

EXTENDING VASE LIFE OF CUT *Strelitzia reginae* Aiton FLOWERS BY COBALT CHLORIDE, CERIUM NITRATE, SILVER NANOPARTICLES AND NANOSIL

Jahangir Azarhoosh¹, Davood Hashemabadi¹✉, Leila Asadpour², Behzad Kaviani¹

¹Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran

²Department of Microbiology, Rasht Branch, Islamic Azad University, Rasht, Iran

ABSTRACT

Cut flowers of *Strelitzia reginae* Aiton (Strelitziaceae) generally have a short vase life. Vascular blockage is a major reason for this. In this paper, we evaluated the effects of pulse treatment with disinfectants including cobalt chloride (CoCl₂), cerium nitrate (Ce(NO₃)₃), silver nanoparticles (SNP) and Nanosil on the vase life and physiological characteristics of cut *S. reginae* flowers stems. Cut flowers kept in the vase solution containing these disinfectants showed significant increase in solution uptake, the content of total protein and pigments of petals, the activities of antioxidative active enzymes superoxide dismutase (SOD) and ascorbate peroxidase (APX). Also, the number of stem-end bacteria and malondialdehyde (MDA) content in cut flowers were decreased as compared to control. Based on obtained results, we introduce Ce(NO₃)₃ as the most effective treatment to extend the vase life of cut *S. reginae* flowers. More so with the concentration of 300 μM which induced the maximum solution uptake and SOD and APX activities that resulted in the longest vase life. Findings of the present study suggested that Ce(NO₃)₃ prolonged postharvest longevity of *S. reginae* by increasing the solution uptake and SOD and APX activity and decreasing the MDA content. The use of Ce(NO₃)₃ reduces the use of chemicals and make saving in costs. The highest bacterial population of micro-organisms on cut stem ends were *Escherichia coli*, *Bacillus*, *Staphylococcus* and *Streptococcus*. Cerium nitrate had the strongest effect on reduction of these bacterial population and yeast.

Key words: antimicrobial compounds, lipid peroxidation, postharvest longevity, vascular blockage, microbial population, stem end

INTRODUCTION

Bird-of-paradise (*Strelitzia reginae*, Strelitziaceae) is a major cut flower crop in warmer regions. Irregular opening of florets and premature wilting of florets are causes of its short vase life and its non-adoption by consumers [Finger et al. 1999]. Vase solutions contain sugars and disinfectants. Sugars act as an energy source and a respiration substrate and supply the energy required to delay senescence and death. However, sugar-containing solutions provide an ideal environ-

ment for the growth of microorganisms [Nair et al. 2000, Amin 2017]. The increase in the microbial population in vase solutions shortens vase life in different ways, e.g. the blockage of xylems and the disruption in water uptake. Therefore, it is necessary to apply disinfectants along with sugar compounds in preservative solutions in order to prevent the growth and propagation of microbes in vase solution and the accumulation of microorganisms in the vessels of the cut stem. One

of the sources of acid production in the vase solution is metal salts, the anion of which produces acid with water. Nitric acid from cerium nitrate is a weak acid and its pH is around 5.70 to 6.15. But, acids such as sulfuric acid (from aluminum sulfate) and hydrochloric acid (from cobalt chloride) produce strong acids and can prevent the growth of bacteria. Thereby, solution uptake and the freshness of cut flowers are maintained for a longer time.

Various disinfectants have traditionally been used to limit microbial growth in vase solutions. Cobalt chloride is a metal salt that is effective in extending the vase life of cut flowers. Cobalt has antimicrobial and anti-ethylene activities. The positive effect of cobalt has been reported on inhibiting the growth of microorganisms, vascular blockage, ethylene synthesis, and stomatal closure during water deficit [Murali and Reddy 1993, Amin 2017]. As well, there are reports as to the effect of cobalt application in vase solution on prolonging the vase life of cut tuberose [Mohammadi et al. 2012], carnation (*Dianthus caryophyllus* L.) [Kazemi et al. 2012], roses (*Rosa*) [Tiwari and Singh 2002], and chrysanthemum [Amin 2017] flowers. Aslmoshtaghi et al. [2014] reported that the application of cobalt chloride up to 200 mg l⁻¹ reduced vascular blockage, increased water uptake, decreased electrolyte leakage, and prolonged vase life of cut roses.

Silver has antibacterial effects and its application in vase solution hinders microorganism growth and vascular blockage [Nowak 1990, Damunupola and Joyce 2006] and thereby extended the vase life [Hasan and Schmidt 2004]. Silver nanoparticles have long replaced conventional silver compounds (silver nitrate and silver thiosulfate) in vase solutions due to their higher area-to-volume ratio, higher efficiency, and lower toxicity. Silver nanoparticles penetrate into the cells of bacteria, disrupt their respiration chain, and cause disorder in their cell division, thereby killing them [Park et al. 2005, Maneerung et al. 2008]. They also inhibit the accumulation of bacteria in vase solution and stem end of cut flowers. Various studies have reported the positive impact of silver nanoparticles on decreasing the microbial load [Oraee et al. 2011], reducing transpiration from leaf surface [Lu et al. 2010], and preserving water uptake [Ansari et al. 2011]. The application of silver nanoparticles in the vase solution increased their vase life of gerbera cut stem [Solgi et

al. 2009], *Lilium* spp. [Kim et al. 2005] and rose [Hasan et al. 2014]. Liu et al. [2009] revealed that silver nanoparticles doubled the vase life of cut *Gerbera* flowers versus the control.

Nanosil is composed of hydrogen peroxide and silver nanoparticles and is used to disinfect water. There is little research on the effect of Nanosil on the vase life of cut flowers [Shadbash and Keshavarzshal 2018]. However, there are various reports as to the effect of its components including hydrogen peroxide [Shadbash and Keshavarzshal 2018] and silver nanoparticles [Kim et al. 2005, Solgi et al. 2009] on the vase life of different cut flowers. Shadbash and Keshavarzshal [2018] reported that the treatment of cut roses ‘Grand Press Angela’ with Nanosil prolonged the vase life significantly by reducing the microbial load of stem ends during vase life, enhancing water uptake, and preserving fresh weight.

Cerium is a trace element on the earth’s crust whose positive effect has been reported on improving plant oxidative activity [Wu et al. 2014]. Antioxidant enzymes increase the vase life of cut flowers by scavenging reactive oxygen species (ROS) and maintaining membrane integrity [Alaey et al. 2011, Shan and Zhao 2015, Wang et al. 2017]. Therefore, cerium can improve the vase life of cut flowers by increasing the activity of antioxidant enzymes. However, little research has been conducted on the effect of cerium on the vase life of cut flowers. Wang et al. [2017] reported that cerium nitrate had a positive impact on improving vase life, reducing wilted flowers, increasing the number of open florets, preserving pigments, and reducing MDA and H₂O₂ content. Also, its application at the rate of 30 µM significantly increased the activity of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX) and glutathione S-transferase (GST) enzymes. There are some reports that the application of cerium nitrate prolongs the vase life of *Lilium longiflorum* [Houa et al. 2018] and *Dianthus caryophyllus* [Zheng and Guo 2018] by enhancing their antioxidant system and maintaining their membrane integrity.

The most important factor in reducing the vase life of cut bird-of-paradise flowers is the deficiency of carbohydrate for the development of florets and the obstruction of xylems by microorganisms [Finger

et al. 1999, Ali and Hassan 2014]. The present study aimed to investigate the effect of silver nanoparticles, Nanosil, cobalt chloride, and cerium nitrate with 3% sucrose on the control of microbial load, the activity of antioxidant enzymes, and the vase life of the cut flowers of bird-of-paradise and finally to introduce the most appropriate preservative for prolonging the vase life of this cut flower. No study has been performed on the effect of Nanosilver on postharvest longevity of bird-of-paradise. There have also been very few studies on the antimicrobial effect of cerium nitrate on cut flower microbes. These statements reveal the novelty of this research.

MATERIALS AND METHODS

The effect of chemicals and disinfectants on the vase life and related traits of bird-of-paradise cut flower was explored in an experiment based on a completely randomized design with three replications. The cut flowers were purchased from the growers at the harvest stage for commercialization. Then, they were immediately packaged and transferred to the laboratory. After making the flowers-stems uniform, they were re-cut to a length of 45 cm under running tap water. To prepare uniform cut flowers, as soon as the flowers were received from the greenhouse, first the stems were cut 47 cm long out of the water and then

2 cm of them were cut under water and thus all the flowers were set to 45 cm. Then, they were pulse treated with solutions containing cobalt chloride (CoCl_2 ; 250 and 500 mg l^{-1}), cerium nitrate ($\text{Ce}(\text{NO}_3)_3$; 100, 300 and 600 μM), silver nanoparticles (SNP; 20 and 40 μM), and Nanosil (2000 and 4000 μM). Distilled water was used as the control treatment. After the pulse treatments, the flowers were transferred to vases containing 250 ml of distilled water and 3% sucrose and were stored in a room with a controlled environment conditions ($20 \pm 2^\circ\text{C}$, 60–70% relative humidity, 12 h of light at an intensity of $15 \mu\text{M m}^{-2} \text{s}^{-1}$) until the end of the experiment.

Assessment of traits

Vase life. It was calculated by counting the days from treatment with disinfectant and antioxidant until wilting of 75% of florets [Gendy and Mahmoud 2012] (Fig. 1).

Solution uptake. It was measured by the following equation:

$$\text{Solution uptake (ml/g FW)} = \frac{V_{i0} - (E_t + V_{tl})}{\text{FW}}$$

where: V_{i0} is the initial solution volume, V_{tl} is the solution volume on the final day, E_t is the final quantity of evaporation from the solution level, and FW is the flower fresh weight on the first day. In the vase

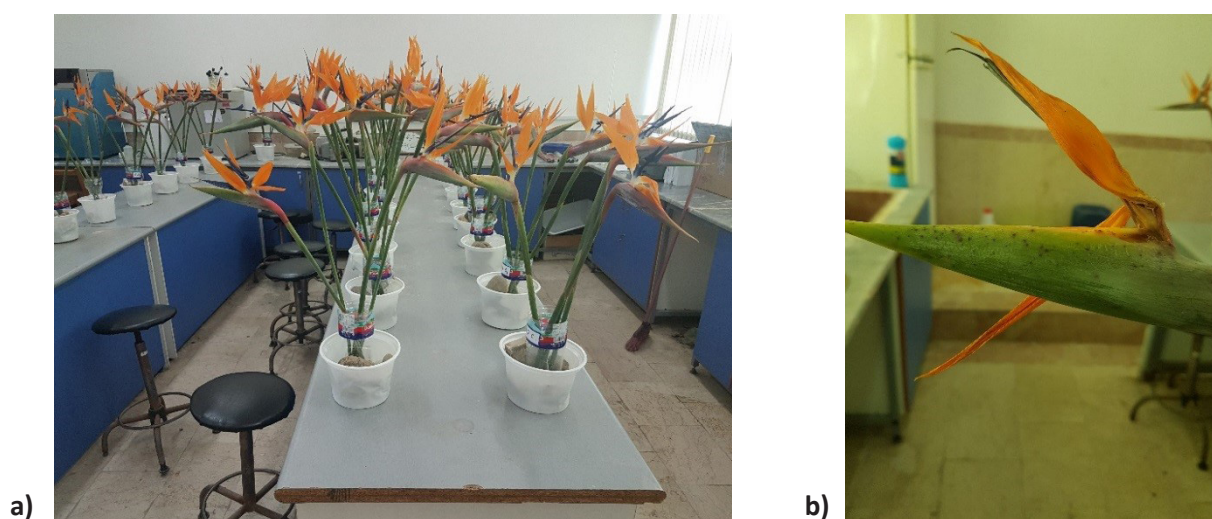


Fig. 1. Sterilized cut flowers on the first (a) and final (b) days of the experiment

life room, 4 vases containing 500 ml of vase solution (without flowers) were kept in different places and their average evaporation were calculated in the water absorption formula.

Stem-end bacteria colony. The number of bacteria colonies was measured at two levels including first and second cm of cut stem ends. The 24 hours after the application of pulse treatments, the stem ends were sampled, separately. They were rinsed with distilled water followed by extracting with 0.9% normal saline serum. Then, 0.1 mL of the solution was cultured on agar and was put in an incubator at 37°C for 24 h. Then, bacteria colonies were counted by a microscope [Liu et al. 2009].

Microbial identification. After plate counting, obtained colonies were studied based on their morphology and different colonial types were randomly selected for further investigation. Some morphological and biochemical characteristics such as Gram-stain morphology, growth and colony morphology on MacConkey agar, motility, oxidase and catalase production, gelatin hydrolysis, starch hydrolysis, indol production, urease production, methyl red reaction, acetoin production (VP), citrate utilization, nitrate reduction and H₂S production were analysed [Schaad et al. 2001, Jowkar et al. 2017]. Yeast were only recognized based on morphology of colony and Gram staining appearance.

Sepal carotenoids. Seeing the first signs of wilting in the first florets in the room, samples were taken from all plots and chemical analysis was performed on the samples. Sepals were extracted with 80% acetone. The absorbance of the infiltrated extract was read at 440, 645 and 663 nm with an APEL, PD-103UV spectrophotometer. Then, carotenoids content was measured in µg g⁻¹ FW by the following equation [Mazumdar and Majumder 2003].

$$\begin{aligned} \text{Carotenoids in sepal} = \\ = (4.69 \times A_{440}) - (0.286 \times 20.0 \times A_{645}) + (8.02 \times A_{668}) \end{aligned}$$

Sepal proteins. It was measured by the Kjeldahl indirect method. Sepal tissues were extracted with sulfuric acid, salicylic acid, and hydrogen peroxide. Following digestion, nitrogen percentage and total protein content were calculated by following formula.

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

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

where: *t* represents the concentration of acid used for titration, *a* represents the amount of acid used for sample, *b* represents the amount of acid used for control, *V* represents the volume of extract derived from digestion, *W* represents the weight of plant sample for digestion, and DM represents the plant dry matter percentage.

Petal flavonoids. To measure the amount of petal flavonoids, 0.1 of sepal was extracted with 2.5 mL of 1% acidic ethanol and was centrifuged for 15 min. The supernatant was extracted with a sampler and was placed in a hot water bath (85°C) for 10 min. The absorbance was then read at 270 and 300 nm [Krizek et al. 1998].

Malondialdehyde (MDA). The content of MDA was measured by Heath and Parker method [1968]. Based on the method, 0.5 g of petal tissue was first extracted with liquid nitrogen and potassium phosphate buffer. The extract was centrifuged several times and each time the supernatant was separated with a sampler. Then, 200 µL of the supernatant was added with 1000 µL of TCA and TBAS. The sample was placed in a hot water bath for 30 min and then, it was transferred into an ice container for 30 min. The cooled solution was centrifuged at 4°C for 10 min. The absorbance of the solution was read at 532 and 600 nm with a spectrophotometer. Finally, the following equation was used to calculate the MDA content of the petal tissue.

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

Antioxidant enzymes. To measure the activity of antioxidant enzymes, the sepals of the replications were separately sampled. Then, their tissues were extracted with potassium phosphate buffer. The extract was centrifuged at a low temperature. After a centrifuge period, the transparent supernatant was used as the enzymatic extract to measure SOD (EC 1.15.1.1) and APX (EC 1.11.1.11) activity.

Superoxide dismutase (SOD) activity. The reaction mixture to measure SOD activity was composed of enzymatic extract (0.1 mL), nitro blue tetrazolium chloride (NBT) (25 mM), methionine (13 mM), EDTA (0.1 mM), sodium carbonate (50 mM), and potassium phosphate buffer (50 mM), which was shaken under fluorescent light at 22°C. The samples were kept in

a dark room for 30 min. Then, their absorbance was read at 560 nm with a Shimadzu UV-120-02 spectrophotometer [Giannopolitis and Ries 1997].

Ascorbate peroxidase (APX) activity. APX activity was measured by Chen and Asada [1989] method. To this work, 200 μL of enzymatic extract, 1280 μL of 50 mM potassium phosphate buffer, 500 μL of 1 mM ascorbate, and 20 μL of 10 mM hydrogen peroxide were gently mixed. Then, the sample absorbance was read at 290 nm in 2-min intervals with a Shimadzu UV-120-02 spectrophotometer.

Experimental design and statistical analysis

The experiment was done based on a completely randomized design with three replications, 30 plots and 4 flowers per plot (totally; 120 flowers). Data were analyzed by MSTATC statistical software package and

means were compared with the Least Significant Difference (LSD) test.

RESULTS

Vase life. The chemicals used in this study influenced the vase life of the bird-of-paradise significantly at the $P < 0.01$ level (Tab. 1). Among the treatments, the control exhibited the shortest vase life (8.06 days). The longest vase life (11.68 days) was obtained from the flowers treated with 300 μM $\text{Ce}(\text{NO}_3)_3$, differing from that of the flowers treated with 600 and 100 μM $\text{Ce}(\text{NO}_3)_3$, 20 mg l^{-1} SNP, and 500 mg l^{-1} CoCl_2 , insignificantly (Tab. 2).

Solution uptake. The application of disinfectants increased the solution uptake of the cut flowers, significantly ($P < 0.01$) compared to the control flowers

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

S.o.V	df	Vase life	Solution uptake	Bacterial population in 1 cm of stem end	Bacterial population in 2 cm of stem end	Sepal carotenoids	Total protein	Flavonoid contents at 270 nm	Flavonoid contents at 300 nm	MDA	SOD	APX
Treatments	9	3.085**	0.000551**	12929**	17489**	0.03533*	3.014**	0.0158**	0.0179**	1.110**	38.01**	13.36**
Error	20	0.421	0.000	215.839	3616.936	0.012	0.600	0.002	0.004	0.009	4.900	0.400
CV (%)	–	6.290	5.609	18.719	78.356	58.205	23.710	9.575	12.306	12.942	8.160	18.117

* and ** significant at $P < 0.05$ and $P < 0.01$ respectively

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

Treatments	Vase life (day)	Solution uptake (ml g^{-1} FW)	Sepal carotenoids ($\mu\text{g g}^{-1}$ FW)	Total protein (%)	Flavonoid contents at 270 nm (%)	Flavonoid contents at 300 nm (%)	MDA (nmol g^{-1} FW)	SOD (IU g^{-1} FW)	APX (IU g^{-1} FW)
250 mg l^{-1} CoCl_2	10.57 ^{bd}	0.094 ^{de}	0.130 ^{bc}	2.94 ^c	0.586 ^a	0.490 ^{bc}	0.44 ^{de}	28.29 ^{ab}	5.40 ^{ab}
500 mg l^{-1} CoCl_2	10.63 ^{ad}	0.110 ^{bc}	0.164 ^{bc}	4.51 ^a	0.433 ^{cd}	0.526 ^b	0.40 ^e	30.37 ^a	5.18 ^{ab}
100 μM $\text{Ce}(\text{NO}_3)_3$	10.87 ^{ac}	0.095 ^{de}	0.351 ^a	4.16 ^{a-c}	0.586 ^a	0.510 ^b	0.39 ^e	29.88 ^{ab}	4.85 ^b
300 μM $\text{Ce}(\text{NO}_3)_3$	11.68 ^a	0.128 ^a	0.381 ^a	4.37 ^{ab}	0.546 ^{ab}	0.470 ^{bc}	0.09 ^f	30.87 ^a	6.03 ^a
600 μM $\text{Ce}(\text{NO}_3)_3$	11.08 ^{ab}	0.125 ^a	0.284 ^{ab}	3.20 ^{a-c}	0.496 ^{bc}	0.693 ^a	0.35 ^e	30.47 ^a	5.55 ^{ab}
20 mg l^{-1} SNP	10.89 ^{ac}	0.117 ^{ab}	0.141 ^{bc}	3.07 ^{bc}	0.426 ^{cd}	0.533 ^b	0.57 ^d	27.20 ^{abc}	3.00 ^c
40 mg l^{-1} SNP	9.76 ^d	0.102 ^{cd}	0.121 ^{bc}	2.85 ^c	0.433 ^{cd}	0.530 ^b	1.63 ^b	23.42 ^{de}	1.24 ^d
2000 μM Nanosil	9.74 ^d	0.118 ^{ab}	0.107 ^{bc}	3.51 ^{a-c}	0.453 ^c	0.503 ^b	1.25 ^c	26.30 ^{bd}	1.08 ^d
4000 μM Nanosil	9.80 ^{cd}	0.118 ^{ab}	0.116 ^{bc}	3.04 ^c	0.453 ^c	0.573 ^b	0.37 ^e	24.12 ^{c-e}	1.65 ^d
Control	8.06 ^e	0.088 ^e	0.084 ^c	1.02 ^d	0.373 ^d	0.390 ^c	1.84 ^a	20.35 ^e	0.93 ^d

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

(Tab. 1). Based on the mean comparison, two treatments of 300 and 600 μM $\text{Ce}(\text{NO}_3)_3$ had the highest solution uptake ($0.125 \text{ ml g}^{-1} \text{ FW}$), but they did not differ from the treatments of 2000 and 4000 μM Nanosil and 20 mg l^{-1} SNP, significantly. The lowest solution uptake was $0.088 \text{ ml g}^{-1} \text{ FW}$ related to the control (Tab. 2).

Stem end bacteria populations. The bacteria colonies were counted in 1 and 2 cm-segments of the end of the stems. The results of the analysis of variance revealed that the effect of the treatments was significant ($P < 0.01$) on the number of bacteria colonies in these two points (Tab. 1). The highest number of colonies was observed in the control. The least number was 9.47×10^6 counted in the 1st cm stem end and 5.04×10^6 counted in the 2nd cm stem end. They were related to the flowers treated with 600 μM $\text{Ce}(\text{NO}_3)_3$, but not differing from that of the treatments of 300 μM $\text{Ce}(\text{NO}_3)_3$ and 20 and 40 mg l^{-1} SNP, significantly (Fig. 2).

Microbial identification. The identified bacterial in the stem are shown in Table 3. The highest bacterial population of cut stem ends belongs to *Escherichia coli*, *Bacillus*, *Staphylococcus* and *Streptococcus*. As can be seen, Nanosilver and Nanosil have prevented yeast growth, while in cut flowers treated with $\text{Ce}(\text{NO}_3)_3$, the presence of yeast has been reported.

The lowest diversity of microbial agents in the 1 cm of end stem belonged to the treatments of 300 μM $\text{Ce}(\text{NO}_3)_3$ and 4000 μM Nanosil that only one microbial agent was identified in the stems treated with these compounds (Tab. 3).

Sepal carotenoids. The effect of the treatments was significant ($P < 0.05$) on sepal carotenoids-concentrations (Tab. 1). It was found that the treatments with 300 and 100 μM $\text{Ce}(\text{NO}_3)_3$ showed the highest sepal carotenoids content (0.381 and $0.351 \mu\text{g g}^{-1} \text{ FW}$), respectively, but they were in the same statistical group with the treatment of 600 μM $\text{Ce}(\text{NO}_3)_3$. The lowest sepal carotenoids content ($0.084 \mu\text{g g}^{-1} \text{ FW}$) was observed in the control flowers (Tab. 2).

Sepal proteins. All disinfectants used in the study increased sepal proteins content significantly at the $P < 0.01$ level (Tab. 1). The comparison of the means revealed that sepal protein content was the lowest (1.02%) in the control. The highest was 4.51% observed in the flowers treated with 500 mg l^{-1} CoCl_2 , not differing from that of 100, 300, and 600 μM $\text{Ce}(\text{NO}_3)_3$ and 2000 mg l^{-1} Nanosil, significantly (Tab. 2).

Petal flavonoids. Petal flavonoids was measured at the 270 and 300 nm absorbance level. The analysis of variance showed that the effect of chemical compounds was significant ($P < 0.01$) on the petal flavo-

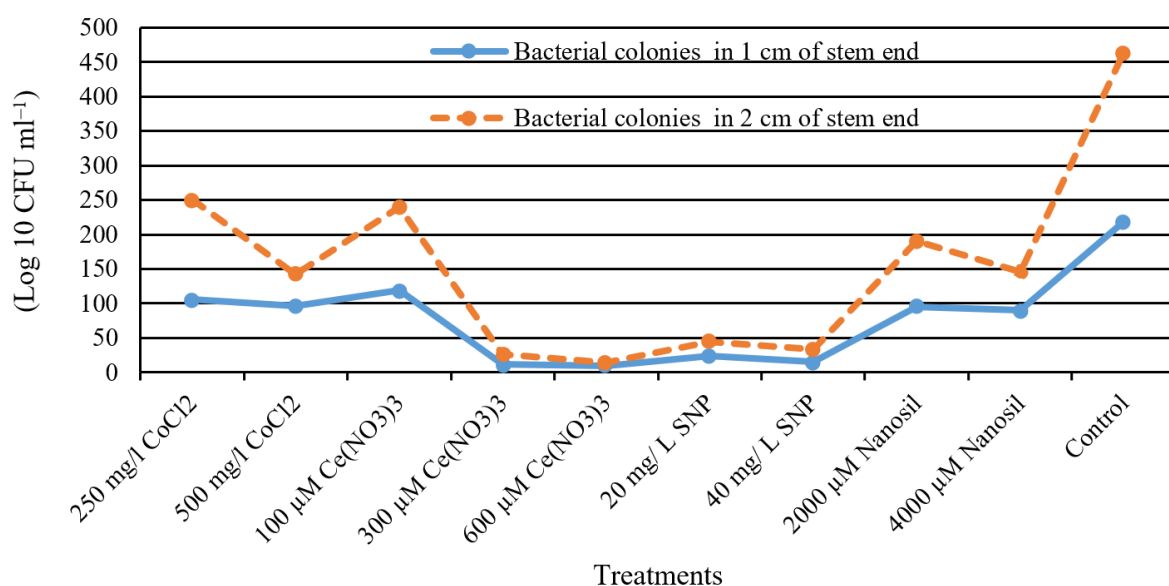


Fig. 2. Effect of different treatments on the stem-end bacteria (first and second centimeters)

Table 3. Microbial identification (identification of bacteria) of first and second cm of cut stem end

Treatments	First cm of cut stem ends	Second cm of cut stem ends
250 mg l ⁻¹ CoCl ₂	<i>Escherichia coli</i> , yeast, <i>Bacillus</i>	<i>Escherichia coli</i>
500 mg l ⁻¹ CoCl ₂	<i>Bacillus</i> , <i>Staphylococcus</i> , yeast	<i>Escherichia coli</i> , <i>Enterobacter</i>
100 μM Ce(NO ₃) ₃	<i>Bacillus</i> , <i>Citrobacter</i> , <i>Escherichia coli</i>	<i>Staphylococcus</i> , yeast
300 μM Ce(NO ₃) ₃	yeast	<i>Staphylococcus</i> , yeast
600 μM Ce(NO ₃) ₃	<i>Actinobacillus</i> , <i>Escherichia coli</i>	<i>Staphylococcus</i> , <i>Escherichia coli</i>
20 mg l ⁻¹ SNP	<i>Bacillus</i> , <i>Escherichia coli</i>	<i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Escherichia coli</i>
40 mg l ⁻¹ SNP	<i>Staphylococcus</i> , <i>Bacillus</i> , <i>Escherichia coli</i> , <i>Pseudomonas</i>	<i>Streptococcus</i>
2000 μM Nanosil	<i>Bacillus</i> , <i>Staphylococcus</i>	<i>Streptococcus</i> , <i>Staphylococcus</i>
4000 μM Nanosil	<i>Streptococcus</i>	<i>Streptococcus</i> , <i>Staphylococcus</i>
Control	<i>Bacillus</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>	<i>Staphylococcus</i> , <i>Escherichia coli</i>

noids of bird-of-paradise (Tab. 1). Flavonoids was the lowest in the control at both 270 and 300 nm levels. The highest flavonoids content at the 270 nm level was related to the treatment of 250 mg l⁻¹ CoCl₂ and 100 and 300 μM Ce(NO₃)₃. These treatments did not differ from one another, significantly. The highest flavonoids content at the 300 nm (0.693%) was obtained from the application of 600 μM Ce(NO₃)₃ (Tab. 2).

Malondialdehyde (MDA). The application of disinfectants to the vase solution reduced MDA accumulation significantly at the P < 0.01 level (Tab. 1). The comparison of the means showed that 300 μM Ce(NO₃)₃ was the most successful treatment in reducing MDA accumulation (0.09 nmol g⁻¹ FW). The highest MDA accumulation was 1.84 nmol g⁻¹ FW observed in the control (Tab. 2).

Superoxide dismutase (SOD) enzyme. Based on the comparison of the means, the treatment of 300 μM Ce(NO₃)₃ had the highest SOD activity (30.87 IU g⁻¹ FW), but it did not differ from the treatments of 100 and 600 μM Ce(NO₃)₃, 250 and 500 mg l⁻¹ CoCl₂, and 20 mg l⁻¹ SNP, significantly. The lowest SOD activity (20.35 IU g⁻¹ FW) was obtained from the control cut flowers (Tab. 2). The effect of the treatments was significant (P < 0.01) on SOD activity (Tab. 1).

Ascorbate peroxidase (APX) enzyme. The impact of disinfectants was found to be significant (P < 0.01) on APX activity (Tab. 1). As the means comparison showed, the flowers treated with 300 μM Ce(NO₃)₃

had the highest APX activity (6.03 IU g⁻¹ FW). However, it did not differ from the treatments of 600 μM Ce(NO₃)₃ and 250 and 500 mg l⁻¹ CoCl₂, significantly. The lowest APX activity was related to the control, which was in the same statistical group with the treatments of 2000 and 4000 mg l⁻¹ Nanosil and 40 mg l⁻¹ SNP (Tab. 2).

DISCUSSION

The vase life of cut flowers heavily depends on water uptake through the end-stem cut, the uninterrupted mobilization of water along the stem, temperature, cut flower nutrition, ethylene suppression and so on. The blockage of xylem by microorganisms limits the mobilization of water to higher parts of stems and flowers and accelerates the wilting of flowers [van Doorn 2012, Hassan et al. 2020]. Disinfectants hinder stem blockage by suppressing the microbial load in the vase solutions and help preserve water status of flower tissue by increasing water uptake, thereby maintaining the freshness and vase life of cut flowers for a longer time [Figuerola et al. 2005, Abri et al. 2014]. We found that the application of disinfectants and antioxidant (SNP, Nanosil, CoCl₂, and Ce(NO₃)₃) in the vase solution of the cut flowers of *Strelitzia reginae* reduced stem-end bacteria colony, increased solution uptake, and prolonged vase life, significantly compared to the control. The best treatment in reducing stem-end mi-

crobial population was cerium nitrate, which expectedly exhibited the highest solution uptake and longest vase life among all treatments.

Lin et al. [2019] found that the application of disinfectants in the vase solution of cut carnation flowers enhanced water uptake and vase life by reducing the growth, propagation, and accumulation of bacteria at the end of stems and in the xylems. Amin [2017] showed that the use of metal salts in the vase solution of cut flowers, although they reduce the microbial population and increase vase life, but the desired concentration of these disinfectants is different for different flowers. Al-Humaid [2004] showed that the application of vase solutions containing glucose (5, 10 or 20%) and disinfectants (200 ppm penicillin together with 250 ppm streptomycin) increased vase life and postharvest quality of cut gladiolus flowers significantly, which is in agreement with our findings. Metallic salts, e.g. cobalt chloride, have an inhibitory effect on bacterial growth in vase solutions. Application of cobalt prevented the vascular blockage of roses effectively and enhanced water uptake [Venkatesh Reddy 1988]. The positive effect of cobalt on the preservation of fresh weight and vase life has been reported for cut carnations [Kazemi et al. 2012], cut chrysanthemums [Amin 2017], and cut roses [Aslmoshtaghi et al. 2014]. In the present study, the treatment of *Strelitzia reginae* with CoCl_2 improved vase life significantly versus the control, which is in agreement with the literature referred to above.

As already mentioned, when the flowers of *Strelitzia reginae* were treated with Nanosil and SNP, they exhibited longer vase life, higher water uptake, and lower stem-end bacteria than the control flowers. Silver ions have antimicrobial and anti-ethylene properties and also play a role in accelerating the closure of stomata during stress and maintaining cellular turgor. Therefore, the positive effect of silver-containing compounds on prolonging the vase life of cut flowers can be justified. In the study of Liu et al. [2009], the application of silver nanoparticles reduced microbes at 2 cm from the end of the stem and increased the vase life (5.3 days) of cut gerbera flowers compared to the control. Shadbash and Keshavarzshal [2018] reported that Nanosil reduced stem-end microbial load, increased water uptake, and prolonged vase life of cut roses, significantly. Also, they found that the applica-

tion of 20 mg l⁻¹ SNP was effective in reducing stem-end microbial load, increasing water uptake, and prolonging vase life versus the control. There are reports as to the positive effect of SNP in suppressing stem-end bacteria and increasing the vase life of cut gerberas flowers [Liu et al. 2009] and cut carnations [Naing et al. 2017], which is consistent with our findings. The decrease in leaf and petal pigments is a symptom of senescence.

We revealed that disinfectants increased sepal pigments as compared to the control. Water stress can cause pigment degradation through dysfunction of the photosynthetic apparatus. Disinfectant compounds increase water absorption, maintain cell turgor and preserve pigments by reducing microbial load. Carbohydrates play an important role in maintaining the intensity of color in plant tissues. Therefore, the supply of carbohydrates by the preservative solution plays an important role in preserving the pigments of leaves and petals in cut flowers. The positive effect of disinfectant compounds on the retention of pigments can be related to the effect of these compounds on increasing the absorption of solution and supply of carbohydrates required by the cut stem, as well as maintaining turgor and cellular health. Pigment content has been reported to increase in cut tuberose treated with CoCl_2 [Mohammadi et al. 2012], cut carnations and roses treated with SNP [Shadbash and Keshavarzshal 2018, Lin et al. 2019], cut roses treated with Nanosil [Shadbash and Keshavarzshal 2018], and cut roses treated with $\text{Ce}(\text{NO}_3)_3$ [Wang et al. 2017], which is in agreement with our findings.

Total protein content decreases during senescence because of the increased activity of proteases and the decrease in protein synthesis [van Doorn and Stead 1997]. So, reducing water deficit-stress can be effective in preserving the structure of membranes and proteins and retarding the death of plant tissues [Sood and Nagar 2003, Lerslerwong et al. 2009]. As expressed in the results, the vase solutions used in the study contributed to preserving sepal proteins by increasing water uptake and retarding wilting.

ROS causes lipid peroxidation and MDA accumulation in plant tissues [Arora et al. 2007]. Compounds that prolong the vase life of cut flowers are effective in preserving membrane structure and decreasing lipid peroxidation and MDA by increasing the activ-

ity of anti-oxidative enzymes and scavenging ROS [Ezhilmathi et al. 2007, Gerailoo and Ghasemnezhad 2011, Shan and Zhao 2015]. Anti-oxidative enzymes preserve the vase life of cut flowers [Hassan and Fetouh 2019]. Cerium is a substance with antioxidant property [He and Loh 2000, Yin et al. 2009] and antimicrobial activity [Huang 2002]. The application of $Ce(NO_3)_3$ in the vase solution has increased the vase life of cut roses [Wang et al. 2017], *Lilium longiflorum* [Houa et al. 2018], and carnation [Zheng and Guo 2018] by increasing the activity of antioxidants, which is consistent with our findings. Silver-containing compounds increase vase life by synthesizing proteins and increasing the activity of antioxidants [Zhuo et al. 1994].

According to Shadbash and Keshavarzshal [2018], the application of different levels of Nanosil (200, 400 and 600 μM) and SNP (10 and 20 $mg\ l^{-1}$) increased POD and CAT activity in cut roses. In agreement with our findings, it has been reported that the application of disinfectants in the vase solution of cut roses increased the activity of antioxidant enzymes and reduced MDA accumulation and protein degradation [Abri et al. 2013]. Hossain et al. [2006] revealed that the reduced activity of APX enzyme signals the initiation of senescence or cell death. It seems that disinfectants used in the present research contributed to preserving the vase life of cut *Strelitzia reginae* flowers by maintaining and increasing the activity of antioxidants.

CONCLUSIONS

The results showed that the disinfectants (Nanosil, SNP, $Ce(NO_3)_3$, and $CoCl_2$) were influential in extending the vase life of cut bird-of-paradise (*Strelitzia reginae* AITON) flowers. The most effective compound in increasing the vase life of this flower, which is the most important factor in the marketability of its cut flower, was found to be $Ce(NO_3)_3$ at the rates of 300 and 600 μM . These treatments were the most optimal in reducing microbial load, increasing water uptake, preserving pigments and proteins, reducing MDA accumulation, and enhancing the activity of antioxidant enzymes. Given the significance of chemical compound, the application of 300 μM $Ce(NO_3)_3$ is recommended as the most appropriate treatment to increase the vase life of *Strelitzia reginae*.

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