

SILICA NANOPARTICLES ENHANCES PHYSIO-BIOCHEMICAL CHARACTERS AND POSTHARVEST QUALITY OF *ROSA HYBRIDA* L. CUT FLOWERS

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ABSTRACT

The effect of silica nanoparticles (SiNPs) preservative solutions on the postharvest quality of rose cut flowers was investigated in this study. SiNPs were used at the concentrations of 0, 1, 2, and 3 mg·dm⁻³. Treatments of SiNPs increased flower longevity compared to untreated flowers. Relative fresh weight, relative water content, and water uptake were improved because of SiNPs treatments. The enhancement in chlorophyll content, total soluble sugars, as well as total phenol and flavonoid contents were positively correlated with SiNPs treatments. Malondialdehyde (MDA) content significantly increased by control roses relative to treated ones. Activities of peroxidase (POX) and polyphenol oxidase (PPO) enzymes significantly increased because of SiNPs treatments compared to the control. The treatment of 2 mg SiNPs·dm⁻³ maximized the longevity and improved the postharvest quality of cut roses. The results obtained suggested that SiNPs treatments could improve the longevity and postharvest quality of cut roses by reducing lipid peroxidation and motivating antioxidant machinery, therefore, retaining the membrane integrity.

Keywords: preservative solutions, silica nanoparticles, membrane stability index, phenol, flavonoid, MDA

INTRODUCTION

Prolonging the vase life of cut flowers and maintaining its quality are the main challenges faced by the florists in the flower trade worldwide (Yagi et al. 2014). Flower longevity is influenced by several factors including water stress. Rose (*Rosa hybrida* L.; family Rosaceae) is considered one of the most important cut flowers traded in the international market (CBI 2017). Early dehydration of cut roses plays an important role in reducing its commercial value (van Doorn 1997). Adequate water relation in harvested cut flowers is positively correlated with the balance between the rate of water uptake and water loss. Water loss occurs rapidly through leaves and stomata during postharvest handling (Halevy & Mayak 1979).

Nanoparticles (NPs) can easily cross/penetrate plant tissues and be transported to different organs faster than bulk materials (Nair et al. 2010) because its diameter is lesser than the leaf stomata and cell

wall pores. Silica nanoparticles (SiNPs) have highly reactive surface-to-volume ratio property (Wang & Naser 1994). It is considered more effective than other Si forms because of their higher density at reactive areas (El-Serafy & El-Sheshtawy 2017). In plants, silicon plays a key role in inducing the plant resistance against biotic and abiotic stresses (Liang et al. 2005). SiNPs increase turgor pressure and plant growth by improving water use efficiency, water translocation, and leaf relative water content (Wang & Naser 1994), in addition to reducing the transpiration rates (Ma & Takahashi 2002). Our previous study showed that SiNPs treatments significantly influenced the relative water content of hollyhock seedlings (El-Serafy & El-Sheshtawy 2017).

Silicon applications prolonged the vase life of carnation and gerbera cut flowers (Kazemi et al. 2012b, c). In case of lisianthus, silicon reduced chlorophyll degradation and preserved total chlorophyll content, which could be involved in the extension of cut flowers

vase life (Kazemi et al. 2012a). The longevity of carnation and chrysanthemum cut flowers was improved because of Si supplementations that enhanced chlorophyll content and total carbohydrates (El-Serafy 2015; Ali & Hassan 2016). Several reports have documented the response of cut roses to other NPs such as silver NPs (Hassan et al. 2014). However, the effect of SiNPs on cut rose flower longevity and postharvest quality is not well-investigated and this study has been conducted to bridge this gap. Therefore, the current study was carried out for evaluating the effects of SiNPs on the longevity and postharvest quality of cut rose 'Gold Star'.

MATERIALS AND METHODS

Plant material

The cut flowers of *R. hybrida* 'Gold Star' (yellow) were obtained from a commercial growers at bud opening stage (petals starting to reflex) and were brought directly to the Laboratory of Horticulture Department, Faculty of Agriculture, Tanta University in February 2017. Lower leaves were removed and the flower stems were washed with distilled water and trimmed to a length of 30 cm under water to avoid air embolism.

SiNPs treatments

Rose flowers were treated with four levels (0, 1, 2, and 3 mg·dm⁻³) of SiNPs preservative solution (15–45 nm, 99.5% purity, Nano-technology Laboratory, Faculty of Science, Tanta University, Tanta, Egypt). Cut flowers were kept in bottles containing 300 ml of solutions and 5 g·dm⁻³ sucrose w/v at 19 ± 2 °C, 63 ± 5% RH and 12-h photoperiod at light intensity of 10–12 μmol·m⁻²·s⁻¹ irradiance that was maintained using white and cool fluorescent lamps. The mouths of bottles were covered with plastic film to prevent contamination and minimize the evaporation (Liu et al. 2012). Four treatments were performed; each treatment consisted of eight replicates (one flower per replicate). The experiment was repeated twice.

Cut flowers longevity

Flowers were assessed daily for longevity determination, and the vase life was terminated when the flowers lost its ornamental appearance (wilting, losing the turgidity, or flower bent neck).

Relative fresh weight

Rose stems were weighed at the beginning of the experiment and weighed again daily. Relative fresh weight was measured according to He et al. (2006).

Relative fresh weight (%) = $(W_t/W_{t-0}) \times 100$; where W_t is the weight of flower stem (g) at $t =$ days 1, 2, 3, 4, ... and W_{t-0} is the weight of the same flower stem (g) at $t-0 =$ the first day of experiment.

Dry matter

Flower dry matter was determined at the end of control vase life after oven-dried at 60 °C for 48 h. Dry matter was expressed to the fresh weight and presented as a percentage.

Water uptake

Water uptake was counted as the amount of water taken by each flower from bottles during vase life evaluation (ml/stem).

Relative water content (RWC)

RWC was performed as reported by Weatherley (1950) using the following equation:

$RWC (\%) = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100$; where the turgid weight is the leaf weight after saturation with double distilled water for 24 h at 4 °C, and the dry weight is the sample weight after oven drying at 70 °C for 48 h. The samples were collected on 0, 1, 3, 5, and 7 days post-treatment (dpt).

Membrane stability index (MSI)

MSI was determined for the third leaf at stem base on 0, 1, 3, 5, and 7 dpt according to Sairam et al. (1997) using the following formula:

$MSI = [1 - (C_1/C_2)] \times 100$; where C_1 is the electric conductivity kept at 40 °C for 30 min and C_2 is the electric conductivity kept at 100 °C in boiling water bath for 15 min.

Total chlorophyll content

Leaf samples were used for chlorophyll determination using chlorophyll meter (SPAD-502, Minolta Co., Japan) and represented by SPAD value at 0, 1, 3, 5, and 7 dpt.

Total soluble sugars

The total soluble sugars in rose leaves at the end of control vase life was determined; samples were oven-dried at 60 °C till constant weight and used for determining total soluble sugars percentage according to Herbert et al. (1971).

Total phenol content assay

By the end of control vase life, total phenol content in dried leaves was determined spectrophotometrically using the Folin–Ciocalteu's reagent with gallic acid as the quantification standard according to McDonald et al. (2001) and presented as mg GAE·g⁻¹ DW.

Total flavonoid content assay

Briefly, total flavonoid content was determined in the dried leaves at the end of control vase life using aluminum chloride method by McDonald et al. (2001). Rutin acid was used as quantification standard and presented as $\text{mg RUT} \cdot \text{g}^{-1} \text{ DW}$.

Malondialdehyde determination

The content of malondialdehyde (MDA) was measured according to Peever and Higgins (1989) at the end of control vase life in fresh leaves sample. MDA concentration was determined using the formula: $\text{MDA content} = 6.45 \times (\text{A532} - \text{A600}) - 0.56 \times \text{A450}$; where A450, A532, and A600 are the absorbance at 450, 532, and 600 nm, respectively, and expressed as $\text{nmol} \cdot \text{g}^{-1} \text{ FW}$ (Bao et al. 2009).

Antioxidant enzyme assays

The activities of antioxidant enzymes were assessed at the end of control vase life. Total soluble enzyme activities were measured in the supernatant spectrophotometrically according to Bradford (1976). Peroxidase (POX) activity was directly assayed in the crude enzyme extract according to Hammerschmidt et al. (1982), whereas polyphenol oxidase (PPO) activity was determined as described by Malik and Singh (1980). For both POX and PPO, changes in absorbance (at 470 and 495 nm, respectively) were recorded for every 30-s intervals for 3 min. Enzyme activity was expressed as the increase in absorbance $\text{U} \cdot \text{mg}^{-1} \text{ protein}$.

Statistical analysis

This experiment was designed in completely randomized design with eight replicates and repeated twice. Data were normally distributed. Data were statistically analyzed according to the analysis of variance (ANOVA). Post hoc pair wise comparisons between the studied treatments were performed using Duncan's multiple (Waller & Duncan 1969), statistical significance was established at $p \leq 0.05$; and were presented as mean \pm SD.

RESULTS

Cut flowers longevity

SiNPs application significantly prolonged rose longevity comparing to control treatment. The longest vase life was observed when SiNPs were applied at $2 \text{ mg} \cdot \text{dm}^{-3}$. Vase life was increased by about 39.9%

over the control (Fig. 1A). Increasing SiNPs level for $2 \text{ mg} \cdot \text{dm}^{-3}$ reduced the longevity of cut roses but still the effects were positive compared to control plants.

Relative fresh weight

During the longevity evaluation, relative fresh weights at all SiNPs treatments were significantly higher than control roses (Fig. 1B). Moreover, the relative fresh weight of control flowers had a sharp reduction after three days of the experiment. On the other hand, SiNPs applications retained relative fresh weight at higher level compared to control flowers particularly by $2 \text{ mg SiNPs} \cdot \text{dm}^{-3}$ treatment.

Dry matter

Generally, all SiNPs applications significantly increased the dry matter values as compared to control flowers. The gradual increase was obtained with increasing SiNPs levels to reach the highest value at $3 \text{ mg SiNPs} \cdot \text{dm}^{-3}$. The lowest value was obtained by untreated cut flowers (Fig. 1C).

Water uptake

SiNPs treatments significantly increased the volume of water uptake by cut roses compared to untreated flowers. Application of SiNPs at a concentration of $2 \text{ mg} \cdot \text{dm}^{-3}$ resulted in the highest volume of water uptake (Fig. 1D) followed by $3 \text{ mg} \cdot \text{dm}^{-3}$. The results also indicate that the untreated flowers resulted in the lowest value in this respect.

Relative water content

The trend of the leaf relative water content of cut roses was similar to that of fresh weight changes, revealing dominating results because of SiNPs applications. SiNP-treated roses retained relative water content at a higher level than control roses during the process of flower senescence (Fig. 2A). The reduction in relative water content of control flowers was sharp after 3 days, whereas treatment with $2 \text{ mg SiNPs} \cdot \text{dm}^{-3}$ retarded this decline.

Membrane stability index

Membrane deterioration was significantly reduced under SiNPs applications as compared to control flowers that lost their membrane permeability rapidly during the vase life period (Fig. 2B). The treatment with $2 \text{ mg SiNPs} \cdot \text{dm}^{-3}$ significantly increased membrane stability index compared to control. Sharp decrease was observed in control roses at the fifth day by 31.28% with reference to $2 \text{ mg SiNPs} \cdot \text{dm}^{-3}$ treatment.

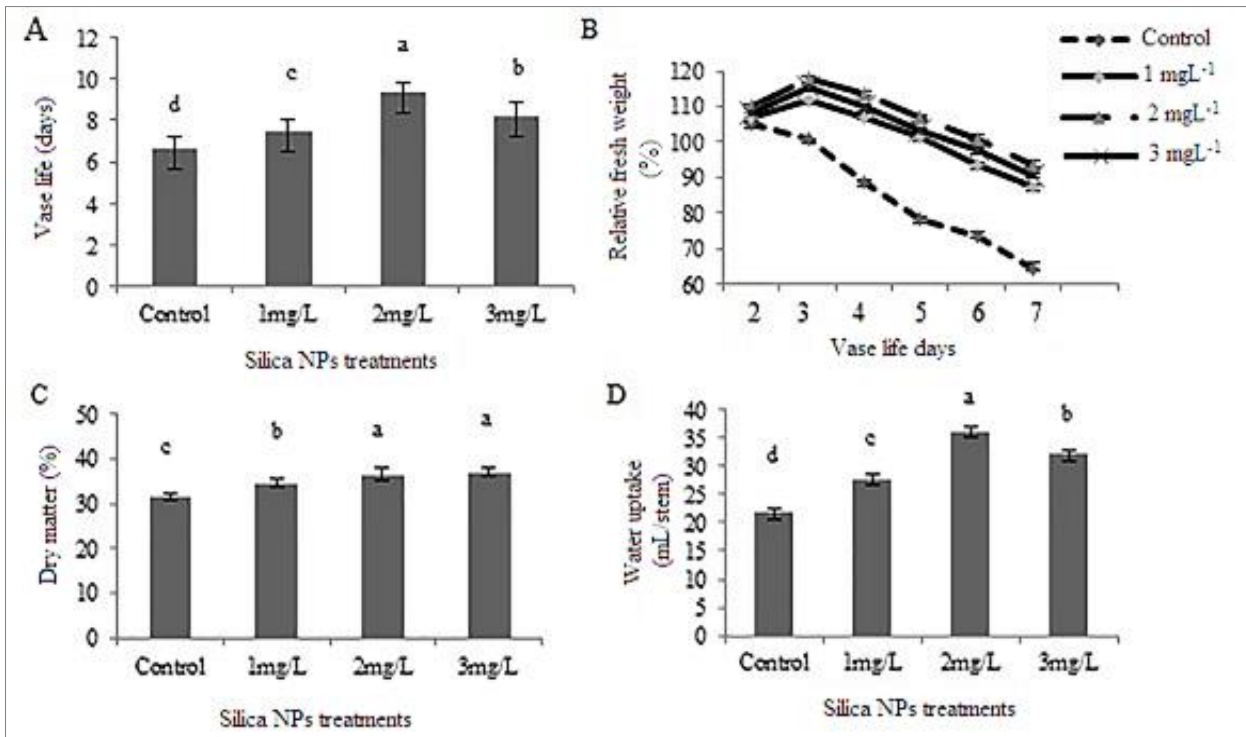


Fig. 1. Vase life (A), relative fresh weight (B), dry matter (C), and water uptake (D) of cut rose 'Gold Star' treated with SiNPs at the concentrations of 0, 1, 2, and 3 $\text{mg}\cdot\text{dm}^{-3}$. Values are mean \pm SD ($n = 16$). Bars with the same letters are significantly different from each other ($p \leq 0.05$).

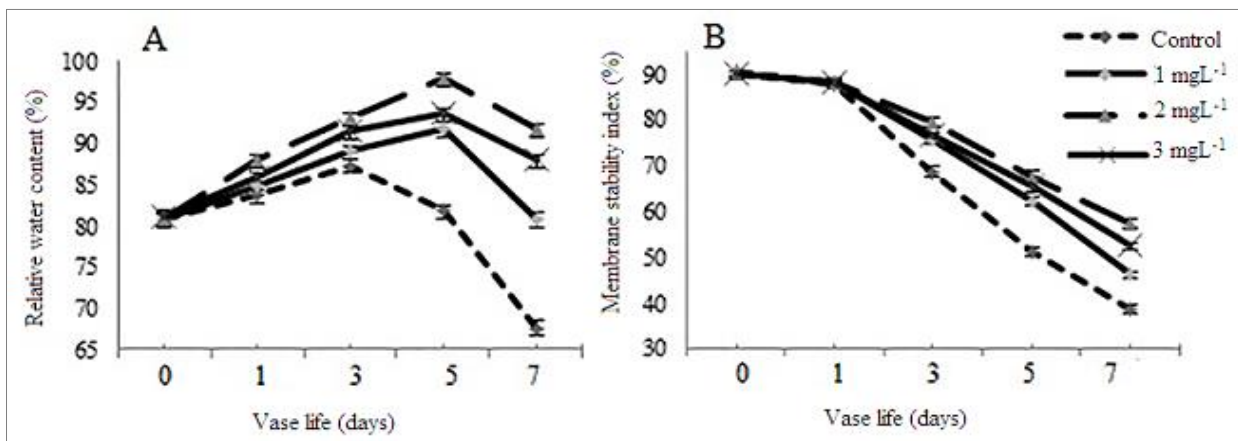


Fig. 2. Relative water content (A) and membrane stability index (B) of cut rose 'Gold Star' treated by SiNPs at the concentrations of 0, 1, 2, and 3 $\text{mg}\cdot\text{dm}^{-3}$ over vase life period. Values are mean \pm SD ($n = 16$).

Total chlorophyll content

Leaf chlorophyll content was slightly increased for 2 days and decreased thereafter. However, the decrease in chlorophyll content in control roses was more critical, whereas all SiNPs treatments retarded

the chlorophyll reduction with the highest positive effect observed at a concentration of 2 $\text{mg}\cdot\text{dm}^{-3}$ (Fig. 3A). On the seventh day, the chlorophyll content in control roses was lower by 25.43% than that in those treated with 2 mg SiNPs $\cdot\text{dm}^{-3}$.

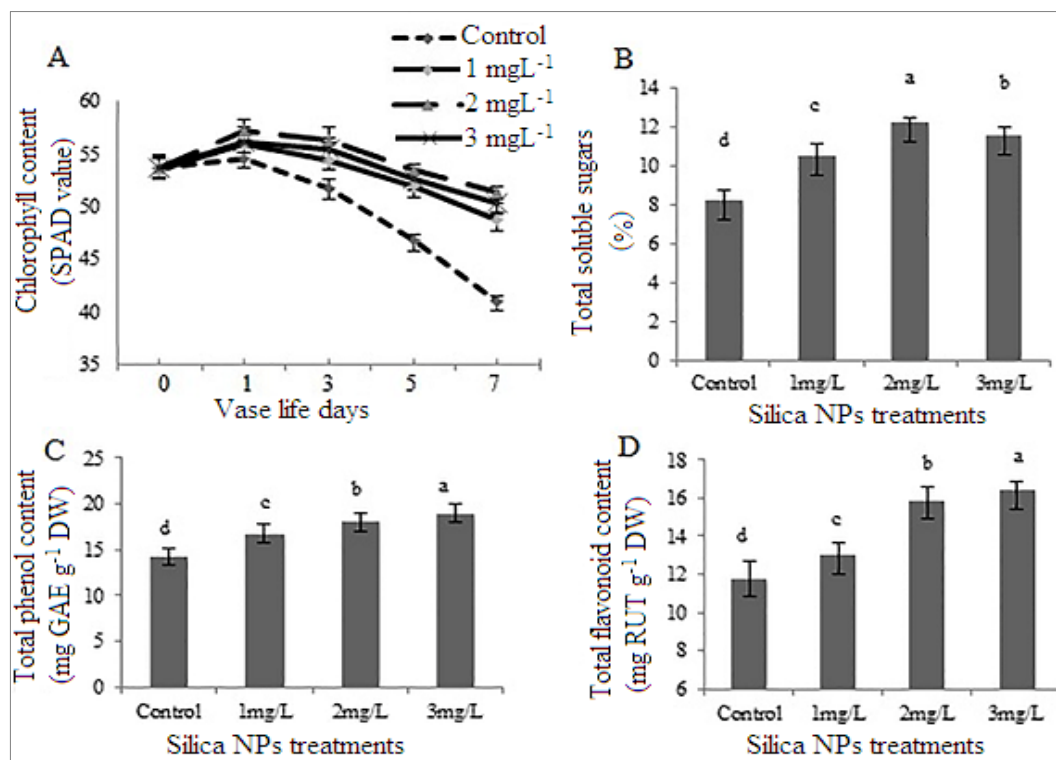


Fig. 3. Chlorophyll content (A), total soluble sugars (B), total phenol content (C), and total flavonoid content (D) of cut rose 'Gold Star' treated with SiNPs at the concentrations of 0, 1, 2, and 3 $\text{mg}\cdot\text{dm}^{-3}$. Values are mean \pm SD ($n = 16$). Bars with the same letters are not significantly different from each other ($p \leq 0.05$).

Total soluble sugars

Total soluble sugars content in SiNPs treatments as compared to control flowers is presented in Figure 3B. A significant and gradual increase in total soluble sugars content was observed with SiNPs application in comparison with control roses to reach the highest value by 2 $\text{mg SiNPs}\cdot\text{dm}^{-3}$. Increasing the level to 3 $\text{mg SiNPs}\cdot\text{dm}^{-3}$ reduced the total soluble sugars. The lowest value of total soluble sugars was observed for control roses.

Total phenol content

The effect of SiNPs treatments on the total phenol content of cut roses was determined and presented in Figure 3C. SiNPs significantly increased the accumulation of phenolic compounds in leaf epidermis compared to untreated flowers. Phenols accumulation gradually increased with increasing SiNPs level to reach the highest value with cut roses treated with 3 $\text{mg SiNPs}\cdot\text{dm}^{-3}$.

Total flavonoid content

Total flavonoid content had the same trend of total phenol as it was impacted positively by SiNPs applications. Flavonoid content was significantly increased

using SiNPs comparing to untreated roses (Fig. 3D). A gradual increase was observed with increasing SiNPs level to reach the highest value in cut roses treated for SiNPs at a concentration of 3 $\text{mg}\cdot\text{dm}^{-3}$. Instead, the lowest flavonoid content was recorded in control flowers.

Malondialdehyde determination

Generally, all levels of SiNPs significantly inhibited MDA accumulation as compared to the control (Fig. 4A). The lowest MDA content was obtained at treatment with 2 $\text{mg SiNPs}\cdot\text{dm}^{-3}$, whereas increasing the SiNPs level to 3 $\text{mg}\cdot\text{dm}^{-3}$ resulted in increased MDA value.

Antioxidant enzyme assays

Antioxidant enzyme activities positively responded to SiNPs applications. Both POX and PPO enzymatic activities significantly increased because of any level of SiNPs relative to control roses (Fig. 4B & 4C). The highest antioxidant enzymatic activities were recorded when the flowers were treated with 2 $\text{mg SiNPs}\cdot\text{dm}^{-3}$, whereas increasing the level of SiNPs to 3 $\text{mg}\cdot\text{dm}^{-3}$ reduced the activities of both the antioxidant enzymes PPO and POX in the leaves, however, to a lesser extent than in control.

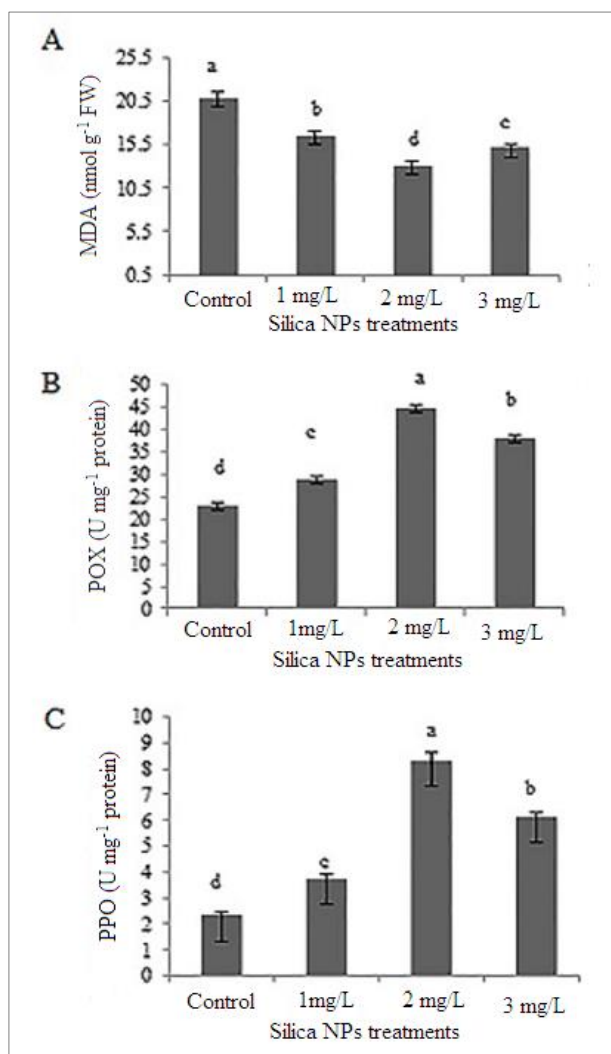


Fig. 4. MDA (A) and antioxidant enzyme activities, POX (B) and PPO (C), of cut rose 'Gold Star' treated with SiNPs at the concentrations of 0, 1, 2, and 3 mg·dm⁻³ at the end of control vase life. Values are mean ± SD (n = 16). Bars had same letters are not significantly different from each other (p ≤ 0.05).

DISCUSSION

Our finding of this study suggests that SiNPs applications could improve the longevity and post-harvest quality of cut roses, particularly when flowers were treated with SiNPs at a concentration of 2 mg·dm⁻³. SiNPs application enhanced the relative fresh weight of cut flowers at any time of the vase life period. Our results were in agreement with previous studies of Jamali and Rahemi (2011) and Kazemi et al. (2012b, 2012c) who found that using silicon in the

vase water solution increased the longevity as well as decreased the fresh weight changes of carnation and gerbera cut flowers.

In addition, SiNPs application significantly increased dry matter of cut roses relative to untreated ones. Si stimulates photosynthesis and strengthen the tissue of several plants and finally increased dry matter (Ma & Takahashi 2002; El-Serafy 2015). We observed the enrichment of water uptake by SiNPs in the treated flowers resulted in good flower turgidity and ornamental appearance compared to control flowers. These results could be ascribed to the role of Si in reducing the transpiration rate and water loss and then maintaining the adequate water relations, which reflected on plant turgidity or postharvest quality (Galati et al. 2015).

Si is transported to shoots and leaves by evapotranspiration and polymerized to polysilicic in plant tissues to form a double layer of thickened silicon cellulose membrane; these layers protect plants from water loss and strength the plants mechanically (Hodson & Sangster 1988). Si deposition might occur in epidermal, strengthening, storage and vascular tissues as a silica bodies (phytoliths), which occupy the spaces between apoplast of the cortex and the cell wall. Si increased the stem diameter because of an enhancement in both cuticle thickness and vascular bundle diameter (El-Serafy 2015). Plants with firmer xylem vessel walls are more resistance to problems in these structures during water stress, besides increasing water volume taken up by plants (Sperry et al. 2002). Si associated with organic compounds formed complexes in the cell walls of epidermal cells, consequently increasing their resistance to degrading enzymes (Snyder et al. 2006).

Our results showed that SiNPs treatments reduced the deterioration of membrane stability index and reduced the degradation of total chlorophyll or carbohydrate contents during vase life period compared to control rose. Similar results were reported by Al-aghabary et al. (2005) and El-Serafy and El-Sheshtawy (2017) who revealed that Si maintained membrane stability index because it reduced MDA. Si decreased chlorophyll degradation (Kazemi et al. 2012a, 2012b) and increased net photosynthetic rate as well as net assimilation (Ahmed et al. 2014). Phenols and flavonoids are major defensive compounds

that protect plants against various stressors (Shetty et al. 2011) as phenols have an important role in absorbing and neutralizing free radicals and decomposing peroxides (Shah et al. 2010). In this study, SiNPs treatments significantly increased the accumulation of phenolic and flavonoid compounds in leaf epidermis compared to untreated flowers. The mechanism of SiNPs-induced phenols may be due to the insoluble SiNPs, which is accumulated in epidermis, that induce the enrichment of constitutional phenols in epidermal cells by their super high adsorption surface (Li et al. 2004), where 1-g SiO₂ particles with a diameter of 20 nm possess adsorption surface of 400 m² (Wang & Naser 1994).

The used treatments inhibited MDA accumulation and, hence, retained the membrane permeability that reflected in improving the postharvest quality/ornamental appearance of cut rose. SiNPs treatments increased the activities of POX and PPO in response to water stress after harvesting, these enzymes increased the defense capacity against oxidative damage (Pandey et al. 2010). Si influences on antioxidative enzymes activity involved in plant defense reactions to biotic and abiotic stresses (Liang et al. 2005; Shi et al. 2005). SiNPs applied at a concentration of 2 mg·dm⁻³ was the most effective concentration in maximizing the longevity and improving the postharvest quality of cut roses.

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