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Calcium Nanoparticles Intermixed with Salicylic Acid Affect Enzymes Activities and Postharvest Attributes of Cut Rose (*Rosa hybrida* cv. Black Magic)

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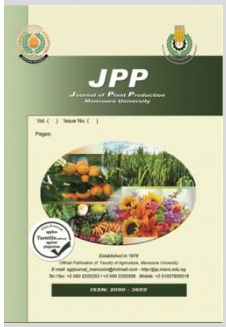

ABSTRACT

The vase life of a flower is determined by a variety of factors, including the type of flower, the environment in which it is kept, and the care it receives. The environment in which a flower is kept can also affect its vase life. Two separated experiments were established for examining the efficacy of calcium nanoparticles (CaNPs) intermixed with or without salicylic acid (SA) at different treatments (Control, CaNPs 2 mM, CaNPs-SA 1 mM, and CaNPs-SA 2 mM), as holding solutions on the vase life and postharvest attributes of *Rosa hybrida* cv. Black Magic cut flowers. The investigation mentioned the influences of CaNPs-SA on some physiological characteristics in roses, including (vase life, flower diameter, relative fresh weight%, flower stem bending index, and water relations). In addition, total phenol content, membrane stability index (MSI), catalase (CAT) antioxidant activity, and cell wall degrading enzymes (CWDAs) such as xylanase (XYL), polygalacturonase (PG), pectinase (PT), and cellulase (CEL) were determined during 10 days of vase life. As well as the generation rates of H_2O_2, malondialdehyde (MDA), and reduction of DPPH. CaNPs-SA at 2 mM prolonged the vase life and maintained higher relative fresh weight % and water relations. In addition, this superior treatment suppressed the CWDAs, and MDA by promoting the DPPH, and CAT, which increased the MSI of rose-cut flowers. Our study recommends the usage of calcium nanoparticles intermixed with salicylic acid as holding solutions on the vase life and postharvest attributes of *Rosa hybrida.*

Keywords: Vase life; rose; bent neck; cell wall degrading enzyme; calcium nanoparticles, salicylic acid

INTRODUCTION

Rosa hybrida L. cv. "Black Magic" is classified as a member of the Rosaceae family. It is topping up the cut flower international exhibitions (Butt, 2005). Although it is one of the most prominent decorative and cut flower plants in the global flower trade, it has a very short vase life, as shown by the petals falling off and the stems of the flowers bending. Additionally, it is believed that the demand for cut flowers is the highest and most exceptional on the global market (van Doorn, 1997). In Egypt, the climate and environmental conditions make it the most convenient country in the world to produce cut rose flowers. However, it faces fierce competition in the global market, especially from the Mediterranean and Western European nations (El-Nabarawy *et al*., 2018). Also, throughout their short vase life (Kazaz *et al*., 2019), rose flowers experience numerous postharvest issues (Ichimura and Shimizu-yumoto, 2007). The vase life of cut flowers prolongation is determined by the lateness of neck bending, hence the flower's welting. After stem flowers cutting, the growth of bacteria results in vascular system blockages that prevent water from reaching the flowers and speed up their wilting (van Meeteren *et al*., 2001).

Weakness of mechanistic support (such as a thin sclerenchyma cylinder or low lignin levels) in the stem may also contribute to bending. So, the cut flower vase life is constrained by mechanical constraints and xylem vascular blockage (Perik *et al*., 2014). To delay the senescence of cut flowers, it has been increasingly fashionable in recent years to apply harmless compounds like Ca. The middle lamella and cell wall structures of plants require calcium ions (Ca^{+2}) to maintain their structural rigidity and stiffness. It also slows down or prevents cell wall deterioration, therefore having access to it is crucial for the durability of cell membranes. The increase of Ca^{+2} could lengthen the shelf life. For instance, a calcium chloride-containing vase solution made gerbera plants take longer to bend (Perik *et al*., 2014). More antioxidant enzyme activity was also preserved, and senescence was postponed in cut gladiolus that had been treated with the Ca treatment (Sairam *et al*., 2011). A decrease in ethylene has a similar effect on prolonging vase life. More pectin in the cellular walls slows down ethylene generation and encourages water to pass through the stems (Aghdam *et al*., 2019).

Due to the special characteristics of nanoparticles (NPs), nanotechnology presents a promising alternative to conventional plant protection methods. These advantages include increased efficacy, decreased input, and lower ecotoxicity (Muraisi *et al*., 2022). Using nanoparticles (NPs) as a method to extend the vase life of cut rose flowers is one of many current strategies. They are more reactive due to their increased surface area and density of reactive areas (Ranjbar *et al*., 2018). Nanoparticles gather in diverse

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tissues due to their smaller diameter, which allows them to pass through cell wall holes and stomatal apertures in leaves (Nair *et al*., 2010). Many researchers have looked on how well NPs work to prolong the shelf life of cut flowers like gerberas, acacias, and roses (Lü *et al*., 2010; El-Shawa *et al*., 2019; El-Shawa *et al*., 2022).

As a non-enzymatic antioxidant and plant growth regulator, salicylic acid (SA) is a phenolic molecule that contributes to the coordination of physiological processes in plants (Arfan *et al*., 2007). It plays evident roles in ion absorption and transfer, transpiration, stomatal conductance, and photosynthetic rate (Hayat *et al*., 2010). By enhancing antioxidant enzyme activity and reducing fresh weight losses, SA prolonged vase life of cut gladiolus spikes, which lead to retardation of senescence (Hassan and Ali, 2014). Additionally, SA reduced gerbera flower wilting, anthocyanin loss, and stem bending (Singh *et al*., 2018). Applying SA had extended shelf life and slowed down wilting in cut roses by decreasing the production of reactive oxygen species (ROS), maintaining higher water balance, and antioxidant enzyme activity (Alaey *et al*., 2011). Furthermore, the research aims to investigate the efficiency of using calcium nanoparticles intermixed with or without salicylic acid to improve cut rose flowers' physiological and biochemical postharvest characteristics.

MATERIALS AND METHODS

Cut flower materials

Cut rose flowers (*Rosa hybrida* L. cv. Black Magic) were obtained from a greenhouse at EL-Kanater El-Khaireia city, Governorate of Qaluobiya, Egypt, at the bud break stage. To reduce water loss, all cut flowers of 50 ±2 cm in height were wrapped in plastic sheets, cooled for three hours at 15°C, and then transported. Upon arrival at the lab, flower stems were recut five centimeters to reach 45 cm in length. The cutting technique was done beneath a surface of distilled water, to prevent an air embolism. The experiment was carried out in a room with ambient air temperature (24± 2ºC), humidity (60±5 RH%), and light levels below cool white fluorescent tubes (12 µmol m^{-2} S^{-1} light intensity) at the Laboratory of Vegetables and Floriculture Department, Faculty of Agriculture, Mansoura University, Egypt, on the 5th and 8th January during both seasons of 2021 and 2022, respectively (Figure 1).

Figure 1. *Rosa hybrida L.* cv. Black Magic the examined variety in the experimental laboratory.

Experimental arrangement

Rose-cut flowers were processed into four treatments simultaneously at the lab. The holding solution treatments were as follows: T_1: control, T_2: CaNPs 2 mM, T_3: CaNPs-SA 1 mM, and T_4: CaNPs-SA 2 mM. Flowers were occupied in CaNPs-SA treatments during their shelf life as holding solutions at Lab temperature. A total of 80 cut flowers were divided into two groups. Both were distributed into the different holding solutions (four treatments) and placed in a glass slender (100 ml). Since the first one contains 40 flowers, each treatment contains 10 flowers for vase life physiological measurements. The second group was characterized by chemical and enzyme activities analysis. In addition, all holding solution treatments were fortified with 20 g L^{-1} sugar and 200 mg L^{-1} 8-hydroxyquinoline sulfate. Flowers were occupied in different holding treatments during their shelf life at Lab temperature (24 ± 2 °C and relative humidity 60 ± 5), with refreshing all the holding solution treatments every 2 days. Furthermore, the obtained data of this investigation is the mean of two separated experiments.

Formation of CaNPs intermixed with SA

The preparation of calcium nanoparticles (CaNPs) according to (Yugandhar and Savithramma, 2013) with little modification. Since it was created by mixing SA at rates of 0, 1, and 2 mM together beside the control. All SA fractions were introduced with distilled water and a $CaCl_2$ solution at 50 mM. The mixture was maintained at ambient temperature for 3 days after first being kept on the checker for an hour at 5000 rpm.

NPs property through UV–vis spectroscopy

By perceiving the UV-Vis spectra of the mixture at several wavelengths, the reduction of the purified Ca^{++} and preside the ensuing CaNPs were detected using ATI Unicom UV-Vis spectroscopic analysis vision programming. The combined CaNPs' UV-vis spectra were reported to be in the 240-450 nm range (Figure 2A).

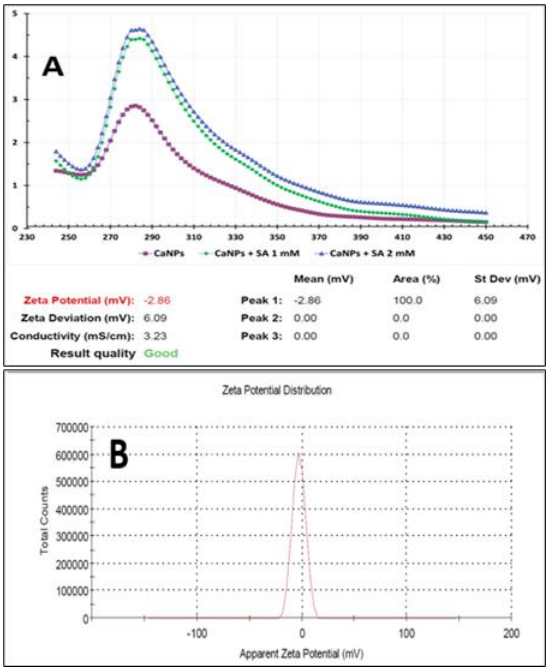

Figure 2. UV-visible absorption spectra of CaNPs intermixed with SA (SA 0, 1, and 2 mM) (A), and zeta potential distribution (B).

CaNPs property through zeta potential

The CaNPs surface situation was described through zeta potential analysis and forecast the stability of the final NPs solution. Through using Malvern Instruments Ltd Zeta Potential Ver. 2.3 at the Central Lab of Electron Microscope

of Mansoura University, Egypt, the method was applied to define the CaNPs intermixed with SA surface charge. A thin layer of ions with an opposing charge is attracted to the surface of CaNPs-SA due to its surface charge. As CaNPs diffuse through the solution, two layers of ions move. The particles' electric potential, or Zeta potential, is measured at the end of the double layer and ranges from +100 mV to 100 mV (Figure 2B). After integration, the CaNPs containing SA had a zeta potential of -2.86 mV (more stability).

Increased levels of stability are frequently observed in CaNPs with Zeta potential values more than +25 mV or less than -25 mV (Honary and Zahir, 2013).

CaNPs characteristics by transmission electron microscopy technique (TEM)

By using transmission electron microscopy (JEOL TEM-2100) connected to a CCD camera, the size, form surface area, molecule's structure, and morphological data of the generated CaNPs were determined. All components of the intermixed CaNPs were set up by holding in copper-coated carbon nets and enabling the dissolvable to flow constantly before taking into consideration the TEM images (Figure 3). TEM photos were conducted in the Central Lab., Faculty of Agric., Mansoura Univ., Egypt.

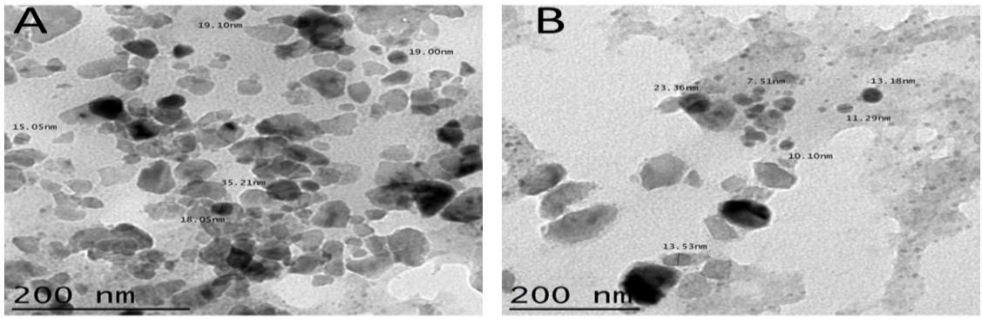

Figure 3. TEM images of CaNPs (A) and CaNPs intermixed with SA (B), show that particles size ranging from 15.05 to 35.21 nm, and 10.10 to 23.36 nm, respectively. Most of NPs were spherical with a few tetragonal particles mixed in .

Measurements

The physiological characters of cut flowers were estimated every two days starting from the 2nd to 10th days of vase life, while the biochemical attributes and enzymes activity were estimated from the initial day (0) with 2 days interval until the 10th day of vase life.

Vase Life, flower diameter, relative fresh weight (%) and Flower stem bending index (SB-index)

The vase life of flowers was calculated every day at 24 ± 2ºC, 60 ± 5% RH, and 12h photoperiod of 20-22 µmol m^{-2} s^{-1}. The vase life was determined as the duration from the start of the treatment until the semblance of a bent neck or the withering of 50% of the petals (Hassan *et al*., 2020). Flower diameter (cm) was determined each 2 days through the vase life by means of two orthogonal diameter measurements. Applying the following formula RFW(%) = $(W_t/W_0)\times100$, the relative fresh weight (RFW) of rose cut flowers was calculated (Lü *et al*., 2010) since W_t is the weight of the stem (g) at t=day 2, 4, 6, etc. and W_0 is the weight of the same stem (g) at t=day 0. On a scale from 0 to 3, the flower stem bending of 10 rose-cut flowers from each treatment was evaluated as follows: 0 = normal flower, 1 = slightly bent, 2 = moderate, and 3 = severely bent neck (Izumi *et al*., 2000).

Water relations (water uptake, total water uptake, water loss, and water balance)

Water uptake (WU) was measured since the weight of vases with and without cut flowers was recorded every 2 days. WU (g $flower^{-1}$ 2 $days^{-1}$) was computed following the next formula, $WU=((S_{t-1})-S_t / FW_{d1})$, where S_t is the weight of the vase solution (g) at t= day 2, 4, 6, etc.; S_{t-1}: solution weight (g) in the previous day, and FW_{d1}: cut flower fresh weight in the first day (the initial day). Total water uptake (TWU) was calculated by collecting all amounts of water uptake for each flower during the vase life (ml $flower^{-1}$ vase $life^{-1}$). The water loss (g $flower^{-1}$ 2 $days^{-1}$) was determined by applying this formula $WL = (WU_d – (\pm CFW_d)/ FW_{d1})$, Whereas WL= Water loss, WU= Water uptake, CFW_d = Change in fresh weight of cut flower at day 2, 4, 6, etc. and FW_{d1} = Fresh weight of cut flower in the first day (the initial weight) according to (Ghale-shahi *et al*., 2015). In addition, water balance (g $flower^{-1}$ 2 $days^{-1}$) was computed as follows ($WB = CWU_d – CWL_d$), whereas WB= Water balance, CWU_d= Change in water uptake, and CWL= Change in water loss.

Total phenol content (TPC)

The method developed by (McDonald *et al*., 2001) was used to calculate total phenol content. An extract was made by stirring a 0.5 g sample of petals coming from the second outside circumference in 50 ml of methanol for two days at 4 ^{0}C. After that, the extract was mixed with Folin-Ciocalteu reagent (5 ml, 1:10) and 4 ml of 1 M aqueous sodium carbonate before being diluted (0.5 ml of 0.1 kg L^{-1}). A spectrophotometer (UK, ST15 0SA Model 7205) was then used to measure the total phenol content at 765 nm, and g GAE kg^{-1} DW was used to represent values.

Membrane Stability Index (MSI)

It was done as stated by (Sairam *et al*., 1997) using two samples of petals (0.2 g) obtained from the second outside circumference of petals in two distinct vials (100 ml each) contained 20 ml double distilled water. The first vial was preserved for 30 minutes at 40 °C, while the second one was kept for 15 minutes in a hot water bath at 100 °C. A conductivity meter was used to measure the conductivity of C_1 and C_2 samples, and ion leakage was used to calculate MSI as follows: $MSI = [1 - (C_1/C_2)] \times 100$.

Hydrogen Peroxide (H_2O_2) Evaluation

In petals samples from the second outer row, the generation of H_2O_2 was also examined (Patterson *et al*., 1984). After mixing the petals sample (0.5 g) with 6 ml of

coldish acetone (100%), the blend was centrifuged (12,000 rpm) for 10 minutes at 4 °C. The extract was centrifuged at 3000 rpm for 10 min after being combined with 0.2 ml NH_4OH and 0.1 ml $Ti(SO4)_2$ (5%). After the pellets had been dissolved in 4 ml (2 M) H_2SO_4, the titanium-peroxide complex's absorbance was measured at 412 nm. The H_2O_2 concentration was shown in mmol.kg^{-1} FW and the absorbance was corrected by a reference curve based on known H_2O_2 values.

Enzyme's activities (PG, XYL, CEL, PT, and CAT)

A piece of rose flower neck (1 g) was crushed and homogenized using 20 mM Tris-HCl buffer, pH 7. After that, the blend was centrifuged at 16,000 rpm for 6 minutes at 4°C with cooling. For testing cellulase (CEL), polygalacturonase (PG), xylanase (XLN), and pectinase (PT), the supernatant was preserved at 20°C. The mixture was left at 37 °C for just an hour while one milliliter of polygalacturonic acid and enough enzyme extraction was added. Then, 500 µl of the dinitro-salicylic acid reagent was mixed and heated for 10 minutes in a water bath. The mixture was abruptly chilled until it reached lab temperature. On a spectrophotometer, the enzymes were measured at 540 nm for the CEL and 560 nm for PG and XLN substrate (Miller, 1959).

According to (Collmer *et al*., 1988), pectinase (PT, EC: 3.2.1.15) activity was estimated, following the extraction method of (Payasi and Sanwal, 2003). A blend of 500 µl of polygalacturonic acid (0.36% w/v), 200 µl of 4 mM $CaCl_2$, and 500 µl of distilled water was utilized for testing the enzyme's performance. The responsive blend was heated to 36 °C for three hours. Identification of enzyme was achieved at 232 nm absorbance. It was decided to use the approach of (Bradford, 1976) to reduce the chemical concentrate's solvent protein concentration. The enzyme's specific activity was typical of in-unit mg^{-1} protein.

Catalase (CAT) activity was estimated using the (Chandlee and Scandalios, 1984) technique [EC 1.11.1.6]. In 5 ml of 50 mM Na_3PO_4 buffer (pH 7.5) including 1 mM phenylmethylsulfonyl fluoride ($C_7H_7FO_2S$), a 0.5 g petals sample was submersed. After that, the extract was centrifuged at 4 ^{0}C for 20 minutes at 12,000 rpm, and the enzyme was measured in the supernatant that was produced. H_2O_2 (0.4 ml, 15 mM) and KH_2PO_4 buffer (2.6 ml, 50 mM, pH 7.0) were combined with the enzyme extract (0.04 ml). The CAT activity (U.mg^{-1} protein), where 1 U represents the decline of 1 mM H_2O_2.min^{-1}.mg^{-1} protein, was used to measure the decomposition of H_2O_2.

Malondialdehyde (MDA) and Radical Scavenging Activity (DPPH)

Lipid peroxidation was evaluated using the MDA content (Hodges *et al*., 1999). A petals sample weighing 0.2 grams from the second outer row was homogenized by centrifugation at 14,000 rpm for 15 minutes in 2 ml $C_2HCl_3O_2$ (0.1%). A sample aliquot (2 ml) was combined with 3 ml $C_2HCl_3O_2$ (5%) and $C_4H_4N_2O_2S$ (0.5%), and the mixture was set aside for 30 minutes. The blend was then centrifuged for 15 minutes at 5000 rpm while being chilled in ice. The following equation was used to determine the MDA content (mM kg^{-1} FW); MDA content = $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$, where A is the optical density of the supernatant at 450, 532, and 600 nm. The approach taken by (Brand-Williams *et al*., 1995) was applied to determine the free radical scavenging activity (DPPH). Weighing out a 0.2 g sample of petals from the second outer row, 200 ml of CH_3OH was then added and to acerate, it was shaken for 24 hours at room temperature. The sample was then filtered (Whatman No. 1), and evaporation at room temperature in a fume hood was used to get rid of the methanol. The subsequent concentrate was saved for later examination. This assay was performed with $C_{18}H_{13}N_5O_6$ (DPPH) reagent. The extract was thoroughly stirred into a 1.5 ml methanolic solution of DPPH (20 g ml^{-1}) before being added. At 517 nm, the decolorizing processes were evaluated and compared to the blank thirty minutes after the reaction and the DPPH activity was calculated as an inhibition percentage (I%): $I\ (\%) = 100 \times (A_{blank} - A_{sample})/A_{blank}$, in which the sample and blank absorbances after 30 minutes of the reaction are represented by A_{sample} and A_{blank}, respectively. The activity of the antiradical is expressed as mmol.kg^{-1} FW was given for the extracted sample with 50% inhibition.

Statistical Analysis

Data of this investigation represent the mean of two separate experiments which were pooled. Costat v. 6.303 program was used for performing the analysis of variance (One-way ANOVA) in a Complete Randomized Design (CRD). Comparison between means were achieved by using Tukey's HSD test at probability of 5% according to (Snedecor and Cochran, 1990). Data had been shown in means ± SE.

RESULTS AND DISCUSSION

Results

Vase Life, flower diameter, relative fresh weight(%) and Flower stem bending index (SB-index)

Vase life

The vase life of *Rosa hybrida* cv Black Magic was significantly prolonged because of applying all calcium nanoparticles (CaNPs) intermixed with or without salicylic acid (SA) comparing with the control (Figure 4A). The highest vase life the roses (15.75 and 14.25 d) was obtained from CaNPs-SA at 2 and 1 mM holding solutions, respectively with insignificant differences between them. Relative to the control rose cut flower, held in solutions fortified with CaNPs intermixed with SA at 2 or 1 mM increased the vase life by 53.66 and 39.02%, respectively.

Flower diameter (cm)

Gradual increase for all holding solution treatments including the control was observed in the flower diameter until the 10th day from the vase life (Figure 4B). It was quite clear that the control rapidly with a positive sharp curve increased the flower diameter compared with the rest treatments, especially from the beginning of the 8th day to the 10th day. This indicates the speed at which the flowers open and enters the stages of deterioration faster than the rest of the treatments. CaNPs at 2 mM without SA came in second place after the control in the flower diameter until the 8th day. It is worth noting that the CaNPs-SA at 2 mM gave a slow increase in flower diameter through the vase life interval, compared to most treatments except for the CaNPs-SA at 1 mM, thus, there was an increase in the vase life periods of these treatments.

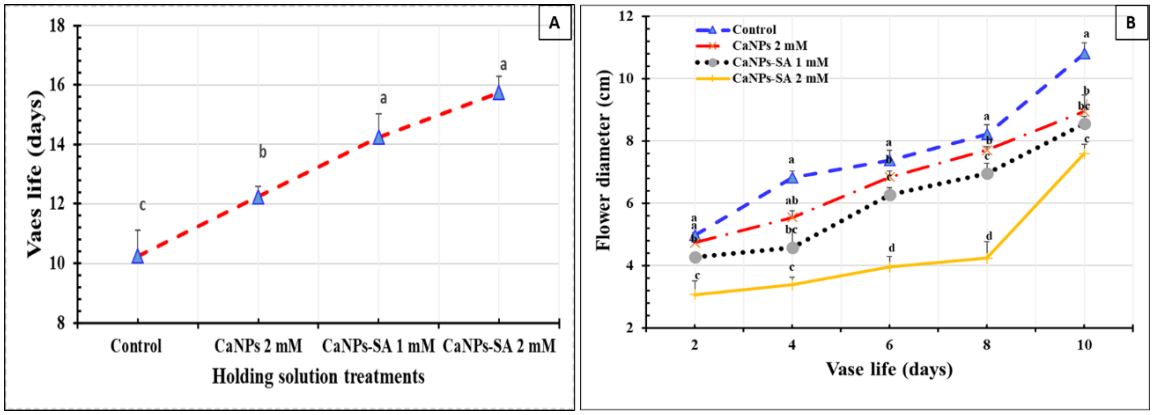

Figure 4. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on vase life (A) and flower diameter (cm) (B) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Relative fresh weight (%)

As it is clear from Figure 5A, the control treatment had a sharp decline curve occurred starting from the 2nd day until the 10th day compared with all other treatments.

Meanwhile, the holding solution supplemented with CaNPs-SA at 2 mM maintained significantly higher and stable relative fresh weights percentages of cut rose flowers during the vase life.

Flower stem bending (SB-index)

As presented in Figure 5B, the different holding solutions had a significant effect on stem bent neck index. The cut rose flowers held in solution fortified with 2 mM CaNPs-SA prevented the bent neck occurrence until the 4th day of vase life.

Also, applying CaNPs-SA at 1 mM or 2 mM from CaNPs without intermixed SA, prevented appearing of a bent neck until the 4th day.

In contrast, the control treatment significantly accelerated the appearance of the bent neck, starting from the 2nd day till the last day of the vase life.

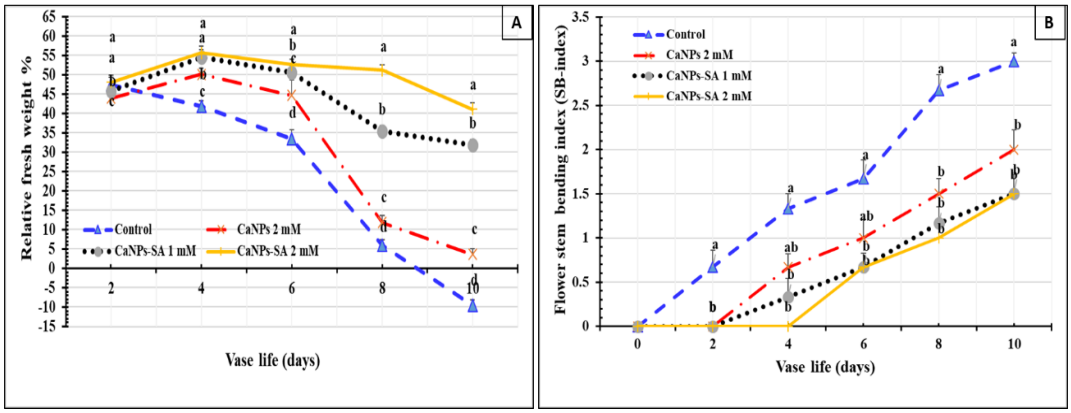

Figure 5. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on relative fresh weight % (A) and flower stem bending index (B) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Water relations

Water uptake g flower^{-1} 2 days

During the vase life, the water uptake (WU) steadily reduced in CaNPs intermixed with or without SA and the control flowers. This decrement was noticed after the 4th day of vase life. However, the decrease was very sharp with the control treatment after the 2nd day compared to the rest of the treatments, and it reached the lowest rate of water uptake (6.31 g flower^{-1} 2 days) on the 10th day of vase life (Figure 6A). Otherwise, CaNPs intermixed with SA at 2 mM markedly maintained the water uptake stable with a slight reduction during the vase life to reach a higher WU value of 12.28 g flower^{-1} on the 10th day, followed by holding solution fortified by CaNPs-SA at 1 mM on 10th day (10.45 g flower^{-1}).

Total water uptake ml flower^{-1} during vase life^{-1}

The maximum total water uptake (TWU) significant value was recorded for rose cut flower held in a preservative solution containing CaNPs intermixed with SA at 2 mM, since it was 66.62 ml flower^{-1} during the vase life (Figure 6B), with a 56.27% increase higher than the control. the second order in that respect was obtained from the treatment of 1 mM CaNPs-SA, as it stimulated the TWU to reach 44.29% more than the control. The lowest TWU value appeared with the control rose cut flower (42.63 ml flower^{-1}).

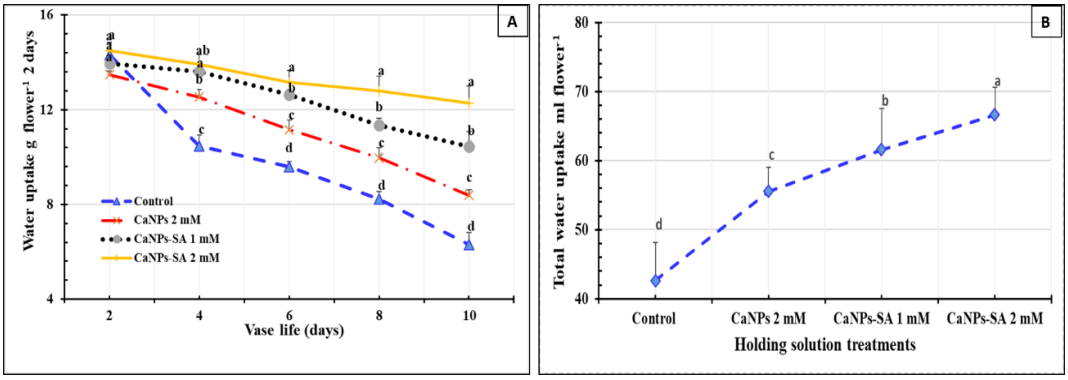

Figure 6. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on water uptake g flower^{-1} 2 days (A) and total water uptake ml flower^{-1} (B) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Water loss g flower^{-1} 2 day

It is obvious from Figure (7A) that CaNPs-SA at 2 or 1 mM which was applied in the holding solutions significantly reduced the water loss values during the evaluated period of rose vase life compared with the other treatments. Otherwise, the treatment of CaNPs-SA at 2 mM had a stable decreased curve in water loss than all other holding solutions. On contrary, the control and CaNPs at 2 mM preservative solutions recorded the maximum water loss values during the vase life period.

Water balance g flower^{-1} 2 day

The highest positive water balance values were obtained from both CaNPs-SA at 2 and 1 mM through the vase life interval (Figure 7B). Meanwhile, less water balance values were recorded with the control and CaNPs 2 mM holding solutions through the vase life, since a sharp decrease in the rose cut flowers' water balance was observed especially with the control cut flowers, starting from the 2nd day until the 10th day.

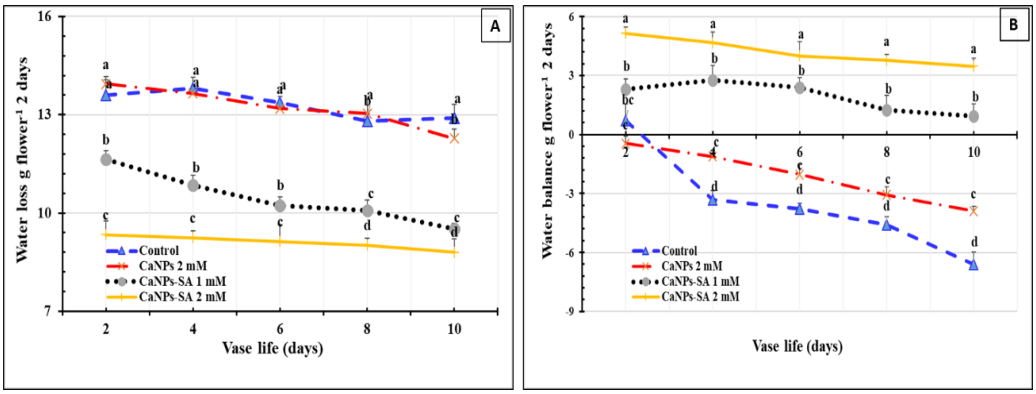

Figure 7. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on water loss g flower^{-1} 2 days (A) and Water balance g flower^{-1} 2 days (B) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Total phenol content (TPC)

TPC rapidly increased especially from the 4th day to the 8th day, but it started to decrease thereafter within the 10th day of the vase life for all CaNPs treatments (Figure 8A). However, as compared to the control, CaNPs combined with or without SA at 1 and 2 mM considerably improved the total phenol content and this improvement was much more pronounced at 2 mM from CaNPs-SA. Otherwise, CaNPs-SA at 1 and 2 mM or CaNPs at 2 mM gained 208.69 %, 165.22 %, and 176.09 % relative to the control treatments on the 8th day of vase life, respectively. In contrast, the control treatments showed a sharp decrease in phenols content compared with all treatments during the vase life.

Membrane stability index (MSI)

CaNPs holding solutions that intermixed with or without salicylic acid preserved the MSI compared with the control, more so with the CaNPs-SA concentration of 2 or 1 mM (87.59 % and 84.23 %, respectively). The control rose cut flowers lost their membrane stability more rapidly starting from day 0 to day 10, as shown in Figure (8B).

Hydrogen peroxide (H_2O_2) assessment

The control rose cut flowers rapidly increased the production of H_2O_2 and reached the highest production on the 8th and 10th day (26.56 and 26.55 mmol Kg^{-1}). However, CaNPs at 2 mM or CaNPs-SA at 1 and 2 mM in the holding solutions had the greatest influence in reducing the production of H_2O_2 (Figure 8C), but it was clear that CaNPs-SA at 2 mM produced the lowest significant H_2O_2 production comparing with all other holding treatments during the vase life.

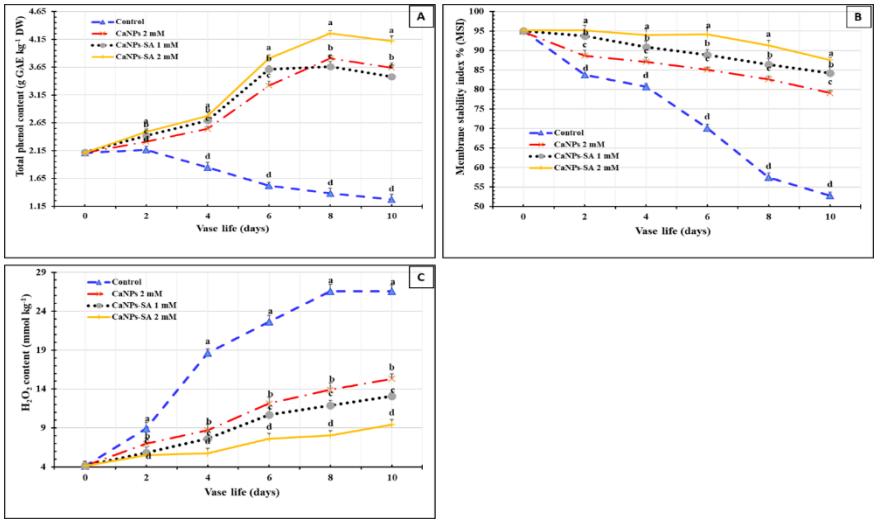

Figure 8. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on total phenol content (A), membrane stability index (B), and H_2O_2 content (C) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Enzyme's activities (PG, XYL, CEL, PT, and CAT)

Through the vase life interval, the activity of PG, XYL, CEL, and PT cell wall-degrading enzymes steadily increased (Figure 9A: 9D). Nevertheless, the control treatment showed a rapid increase in rates of enzyme activity relative to all other holding treatments. CaNPs-SA at 2 mM minimized the cell wall degrading enzyme activities significantly rather to other treatments. As this superior treatment on the 10th day recorded the lowest activities for PG (24.00-unit mg^{-1} protein), PE (21.31-unit mg^{-1} protein), XYL (6.89-unit mg^{-1} protein), and CEL (2.85-unit mg^{-1} protein). On the other hand, the production of CAT as an antioxidant enzyme was rapidly increased during the vase life period, especially with rose cut flower held in a preservative solution supplemented with CaNPs-SA at 2 mM, followed by CaNPs without intermixed SA at 2 mM and CaNPs-SA at 1 mM (Figure 9E).

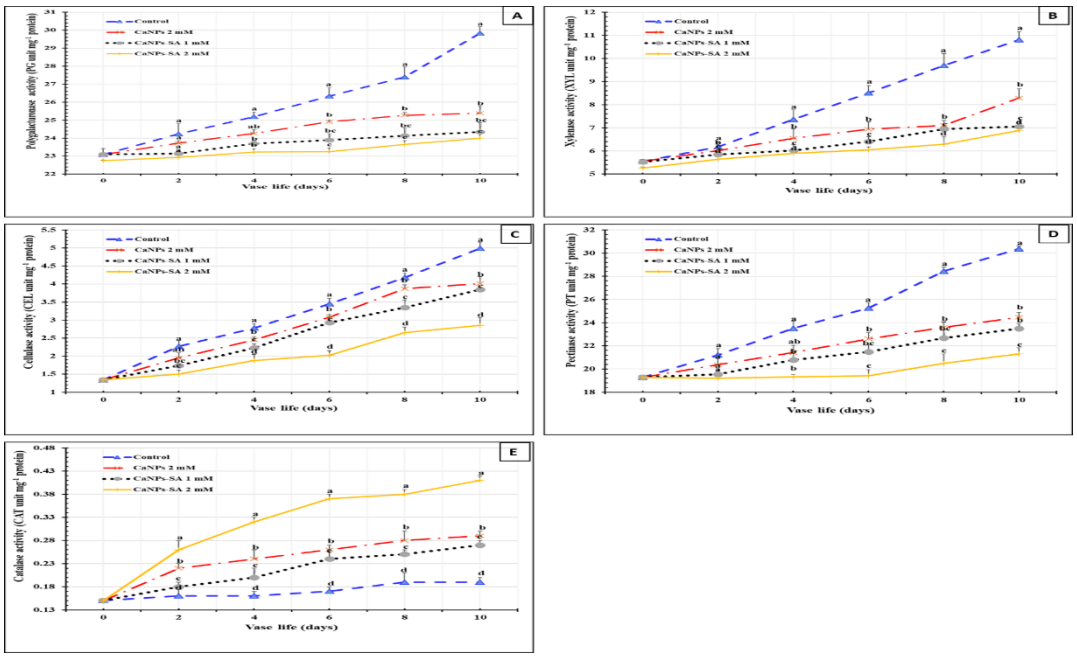

Figure 9. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on PG (A), XYL (B), CEL (C), PT (D), and CAT (E) enzyme activities of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Malondialdehyde (MDA) and Radical Scavenging Activity (DPPH)

MDA content was raised progressively through the vase life interval, starting from the first day from holding in the preservative solution until the 8th day with the control rose cut flowers. On the contrary, CaNPs intermixed with or without salicylic acid treatments laid to a slight increase in MDA contents in comparison with the control, especially

with CaNPs-SA at 2 mM treatment starting from the 6th day until the 8th day. The maximum MDA contents were observed with all holding treatments on the 8th day of vase life (Figure 10A). The radical scavenging activity (DPPH) was significantly decreased with CaNPs intermixed with or without salicylic acid treatments (Figure 10B). The highest DPPH capacity was recorded for rose cut flowers held in the control treatment on the 6th day of vase life.

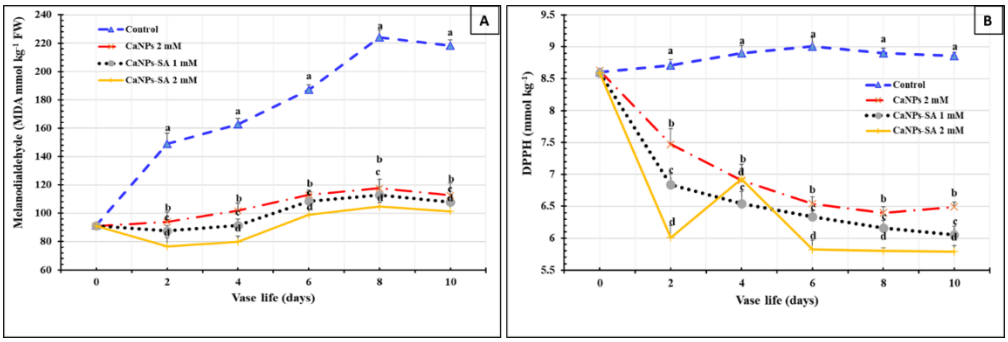

Figure 10. Impact of CaNPs at 2 mM intermixed with or without SA at 1 and 2 mM as holding solutions on MDA (A) and DPPH (B) of cut rose flowers during the vase life periods. Every value is the average of two separate and consecutive experiments ± SE. The vertical bars indicated the SE. Values with different letters at the same day of vase life are significantly different upon Tukey's HSD test at probability of 5%.

Discussion

Postharvest researchers face the most pressing challenge of delaying the mechanisms that control flower senescence to enable cut flowers with the highest quality and longest vase life to reach distant markets (Ahmad and Tahir, 2017). The most significant issues with cut rose flowers in general are neck bending and a short vase life. The primary reason for these issues is that cut flowers lose more water than its uptakes (El-Shawa *et al*., 2022), in addition to the low percentage of lignin in the region immediately below the flower bud (Perik *et al*., 2012), and with an increase in ROS and enzyme activity that breaks down cell walls (Reezi *et al*., 2009), which resulted in an intensification of the problem. So, the main objective of this study was to examine the ability of CaNPs intermixed with SA (1 and 2 mM), besides CaNPs at 2 mM without SA, and the control for enhancing the vase life and visual aesthetic value of R. *hybrida* cv. Black Magic flowers. In the current study, cut rose flowers held in solutions fortified with CaNPs intermixed with SA at 1 or 2 mM were proved to be most effective treatments in improving the vase life by 39.02 % and 53.66 % relative to the control. The highest vase life (15.75 days) was obtained from applying CaNPs-SA at 2 mM (Figure 4A). Increased vase life span due to solely CaNPs at 5 mM as pulsing treatments and SA at 2 mM has also been reported in cut roses (El-Shawa *et al*., 2022), as it might be connected to how calcium increases cell resistance by limiting the creation of ethylene (Sardoei, 2014). Additionally, SA functions as an ACC oxidase inhibitor, a direct ethylene precursor (Bayat and Aminifard, 2017; Heidarnezhadian *et al*., 2017). Likewise, calcium's capacity to slow down the respiration and alter osmotic pressure contributes to these actions. Water moves easily from the cut stems as a result of the pectin buildup in the xylem cellular walls, which also prevents the stems from bending (Van Ieperen and Van Gelder, 2006), creating a compound in the middle lamella between the membrane cell wall and poly-galacturonic acid to stiffen the cellular membranes (Aguayo *et al*., 2006). The control treatment accelerated the flower opening during the vase life, which appeared in increasing flower diameter during the first days of the vase life more than the rest holding treatments and this had the effect of reducing the vase life of it (Figure 4B).

Concerning the effect of holding solutions on relative fresh weight, flower stem bending index, and water relations (water uptake, total water uptake, water loss, water balance), CaNPs-SA at 2 mM in the holding solutions significantly maintained higher relative fresh weight, inhibited stem bending until the 4th day of vase life, increased the total water uptake and water uptake 2 day^{-1}, minimized the water loss, and preserved the highest water balance of rose cut flower during the vase life period (Figure 5, 6, and 7). For preventing any fresh weight loss and delay senescence, SA may be able to reduce respiration and transpiration rates by closing the stomata and/or enhancing the cut flower's water intake (Balas *et al*., 2006). This would lead to an increase in relative fresh weight, in another study, SA at a concentration of 2 mM alone in holding solutions preserved the cut roses' quality for the duration of their vase life by preventing fresh weight loss, promoting water absorption, and preventing stem bending in gerbera flowers (A. Singh *et al*., 2018). Additionally, SA boosted fresh weight and slowed dehydration of cut gladiolus flowers, according to (Saeed *et al*., 2016). Likewise, SA has an acidifying and antibacterial properties cause cut flowers to absorb more water while losing less water through transpiration, enhancing their water balance. Since SA might lower the pH of the preservative solution, which increases water absorption and inhibits the growth and spread of bacteria (Soleimany-Fard *et al*., 2013).

In addition, Ca makes plant cell walls significantly more structurally rigid, serving as the major mechanical support for the entire plant, which might explain the beneficial benefits of CaNPs combined with SA on postponing the cut rose bent neck (Li *et al*., 2012). Additionally, Ca bound via Ca-pectate in the middle lamella is necessary for the augmentation of plant tissues and cell walls (Hawkesford *et al*., 2012). According to (García-González *et al*., 2022), in the same context, gerbera flowers treated with 50 mg L-1 CaO-NP sustained a minimal stem bending for the duration of their vase life. Likewise, (Moallaye Mazraei *et al*., 2020) observed that gerbera in hydroponics absorbs more water for a high concentration of Ca nano-chelates (3 g L^{-1}).

Our data recorded higher total phenol content and membrane stability index with the minimum H_2O_2 content during the vase life of cut roses (Figure 8) which were held in

preservative solutions supplemented with CaNPs-SA at 2 mM, followed by CaNPs-SA at 1 mM. The antioxidant defense mechanisms of phenolic enrichment are known to be strengthened, and free radical scavenging protects flowers from oxidative stress (Shabanian *et al*., 2019). The increased membrane stability that introduced from CaNPs-SA treatment at 2 mM is consistent with (Ilyas *et al*., 2021) who explain that neutral SA entering the cell wall becomes more favorable in the acidic cell wall and more negatively charged in the cytoplasm, leading to an "ion trap" uptake mechanism of translocation.

The current study showed that intermixed CaNPs at 2 mM with SA delayed cell wall degrading by modulating XYL, PG, CEL, and PT, and promoting the production of antioxidant enzyme activity (CAT) (Figure 9) which decreased the MDA relative to the control (Figure 10A) over the vase life period (Youwei and Yinzhe, 2013) and increase CAT activity could aid in the scavenging of H_2O_2 during the vase life period (Abdelkader *et al*., 2022). Additionally, the presence of Ca^{+2} causes the middle lamella to degrade by lowering the activity of the polygalacturonase enzyme (Wehr *et al*., 2004). Also, (Ditta and Arshad, 2016) revealed that the application of CaNPs improves the antioxidative defense and lowers the accumulation of ROS inside plant. Moreover, previous researches have cleared that CaNPs increased the antioxidant enzyme activities and lowering MDA and H_2O_2 concentrations inside plants (Anantharaman and George, 2016; Shukla *et al*., 2019). Otherwise, the plant uses the antioxidative enzyme defense like CAT for protecting its cells from the negative impacts of ROS, that causes the senescence of cut flowers petal (Gill and Tuteja, 2010). In previous studies, Applying SA at pre or postharvest had improved the activity of catalase and extending the vase life of cut flowers such as gladiolus and roses (Saeed *et al*., 2016; Kazemi *et al*., 2018). Moreover, applying SA at 2.0 mM induced maximum MSI index and reduced the electrolyte leakage in *Crocus sativus* (Khayyat *et al*., 2018). Also, (Nisar *et al*., 2021) applied SA at 0.05 mM and found that SA was most effective in improving the *Nicotiana plumbaginifolia* flower vase life, and exogenous SA could preserve membrane integrity by enhancing the antioxidant system activity. In addition, SA boosts the activity of antioxidant enzymes by briefly storing abscisic acid, delaying the hydrolysis of cell components, and reducing ROS production (Hayat *et al*., 2010). Additionally, the primary function of calcium ions (Ca^{+2}) is to interact with the free carboxyl groups of galacturonic acids at the middle lamella and pectin, making cell walls more durable and better able to fend off enzyme-induced degradation (White and Broadley, 2003).

CONCLUSION

The investigation was an attempt to study the role of CaNPs intermixed with or without SA as holding solutions in enhancing the vase life characteristics of rose cut flowers (*Rosa hybrida* cv. Black Magic). CaNPs-SA at 2 mM extended the cut flowers vase life by improving the water relationships, delayed the stem flower bent neck, and increased the membrane stability and antioxidant enzyme (CAT), which led to diminishing H_2O_2, MDA accumulation, and the cell wall degrading enzymes (PG, XYL, CEL, and PT). The current research clears that there is a lot of potential to examine the ability of CaNPs intermixed with SA in post-harvest treats of such important cut flowers.

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تأثير جزيئات الكالسيوم النانوية الممزوجة بحامض الساليسيليك على نشاط الإنزيمات وصفات ما بعد الحصاد لأزهار الورد المقطوفة (صنف بلاك ماجيك)

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الملخص

يعتبر الورد أحد أهم نباتات الزينة وأزهار القطف، والتي تستخدم في العديد من مشاريع التنسيق الخارجية والداخلية. العيب الرئيسي هو فترة ما بعد الحصاد القصيرة وانحناء عنق الزهرة. تم إجراء تجربتين منفصلتين لفحص فعالية جسيمات الكالسيوم النانوية (CaNPs) الممزوجة مع حامض الساليسيليك (SA) أو بدونه فى أربعة معاملات (الكنترول ، الكالسيوم النانوى بتركيز ٢ ملليمول ، الكالسيوم النانوى الممزوج بحامض الساليسيلك بتركيز١ ملليمول، والكالسيوم النانوى الممزوج بحامض الساليسيلك بتركيز ٢ ملليمول، كمحاليل تثبيت حتى نهاية فترة حياة أزهار الورد المقطوفة صنف بلاك ماجيك. اهتم البحث بدراسة تأثيرات محاليل التثبيت السابقة على بعض الصفات الفسيولوجية للورود بما في ذلك (عمر الزهرة بعد القطف، قطر الزهرة، نسبة الوزن الطازج النسبي، مؤشر انحناء عنق الأزهار، والعلاقات المائية). بالإضافة إلى ذلك، محتوى الفينول الكلي، مؤشر ثبات الغشاء(MSI) ، نشاط انزيم الكتاليز المضاد للأكسدة (CAT)، وإنزيمات تحلل الجدر الخلوية (CWDAs) مثل الزيلاناز(XYL) ، البولى جلاكتوربنيز (PG)، البكتيناز (PT) ، والسليولاز (CEL)خلال ١٠ أيام من عمر الأزهار. بالإضافة إلى معدلات توليد فوق أكسيد الهيدروجين (H_2O_2) و malondialdehyde (MDA) وخفض DPPH . حيث أدى استخدام جسيمات الكالسيوم النانوية الممزوجة بحامض الساليسيلك بتركيز ٢ ملليمول إلى إطالة عمر الأزهار المقطوفة والحافظ على نسبة أعلى من الوزن الطازج النسبي والعلاقات المائية. بالإضافة إلى ذلك، نتج عن تلك المعاملة تقليل وتثبيط نشاط انزيمات تحلل جدر الخلايا CWDAs و MDA من خلال تعزيز نشاط كسح الجذيرات الحرة DPPH وزيادة نشاط الإنزيمات المضادة للأكسدة CAT مما أدى إلى زيادة مؤشر ثبات الغشاء MSI لأزهار الورد المقطوفة.