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Research Paper

The Effect of Cerium Nitrate and Salicylic Acid on Vase Life and Antioxidant System of Cut Lisianthus (*Eustoma grandiflorum* cv. Pink Picotte) Flowers

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Abstract

To reduce the postharvest loss of cut lisianthus 'Pink Picotte' flowers, an experiment was conducted based on a randomized complete design with three replications. The experimental treatments were composed of salicylic acid (SA) at three rates of 50, 100 and 200 mg L^{-1} and cerium nitrate ($Ce(NO_3)_3$) at four rates of 20, 40, 80 and 200 µM and distilled water (control) applied at 24-h pulses with 3% sucrose. It was found that SA and Ce $(NO_3)_3$ influenced all recorded traits significantly except for dry matter. The treatments of 40 µM Ce $(NO_3)_3$ and 100 mg L^{-1} SA performed the best in extending vase life so that they were associated with the longest vase lives of 15.42 and 15.20 days, respectively. In addition to improving vase life, these two treatments outperformed the other treatments in inhibiting the loss of fresh weight, reducing bacterial colony in vase solution, and increasing leaf chlorophyll. The highest catalase activity (8.57 IU g^{-1} FW min^{-1}) was observed in the plants treated with 40 µM Ce $(NO_3)_3$, not differing from the treatments of 50 and 100 mg L^{-1} SA significantly. Furthermore, these treatments were effective in increasing superoxide dismutase activity. The results revealed that the application of 200 mg L^{-1} SA had adverse impacts on the vase life and the related traits. Overall, it is not recommended to apply high concentrations of Ce $(NO_3)_3$ (80 and 200 µM) and SA (200 mg L^{-1}) in the vase solution of cut lisianthus 'Pink Picotte' flowers.

Keywords: Catalase, Cerium nitrate, Senescence, Superoxide dismutase, Vase solution.

INTRODUCTION

Cut flowers are a luxury commodity of the flower industry, and the retention of their economic value in target markets depends on the preservation of their post-harvest quality and freshness. As soon as flowers are separated from their mother plants, the activity of senescence accelerating factors increase in them, so a way to preserve the quality and freshness of cut flowers for a prolonged period is to stop or retard the activity of these factors. Researchers have always used various methods to postpone the activity of aging accelerating factors and extend the longevity of cut flowers. One of the simplest ways is to treat them with specific solutions to extend their vase life (Edrisi, 2009; Rahemi, 2011).

Salicylic acid (SA) is a plant growth regulator that is found throughout the entire plant kingdom and is involved in many biochemical and physiological processes. The effect of SA has been well documented on the expression of aging-related genes and plant responses to different stresses (Raskin, 1992; Alaey *et al*., 2011; Buchanan Wollaston *et al*., 2003). The positive role of SA has been reported in preserving postharvest quality and extending the postharvest life of various cut flowers (Alaey *et al*., 2011; Ezhilmathi *et al*., 2007; Nikkhah Bahrami *et al*., 2013). Researchers suggest that SA increases postharvest life of cut flowers through increasing the uptake of vase solution (Nikkhah Bahrami *et al*., 2013; Capdeville *et al*., 2003), hindering the loss of fresh weight (Ezhilmathi *et al*., 2007; Mori *et al*., 2001; Aelaei *et al*., 2017), preserving and increasing chlorophyll (Mei-hua *et al*., 2008; Aelaei *et al*., 2017), reducing ethylene biosynthesis (Serek, 1992), increasing the activity of antioxidant enzymes, reducing reactive oxygen species (ROS), and decreasing the peroxidation of membrane lipids (Ezhilmathi *et al*., 2007; Kazemi *et al*., 2011).

Cerium (Ce) is a trace element on the earth with an established role in increasing the activity of antioxidant enzymes in plants (Wu *et al*., 2014; Wang *et al*., 2017). There are reports about the impact of Ce on plant resistance to oxidative stresses (Wu *et al*., 2014; Liang *et al*., 2006) and the reduction of lipid peroxidation (Liu *et al*., 2016; Houa *et al*., 2018). Since reinforcing the antioxidant system of cut flowers is effective in suppressing ROS, maintaining membrane structure, and subsequently, extending their postharvest life, interests have been recently drawn to the treatment of cut flowers with Ce, which has an antioxidant property (Houa *et al*., 2018; Wang *et al*., 2017; Zheng and Guo, 2018).

Wang *et al*. (2017) reported that the treatment of cut rose flowers with cerium nitrate increased the number of open buds, reduced the number of withered flowers, and increased chlorophyll pigments and carotenoids. Furthermore, cerium increased the activity of antioxidant enzymes, reduced H_2O_2, and decreased malondialdehyde (MDA), thereby extending the vase life of the cut rose flowers versus the control plants significantly. The positive effect of Ce on improving the shelf life of cut flowers has also been reported for *Lilium* (Houa *et al*., 2018) and carnations (Zheng and Guo, 2018).

Lisianthus (*Eustoma grandiflorum*) is a major cut flower from the family Gentianaceae. This cut flower has a special place in global markets owing to its beauty, color diversity, flower shape, and its similarity to roses so that the demand for it is increasingly on the rise. However, like all other cut flowers, short vase life is the chief factor limiting its supply to target markets. Therefore, increasing quality and postharvest vase life of cut lisianthus can be a simple and inexpensive way to increase its supply and demand in national and international markets (Kiamohammadi and Hashemabadi, 2011; Nikkhah Bahrami *et al*., 2013). The present study aimed to increasing the vase life of cut lisianthus 'Pink Picotte' flowers by using preservatives containing cerium nitrate ($Ce(NO_3)_3$) and salicylic acid (SA).

MATERIALS AND METHODS

The effect of salicylic acid (SA) at three rates of 50, 100 and 200 mg L^{-1} and cerium nitrate

($Ce(NO_3)_3$) at four rates of 20, 40, 80 and 200 µM was explored on the vase life of cut lisianthus flowers in an experiment based on a randomized complete block design with eight treatments on 120 flower with three replications. The cut flowers of lisianthus 'Pink Picotte' were purchased at a greenhouse in Tehran at the commercial harvest stage and were transferred to the laboratory by taking care of all hygiene and transportation principles. The cut flowers were uniformly cut under running water to a length of 45 cm and were treated with the solutions for 24 hours. After the pulsed treatments, the flowers were placed in vases containing 500 mL of 3 % sucrose and were kept in a room at a temperature of 20±2°C, relative humidity of 60-70%, and a light regime of 12 hours at an intensity of 15 µM m^{-2} s^{-1} until the end of the experiment.

Trait Assessment

Vase life was calculated by counting the days from the flower treatment with SA and Ce $(NO_3)_3$ until the wilting of 50% of florets (Cho *et al*., 2001). To record flower fresh weight, their fresh weight on the last day of the experiment was subtracted from that measured on the first day. Dry matter percentage was obtained from dividing the dry weight of flowers by their fresh weight on the last day and multiplying the result by 100. To determine the number of vase solution bacteria, the method described in Oraee *et al*. (2011) was applied for which 24 hours after the treatments, 2 cc of the vase solution was diluted with 0.9% normal saline serum and was cultured on nutrient agar. 24 hours after the incubation of the cultured samples at 37°C, the number of bacterial colonies was counted.

Chlorophyll a, b, and total of the leaves were measured by Mazumdar and Majumdar (2003)'s procedure. So, the leaves were sampled on the fifth day of the experiment and they were extracted in 80% acetone. The absorption of the filtered samples was read at 643 and 660 nm with an Apple PD-330UV spectrophotometer, and chlorophyll a, b, and total were calculated by the following equations:

$$\text{Chlorophyll a} = 9.93\ (A_{660}) - 0.777\ (A_{643})$$
$$\text{Chlorophyll b} = 17.6\ (A_{643}) - 2.81\ (A_{660})$$
$$\text{Total chlorophyll} = 7.12\ (A_{660}) + 16.8\ (A_{643})$$

To determine the activity of antioxidant enzymes, we focused on the activity of catalase (CAT) and superoxide dismutase (SOD) for which the healthy petals were sampled on the fifth day of the experiment. They were, then, extracted by 50 mM potassium phosphate buffer and were centrifuged at 4°C at 10500 rpm for 20 minutes. The resulting clear solution was used as the enzymatic extract.

SOD enzyme activity was measured by the method described in Giannopolitis and Ries (1997). The reaction solution to measure SOD activity was composed of 0.1 mL of the enzymatic extract, 25 mM of nitro blue tetrazolium chloride, 13 mM of methionine, 0.1 mM of EDTA, 50 mM of carbonate sodium, and 50 mM of potassium phosphate buffer. It was slowly shaken in specific tubes for 15 minutes under florescent light exposure at 22°C. Then, the samples were placed in a dark room. After that, their absorption was read at 560 nm, and the SOD activity was expressed in the IU g^{-1} FW.

To measure CAT activity, 20 µL of the enzymatic extract was mixed with 3 mL of 50 mM potassium phosphate buffer and 15 µM of hydrogen peroxide. Then, the decline of absorbance at 240 nm was recorded by an Apple PD-330UV spectrophotometer at certain intervals for two minutes. Finally, CAT activity was calculated by the coefficient of hydrogen peroxide absorbance (0.036 Mm cm^{-1}) and was reported in IU g^{-1} FW min^{-1} (Aebi, 1984).

Data analysis

Data collected from daily visits and laboratory analysis were statistically analyzed in the SPSS software package. Means were compared by the LSD test.

RESULTS AND DISCUSSION

Vase life

The effect of SA and $Ce(NO_3)_3$ was significant ($P < 0.01$) on the vase life of cut lisianthus flowers (Table 1).

The comparison of means revealed that the cut lisianthus treated with 40 µM Ce $(NO_3)_3$ and those treated with 100 mg L^{-1} SA had the longest vase life of 15.42 and 15.20 days, respectively. But, they did not differ from the treatment of 50 mg L^{-1} SA (14.78 days) significantly. The plants treated with 200 mg L^{-1}SA exhibited the shortest vase life (10.63 days), which was one day shorter than that of the control (Fig. 1).

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

S.o.V	df	Vase life	Bacteria coloniesin vase solution	Fresh weight loss	Dry matter	Chl. a	Chl. b	Total chl.	SOD	CAT
Replication	2	0.428^{ns}	690^{ns}	0.592^{ns}	4.0212^{ns}	0.0044^{ns}	0.3616^{ns}	0.332^{ns}	6.12^{ns}	2.42^{ns}
Treatment	7	9.557^{**}	4432^{**}	6.20^{**}	0.878^{ns}	2.706^{**}	1.891^{*}	9.136^{**}	20.66^{*}	15.02^{**}
Error	14	0.633	722	0.954	2.0722	0.4725	0.445169	0.619	6.125	2.001
CV (%)		6.01	25.28	22.17	8.517	12.81	31.96	10.60	8.48	27.6

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

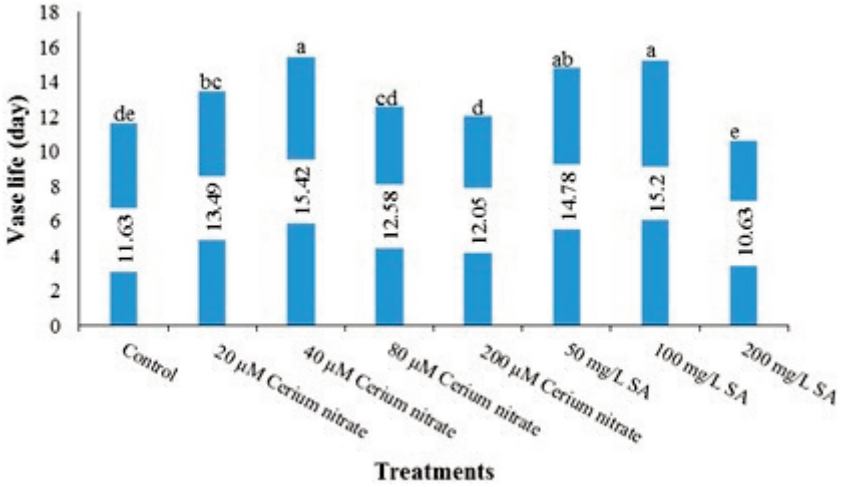

Fig. 1.The effect of SA and Ce $(NO_3)_3$ on the vase life of cut lisianthus flowers.

Kazemi *et al*. (2011) reported that the application of 1.5 mM SA increased the post-harvest vase life of cut carnations, but the application of 3 mM SA decreased it significantly. In a similar finding, we observed that the increase in the SA level reduced the vase life of lisianthus. Researchers argue that SA has an antimicrobial effect whose application in vase solution stunts the growth of microorganisms, hinders vascular blockage, improves water uptake, and prolongs vase

life. In addition, they suggest that SA prevents water loss by influencing stomatal conductance, thereby preserving the freshness of cut flowers for a prolonged period (Alaey *et al*., 2011; Mori *et al*., 2001). Fan *et al*. (2008) found that the treatment of gerbera cut flowers with SA retarded senescence via preserving membrane structure and reducing MDA accumulation.

Cerium is an antioxidant (He and Loh, 2000) and antimicrobial (Huang *et al*., 2002) compound whose positive impact at low dosages has been reported on extending post-harvest longevity of cut roses (Wang *et al*., 2017) and lilium (Houa *et al*., 2018). These researchers reported that the application of Ce $(NO_3)_3$ at higher concentrations reduced vase life, which is consistent with our findings.

Fresh weight loss

Based on the results of variance analysis, the effect of SA and Ce $(NO_3)_3$ was significant ($P < 0.01$) on fresh weight loss (Table 1). Fig. 2 illustrates that the highest fresh weight loss (6.483 g) was related to the treatment of 200 mg L^{-1} SA, but it did not differ from that of the control (6.013 g) and 80 µM $Ce(NO_3)_3$ (5.853 g) significantly. The plants treated with 100 mg L^{-1} SA exhibited the lowest fresh weight loss (3.093 g), not significantly different from that of the flowers treated with 20, 40 and 200 µM $Ce(NO_3)_3$ or 50 mg L^{-1} SA (Fig. 2).

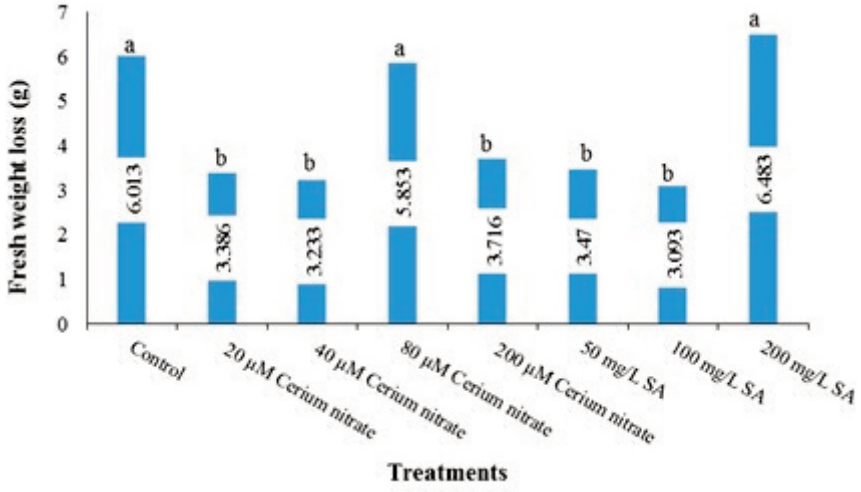

Fig. 2. The effect of SA and Ce $(NO_3)_3$ on the fresh weight loss of cut lisianthus flowers.

These findings reveal that the treatments that perform best for vase life are associated with lower fresh weight loss. In other words, vase life prolonging compounds that were applied in the present study contributed to preserving the postharvest longevity and freshness of lisianthus by inhibiting the decline of fresh weight. Consistent with our findings, it has been reported by other studies that the application of SA in vase solutions significantly hindered the loss of fresh weight in cut roses (Alaey *et al*., 2011; Aelaei *et al*., 2017) and lisianthus (Nikkhah Bahrami *et al*., 2013).

Dry matter percentage

Dry matter percentage was not significantly influenced by different levels of SA and $Ce(NO_3)_3$ (Table 1). It is evident in Fig. 3 that the highest dry matter (18.03%) was related to the application of 100 mg L^{-1} SA, and the lowest (16.33) was commonly recorded by the treatments of 20 and 80 µM $Ce(NO_3)_3$ (Fig. 3). Roodbaraky *et al*. (2012) reported that the application of SA

at a rate of 150 mg L^{-1} increased dry matter of carnation 'Liberty Abgr'. The positive effect of SA on increasing dry weight of cut flowers has also been reported for roses 'Black Magic' (Aleay *et al*., 2011) and roses 'Hater Class' (Aelaei *et al*., 2017).

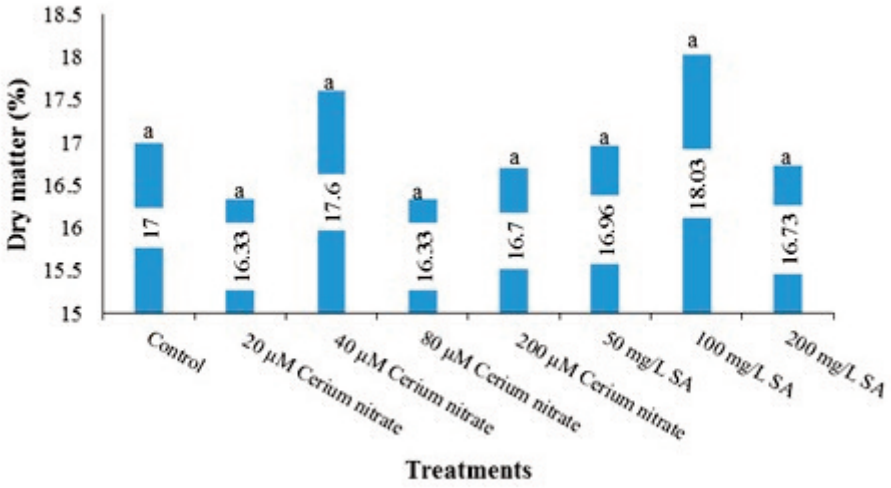

Fig. 3. The effect of SA and $Ce(NO_3)_3$ on the dry matter percentage of cut lisianthus flowers.

Bacterial content of vase solution

The results of the analysis of variance revealed the significant effect of SA and Ce $(NO_3)_3$ on the bacterial population in the vase solution at the $P < 0.01$ level (Table 1). The comparison of means showed that the control plants and the plants exposed to 200 µM $Ce(NO_3)_3$ were related to the most populated bacterial colony in the vase solution (154 Log_{10} CFU mL^{-1}), but they did not differ from that of the treatments of 200 mg L^{-1} SA and 80 µM $Ce(NO_3)_3$. The treatments of 100 mg L^{-1} SA (53.33 Log_{10} CFU mL^{-1}) and 40 µM Ce $(NO_3)_3$ (64 Log_{10} CFU mL^{-1}) performed best in suppressing the microbial load of the vase solution (Fig. 4).

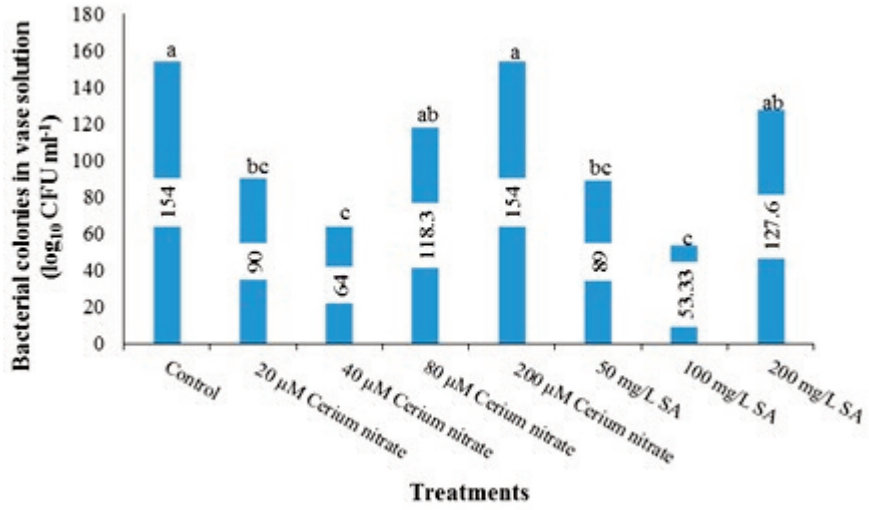

Fig. 4. The effect of SA and Ce $(NO_3)_3$ on the bacterial population in the vase solution of cut lisianthus flowers.

The activity of microorganisms in vase solutions cause the release of toxic compounds, an increase in ethylene synthesis, vascular blockage, and the induction of reactions that lead to cell death (Edrisi, 2009). The application of antiseptics to vase solutions prolongs the vase life of cut flowers by removing microorganisms and their detrimental effects (Anjum *et al*., 2001). Antibiotic properties have been reported for cerium (Huang, 2002) and SA (Mori *et al*., 2001; Kazemi *et al*., 2011). As mentioned in results, the application of antiseptic compounds at appropriate rates in the vase solution of lisianthus reduced bacterial population and extended vase life. Kazemi *et al.* (2011) found that the application of SA alone or in combination with sucrose in the vase solution of cut carnations reduced the growth and propagation of bacteria in vase solution. As well, Roodbaraky *et al*. (2011) reported that the application of SA in the preservative of cut carnation flowers stunted the growth of microbes, which is in agreement with our findings.

Chlorophyll a, b, and total

The effect of SA and Ce $(NO_3)_3$ was significant on chlorophyll a ($P < 0.01$), chlorophyll b ($P < 0.05$), and total chlorophyll ($P < 0.01$) (Table 1). Based on the results of means comparison, the highest chlorophyll a content (6.893 mg g^{-1} FW) was related to the treatment of 100 mg L^{-1} SA, but it did not show a statistically significant difference to the treatment of 40 µM $Ce(NO_3)_3$ (6.706 mg g^{-1} FW) (Fig. 5).

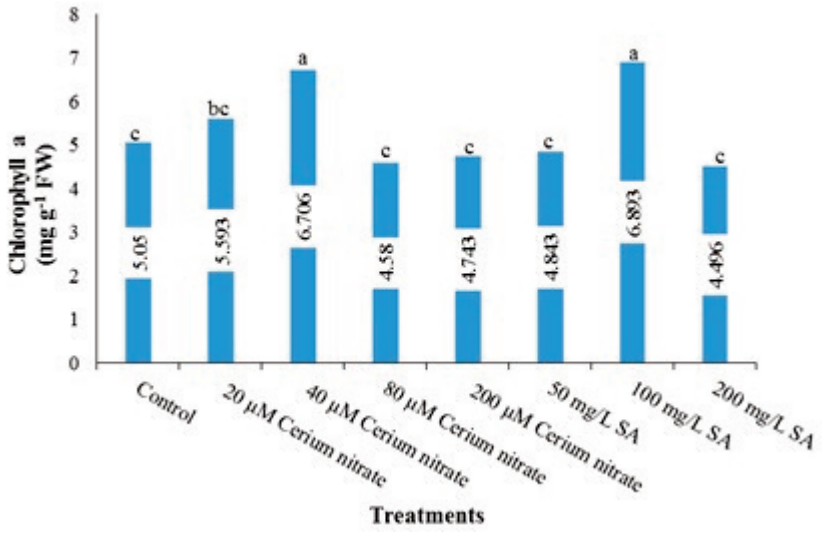

Fig. 5. The effect of SA and Ce $(NO_3)_3$ on chlorophyll a content in cut lisianthus flowers.

Fig. 6 depicts that the treatments of 40 µM $Ce(NO_3)_3$ and 100 mg L^{-1} SA produced the highest chlorophyll b contents of 3.59 and 2.946 mg g^{-1} FW, respectively. The lowest was 1.32 mg g^{-1} FW observed in the plants treated with 200 µM Ce $(NO_3)_3$, which was in the same statistical group with the treatments of 80 µM $Ce(NO_3)_3$ and 50 and 200 mg L^{-1} SA (Fig. 6).

As is seen in Fig. 7, total chlorophyll content was significantly increased when 40 µM $Ce(NO_3)_3$ or 100 mg L^{-1} SA were applied in which it was 10.296 and 9.84 mg g^{-1} FW, respectively. The lowest total chlorophyll content of 5.926 mg g^{-1} FW was recorded in the plants treated with 80 µM $Ce(NO_3)_3$, which did not differ from that of the treatments of 200 µM $Ce(NO_3)_3$ and 200 mg L^{-1} SA significantly (Fig. 7).

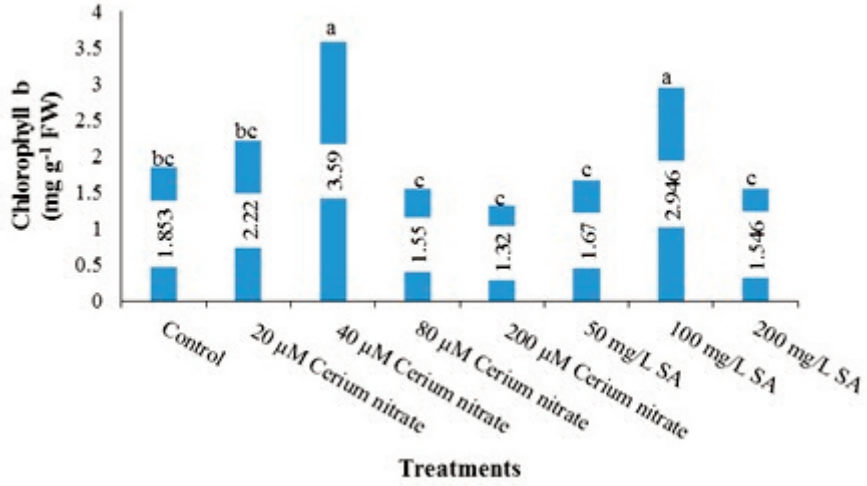

Fig. 6. The effect of SA and Ce $(NO_3)_3$ on chlorophyll b content in cut lisianthus flowers.

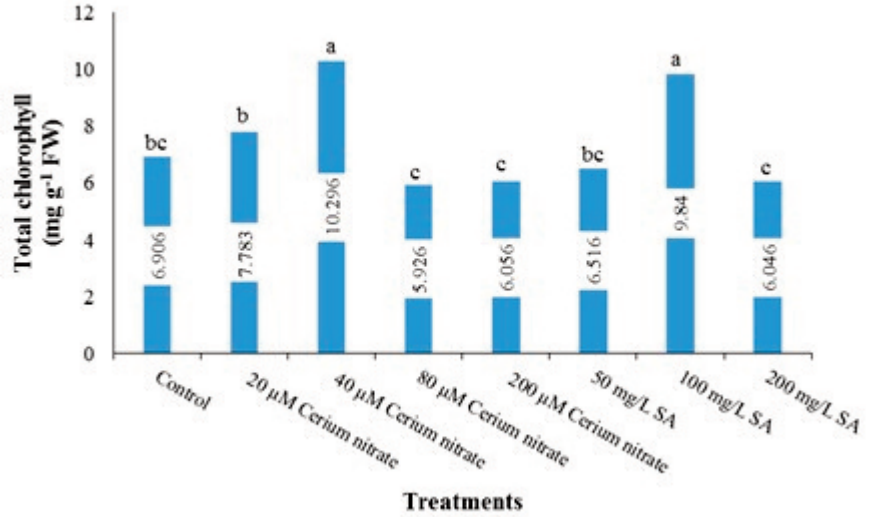

Fig. 7. The effect of SA and Ce $(NO_3)_3$ on total chlorophyll content in cut lisianthus flowers.

Aelaei *et al*. (2017) reported that the application of SA postponed leaf chlorophyll degradation in cut rose flowers. Some researchers argue that by reducing ethylene activity, SA prevents the degradation of chlorophyll and thereby it increases leaf chlorophyll content (Mei-hua *et al*., 2008). In a study by Kazemi *et al*. (2011), the application of 1.5 µM SA increased total chlorophyll content of cut carnation flowers whereas it reduced their total chlorophyll when it was applied at a rate of 3 µM. A positive effect of SA on preserving and increasing leaf chlorophyll was also reported for cut gladiolus flowers (Hassan and Ali, 2014), which is consistent with our findings.

According to Wang *et al*. (2017), the application of Ce $(NO_3)_3$ in the vase solution of cut rose flowers increased chlorophyll a and b and total chlorophyll. They used Ce $(NO_3)_3$ at four rates of 10, 30, 50 and 100 µM and the most effective for chlorophyll improvement was 30 µM. They suggest that the degradation of photosynthesizing pigments, like chlorophyll a and b, is a symptom of aging. According to them, Ce $(NO_3)_3$ prevented the aging of cut roses by maintaining and increasing these pigments.

Antioxidant enzymes

The effect of SA and Ce $(NO_3)_3$ was significant on the activity of superoxide dismutase (SOD) at the $P<0.05$ level and on the activity of catalase (CAT) at the $P<0.01$ level (Table 1).

Fig. 8 displays the effect of different treatments on SOD activity. It is evident that the highest SOD activity (32.75 IU g^{-1} FW min^{-1}) was related to the treatment of 100 mg L^{-1} SA, but it did not differ from the treatments of 50 mg L^{-1} SA and 20, 40 and 80 µM Ce $(NO_3)_3$ significantly. The lowest SOD activity (25.61 IU g^{-1} FW min^{-1}) was observed in the treatment of 200 µM Ce $(NO_3)_3$ (Fig. 8).

Means comparison for the effect of the treatments on CAT activity indicated that it was the highest in plants treated with 40 µM Ce $(NO_3)_3$, those treated with 100 mg L^{-1} SA, and those treated with 50 mg L^{-1} SA. The lowest was associated with the application of 80 µM Ce $(NO_3)_3$ and 200 µM SA. These two latter treatments did not differ significantly (Fig. 9).

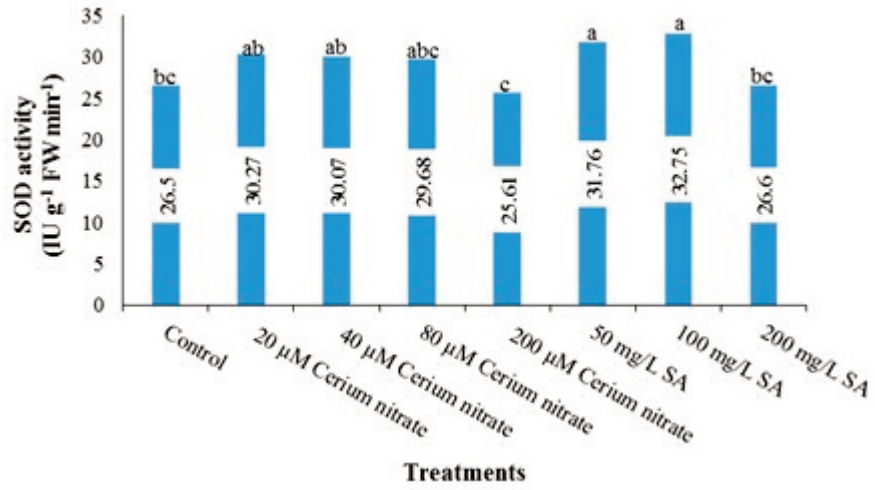

Fig. 8. The effect of SA and Ce $(NO_3)_3$ on superoxide dismutase (SOD) activity in cut lisianthus flowers.

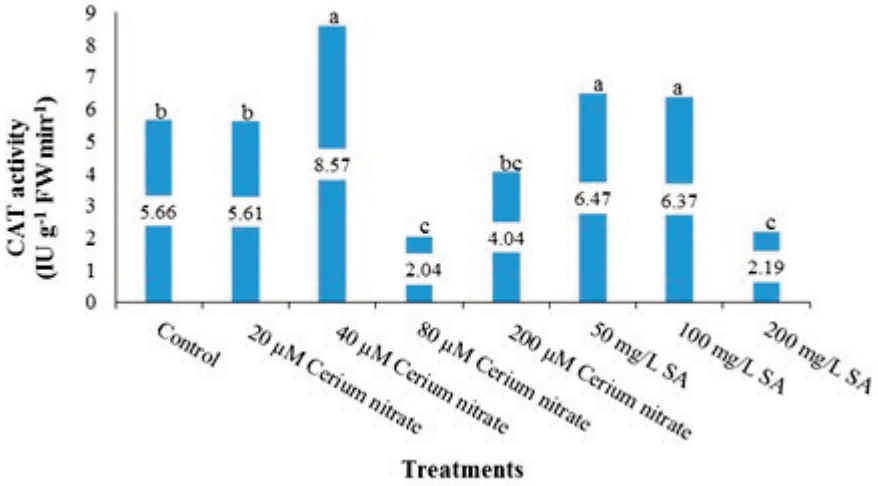

Fig. 9. The effect of SA and Ce $(NO_3)_3$ on catalase (CAT) activity in cut lisianthus flowers.

SOD and CAT are antioxidant enzymes of plants' defensive system with a significant role in scavenging ROS and prolonging the vase life of cut flowers (Wang *et al*., 2017; Shan and Zhao, 2015). It is argued that by increasing the activity of antioxidant enzymes, SA contributes to scavenging ROS and extending the vase life of cut flowers (Ezhilmathi *et al*., 2007; Ansari and Misra, 2007). Fan *et al*. (2008) reported that SA increased the activity of antioxidant enzymes and thereby it prevented the activity of ROS and prolonged the postharvest longevity of gerbera cut flowers by contributing to the preservation of membrane stability. Similar results have been reported by Yuping (2009), Capdeville *et al*. (2003), and Alaey *et al*. (2011).

Aelaei *et al*. (2017) stated that SA had an antioxidant property and its application in the vase solution of cut roses 'Hater Class' increased CAT and POD activities and suppressed ROS activity and aging. Nikkhah Bahrami *et al*. (2013) reported that the application of 100 mg L^{-1} SA increased SOD activity in cut lisianthus flowers significantly. We observed the same effect.

According to Shan and Zhao (2015), lanthanum (La) is a trace element that is involved in the activity of antioxidant enzymes. They found that the application of La increased the activity of these enzymes in cut lilium flowers and prolonged their vase life. Cerium is a trace element from the lanthanide series. Wang *et al*. (2017) reported that the application of $Ce(NO_3)_3$ increased SOD and CAT activity in cut roses so that their highest activity was observed when $Ce(NO_3)_3$ was applied at a rate of 30 µM, but the highest levels of $Ce(NO_3)_3$ (50 and 100 µM) decreased the activity of these enzymes, which is in agreement with our findings.

CONCLUSIONS

We found that the application of SA at the rate of 100 mg L^{-1} and the application of $Ce(NO_3)_3$ at the rate of 40 µM reduced the microbial load of the vase solution, thereby preserving fresh weight and maintaining and increasing leaf chlorophyll content. They also increased the activity of antioxidant enzymes and contributed to prolonging the vase life of cut lisianthus flowers. Therefore, it is recommended to apply them to increase the postharvest vase life of the cut lisianthus 'Pink Picotte'.

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