# Characterization of the role of sodium nitroprusside, a nitric oxide donor, on vase life and postharvest attributes of Alstroemeria aurea 'Orange Queen'

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**Article**

**Keywords:** Alstroemeria, nitric oxide, malondialdehyde, solution uptake

**Posted Date:** April 20th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1563424/v1

# Abstract

Senescence is the important physiological process that impact the quality of cut flowers. Alstroemeria is one of the major cut flowers that has received a lot of attention in recent years due to its high yield, extremely beautiful flowers and variety of colors. In order to improve the vase life and other postharvest characteristics of Alstroemeria cut flowers 'Orange Queen', preharvest foliar application of sodium nitroprusside, nitric oxide (NO) donor at concentrations of 0, 50, 100 and 200 µM and short-term treatment (pulsing) after harvest at the same concentration were performed as a factorial trial base on a completely randomized design. In this experiment, vase life, solution uptake, relative fresh weight, relative water content, malondialdehyde and catalase activity were measured. It was observed that nitric oxide improved postharvest characteristics. Foliar application of 100 µM sodium nitroprusside (SNP) along with pulsing of 50 µM SNP interdependent on vase life time period was observed to be the optimal dose for improving relative fresh weight (RFW), relative solution uptake (RSU), relative water content (RWC), catalase (CAT), and malondialdehyde (MDA) in Alstroemeria cut flowers. Vase life markedly extended by preharvest application of 100 µM SNP + 50 µM SNP as pulsing from 7 day to 16 day and thus those inducer effects were dose- and time-dependent. Taken together, these results suggest that SNP plays a critical role in multiple modes of action to prolong vase life via maintaining RFW, RWC, scavenging free radical in term of enzymes antioxidant, inhibiting lipid peroxidation in 'Orange Queen' of Alstroemeria cut flowers.

# Introduction

*Alstromeria hybrida* L. commonly called the Peruvian lily or lily of the Incas, is a genus of flowering plants in the family Alstroemeriaceae. They are all native to South America although some have become naturalized in the United States, Mexico, Australia, New Zealand, Madeira and the Canary Islands. Alstroemeria is a recent introduction into the world's floriculture scene and has become a major cut flower. Flowers are simple or compound. Flower colors range from white to dark yellow to various pinks, violets, purples and reds. Yellow throats and black dots at the base of the petals and throats are a trademark of alstroemeria. It is a perennial herbaceous rhizomatous plant. Fibrous roots and aerial parts originate from a sympodially branching rhizome [1].

Senescence occurs in cut flowers by several factors, disruption of water uptake and transport, due mainly to microbial vessel blockage; depletion of the respiratory substrate, which limits the energy available to sustain life processes; and harmful effects of the reactive oxygen species (ROS) emerging during the oxidative stress after the flowering stems are detached from mother plants as well as the senescence-accelerating ethylene action in the ethylene-sensitive species [2]. The important problem in Alstroemeria flowers is leaf yellowing [3] which may occur over several days and progress rapidly [4] and it is due to its high sensitivity to ethylene, which grows rapidly under postharvest conditions and reduces the quality of flowers and the economic value of the product [5].

Nitric oxide (NO) is a gaseous lipophilic free radical [6]. NO not only easily migrates in the hydrophilic domains of the cell such as cytoplasm, but also freely disperses through the lipid phase of membranes [7]. No is a bioactive molecule that exerts a number of diverse signal functions [8]. This multifunctional plant signal molecule plays role in various plant processes including senescence [9], stomatal closure and aperture [10], chlorophyll production and anthocyanin biosynthesis [11], plant growth and development [12], plant defense signal against pathogen influence [9], plant death, regulation of ion channels of guard cells, mitochondrial and chloroplast functionality [12], seed germination [10], hypocotyl elongation, xylem differentiation, root development and regulation of photosynthesis and fruit ripening [9]. Nitric oxide can stimulate the production of many antioxidants in postharvest stages [13]. NO has some interactions with phytohormones. The interaction between NO and ethylene during the maturation and senescence of plant tissues suggested an antagonistic excitement of two gases [12]. It was hypothesized that ethylene biosynthesis in growing plants may be regulated by NO. NO inhibits ethylene action and synthesis in higher plants [14]. It has been offered that NO acts as a natural senescence delaying plant growth regulator primarily by down regulating of ethylene production [15]. NO could be a novel means of postharvest control to maximize shelf life of fruits, vegetables and flowers.

The role of NO in improving postharvest attributes of cut flowers has received increased attention across a number of disciplines in recent years. Recent evidence suggests that application of 0, 50 and 100 µM of sodium nitroprusside (SNP) - an external nitric oxide (NO) donor- on cut flowers of rose and gladiolus increased proteins, solution uptake, vase life and fresh weight of flowers compared to the control [14, 16]. It has previously been observed that application of sodium nitroprusside at concentrations of 0, 50, 100, 150, 200 and 500 µM on cut lilies resulted in increasing vase life compare to control [17]. There is evidence that NO plays a crucial role in vase life and physiological attributes of *Dianthus caryophyllus* L. 'Tempo'. The results showed that exogenous NO could significantly extend the vase life of cut carnation flowers (16.9 days) and could delay petal wilting, maintain water metabolism, the antioxidative enzymes activity and eliminate reactive oxygen species (ROS) and as well as cell membrane stability [18]. In another research in this area, which examined the effect of SNP as a vase solution in two species of gerbera, it was found that a concentration of 150 µM as a preservative solution increased the fresh weight of both species of gerbera and increased the vase life of flowers, as well as increased the activity of ascorbate peroxidase, peroxidase and phenylalanine ammonialyase enzymes and reduced the malondialdehyde [19]. Sodium nitroprusside at a concentration of 150 µM with abscisic acid was applied as a foliar spray on cut rose and it was observed that both abscisic acid and SNP increased the activity of superoxide dismutase, peroxidase and ascorbate enzymes [20]. Recent evidence suggests that SNP improves the postharvest longevity of cut rose (*Rosa hybrida* L.), lisianthus (*Eustoma grandiflorum*), sunflower (*Helianthus annuus* L.) by suppressing ethylene synthesis and protecting proteins [21].

One of the most important problems of Alstroemeria postharvest, is the rapid yellowing of the leaves of stem before abscision the petals, which is due to its high sensitivity to ethylene. Ethylene develops rapidly under postharvest conditions and reduces the quality and economic value of flowers, so the use of treatments that act as inhibitors of biosynthesis and activity of ethylene, can increase the vase life of cut flowers. One of these inhibitors, is nitric oxide, which acts as an inhibitor of ethylene production and its effect. SNP can be used as a healthy and very effective compound to maintain the quality and vase life of harvested cutflowers and delay the senescence of them and increase their marketing. This study uses recent data from NO to evaluates the effectiveness of pre- and postharvest application of SNP on vase life and postharvest characteristics of Alstroemeria as well as determines its appropriate concentrations.

# Materials And Methods

### Plant materials and growing conditions

Alstroemeria plants (*Alstroemeria aurea*) cv. Orange Queen (Royal Van Zan Ten – Netherlands) were obtained from a commercial greenhouse. Plants were cultured in plastic pots with a diameter of 24 cm and a height of 19 cm. The soilless growth medium was composed of perlite : cocopeat (1:3 v/v). The greenhouse day/night temperature was set at 18–21/10–12 °C, light duration was 10-12 h and light intensity at 400-500 µmol $m^{-2}$ $s^{-1}$. The plants were fertilized three times a week based on the composition of the nutrient solution shown in Table 1.

**Table 1-nutritional program used for Alstroemeria for 100 L nutrient solution.**

| $Mg(SO_4)_2$ | $KNO_3$ | $NH_4NO_3$ | $Na_2MoO_4$ | $Na_2[B_4O_5](OH)_4$ | MAP | $MnSO_4$ | $ZnSO_4$ | $K_2SO_4$ | Fe chelate 6% | $5Ca(NO_3)_2\text{-}NH_4NO_3.10H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 g | 32 g | 4 g | 0.035 g | 0.03 g | 5 g | 0.2 g | 0.15 g | 8 g | 5 g | 10g |

### Treatment with sodium nitroprusside (SNP)

In this experiment preharvest foliar application of SNP (Fluka company- Switzerland) at concentrations of 0, 50, 100 and 200 µM was applied jointly with post-harvest application of SNP (as pulsing) with the same concentrations, 0, 50, 100 and 200 µM.

For preharvest application, about one month after the establishment of plants and growth in greenhouse conditions, foliar spraying of plants was performed at intervals of two weeks for four months. For postharvest application, pulse treatments were applied in the laboratory for 24 hours after harvesting the cut flowers. For this purpose, cut flowers were harvested early in the morning and immediately were placed in buckets containing tap water and directly transported to the laboratory where flower stems were re-cut under distilled water to a 40-cm length and treated with SNP as a NO donor compound. This red solid compound was dissolved in distilled water in concentrations of 50, 100 and 200 µM. Distilled water was used as control treatment (0 µM). The solution was applied immediately because of its short half-life. Four stems were placed in each 500 mL flask. Flowers were held in solutions at laboratory for 24h to permit compound uptake by stems. After this period, flower stems were transferred to distilled water and were held in this situation until the end of vase life. At the experimental period, the temperature was 22±1ºC, relative humidity was about 70%, and a 12 h photoperiod was maintained using fluorescent lamps with light intensity of 13 µmol $m^{-2}$ $s^{-1}$.

### Relative fresh weight (RFW) of flowers

Fresh weight change of flowers was measured as relative fresh weight (g $g^{-1}$ initial fresh weight $day^{-1}$) based on Joyce and Jones [22] by the following formula:

$RFW = FW_i / FW_0$

**$FW_i$=** is the weight of stem (g) at desired day

**$FW_0$**= the weight of the same stem (g) at first day

### Vase life

The vase life of Alstroemeria cut flowers was determined by counting the number of days from harvest to full opening of the primary florets, 50 % petal fall and 50 % leaf yellowing from daily observation [3,23].

### Relative water content (RWC) of petals

The RWC from florets at different stages was determined by Anderson et al. [24]. The fresh weight (FW) of detached florets was recorded by placing them in pre-weighed test tubes with distilled water and again recording the weight of test tubes. The increase in weight of the test tube was FW of florets. The tubes were again weighed after 4 h and now the increase in weight was turgid weight (TW). The florets were then oven dried at 72 °C for 48 h and weight was recorded as dry weight (DW). The RWC was calculated as:

$$RWC = \frac{Fw - DW}{TW - DW} \times 100$$

### Relative solution uptake (RSU)

Solution uptake rate was determined as the ratio of absorbed solution by flower and primary fresh weight of the flower stem. The amount of relative uptake solution was measured using the method of Alaei et al. [25] and was expressed in ml per gram of initial fresh weight by the following formula:

$RSU = WU_i / FW_0$

RSU= Relative solution uptake

WUi= vase solution weight (g) on t = day 5 and 10.

$FW_0$= Primary fresh weight

### Malondialdehyde (MDA)

Petal samples (0.2 g) were homogenized in 5 mL of 1% trichloroacetic acid (TCA). The homogenate was centrifuged at 8,000 g for 10 min, and then 1 mL of supernatant was added to 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was calculated at 532 and 600 nm. The MDA content was measured when the extinction coefficient of the sample was 155 mM $cm^{-1}$ [26].

MDA (μmol/g FW) = (A532 – A600 / 155)×100

### Catalase (CAT) activity assay

For the catalase enzyme assay, 500 mg of tissue was extracted in 2.5 mL of 50 mM phosphate buffer (pH 7) at 0 °C by centrifuging at 10,000 g for 15 min and then collecting supernatant to make the final volume to 100 mL. Catalase activity was determined by Aebi [27]. The absorbance of the supernatant was calculated at 240 nm The CAT activity was measured when the extinction coefficient of the sample was 43.6 mM $cm^{-1}$.

$$unit\ \frac{mM}{min} = \frac{do\ D/\min(slope) \times vol.of\ assay}{Extinction\ coefficient\ (43.6)}$$

### Statistical analysis of data and software used

This experiment was conducted as a factorial based on the completely randomized trial with three factors, the first factor was preharvest application of SNP with concentrations of 0, 50, 100 and 200 μM, the second factor was postharvest application of SNP with concentrations of 0, 50, 100 and 200 μM and the third factor was sampling time of cut flowers during vase life period ($0^{th}$, $5^{th}$ and $10^{th}$ day). Data management and analysis were performed using SAS version 9.2. The means were compared using Tukey's multi-domain method at a probability level of 1 %.

## Results

Effect of SNP as holding solution on vase life, postharvest quality parameters and biochemical attributes of Alstroemeria is explained below. The analysis of variances revealed that pre- and postharvest application of different concentrations of SNP significantly influenced the postharvest quality of Alstroemeria cut flowers.

### Relative fresh weight (RFW)

Comparing the results, it can be seen that among the treatments used in this study, preharvest application of 100 μM sodium nitroprusside incombination with pulse treatment use of 50 μM SNP at postharvest stage cause a significant effect on the relative fresh weight of Alstroemeria cut flowers during vase life period (Figure 1).

Different letters indicate statistically significant differences between the treatments and control in (P≤0.01).

### Relative solutionuptake (RSU)

With respect to the relative solution uptake of cut flowers of Alstroemeria, it can also be observed that the application of all concentrations of sodium nitroprusside in two stages (pre and post-harvest) led to an increasing trend in the relative solution uptake compared to the control. Over time, the relative solution uptake showed a decreasing trend. According to Figure 2, although the treatment of 100 μM sodium nitroprusside in the pre-harvest stage and 200 μM in the post-harvest stage on the 5th day had the highest relative solution uptake, but on the 10th day showed a sharp decrease. It is apparent from this figure that foliar application of 100 μM SNP in the pre-harvest stage along with 50 or 100 μM SNP as pulsing in the post-harvest stage had a greater effect on increasing the relative solution uptake during vase life period compared to the control and other treatments (Figure 2).

Different letters indicate statistically significant differences between the treatments and control in ($P \leq 0.01$).

**Relative water content of petals (RWC)**

Comparing the results, it can be seen that the relative water content of petals showed a decreasing trend over time, but application of 50 or 100 μM SNP at preharvest stage alongside postharvest application of 50 μM SNP cause to maintain peatals water content during the vase period compared to control (Figure 3).

Different letters indicate statistically significant differences between the treatments and control in ($P \leq 0.01$).

**Malonedialdehyde (MDA)**

The results, as shown in figure 4, indicate that application of sodium nitroprusside in both pre and post-harvest stages over the vase life period significantly reduced malondialdehyde compared to the control but there was no significant difference between control and using 200 μM sodium nitroprusside in the pre and post-harvest stages. It should be noted that over time, the amount of malondialdehyde in all treatments showed an increasing trend, although this amount was lower than the control. According to figure 4, the highest amount of malondialdehyde was observed in control and the lowest amount of it was observed in foliar application of 100 μM sodium nitroprusside jointly with pulsing of 50 μM SNP at postharvest stage.

Different letters indicate statistically significant differences between the treatments and control in ($P \leq 0.05$).

**Catalase enzyme activity (CAT)**

An inspection of the data in figure 5, reveals that among the treatments used in this study, preharvest application of 100 μM SNP along with postharvest application of 50 μM of it was able to create a significant difference compared to the control. It should be noted that, as shown in figure 5, increasing the time of keeping cutflowers in vase solution, leads to a significant increase in catalase activity.

Different letters indicate statistically significant differences between the treatments and control in ($P \leq 0.01$).

**Vase life**

From the data in figure 6, it is apparent that the vase life of Alstroemeria cut flowers varies between different concentrations of SNP.  In figure 6 there is a clear trend of increasing the vase life with application of SNP at pre- and post-harvest stages to 100 μM compared to control, but with increasing the concentration of SNP, to 200 μM, vase life was decreased. According to figure 6, preharvest application of 100 μM SNP and pulse treatment of 50 μM SNP, cause to 44% increase in vase life (from 7 days to 16 days) compared to the control.

# Discussion

The results of the present study show that the application of sodium nitroprusside in two stages (pre and post-harvest) leads to less reduction of weight of flowers as compared to control. Normally, RFW is also strongly associated with the vase life of cut flowers. Maintaining the fresh weight of cut flowers during vase life period is an important parameter to evaluate the vase life and the postharvest quality of flowers and it has been proven that the heavier the cut flowers, the longer the vase life [28]. Prior studies have noted the importance ofnitric oxide in increasing water absorption, as a result, increasing the relative fresh weight of cut flowers by influencing the regulation of ion channels of guard cells,  closing stomata [10], decreasing osmotic pressure, transpiration and an effect on abscisic acid [14]. In this study, sodium nitroprusside was also used in the pre-harvest stage, it is possible that sodium nitroprusside, as a nitric oxide-releasing compound, increased endogenous β-gluconase activity. The enzyme β-gluconase is involved in the hydrolysis of glycosidic bonds between glucose units within the cell wall, thereby leading to cell wall relaxation and increased cell development. In a study, it was stated that nitric oxide deficiency in mutant Arabidopsis plants reduces the activity of β-gluconase enzyme, so plant growth remains limited. Since the growth process is associated with an increase in turpentine pressure and consequently an increase in the relative water content, nitric oxide deficiency leads to a decrease in turpentine and fresh weight in plants [29]. The effect of nitric oxide on many other processes, including stem elongation, meristem development, and relationship between organs, has been reported by the vascular system. The presence of nitric oxide in vascular tissues and epidermal cells of roots, stems and leaves has been proven [30], Therefore, it seems that the application of sodium nitroprusside in the pre-harvest stage in this study could be effective through the development and improvement of the vascular system and also the positive effect on plant water relations on the fresh weight of the plant. In confirmation of the results of the present study in Abdi's [31] study on roses of 'Avalanch', they reported the effect of nitric oxide on increasing the relative fresh weight of flowers in the post-harvest stage. In accordance with the present results, previous studies have demonstrated the positive effects of sodium nitroprusside or other nitric oxide releasing compounds on the improvement of relative fresh weight. For example, Mittal and Shalini [32] reported the effect of sodium nitroprusside on gladiolus, Rahimian Booger and Salehi [33] on gladiolus, Mirzaei Esgandian and Jabbarzadeh [34] on rose 'Utopia' and 'Dolce Vita', Karamian et al. [35] on gerbera flowers and Dwivedi et al. [16] reported on gladiolus.

In our study, the Alstroemeria cut flowers absorbed more solution and performed better when sprayed with SNP and then pulse treated with SNP than control (Figure 2). Vase life of cut flowers is dependent upon the water status in flowers i.e., turgidity. The flowers are acceptable only when petals are turgid. When a flower is harvested, the uptake of water from parent plant ceases but the transpiration loss continues that results in water deficit followed by petal wilting [36] . Flowers with wilted petals become unacceptable and their vase life is over. So, water status plays an important role in determining the vase life of flowers [37, 38, 39, 40]. Uptake of water by the plant is one of the important factors to increase the vase life of cut flowers [18]. Many studies have reported that nitric oxide causes the stomata to close, which in turn leads to the production of the  abscisic acid, and as we know, this hormone plays a key role in responding to water stress and increases water absorption [41]. Nitric oxide has a lead role in xylem differentiation which could be accounted for a higher amount of solution/water uptake by flowers with the application of SNP, NO donor, [42]. SNP responses are dose dependent so at low concentration, it resulted in better uptake through stem whereas at high concentration, it interfered with translocation channel. The decrease in the ability to absorb solution at a high concentration of SNP could also be ascribed to microbial growth in vase solution as well as on the stem surface and air embolism of xylem tissues [43]. In addition, nitric oxide leads to improved quality through stem strength and chlorophyll retention [44]. It is indirectly effective in water uptake.

The current work clearly reported the ability of SNP to significantly prolong the flowers longevity and maintain the postharvest quality of them. The mechanisms thereby SNP treatments exhibited these effects most probably ascribed to water relation maintenance, antimicrobial effect, alleviating the oxidative stress, membrane integrity and chlorophyll maintenance of cut flowers. These mechanisms altogether regulated and delayed the senescence of Alstroemeria cut flowers. SNP treated flowers showed greater ability to maintain water as indicated by higher RWC and RFW of the flowers. Maintaining water relations during postharvest has shown to be very important to extend the vase life while disturbed water balance leads to senescence of flowers [45, 46, 47], also reported that maintaining the fresh weight of flowers may be ascribed to increased water uptake and/or transpiration reduction, thus preventing the fresh weight loss. In accordance with our data, previous publications indicated that SNP treatment improved water relations and increased the RWC during vase life period [48, 49, 50].

The application of sodium nitroprusside in both pre and post-harvest stages reduced the amount of malondialdehyde, although over time, the amount of malondialdehyde showed an increasing trend. Malondialdehyde is a product that is produced by peroxidation of membrane lipids and its amount inside the cell can indicate the amount of oxidative stress in plants as well as cell membrane homeostasis. So, the higher membrane stability plays a key role in inhibiting leakage of electrolytes, sugars, pigment, solute leakage, and also lipid peroxidation as well as in delay senescence during cut flowers postharvest [51]. Our results showed that the change in lipid peroxidation occurrence resulting from the MDA production were alleviated by SNP concentrations (50, 100 μM). These results are in agreement with those reported earlier by Mansouri [52]Kazemzadeh-Beneh et al. [53], Mohasseli and Sadeghi [54]; who suggested that SNP prolonged the vase life of flowers. Cut flower senescence is linked to a sequence of highly regulated physiological and biochemical processes such as degradation of proteins, DNA content, peroxidation of lipids and membrane leakage, degradation of macromolecules, cellular decompartmentalization, floral abscission, color change, leaf yellowing, and weight loss [55]. These changes are due to the increase in the production of reactive oxygen species, which is due to the imbalance of reactive oxygen radicals and antioxidant enzymes, leading to secondary oxidative damage due to the loss of permeability of the membrane [56]. Lipid peroxidation is a complex process that begins with the removal of hydrogen atoms from unsaturated fatty acids, which in turn leads to the production of lipid radicals. The increase in the lipid peroxidation process is determined by a change in the amount of malondialdehyde. Sodium nitroprusside, as a nitric oxide-releasing compound, can reduce lipid peroxidation by three mechanisms. A) In the first mechanism, nitric oxide reacts rapidly with alloxy lipids and peroxide radicals, thereby stopping the peroxidation chain and thereby reducing the amount of malondialdehyde. B) In the process of lipid peroxidation, the enzyme lipoxygenase is one of the key oxidizing enzymes in this process, and its mechanism is also mentioned by reducing $Fe^{3+}$ to $Fe^{2+}$ in the active site of the lipoxygenase enzyme [57]. C) The third mechanism is through the effect of nitric oxide on the increase of antioxidant enzymes, which leads to the removal of activated oxygen radicals, thereby reducing cell membrane damage and thus reducing the amount of malondialdehyde [54].

Considering that it has been reported in various studies that the application of high concentrations of nitric oxide releasing compounds depending on the plant genotype can lead to toxicity in plants [16], so considering that, at a concentration of 200 μM, malondialdehyde levels showed an increasing trend. It can be assumed that this concentration is high for Alstroemeria and leads to toxicity thus increased MDA.

As shown in figure 5, the application of sodium nitroprusside in both preand post-harvest stages increases the activity of catalase, antioxidant enzyme. In the post-harvest stage of flowers and plants, the production of reactive oxygen species is one of the main causes of damage that changes the structure of membrane compounds and antioxidants. Under natural conditions, reactive oxygen species are constantly produce at low concentration in different parts of the plant, including chloroplasts and mitochondria, plasma membranesand etc., which increase in stress conditions and senescence. Earlier studies have been confirmed that reactive oxygen species ultimately cause damage to proteins, RNA, DNA, and cell membranes, and cause to lipid peroxidation [58]. The action of nitric oxide as an antioxidant and its cytotoxic role due to the reaction with free radicals have been considered [16]. In addition, nitric oxide induces the production of antioxidant enzymes, thereby suppressing free radicals and delaying senescence [14]. Also nitric oxide could delay leaf senescence by enhancing CAT and SOD activities and inducing the production of other enzymes, such as glutathione [59].Various reports indicate that nitric oxide has a high affinity for iron-containing enzymes and improves the activity of these protective enzymes. The application of nitric oxide varies depending on the species, cultivar and concentration used, but it has been shown that the application of nitric oxide in low concentrations leads to the expression of genes involved in the synthesis of protective enzymes [60]. Earlier studies have shown that nitric oxide, after reacting with reactive oxygen species, leads to the production of peroxynitrite and reduces the production of these reactive oxygen species. When the pH is in the physiological range, the peroxynitrite produced can be decomposed into a nitrate and proton anion or react with a radical of hydrogen peroxide, thereby reducing reactive oxygen species [60], which may be the reason for the increase in antioxidant enzymes activity in the present study. The results were in accordance with the findings of Naing et al. [55] that reported a positive effect of nitric oxide on the activity of antioxidant enzymes on gerbera cut flowers and Mittal and Shalini [32] that reported the application of SNP on gladiolus flowers increases the activity of antioxidant enzymes such as catalase and ascorbate peroxidase. In the present study, it was shown that the application of high concentrations (200 µM) of SNP cause a decrease in the activity of antioxidant enzymes and antioxidant capacity. Negative effects induced by high concentrations of SNP in CAT activity were dose- dependent [14], therefore, in the present study, preharvest foliar application of 100 µM SNP jointly with pulse application of 50 µM of it were selected as optimal concentrations for CAT activity.

In the present study, the application of sodium nitroprusside increased the vase life of Alstroemeria flowers. In cut flowers, one of the limitations of their maintenance in the postharvest stage is senescence. In senescence process, the formation of reactive oxygen species leads to oxidative damage, which indicates the inability of the plant to antioxidant defense at this stage [61]. In recent years, nitric oxide has been recognized as an antioxidant molecule in plants [62].It should be noted that the effect of nitric oxide depends on the concentration and species of plants, and high concentrations of nitric oxide, through nitrosative stress in cooperation with oxidative stress, cause more destructive damage [63]. Nitric oxide, in high concentration as a free radical, has the ability to damage membranes, proteins and nucleic acids in plant cells, but in low concentrations is necessary for plant growth and acts as a defense system in plants, therefore, NO has both protective and toxic effects [64]. In this study, preharvest application of 200 µM SNP led to a reduction in vase life, but at a concentration of 50 µM, the highest vase life was observed, which shows the positive effect of low concentrations of sodium nitroprusside on vase life. Sensitivity to ethylene and water stress is the main factor limiting the postharvest life of cut flowers [21]. In the present study, the application of sodium nitroprusside at a concentration of 100 µM in the preharvest stage and a concentration of 50 µM in the postharvest stage, in addition to increasing vase life, led to a reduction in water loss. In Alstroemeria, one of the most important problems in the postharvest stage is yellowing of leaves and reduced vase life. Therefore, SNP application for exogenous NO could act as a promising tool to enhance the postharvest performance of Alstroemeria cut flowers. 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 plant [65]. The results were in accordance with the findings of Salachna and Byczynska [64]on cut Eucomis, Ghaei et al. [50] on Gerbera, Mirzaei Esgandian and Jabbarzadeh [34] on two cultivars of rose, Dwivedi et al. [16] on cut gladiolus and Kazemzadeh-Beneh et al. [53] (2018) on gladiolus.

## Conclusion

In conclusion, vase life period in the *Alstroemeria aurea* cv. Orange Queen cut flower is likely to be associated with many parameters, particularly fresh weight content, water uptake, enzymatic antioxidant activities and lipid peroxidation. Results showed that foliar application of SNP as well as supplementing vase solution with SNP enhanced RFW and water uptake, maintained or increased antioxidant activity, leading to inhibit lipid peroxidation and scavenged free radical, and ultimately causing delay in the senescence of flowers. The most significant in the present study, the results showed that the application of sodium nitroprusside increased the vase life and the postharvest indices in Alstroemeria improved and the concentrations of 50 µM in the postharvest stage and 100 µM in the preharvest stage were more effective than other treatments.

## References

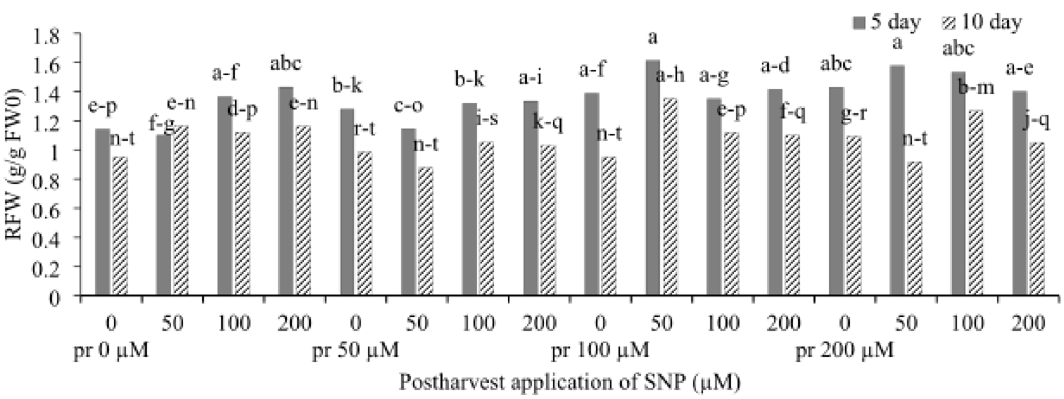
1. Dole, J.M. and Wilkins, H.F. 2004. Floriculture: Principles and Species. Prentice Hall. N.J., USA. 1022p.
2. Skutnik, E., Łukaszewska, A. and Rabiza-Świder, J. 2021. Effects of postharvest treatments with nano silver on senescence of cut Lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) Flowers. Agronomy: 11(2): 215–229.
3. Mutui, T.M., Emongor, V.E. and Hutchinson, M.J. 2006. The effects of gibberellin 4 + 7 on the vase life and flower quality of Alstroemeria cut flowers. Plant Growth Regulation, 48: 207–214.
4. Ferrant, A., Mensuali-Sodi, A. and Serra, G. 2009. Effects of thidiazuron and gibberellic acid on leaf yellowing of cut stock flowers. Eentral European Journal of Biology, 4(4): 461–461.
5. Chamani, A., Ismailpour, B., Pourbirami Hir, Y., Lajair, H. and Saadati, A. 2012. Effects of thidiazuron and humic acid on postharvest life of Alstroemeria cultivar Keniabe. Journal of Horticultural Science, 26(2):147–152. (in Persian)
6. Bartha, B., Kolbert, Z. and Erdei, L. 2005. Nitric oxide production induced by heavy metals in *Brassica juncea* L. Czern. and *Pisum sativum* L. Acta Biologica Szegediensis, 49: 9–12.
7. Hayat, S., Mori, M., Pichtel, J. and Ahmad, A. 2010 b. Nitric oxide in plant physiology. Wileyblackwell. 210p.
8. Tongfei, L., Yuying, W., Boqiang, L., Guozheng, Q. and Shiping, T. 2011. Defense responses of tomato fruit to exogenous nitric oxide during postharvest storage. Postharvest Biology and Technology, 62: 127–132.
9. Rudell, D.R. and Matheis, J.P. 2006. Nitric oxide and nitrite treatments reduce ethylene evolution from apple fruit disks. HortScience, 41(6): 1462–1465.
10. Cevahir, G., Aytamka, E., and Erol, Ç. 2007. The role of nitric oxide in plants. Biotechnology and Biotechnological Equipment, 21(1): 13–17.
11. Bowyer, M.C., Wills, R.B.H., Badiyan, D. and Ku, V.V.V. 2003. Extending the postharvest life of carnations with nitric oxide camparison of fumigation and *in vivo* delivery. Postharvest Biology and Technology, 30: 281–286.
12. Del Rio, L.A., Corpas, F.J., and Barroso, J.B. 2004. Molecules of interest nitric oxide and nitric oxide synthase activity in plants. Phytochemistry, 65: 783–792.
13. Asghari, M.R. 2015. New (non-classical) plant growth hormones and regulators. Urmia University Press, 345 p. (in Persian)
14. Seyf, M., Khalighi, A., Mostofı, Y. and Naderi, R. 2012. Effect of sodium nitroprusside on vase life and postharvest quality of a cut rose cultivar (*Rosa hybrida* 'Utopia'). Journal of Agricultural Science, 4(12): 174–181.
15. Sankhla, N., Mac Kay, W.A. and Davis, T.D. 2005. Effect of nitric oxide generating compounds on flower senescence in cut racemes of pink flowered *Lupinus havardii* Wats. In: Proceedings of Plant Growth Regulation. Society of America-Annual Meeting, 32: 126–132.
16. Dwivedi, K.Sh. Arora, A., Singh, P.V. and Sariam, R. 2016. Effect of sodium nitroprusside on differential activity of SAGS in relation to vase life of gladious cut flowers. Scientia Horticulturae, 210: 158–165.
17. Dhiman, M.R. and Parkash, Ch. 2013. Role of nitric oxide donor compound to extend the vase life of lilium cut flower. International Journal of Agriculture Innovations and Research, 2(2): 258–262.
18. Ashouri Vajari, M. and Nalousi, A.M. 2013. Effect of nitric oxide on postharvest quality and vase life of cut carnation flower. Journal of Ornamental Plants, 3 (3): 183–190.
19. Shabanian, S., Esfahani, M.N., Karamian, R. and Tran, L.S.P. 2018. Physiological and biochemical modifications by postharvest treatment with sodium nitroprusside extend vase life of cut flowers of two gerbera cultivars. Postharvest Biology and Technology, 137: 1–8.
20. Deng, Y., Wang, C., Huo, J., Hu, W. and Liao, W. 2019. The involvement of NO in ABA-delayed the senescence of cut roses by maintaining water content and antioxidant enzymes activity. Scientia Horticulturae, 247: 35–41.
21. Naziri Moghaddam, N., Kaviani, B., Safari Motlagh, M.R. and Khorrami Raad, M. 2021. Effect of sodium nitroprusside on the vase life of cut rose, lisianthus, and sunflower. Journal of Ornamental Plants, 11(3): 185–195.

22. Joyce, D.C. and Jones, P.N. 1992. Water balance of the foliage of cut Geraldton waxflower. Postharvest Biology and Technology, 2: 31–39.
23. Ferrant, A., Hunter, D.A., Hackett, W.P. and Reid, M. 2002. Thidiazuron- a potent inhibitor of leaf senescence in Alestromeria. Postharvest Biology and Technology, 25: 333–338.
24. Anderson, J.M., Pegg, K.G., Dann, E.K., Cooke, A.W., Smith, L.A., Willingham, S.L., Giblin, F.R., Dean, J.R. and Coates, L.M. 2004. New strategies for the integrated control of avocado fruit diseases. In: New Zealand and Australia Avocado Grower's Conference.05: 1–6.
25. Alaei, M., Mirzaei Mashhad, M. and Mortazavi, N. 2018. Effect of post-harvest salicylic acid treatment on physicochemical properties and vase life of cut flowers of Hatchercals cultivar. Plant Production Technology, 17 (1): 47–33. (in Persian)
26. Horst, J.H., and Cakmak, I. 1991. Effects of Aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycin max*). Physiologia Plantarum, 83: 463–468.
27. Aebi, H. 1984. Catalase *in vitro*. Methods in Enzymology, 105: 121–126.
28. Liao, W.B., Zhang, M.L. and Yu, J.H. 2013. Role of nitric oxide in delaying senescence of cut rose flowers and its interaction with ethylene. Scientia Horticulturae, 155: 30–38.
29. Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010 a. Effect of exogenous salicylic acid under changing environment: a review. Environmental and Experimental Botany, 68(1): 14–25.
30. Corpas, F.J., Barroso, J.B., Carreras, A., Valderrama, R., Palma, J.M., Leon, A.M., Sandalio, L.M. and Del Río, L.A. 2006. Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta, 224(2): 246–254.
31. Abdi, R. 2020. The effect of nitric oxide and putrescine on some physiological and morphological characteristics of rose (*Rosa hybrida*) cultivar "Avanche". Master Thesis of the Faculty of Agriculture, Urmia University, 152 p. (in Persian)
32. Mittal, I. and Shalini, J. 2021. Evaluation of sodium nitroprusside (NO donor) as pulsing solution in improving post harvest quality of gladiolus spikes. Indian Journal of Experimental Biology, 59: 467–475.
33. Rahimian Booger, A.R. and Salehi, H. 2013. The effect of nitric oxide and irrigation cycle on quantitative and qualitative characteristics of gladiolus cut flowers. The First National Conference on Agriculture and Sustainable Natural Resources, Iran, Tehran. (in Persian)
34. Mirzaei Esgandian, N. and Jabbarzadeh, Z. 2020. Effects of sodium nitroprusside on improving the vase life of *Rosa hybrida* cvs. Utopia and Dolce Vita. Journal of Plant Process and Function, 38 (9): 77–91. (in Persian).
35. Karamian, R., Nasr Esfahani, M. and Shabanian, S. 2020. Study of the effects of salicylic acid, sodium nitroprusside and ethanol on vase life and flower quality of cut flowers of two gerbera varieties. Journal of Plant Research, 32 (3): 635–646. (in Persian)
36. Lü, P., Cao, J., He, S., Liu, J., Li, H., Cheng, G., Ding, Y. and Joyce, D.C. 2010. Nano-silver pulse treatments improve water relations of cut rose cv: Movie Star flowers. Postharvest Biology and Technology, 57: 196–202.
37. Ebrahimzadeh, A., Jiménez, S., Da Silva, J., Satoh, S. and Lao, M.T. 2008. Post-harvest physiology of cut carnation flowers. Fresh Produce, 2: 56–71.
38. Liu, J., He, S., Zhang, Z., Cao, J., Lv, P., He, S., Cheng, G. and Joyce, D.C. 2009. Nano-silver pulse treatments inhibit stem-end bacteria on cut gerbera cv: Ruikou flowers. Postharvest Biology and Technology, 54: 59–62.
39. Perik, R.R.J., Razé, D., Harkema, H., Zhong, Y. and van Doorn, W.G. 2012. Bending in cut *Gerbera jamesonii* flowers relates to adverse water relations and lack of stem sclerenchyma development, not to expansion of the stem central cavity or stem elongation. Postharvest Biology and Technology, 74: 11–18.

40. van Doorn, W.G. 2012. Water relations of cut flowers: an update. In: Janick J (ed) Horticultural reviews. Wiley, Hoboken, pp. 55–106.
41. Jiang, C.Zh. 2012. Postharvest biology and technology of cut flowers and potted plants. Horticultural Reviews, 40(1): 1–54.
42. Gabaldon, C., Gomez, R.L.V., Pedreno, M.A. and Ros Barcelo, A.R. 2005. Nitric oxide production by differentiating xylem of *Zinnia elegans*. New Phytologist, 163: 121–130.
43. Bhattacharjee, S.K. and De, L.C. 2006. Postharvest technology of flowers and ornamental plants. Point Publishers, Jaipur, p 440.
44. Zhang, Q.T., Zhang, L.L., Geng, B., Feng, J.R., Zhu, S.H. 2019. Interactive effects of abscisic acid and nitric oxide on chilling resistance and active oxygen metabolism in peach fruit during cold storage. Journal of the Science of Food and Agriculture, 99: 3367–3380.
45. Hassan, F.A.S. and Ali, E.F. 2014. Protective effects of 1- methylcyclopropene and salicylic acid on senescence regulation of gladiolus cut spikes. Scientia Horticulturae, 179: 146–152.
46. Hassan, F.A.S., Ali, E.F. and El-Deeb, B. 2014. Improvement of postharvest quality of cut rose cv. 'First Red' by biologically synthesized silver nanoparticles. Scientia Horticulturae, 179: 340–348.
47. Fahmy, H., Esmat, A. and Ragia, M. 2020. Involvement of ethylene synthetic inhibitors in regulating the senescence of cut carnations through membrane integrity maintenance. Journal of Horticultural Research, 28(1): 39–48.
48. Abbasi, J., Hassanpour Asil, M. and. Olfati, J. A. 2020. Effects of melatonin and sodium nitroprusside (SNP) spray on salinity adjustment and improving the morpho-physiological traits of cut flower *Gerbera Jamesonii*. Journal of Science and Technology of Greenhouse Cultures, 11 (1): 29–44. (in Persian)
49. Sadeghi Faragheh, J., Nasibi, F. and Farahmand, H. 2017. Effect of nitric oxide on physiological and antioxidant responses and reduction of curvature of gerbera cuttings. Iranian Journal of Horticultural Science and Technology, 17 (2): 193–208. (in Persian)
50. Ghaei, S. 2018. Pre-harvest evaluation of nitric oxide on qualitative and quantitative characteristics and flowering life of *Gerbera jamesonii* 'Stanza'. Master of Science Thesis. Isfahan University of Technology, 56p. (in Persian)
51. Ghadakchiasl, A., Mozaffari, A.A. and Ghaderi, N. 2017 - Mitigation by sodium nitroprusside of the effects of salinity on the morpho-physiological and biochemical characteristics of *Rubus idaeus* under in vitro conditions. Physiology and Molecular Biology of Plants, 23(1): 73–83.
52. Mansouri, H. 2012. Salicylic acid and sodium nitroprusside improve postharvest life of Chrysanthemum. Scientia Horticulturae, 145: 29–33.
53. Kazemzadeh-Beneh, H., Samsampour, D. and. Zarbakhsh, S. 2018. Biochemical, physiological changes and antioxidant responses of cut gladiolus flower 'White Prosperity' induced by nitric oxide. Advances in Horticultural Science, 32(3): 421–431.
54. Mohasseli, V. and Sadeghi, S. 2017. Exogenously applied sodium nitroprusside improves physiological attributes and essential oil yield of two drought susceptible and resistant specie of Thymus under reduced irrigation. Industrial Crops and Products, 130: 130–136.
55. Naing, A.H., Lee, K., Kim, K.O., Ai, T.N. and Kim, C.K. 2017. Involement of sodium nitroprusside (SNP) in the mechanism that delays stem bending of different gerbera cultivars. Plant Science, 8 (2045): 1–10.
56. Hernández, J.A. and Almansa, M.S. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiologia Plantarum, 115(2): 251–257.
57. Naziri Moghaddam, N., Hashemabadi, D. and Kaviani, B. 2014. Improvement of vase life of cut rose, sunflower and lisianthus with sodium nitroprusside. European Journal of Experimental Biology, 4(3): 162–165.

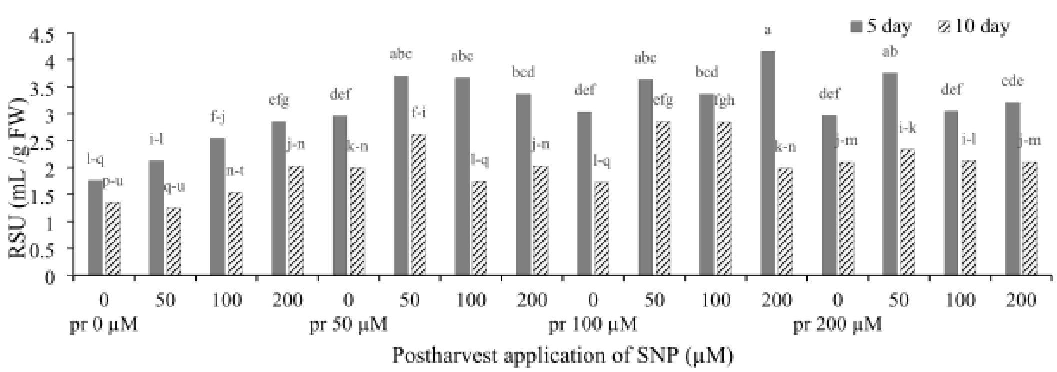
58. Lin, X., Li, H., Lin, S., Xu, M., Liu, J., Li, Y. and He, S. 2019. Improving the postharvest performance of cut spray 'Prince' carnations by vase treatments with nano-silver and sucrose. The Journal of Horticultural Science and Biotechnology, 94: 513–523.
59. Lau, S.E., Hamdan, M.F., Pua, T.L., Saidi, N.B. and Tan, B.C. 2021. Plant nitric oxide signaling under drought stress. Plants, 360(10): 1–29.
60. Fan, H.F., Du, C.X., Ding, L. and Xu, Y.L. 2014. Exogenous nitric oxide promotes waterlogging tolerance as related to the activities of antioxidant enzymes in cucumber seedlings. Russian Journal of Plant Physiology, 61(3): 366–373.
61. Howard, T. 2013. Senescence, ageing and death of the whole plant. New Phytologist, 197: 696–711.
62. Shapiro, A.D. 2005. Nitric oxide signaling in plants. Vitamins and Hormones,72: 393–398.
63. Chang, L., Liu, L., and Guo, X. 2011. The physiological responses of carnation cut flower to exogenous nitric oxide. Scientia Horticulturae, 127: 424–430.
64. Salachna, P. and Byczynska, A. 2017. Effect of nitric oxide and production location on vase life of cut Eucomis 'Sparkling Burgundy' flowers. World Scientific News, 83: 229–234.
65. Shi, J., Gao, L., Zuo, J., Wang, Q., Wang, Q. and Fan, L. 2016. Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. Postharvest Biology and Technology, 116: 98–104.

# Figures



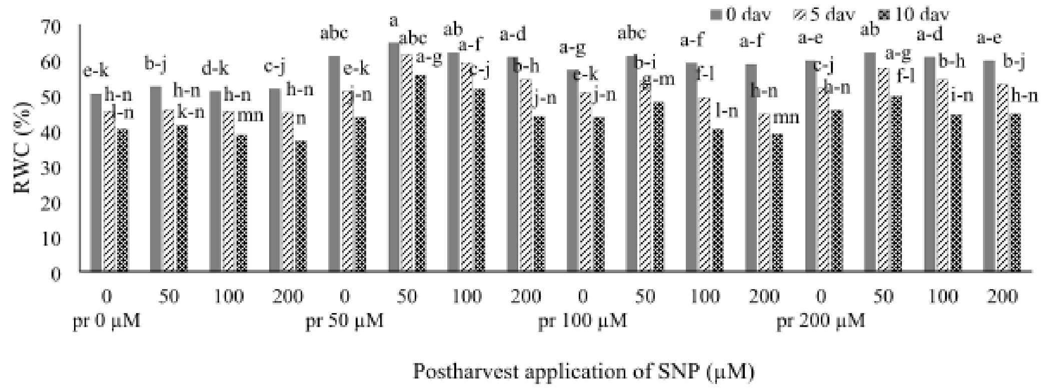

### Figure 1

Effect of pre- and post-harvest application with different concentrations of SNP over vase life period on relative fresh weight of Alstroemeria 'Orange Queen' (pr: preharvest application of SNP).



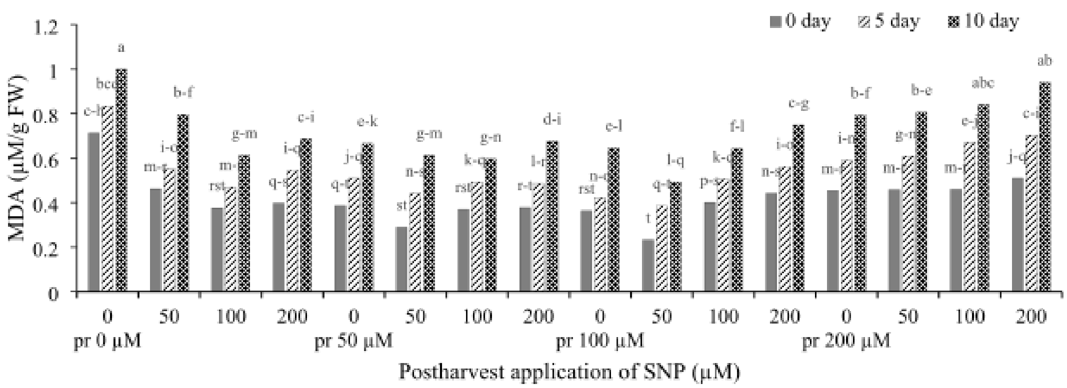

### Figure 2

Effect of pre- and post-harvest application with different concentrations of SNP over vase life period on relative solution uptake of Alstroemeria ‘Orange Queen’ (pr: preharvest application of SNP).



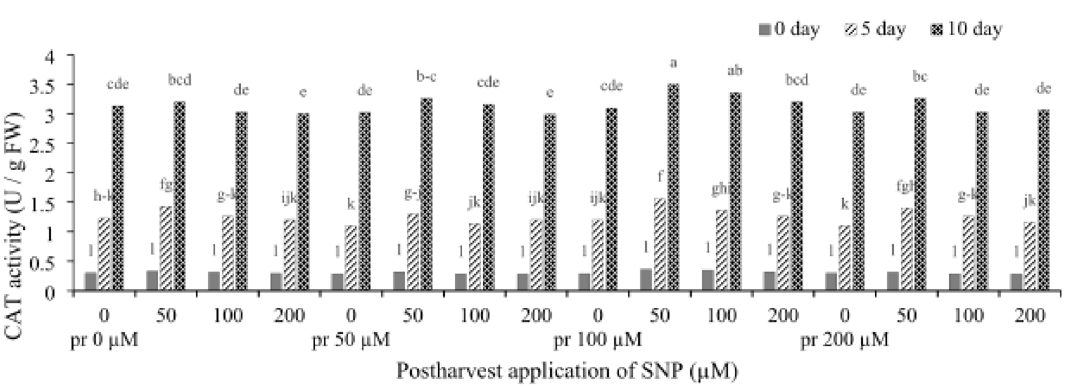
## Figure 3

Effect of pre- and post-harvest application with different concentrations of SNP over vase life period on relative water content of Alstroemeria ‘Orange Queen’ (pr: preharvest application of SNP).



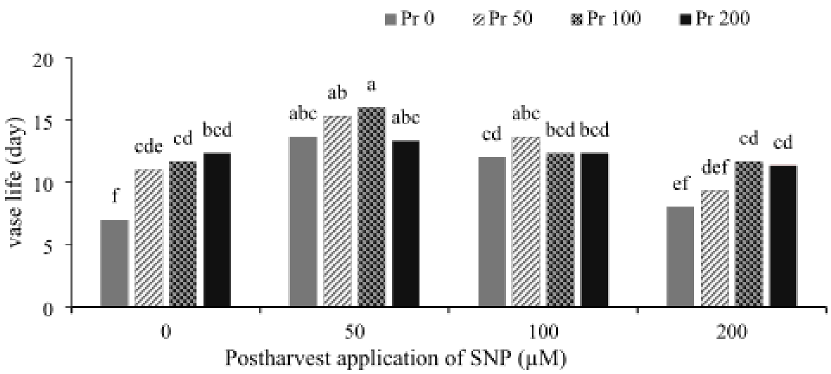
## Figure 4

Effect of pre- and post-harvest application with different concentrations of SNP over vase life period on malondialdehyde content of Alstroemeria “Orange Queen” (pr: preharvest application of SNP).



## Figure 5

Effect of pre- and post-harvest application with different concentrations of SNP over vase life period on CAT activity of Alstroemeria ‘Orange Queen’ (pr: preharvest application of SNP).




## Figure 6

Effect of pre- and post-harvest application with different concentrations of SNP on vase life of Alstroemeria ‘Orange Queen’ (pr: preharvest application of SNP (μM).