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The Effect of Vase Solutions Containing Cobalt, Cerium, and Silver Nanoparticles on Postharvest Life and Quality of Cut Birds of Paradise (*Strelitzia reginae*)

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Abstract

A study was conducted on increasing the postharvest quality and vase life of cut *Strelitzia reginae* flowers based on a randomized complete design with 10 treatments in three replications. The experimental treatments included cobalt chloride ($CoCl_2$) (250 and 500 mg L^{-1}), cerium nitrate ($Ce(NO_3)_3$) (100, 300, and 600 µM), silver nanoparticle (SNP) (20 and 40 mg L^{-1}), and Nanosil (2000 and 4000 µM) applied as the 24-hour pulse. Distilled water was used as the control. Results showed that the longest vase life (11.68 days) was obtained from the application of 300 µM $Ce(NO_3)_3$, but it did not significantly differ from the treatments of 100 and 600 µM $Ce(NO_3)_3$, 500 mg L^{-1} $CoCl_2$, and 20 mg L^{-1} SNP. The best treatments in increasing water uptake and dry matter, preserving fresh weight, and reducing stem-end and vase solution bacteria were 300 and 600 µM $Ce(NO_3)_3$. The lowest malondialdehyde accumulation (0.09 nmol g^{-1} FW) and the highest activity of peroxidase (0.147 nmol g^{-1} FW) and catalase (1.02 nmol g^{-1} FW) were obtained from the plants treated with 300 µM $Ce(NO_3)_3$ in the vase solution. The highest sepal flavonoid (0.493 %) was related to the treatment of 2000 µM Nanosil. The control plants exhibited the greatest loss of fresh weight and the lowest values of the recorded traits. Based on the results, it is recommended to use a vase solution containing 300 µM $Ce(NO_3)_3$ and 3 % sucrose to preserve the quality and increase postharvest vase life of cut *S. reginae* flowers.

Keywords: Antioxidant Enzymes, Hydrogen Peroxide, Nanosil, Vascular Blockage.

INTRODUCTION

Strelitzia reginae commonly known as bird-of-paradise is an important cut flower from the family Strelitziaceae. Vascular blockage by microorganisms and the resulting decline of water uptake are among the factors advancing the early aging of the cut flowers of *S. reginae* (Finger *et al*., 1999; Ali and Hassan, 2014). The removal of microorganisms from vase solutions and stem ends by disinfectants and antibacterials is a well-established method to increase water uptake and retain postharvest fresh weight and longevity of cut flowers (Liu *et al.*, 2009; Mohammadi *et al*., 2012). Metallic salts have traditionally been used as disinfectants in the preservative solution of cut flowers. Cobalt chloride ($CoCl_2$) is a metallic salt that can effectively help maintain postharvest longevity of cut flowers. Cobalt-containing compounds can contribute to sustaining water uptake by cut flowers through preventing the blockage of xylems. Cobalt is also involved in stomatal closure and retention of water potential of cut flowers. There are reports as to the inhibitory impact of cobalt on ethylene biosynthesis (Murali and Reddy, 1993; Halevy *et al*., 2001). Aslomshtaghi *et al*. (2014) investigated the effect of $CoCl_2$ (100, 200, and 300 mg L^{-1}) on the vase life of cut roses and reported that $CoCl_2$ prevented vascular blockage and increased water uptake in this cut flower. Mohammadi *et al*. (2012) reported that the application of 300 mg L^{-1} $CoCl_2$ prolonged vase life, increased water uptake, and helped fresh weight retention of cut tuberoses.

The ions of silver (Ag^+) have antibacterial activities (Nowak and Rudnicki, 1990). So, the application of Ag-containing compounds to the vase solution can improve vase life by inhibiting vascular blockage (Damunupola and Joyce, 2006). Silver nanoparticles (SNPs) prevent DNA propagation of bacteria by penetrating bacteria cells, disrupting their respiration chain, and disordering their cell division, thereby killing the bacteria and hinder vascular blockage of cut flowers (Maneerung *et al*., 2008). Morones *et al*. (2005) suggest that SNP increases water uptake and postharvest longevity of cut flowers by preventing the growth and propagation of bacteria in vase solution and stem end. The application of Ag to the preservative of cut carnation acted as a strong antibacterial and anti-ethylene and extended the postharvest longevity of this cut flower significantly versus the control (Halevy and Mayak, 1981). Liu *et al*. (2009) explored the impact of different levels of SNP on the vase life of cut gerbera and concluded that 5 mg L^{-1} SNP extended vase life and improved water uptake.

Nanosil is a solution composed of hydrogen peroxide and SNP. Like SNP, this solution has an antibacterial activity. There are few studies on the effect of Nanosil on the vase life of cut flowers. But, the positive impact of its constituents, i.e., hydrogen peroxide (Shadbash and Keshavarzshal, 2018) and SNP (Kim *et al*., 2005; Solgi *et al*., 2009), have reported on the vase life of cut flowers. Shadbash and Keshavarzshal (2018) reported that the treatment of cut roses cv. 'Grand Press Angela' with Nanosil prolonged their vase life significantly via reducing stem-end microbial load, increasing water uptake, and preserving fresh weight.

Cerium (Ce) is a trace element in the crust that belongs to the group of Lanthanum, and its desirable impact has been reported on the improvement of antioxidant system activities in plants (Wu *et al*., 2014). The effects that antioxidant enzymes have on the postharvest longevity of cut flowers are associated with their activities in scavenging reactive oxygen species (ROS) and protecting membrane structure (Alaey *et al*., 2011; Shan and Zhao, 2015; Wang *et al*., 2017). According to Wang *et al*. (2017), cerium nitrate (Ce $(NO_3)_3$) extended vase life, reduced flower wilting, increased the number of open florets, preserved pigments, reduced MDA content, and increased the activity of antioxidant enzymes. The literature shows that the application of $Ce(NO_3)_3$ in the vase solution prolonged the vase life of cut carnations (Zheng and Guo, 2018) and *Lilium* (Houa *et al*., 2018) by strengthening their antioxidant systems and protecting their membrane structure.

The present research aimed to examine the effects of silver nanoparticles (SNP), Nanosil, cobalt chloride ($CoCl_2$), and cerium nitrate (Ce $(NO_3)_3$) along with 3% sucrose on the control of

the microbial load, the activity of antioxidant enzymes, and the vase life of cut birds-of-paradise (*S. reginae*) in order to propose the best preservative for the best vase life of this cut flower.

MATERIALS AND METHODS

Plant materials

The cut flowers of *S. reginae* at the commercial harvest stage (the exit of the first floret from the pod and the orange sepals being visible) were procured at a hydroponic greenhouse in Tehran, Iran and were transferred to the study site in commercial packaging and by taking care of all transportation principles in the shortest possible time. In the laboratory, the visually homogenous flowers were re-cut under tap water to a height of 45 cm and were used for the study.

Experimental design and treatments

The study was based on a randomized complete block design with 10 treatments, 3 replications, 30 plots, and 4 branches per plot. The experimental treatments included $CoCl_2$ (250 and 500 mg L^{-1}), Ce $(NO_3)_3$ (100, 300, and 600 µM), SNP (20 and 40 mg L^{-1}), and Nanosil (2000 and 4000 µM) along with 3% sucrose applied as a 24-hour pulse treatment. Distilled water was employed as the control. After the pulse treatments, the flowers were placed in vase solutions containing 250 mL of distilled water and 3% sucrose and were kept in an environmentally controlled room (a temperature of 20 ± 2 °C, relative humidity of 60-70 %, a lightness duration of 12 hours, and a light intensity of 15 µM m^{-2} s^{-1}) until the end of the experiment.

Assessment of traits

Vase life

Postharvest longevity of the cut flowers was calculated by counting the days from the application of treatment until the withering of 75 % of the florets (Gendy and Mahmoud, 2012).

Water uptake

It was measured by the following equation:

$$\text{Solution uptake (ml g}^{-1}\text{ FW)} = \frac{V_{t0}-(E_t+V_{t1})}{FW}$$

in which V_{t0} represents the initial solution volume, V_{t1} represents the last-day solution volume, E_t represents the final value of evaporation from the solution surface, and FW represents the flower branch weight on the first day.

Fresh weight loss

The fresh weight of one flower branch was recorded with a digital scale on the first and last day of the experiment. To avoid vascular blockage, 1 cm from the stem end was re-cut under tap water every other day, and it was re-weighed. Then, the sum of the last-day fresh weight and the weights measured after each re-cut was subtracted from the first-day fresh weight. The figure was reported as fresh weight loss.

Dry matter

At the end of the vase life, the fresh weight of a flower branch from each plot was measured. Then, the same branches were oven-dried at 70 °C for 48 hours. Dry matter percentage was calculated by the following equation:

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

Stem end bacteria count

Twenty-four hours after the pulse treatments, 1 cm was cut from the stem end with sterile scissors and washed with distilled water. Then, the samples were ground and crushed in a china mortar with 0.9 % normal saline serum. Next, 0.1 mL of the mixture was cultured on nutritional agar and incubated at 37 °C for 24 hours. The bacteria colonies were, finally, counted under an optical microscope (Liu *et al*., 2009).

Vase solution bacteria count

Twenty-four hours after the pulse treatments, 5 mL of the vase solution was diluted with 0.9 % normal saline solution. Then, 0.1 mL of the solution was placed on nutritional agar and incubated at 37 °C for 24 hours. Then, the bacteria colonies were counted under an optical microscope (Liu *et al*., 2009).

Identification of stem end and vase solution bacteria

After the colonies were counted, they were studied morphologically and the colonies with different morphologies were randomly selected for further examinations during which they were subjected to the measurement of some morphological and biochemical traits, including stainability and morphological traits in Gram staining, colony growth and morphology on MacConkey agar medium, mobility, oxidase and catalase production, gelatine hydrolysis, starch hydrolysis, indole production, urease production, methyl red reaction, acetoin production, citrate use, nitrate reduction, and H_2S production. Finally, the genera of most bacteria were reported.

Sepal flavonoids

As soon as the first symptoms of withering were observed in the petals, the sepals were sampled to measure flavonoid content. So, 0.1 g of the sepals were extracted with 2.5 mL of acidic ethanol (99 % ethanol + 1% glacier acetic acid) and centrifuged at 3600 rpm for 15 minutes. The resulting supernatant was extracted with a sampler and was placed in a hot-water bath at 85°C for 10 minutes. The absorption of the samples was then read at 330 nm with a PD-3000 UV APEL spectrophotometer (Krizek *et al*., 1998).

Malondialdehyde (MDA)

MDA content was measured by the procedure described in Heath and Parker (1968). So, 0.5 g of the petal tissue was extracted with liquid nitrogen and potassium phosphate buffer. The extract was centrifuged several times; each time, the supernatant was separated with a sampler. Afterward, 200 µL of the extract was added with 1000 µL of trichloroacetic acid (TCA) and thiobarbituric acid (TBAS). The sample was placed in a hot water bath at 85°C for 30 minutes, and then, it was transferred into ice for 30 minutes. The cool solution was centrifuged at 4°C for 10 minutes. Then, its absorption was read at 532 and 600 nm with a spectrophotometer. Eventually, the following formula was used to determine MDA content.

$$\text{MDA (nmol g}^{-1}\text{ FW)} = A_{532nm} - A_{600nm}$$

Measurement of the activity of antioxidant enzymes

Enzymatic extract preparation: As soon as the first symptoms of withering appeared on the first florets, all plots were sampled. Then, 100 mg of the sepal sample was mixed and homogenized with 1 mL of 100 mM potassium phosphate buffer (pH = 7), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM of 1% polyvinylpyrrolidone (PVP) in a china mortar at a low temperature. Next, the samples were centrifuged at 4°C at 15000 rpm for 30 minutes. The supernatant of the

samples (enzymatic extract) was separated and kept at 80 °C until the measurement of catalase and peroxidase activities.

Catalase (CAT) activity

CAT activity was measured by Beers and Sizer's (1952) method. The reaction mixture was composed of 40 µL of 15 mM hydrogen peroxide, 2.6 mM of 50 mM potassium phosphate buffer (pH = 7), and 40 µL of the enzymatic extract, which were well mixed. CAT activity was estimated by reading the absorption of the sample at 240 nm with a Jasco V530 spectrophotometer (Japan) and using an extinction coefficient of 39.4 mM cm^{-1}.

Peroxidase (POD) actvity

The procedure described by In *et al*. (2007) was employed to determine POD activity. To this end, 100 µL of the enzymatic extract, 450 µL of H_2O_2 solution, and 450 µL of guaiacol solution were mixed. Then, the absorption of the sample was read at 470 nm with a Jasro V530 spectrophotometer (Japan), and POD activity was reported in nmol g^{-1} FW.

Data analysis

Statistical data were collected observationally during the experiment and also by the use of laboratory tools. The data were analyzed in the MSTATC statistical software package. Also, the means were compared by the LSD test at the $P < 0.05$ level.

RESULTS

Vase life

All experimental treatments significantly ($P < 0.01$) retarded aging of the cut *S. reginae* flowers versus the control whose aging started in 8.064 days (Table 1). The longest vase life was observed in the plants treated with 300 and 600 µM Ce $(NO_3)_3$ (11.68 and 11.08 days, respectively), but they did not differ from the treatments of 20 mg L^{-1} SNP (10.89 days), 100 µM Ce $(NO_3)_3$ (10.87 days), and 500 mg L^{-1} $CoCl_2$ (10.63 days) significantly (Fig. 1).

Solution uptake

The experimental treatments significantly ($P < 0.01$) influenced solution uptake (Table 1). The lowest vase solution uptake (0.088 mL g^{-1} FW) was related to the control. The treatments of 300 and 600 µM Ce $(NO_3)_3$ exhibited the highest rate of solution uptake (0.125 mL g^{-1} FW), but not differing from that of the treatments of 20 mg L^{-1} SNP and 2000 and 4000 µM Nanosil significantly (Fig. 2).

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

S.o.V	df	Vase life	Solution uptake	Loss fresh weight	Dry matter	Bacterial population in vase solution	Bacterial populationin stem end	Flavonoid contents	Malondialdehyde (MDA)	Peroxidase (POD)	Catalase (CAT)
Treatments	9	3.085**	0.00055**	231**	36.06**	10330**	1292**	0.015**	1.110**	0.0047**	0.241**
Error	20	0.421	0.000	18.184	7.345	42.243	215.83	0.004	0.009	0.001	0.016
CV (%)		6.29	5.60	7.31	9.98	9.49	18.71	15.50	12.94	41.88	17.61

**: Significant at P<0.01.

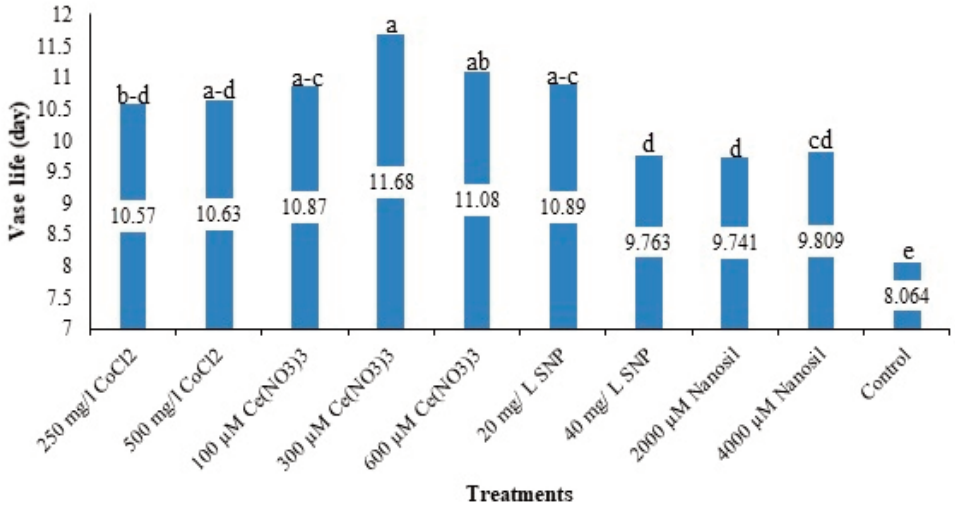

Fig. 1. The effect of $CoCl_2$, Ce $(NO_3)_3$, SNP, and Nanosil on the vase life of cut *S. reginae* flowers.

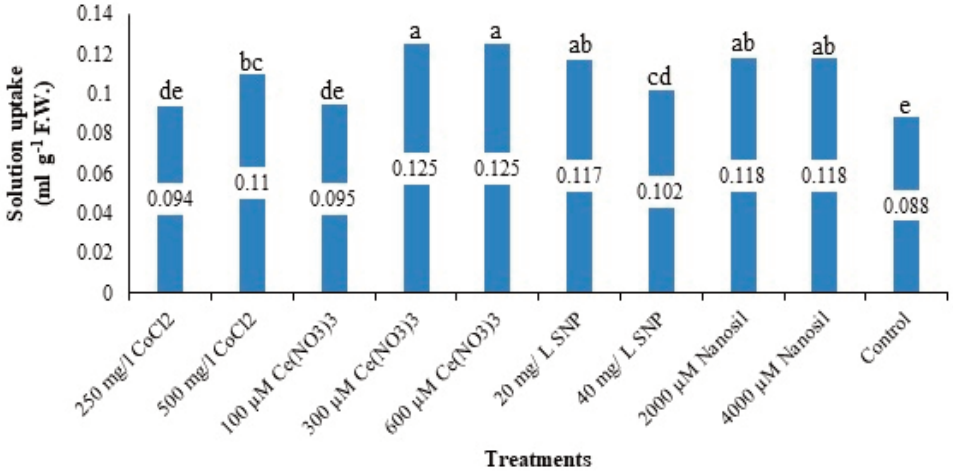

Fig. 2. The effect of $CoCl_2$, Ce $(NO_3)_3$, SNP, and Nanosil on the solution uptake of cut *S. reginae* flowers.

Fresh weight loss

The effects of the experimental treatments were significant ($P < 0.01$) on flower fresh weight loss (Table 1). Fig. 3 demonstrates that all experimental treatments outperformed the control in preserving fresh weight. The highest and lowest fresh weight were related to the control (73.51 g) and 300 µM Ce $(NO_3)_3$ (44.45 g), respectively (Fig. 3).

Dry matter

Based on the analysis of variance, dry matter was significantly ($P < 0.01$) affected by the experimental treatments (Table 1). The comparison of the means revealed that the treatments of 300 and 600 µM Ce $(NO_3)_3$ and 20 mg L^{-1} SNP performed the best in increasing dry matter so that they increased it by 33.46 %, 31.54 %, and 29.26 %, respectively. The lowest dry matter percentage (22.39 %) was related to the control (Fig. 4).

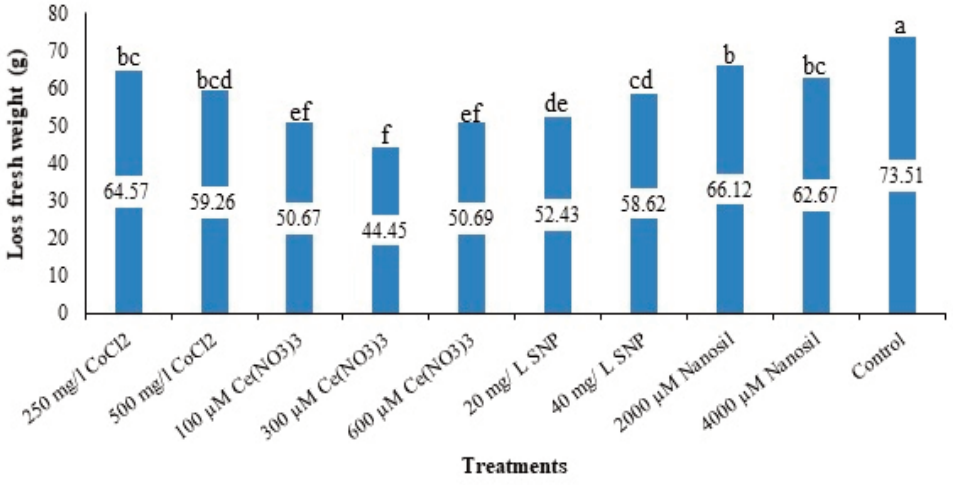

Fig. 3. The effect of $CoCl_2$, Ce $(NO_3)_3$, SNP, and Nanosil on the fresh weight loss of cut *S. reginae* flowers.

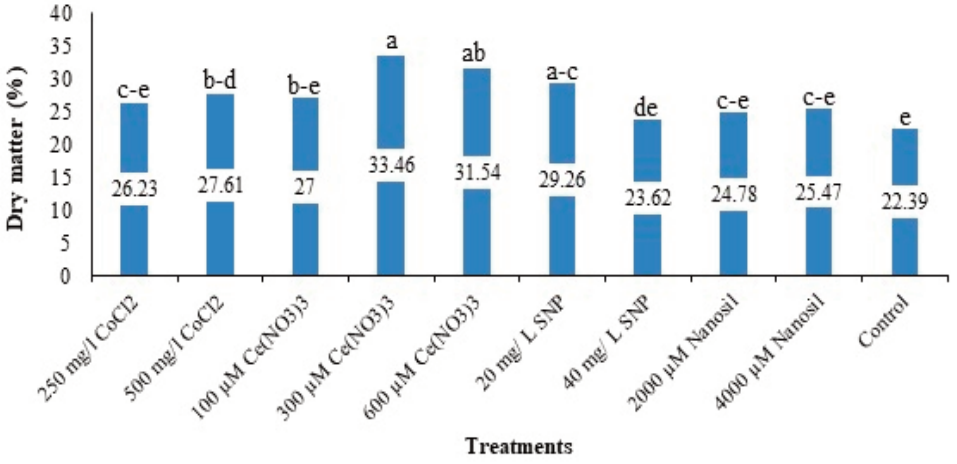

Fig. 4. The effect of $CoCl_2$, Ce $(NO_3)_3$, SNP, and Nanosil on the dry matter percentage of cut *S. reginae* flowers.

Stem-end bacteria count

The effects of the experimental treatments were significant ($P < 0.01$) on the stem-end bacteria population (Table 1). The application of the treatments reduced this population versus the control (218.3×10^6 Log_{10} CFU mL^{-1}) so that the lowest population was related to the treatments of 600 µM $Ce(NO_3)_3$ (9.47×10^6 Log_{10} CFU mL^{-1}) and 300 µM Ce $(NO_3)_3$ (11.9×10^6 Log_{10} CFU mL^{-1}), which were not significantly different from the treatments of 20 and 40 mg L^{-1} SNP (Fig. 5).

Solution bacteria count

All experimental treatments reduced vase solution bacteria population significantly ($P< 0.01$) (Table 1). As is evident in Fig. 6, higher concentrations of the experimental treatments further reduced the bacteria population of the vase solution. The lowest population was observed in the

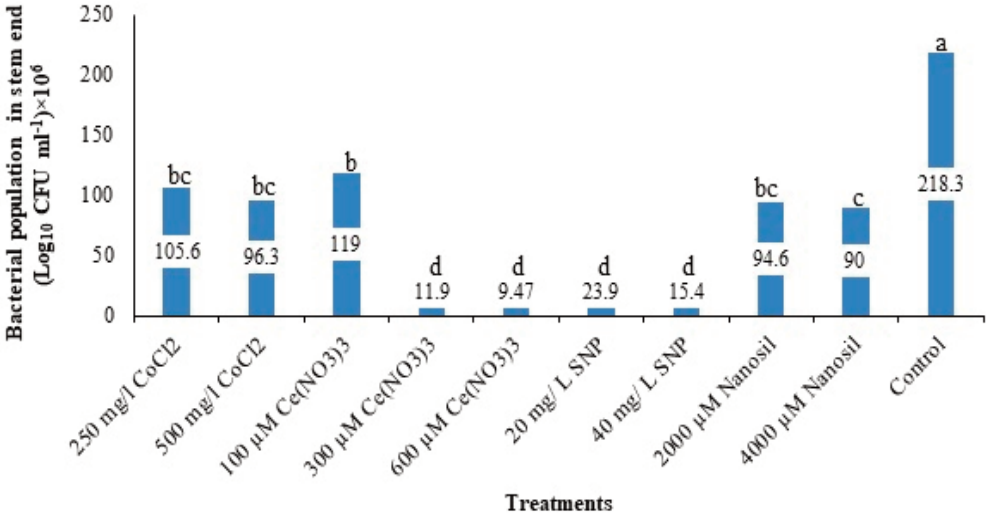

Fig.5. The effect of $CoCl_2$, Ce $(NO_3)_3$, SNP, and Nanosil on the stem-end bacteria population of cut *S. reginae* flowers.

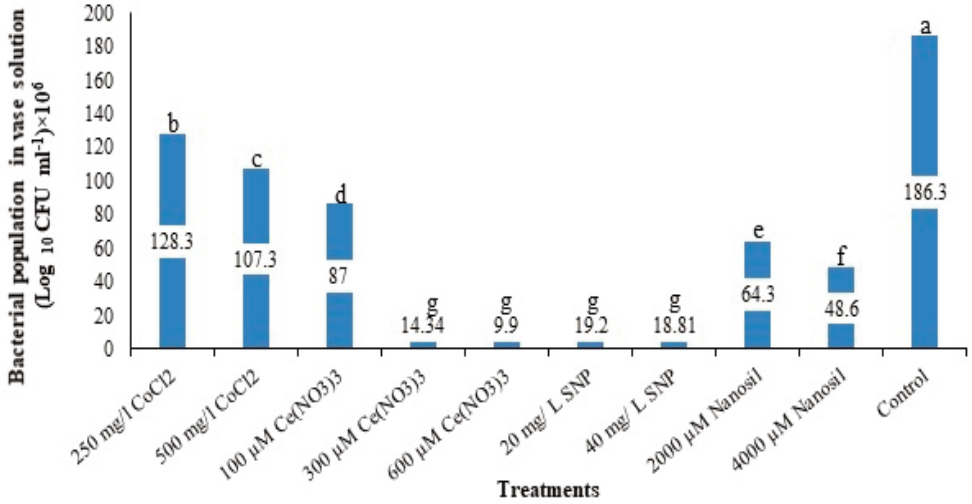

Fig. 6. The effect of $CoCl_2$, $Ce(NO_3)_3$, SNP, and Nanosil on the vase solution bacteria population of cut *S. reginae* flowers.

treatment of 600 µM Ce $(NO_3)_3$ (9.90×10^6 Log_{10} CFU mL^{-1}), but it did not differ from the treatments of 300 µM Ce $(NO_3)_3$ and 20 and 40 mg L^{-1} SNP significantly. The highest population was 186.3×10^6 Log_{10} CFU mL^{-1} observed in the control (Fig. 6).

Identification of stem-end and vase solution bacteria strains

Tables 2 and 3 present the strains of bacterial identified in the stem end and vase solution. Based on Table 2, there were strains of Gram-negative and Gram-positive bacteria and yeast in the stem end of the cut *S. reginae* flowers treated with distilled water (the control). The application of 200 µM $Ce(NO_3)_3$ fully stopped the growth of both Gram-negative and Gram-positive bacteria in the stem end, and only yeasts were detected in this treatment. The treatments of 2000 and 4000 µM Nanosil prevented the growth of Gram-negative bacteria and yeast in the stem end. When 600 µM $Ce(CO_3)_3$ was applied, the growth of yeast and Gram-positive bacteria was stopped.

The growth of Gram-negative bacteria was stopped in the vase solution containing 500 mg L^{-1} $CoCl_2$, 300 µM $Ce(NO_3)_3$, 20 mg L^{-1} SNP, 2000 µM Nanosil, or 4000 µM Nanosil. Among the Gram-positive bacteria, bacillus was the only strain that grew in the vase solution containing 250 or 500 mg L^{-1} $CoCl_2$, 600 µM $Ce(NO_3)_3$, or 20 mg L^{-1} SNP. Yeast was also detected in these solutions. *Escherichia coli*, pseudomonas, and enterobacter from the Gram-negative strains and streptococcus, staphylococcus, and bacillus from the Gram-positive strains were identified in the control. The control had yeast, too (Table 3).

Table 2. List of bacteria identified in the cut stem end.

Treatments	Gram-negative bacteria	Gram-positive bacteria	Fungus
250 mg L^{-1} $CoCl_2$	*E. coli*,	*Bacillus*	Yeast
500 mg L^{-1} $CoCl_2$	—	*Bacillus, Staphylococcus*	Yeast
100 µM Ce $(NO_3)_3$	*Citrobacter*, *E. coli*	*Bacillus,*	—
300 µM Ce $(NO_3)_3$	—	—	Yeast
600 µM Ce $(NO_3)_3$	*Actinobacillus*, *E.coli*	—	—
20 mg L^{-1} SNP	*E. coli*	*Bacillus*	—
40 mg L^{-1} SNP	*E. coli, Pseudomonas*	*Staphylococcus, Bacillus*	—
2000 µM Nanosil	—	*Bacillus, Staphylococcus*	—
4000 µM Nanosil	—	*Streptococcus*	—
Control	*E. coli, Pseudomonas*	*Bacillus, Staphylococcus, Streptococcus*	Yeast

Table 3. List of bacteria identified in vase solution.

Treatments	Gram-negative bacteria	Gram-positive bacteria	Fungus
250 mg L^{-1} $CoCl_2$	*E. coli*	*Bacillus*	Yeast
500 mg L^{-1} $CoCl_2$	—-	*Bacillus*	Yeast
100 µM Ce $(NO_3)_3$	*E. coli, Enterobacter*	*Staphylococcus, Bacillus,*	-—
300 µM Ce $(NO_3)_3$	—-	*Staphylococcus*	-—
600 µM Ce $(NO_3)_3$	*E.coli*	*Bacillus*	Yeast
20 mg L^{-1} SNP	—-	*Bacillus*	Yeast
40 mg L^{-1} SNP	*Pseudomonas*	*Streptococcus, Staphylococcus*	—-
2000 µM Nanosil	—-	*Streptococcus, Staphylococcus*	-—
4000 µM Nanosil	—-	*Streptococcus, Staphylococcus*	-—
Control	*E. coli, Pseudomonas, Enterobacter*	*Streptococcus, Staphylococcus, Bacillus*	Yeast

Sepal flavonoid content

The impact of the experimental treatments was significant ($P < 0.01$) on sepal flavonoid content (Table 1). Flavonoid content was significantly increased with the application of the experimental treatments versus the control (0.296 %). The flowers treated with 2000 µM Nanosil exhibited the highest sepal flavonoid content (0.493 %), whose difference with the treatments of 4000 µM Nanosil, 40 mg L^{-1} SNP, and 100 and 600 µM $Ce(NO_3)_3$ was not statistically significant (Fig. 7).

Malondialdehyde (MDA)

The analysis of variance revealed that the effect of the experimental treatments was significant ($P < 0.01$) on MDA accumulation (Table 1). Fig. 8 displays the positive effect of the experi-

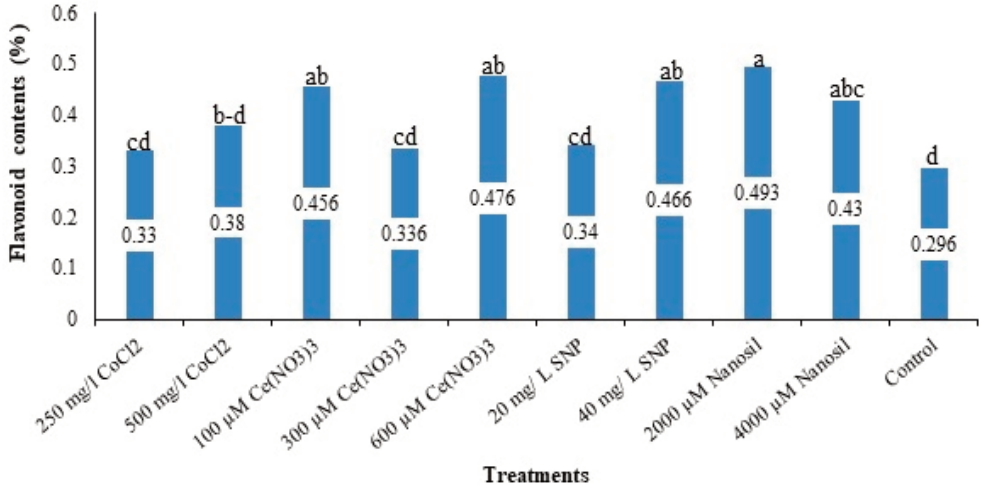

Fig. 7. The effect of $CoCl_2$, $Ce(NO_3)_3$, SNP, and Nanosil on the sepal flavonoid content of cut *S. reginae* flowers.

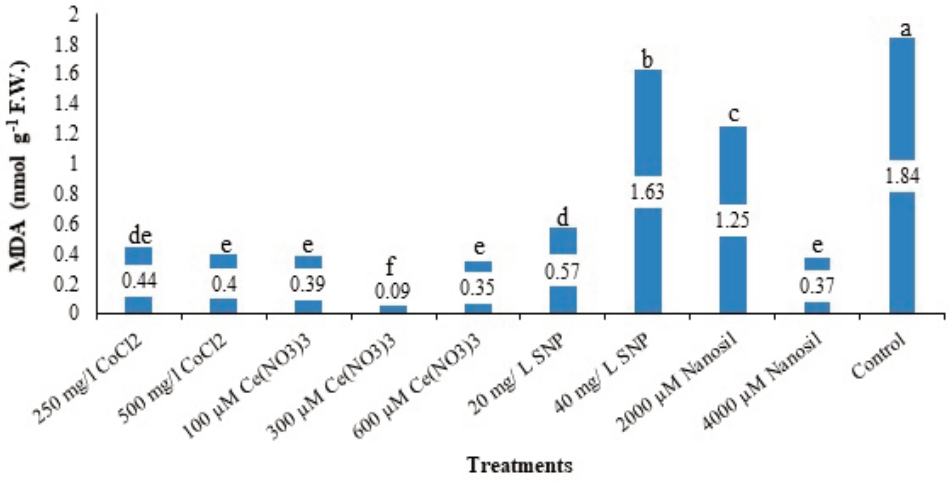

Fig. 8. The effect of $CoCl_2$, $Ce(NO_3)_3$, SNP, and Nanosil on the malondialdehyde content of cut *S. reginae* flowers.

mental treatments on reducing MDA accumulation in sepals. The highest rate of MDA accumulation was related to the control (1.84 nmol g^{-1} FW) and the plants treated with 40 mg L^{-1} SNP (1.63 nmol g^{-1} FW). This implies the negative effect of high concentrations of SNP on membrane health and increasing lipid peroxidation. Among all the treatments, the lowest MDA content was recorded by the treatment of 300 µM Ce $(NO_3)_3$ (0.09 nmol g^{-1} FW), so this treatment was most successful in reducing lipid peroxidation (Fig. 8).

Activity of antioxidant enzymes

Peroxidase (POD) activity

The effect of the experimental treatments was significant ($P < 0.01$) on POD activity (Table 1). Based on the comparison of the means, POD activity was higher in the plants treated with 300 µM $Ce(NO_3)_3$. The POD activity of these plants was estimated at 0.147 nmol g^{-1} FW. However, it did not differ from the treatments of 100 and 600 µM $Ce(NO_3)_3$ significantly (0.115 and 0.113 nmol g^{-1} FW, respectively). The lowest POD activity (0.035 nmol g^{-1} FW) was related to the control and 2000 µM Nanosil (Fig. 9).

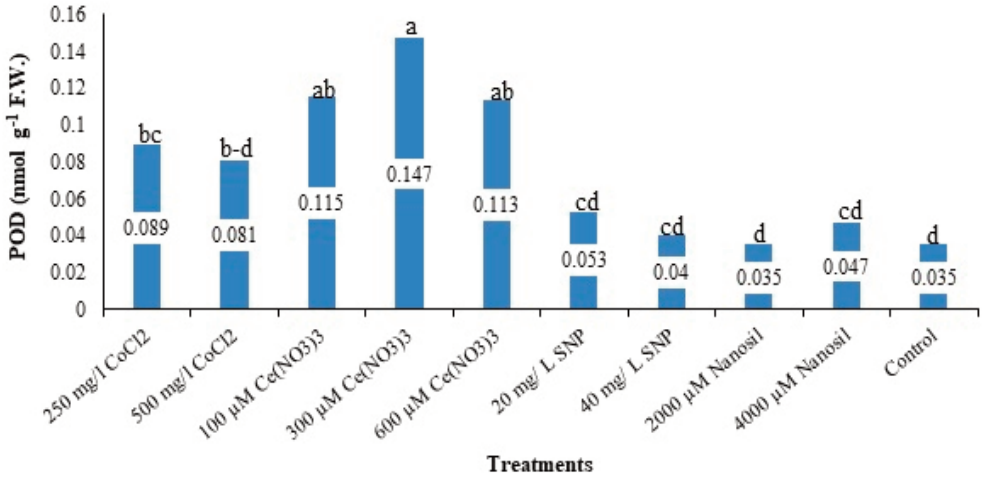

Fig. 9. The effect of $CoCl_2$, $Ce(NO_3)_3$, SNP, and Nanosil on the peroxidase activity of cut *S. reginae* flowers.

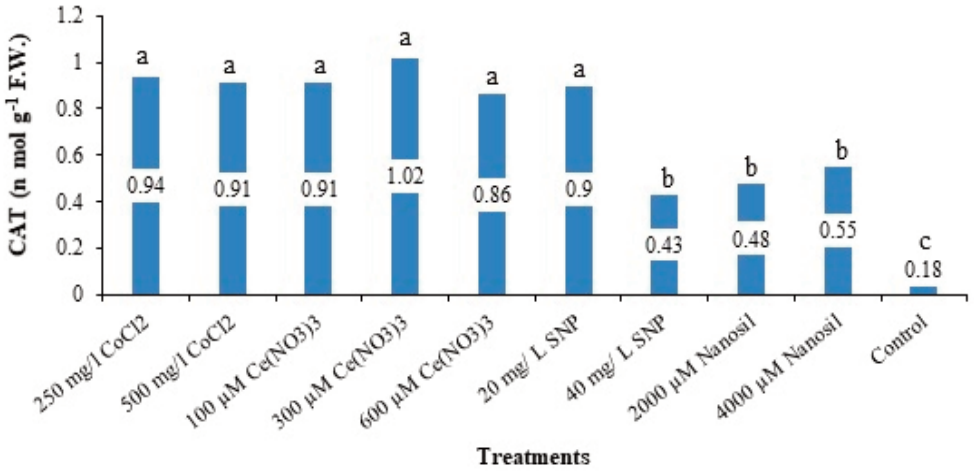

Fig. 10. The effect of $CoCl_2$, $Ce(NO_3)_3$, SNP, and Nanosil on the catalase activity of cut *S. reginae* flowers.

Catalase (CAT) activity

CAT activity was significantly ($P < 0.01$) increased in the treated plants versus the control (Table 1). The highest CAT activity (1.02 nmol g^{-1} FW) was related to the treatment of 300 µM $Ce(NO_3)_3$, which was not significantly different from the treatments of 100 and 600 µM $Ce(NO_3)_3$, 250 and 500 mg L^{-1} $CoCl_2$, and 20 mg L^{-1} SNP. The lowest CAT activity (0.18 nmol g^{-1} FW) was observed in the control (Fig. 10).

DISCUSSION

The capability and persistence of water uptake and its mobilization along the stem are the key factors determining the postharvest vase life of cut flowers. In most cut flowers, xylems are responsible for the supply and translocation of water along the stem. So, the blockage of the xylems in any form would directly affect the postharvest freshness and longevity of cut flowers negatively. One of the primary causes of xylem blockage is the growth and activity of microorganisms. So,

the most common method to protect the health and performance of xylems at the postharvest stage is to apply antibacterials in the vase solution (Damunupola and Joyce, 2006). In the present study, the application of $CoCl_2$, SNP, Nanosil, and $Ce(NO_3)_3$, which all have antimicrobial activities, reduced the bacteria population of the vase solution and stem end significantly versus the control. The lower bacteria population increased water uptake, thereby preserving fresh weight, increasing dry matter, and prolonging vase life of cut *S. reginae* flowers. It can, therefore, be said that the reduction of vase solution and stem-end microbial load in the present study contributed to preserving the health of xylems and persistence of water uptake in cut *S. reginae* flowers, thereby protecting the freshness and longevity of the cut flowers for a longer time through preserving fresh weight and supplying the energy required for the survival (via sucrose uptake from the vase solution).

Preceding studies have also reported that disinfectants increase the vase life of cut flowers by reducing microbial load, sustaining water uptake, and preventing fresh weight loss (Oraee *et al*., 2011; van Doorn, 2012; Hosseinzadeh *et al*., 2014). In Lin *et al*.'s (2019) study, the application of antibacterials in the vase solution reduced the growth and accumulation of microorganisms, increased water uptake, and protected long-term freshness of cut carnations. Similar results were reported by Al Humaid (2005), which are consistent with our findings.

The positive effect of Co in reducing bacteria growth, inhibiting xylem blockage, enhancing water uptake, preserving freshness, and prolonging vase life has been reported in cut roses (Venkatesh Reddy, 1988; Aslmoshtaghi *et al*., 2014), carnations (Kazemi *et al*., 2012), and chrysanthemums (Amin, 2017), which is in agreement with our results.

The application of Nanosil and SNP increased vase life effectively when compared to the control. The antibacterial impact of SNP has been proven on a wide range of microorganisms. SNP kills bacteria by penetrating their cells and disrupting their respiration chain (Damunupola and Joyce, 2006). There are scientific reports that the application of SNP in the vase solution reduces microbial populations, improves water uptake, and prolongs the vase life of cut carnations (Naing *et al*., 2017) and gerbera (Solgi *et al*., 2009). Although research is little on the effect of Nanosil on vase life, Shadbash and Keshavarzshal (2016) reported that the application of Nanosil in the vase solution of cut roses increased water uptake and extended their vase life by reducing stem-end bacteria load, which is consistent with our findings.

$Ce(NO_3)_3$ is a metallic salt that has recently come into use as a constituent of vase solutions of cut flowers. Cerium has antibacterial activities (Huang, 2002). However, its impacts on suppressing vase solution and stem-end microorganisms of cut flowers have not been researched yet. There are, nonetheless, some reports as to its antioxidant impact on increasing vase life. The positive effect of $Ce(NO_3)_3$ has been reported on increasing the postharvest vase life of cut *Lilium* (Houa *et al*., 2018) and carnations (Zheng and Guo, 2018), which corroborates our findings.

Flavonoids are secondary metabolites of plants that are involved in the formation of flower color. These compounds have antioxidant properties (Li *et al*., 2015). Schoner and Krause (1990) reported the effect of flavonoids in scavenging free oxygen radicals. The decline in flower pigments is a symptom of aging in cut flowers. In the present research, the application of disinfectants increased flavonoids versus the control. The favorable effect of these metallic salts on flavonoids can be related to their impact on retarding aging and early withering. Hashemabadi and Bagheri (2013) argue that the application of disinfectants contributed to preserving vase life and flavonoid content of cut flowers through increasing water uptake, which is consistent with our results.

Aging of cut flowers generally starts with an increase in the synthesis of free oxygen radicals (hydrogen peroxide, hydroxide radicals, and superoxide anions). The activity and accumulation of reactive oxygen species (ROS) in plant tissues induces oxidative stress, damages membrane, and results in MDA accumulation. The increase in the peroxidation of lipids in particular and macromolecules in particular, as well as MDA accumulation, leads plants towards death. Plants

employ enzymatic and non-enzymatic defensive systems to cope with free oxygen radicals. So, when cut flowers are exposed to water stress, the activity of their antioxidant system is increased to scavenge free oxygen radicals. The enzymatic defensive systems, e.g., CAT and POD, are among the plants' defensive lines against free oxygen radicals and the detrimental impacts of oxidative stress. Antioxidant enzymes inactivate and remove ROS. These enzymes donate oxygen to free oxygen radicals, thereby neutralizing their oxidating capability and protecting the plants against the damages of oxidative stress and lipid peroxidation (Carlos *et al*., 1996; Mittler, 2002; Rohi *et al*., 2010).

The prolongation of the vase life of cut flowers following the increased level of antioxidant activities has been reported by several researchers (Gerailoo and Ghasemnezhad, 2011; Tanazad *et al*., 2016; Wang *et al*., 2017). Tanazad *et al*. (2016) argue that the activity of antioxidant enzymes signals that cells are active. On the other hand, MDA accumulation and lipid peroxidation are signs of early aging and withering of cut flowers (Shan and Zhao, 2015; Song *et al*., 2014). In the present study, the application of disinfectants reduced MDA and increased the activity of antioxidant enzymes. The desirable impact of these compounds can be ascribed to their antibacterial activity, the reduction of microbial load, the preservation of water uptake, the conservation of cell turgor, and in general, the survival of the cells.

Cerium is an antioxidant compound. The application of $Ce(NO_3)_3$ in the vase solution of cut flowers augments the activity of antioxidant enzymes and reduces MDA accumulation (Houa *et al*., 2018; Zheng and Guo, 2018). Houa *et al*. (2018) reported that the application of $Ce(NO_3)_3$ extended the postharvest vase life of cut Lilium through increasing the activity of antioxidant enzymes and reducing MDA accumulation. Similar results were reported by Zheng and Guo (2018) for cut carnations, which supports our findings. Wang *et al*. (2017) revealed that the highest activity of CAT and POD and the lowest amount of MDA were obtained in cut roses when the vase solution was applied with 30 µM $Ce(NO_3)_3$. In Shadbash and Keshavarzshal's (2018) research, the application of Nanosil and SNP in the vase solution increased the activity of antioxidant enzymes and prolonged the postharvest longevity of cut roses, which is similar to our findings.

CONCLUSION

This research revealed that all treatments ($CoCl_2$, SNP, Nanosil, $Ce(NO_3)_3$) improved the recorded traits versus the control. However, the most influential compound on extending vase life and improving the related traits was $Ce(NO_3)_3$ at two levels of 300 and 600 µM. Given the environmental and economic significance of consuming chemicals at lower rates, it is recommended to apply $Ce(NO_3)_3$ at a rate of 300 µM as the most appropriate treatment for improving the vase life of cut *S. reginae* flowers.

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