducing ethylene production, protecting cells against the detrimental effects of reactive oxygen species (ROS), and protecting proteins. An increase in protein level with the application of NO has been reported by Zhang *et al*. (2007) and Beligni *et al*. (2002), which is consistent with our findings. Dolatabadian *et al*. (2009) suggest that free oxygen radicals have a high affinity for bonding with proteins and degrading them. Since NO has antioxidant activity, it seems it prevents the degradation of proteins in the petals of cut roses, lisianthus, and sunflowers by scavenging free oxygen radicals.

With aging, pigments start to degrade. NO is involved in chlorophyll synthesis and contributes to chlorophyll retention in plant tissues (Bowyer *et al*., 2003; Ashouri Vajari and Nalousi, 2013). ROS is reportedly the main factor responsible for the degradation of photosynthesizing pigments, and NO protects these pigments by its antioxidant effect and by scavenging ROS. Additionally, NO plays a role in increasing Fe availability to plants, thereby contributing to chlorophyll retention (Antoniou *et al*., 2013; Kim and Lee, 2005). In the present study, the application of SNP increased chlorophyll b content versus the control at all levels and in all three species, except for the treatment of the lisianthus with 20 and 60 µM SNP, although the difference was statistically insignificant. According to Mostofi *et al*. (2010) and Seyf e*t al*. (2012), SNP had no significant effect on the chlorophyll content of cut carnations, which agrees with our findings.

CONCLUSIONS

SNP extended the vase life of cut rose, lisianthus, and sunflower by suppressing ethylene production and protecting dry matter and total protein. SNP was most influential on vase life at rates up to 40 µM, but its further increase to 60 µM reduced its efficiency in protecting vase life. SNP had no significant effect on protecting water uptake and reducing the microbial population in vase solutions and stem ends, as well as chlorophyll content. The most appropriate SNP rate was 40 µM for extending the vase life of the cut roses and sunflowers and 20 µM for extending the vase life of the cut lisianthus flowers.

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