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# Postharvest Life of Cut Gerbera (*Gerbera jamesonii*) as Affected by Nano-silver Particles and Calcium Chloride

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**Abstract**
The purpose of this study was to evaluate the effects of Nano-silver (NS), calcium chloride ($CaCl_2$) and their combinations on *Gerbera jamesonii* 'Carambole' cut flowers. The experiment was conducted as a completely randomized design (CRD) with 10 treatments and four replications, with three flowers in each replication. Treatments consisted of 5 mg $L^{-1}$ NS, 1% and 2% $CaCl_2$, 5 mg $L^{-1}$ NS+ 1% $CaCl_2$, 5 mg $L^{-1}$ NS + 2% $CaCl_2$, 0.1% and 0.2% $CaCl_2$, 5 mg $L^{-1}$ NS + 0.1% $CaCl_2$, 5 mg $L^{-1}$ NS + 0.2% $CaCl_2$ and a control. It was revealed that $CaCl_2$ postharvest spray, NS in a vase solution, as well as their combinations could significantly increase the vase life of gerbera flowers. The longest postharvest life of treated flowers was obtained from 5 mg $L^{-1}$ NS and 5 mg $L^{-1}$ NS + 1% $CaCl_2$ treatments. The highest solution uptake was observed in 5 mg $L^{-1}$ NS, 5 mg $L^{-1}$ NS + 2% $CaCl_2$ and 5 mg $L^{-1}$ NS +1% $CaCl_2$ treatments. Furthermore, application of NS and $CaCl_2$ resulted in a reduced loss of relative fresh weight. The application of $CaCl_2$ alone was able to increase all mentioned traits; however, this increase was less than 5 mg $L^{-1}$ NS, 5 mg $L^{-1}$ NS + 1% $CaCl_2$ and 5 mg $L^{-1}$ NS + 2% $CaCl_2$ treatments. Additionally, in vitro cultivation and microscopic counting of microorganisms showed that microorganism growth at the end of flowering stems had been largely restricted by using NS solely or in combination with $CaCl_2$.

**Keywords:** antimicrobial, relative fresh weight, solution uptake, vase life

## Introduction

The quality loss of cut flowers may depend on various factors (Kazemi *et al*., 2011). To be specific, for gerbera flowers, factors such as genetic (Nazari deljou *et al*., 2011), postharvest storage temperature (Celikel and Reid, 2002), phytohormones (Emonger, 2004) and water balance (van Meeteren, 1978) are the main causes of postharvest petal wilting and stem bent neck and/or stem break (Wernett *et al*., 1996). It is well documented that one of the main causes for the inferior quality of cut flowers is the blockage of xylem vessels by microorganisms that accumulate in the vase solution or in the vascular vessels of the plant (Marandi *et al*., 2011). When the vessels of stems become blocked, water uptake and transpiration by leaves cause a net loss of water from the cut flower (Hassan, 2005).

Many substances have been used in cut flower vase solutions to extend the postharvest life of plants by reducing microbial contamination. Bactericides are the most important components in preservative solutions used to control bacteria and to prevent bacterial embolism (Halevy and Mayak, 1981). Other materials

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that have been successfully tested in preservatives include 6% sucrose (Kim and Lee, 2002). Sucrose has been used as a substrate for respiration (Victoria *et al*., 2003), which helps to maintain the osmotic potential of the petal cells (Sujatha *et al*., 2003). Pre-treatment of cut roses with thymol (100 mg $L^{-1}$) have been found effective against some bacteria (Oraee *et al*., 2010). Solgi *et al*. (2009) reported that pre-treatment of *Gerbera jamesonii* ‘Dune’ with nano-silver particles (SNPs) was effective as an antibacterial agent (Morones *et al*., 2005). Vase life of *Gerbera jamesonii* ‘Ruikou’ cut flowers has been prolonged with a 5 mg $L^{-1}$ SNP solution as pulsed treatments for 24 h (Liu *et al*., 2009). The application of 10 mg $L^{-1}$ SNP + 5% sucrose for 24 h extended the vase life of cut *Rosa hybrida* ‘Dolce Vita’ flowers (Oraee *et al*., 2010).

Nano-silver (NS) particles more strongly inhibit bacteria and other microorganisms than normal silver element in various oxidation states, i.e., $Ag^0$, $Ag^+$, $Ag^{2+}$ and $Ag^{3+}$ (Furno *et al*., 2004; Jilang *et al*., 2004). Using nano-silver compounds (NS) as a pulse treatment and vase solution is a relatively new approach for cut flowers (Liu *et al*., 2009; Solgi *et al*., 2009) that has demonstrated its importance as a reliable antibacterial agent (Alt *et al*., 2004; Morones *et al*., 2005). It has been reported that NS releases $Ag^+$ (Lok *et al*., 2007), which interacts with cytoplasmic components and nucleic acids, inhibits respiratory chain enzymes and interferes with the membrane permeability of microorganism cells (Russel and Hugo, 1994; Park *et al*., 2005). The use of NS is becoming increasingly widespread in other areas as well, such as medicine, fabrics, water purification and various other industrial applications (Jain and Pradeep, 2005; Dubas *et al*., 2006; Chen and Schuesener, 2008). The positive effect of NS pulse treatment is attributed to the inhibition of bacterial growth in the vase solution and at the plant's stem end during the postharvest period. $Ag^+$, applied as silver thiosulfate, effectively inhibits ethylene-mediated physiological activities such as flower senescence and abscission (Altman and Solomos, 1995; Ichimura *et al*., 2008). As with other cations (e.g., $K^+$, $Ca^{2+}$), $Ag^+$ has positive effects on plant stem hydraulic conductivity (van Ieperen, 2007). Ohkawa *et al*. (1999) reported that silver-containing compounds extended the vase life of cut roses. Lü *et al*. (2010) reported that pulse treatment of cut roses with 50 and 100 mg $L^{-1}$ NS solutions for 1 h extended its vase life and enabled the reduction of flower fresh weight during the vase period. According to Kim *et al*. (2005), vase life of Asiatic *Lilium hybrid* cv. ‘Dreamland’ and Oriental *Lilium hybrid* cv. ‘Siberica’ were extended by dipping in a mixture of 0.1% nanoparticle pure colloidal $Ag^+$ ion, $H_2O_2$ and natural chitosan. It has been shown that there is a negative correlation between the number of bacteria and water conductivity in the stems of cut flowers of Lisianthus (Kazemi *et al*., 2011). Balestra *et al*. (2005) demonstrated that cut gerbera flowers were sensitive to microbial contamination at their stem base or via the vase solution, which consequently shortened their vase life.

One of the primary physiological disorders that decrease the quality of gerbera flowers is bent neck. Nikbakht *et al*. (2008) reported that calcium (Ca) accumulation in the scapes of gerbera flowers can prevent and delay bent neck incidence. Tissue calcium content affects all development stages during plant growth (Ferguson and Drobak, 1988). The role of intra- and extracellular Ca in altering cell metabolism is often attributed to its influence on cell walls and membrane structure and function (Ferguson, 1984; Konno *et al*., 1984). Calcium is also involved as a second messenger in the regulation of an array of intracellular events (Nabigol, 2012). Combining of NS and Ca substances has to date not been investigated in any research project; this type of work is applicable also to other ornamental cut flowers (such as roses and carnations) that experience this postharvest problem. The purpose of this study was to evaluate the effects of nano-silver, calcium chloride and their combination on 'Carambole' gerbera flowers.

## Materials and Methods

### *Plant material*

Gerbera flowers (*Gerbera jamesonii* Bolus ex. Hook cv. Carambole) were purchased from a research greenhouse and transferred within 1 h to the Postharvest Laboratory of the Horticultural Science Department, College of Agriculture, Shiraz University, Shiraz, Iran. In the laboratory, in order to eliminate air blockage in the stem, flower stems were cut submerged in deionized water (DI), leaving the stems approximately 25cm long. Flowers were then selected for uniformity of size and kept at 20 ± 2°C and a relative humidity of 60 ± 5%.

Ten postharvest treatments, including 5 mg $L^{-1}$ NS, 1% $CaCl_2$, 2% $CaCl_2$, 5 mg $L^{-1}$ NS + 1% $CaCl_2$, 5 mg $L^{-1}$ NS + 2% $CaCl_2$, 0.1% $CaCl_2$, 0.2% $CaCl_2$, 5 mg $L^{-1}$ NS + 0.1% $CaCl_2$, 5 mg $L^{-1}$ NS + 0.2% $CaCl_2$ and a control were used. The upper and lower surfaces of flowers and 5 cm of stem below the flowers were sprayed with $CaCl_2$. Flowers were kept horizontally until runoff stopped. Other treatments were applied to the vase solution.

In the 5 mg $L^{-1}$ NS treatment, flowers were kept in a 5 mg $L^{-1}$ NS vase solution for 24 h and then transferred to a maintenance solution containing 6% sucrose; a 1% and 2% $CaCl_2$ solution were sprayed on the flowers and 5 cm of stem below the flowers, and kept for 24 h in distilled water before finally being transferred to a 6% sucrose maintenance solution. $CaCl_2$ 0.1% and 0.2% were added to distilled water and after keeping flowers for 24 h in this solution, flowers were transferred to a 6% sucrose maintenance solution. Combination treatments (1% $CaCl_2$ + 5mg $L^{-1}$ NS, 2% $CaCl_2$ + 5mg $L^{-1}$ NS, 0.1% $CaCl_2$ + 5mg $L^{-1}$ NS and 0.2% $CaCl_2$ + 5mg $L^{-1}$ NS) were also applied as previously stated, the only difference being the simultaneous use of treatments. The control flowers were put in distilled water for 24 h and then transferred to a 6% sucrose maintenance solution. All flowers were kept in the maintenance solution until the end of experiment.

### *Vase solution uptake*

Relative fresh weight and relative solution uptake were measured daily for all the treatments (Nikbakht *et al*., 2008). The weights of vases without their cut flowers were recorded daily during the vase life evaluation period using a digital balance. Average daily vase solution uptake was calculated by the following formula: vase solution uptake rate (g $stem^{-1}day^{-1}$) = ($S_{t-1}$-$S_t$); where, $S_t$ is the weight of the vase solution (g) at t = day 1, 2, 3, etc., and $S_{t-1}$ is the weight of the vase solution (g) on the previous day.

### *Relative fresh weight*

The fresh weight of cut flowers was recorded daily during the experiment. Relative fresh weight of stems was calculated using the following formula: RFW (%) = ($W_t$/$W_{t0}$) × 100; where $W_t$ is the weight of stem (g) at t = day 0, 1, 2, etc., and $W_{t0}$ is the weight of the same stem (g) at t = day 0.

### *Vase life*

The cut flowering stems were assessed daily for visual appeal during the vase life evaluation period. Vase life was judged to have ended when 50% or more of florets on an inflorescence were deemed unattractive (Joyce *et al*., 2000).

To determine the rate of bacterial pollutions, 0.5 g (~2cm length) segments of stem ends were excised. Explants were washed three times with DI to reduce surface microbial loads and then ground and diluted with 0.9% normal saline. Aliquots (0.1 ml) of extract were spread on nutrient agar plates containing a PCA culture media. Bacterial colonies were measured after incubation for 24 h at 37ºC. A dilution series was made with 0.9% normal saline to achieve 30 to 300 bacterial colonies in each petri dish. All bacteria counts were made on triplicate sub-samples (Balestra *et al*., 2005; Liu *et al*., 2009).

The experiment was conducted as a completely randomized design (CRD) with 10 treatments and four replications, with three flowers in each replication. Finally, data analysis was performed using SAS software and the means were compared using an LSD test at 5% level of probability.

## Results and Discussion

NS in the vase solution and 1% $CaCl_2$ spray and their combinations significantly extended gerbera flowers' vase life and inhibited bent neck, compared to the control treatment; 5 mg $L^{-1}$ of NS treatment and 5 mg $L^{-1}$ NS + 1% $CaCl_2$ had the longest vase life (15.75 and 13 days, respectively), while the vase life of the control flowers was three days (Table 1).

The longer vase life of flowers treated with NS can be attributed to lower bacterial plugging of xylems and their slow senescence rate (Put, 1990; Bleeksma and van Doorn, 2003; Liu *et al*., 2009). On the other hand, it was shown that ion leakage (due to higher membrane permeability) will be reduced by the application of 1% nano-silver, hence increasing the vase life of cut rose flowers (Jowkar *et al*., 2013).

Results showed that during the experiment, flower solution uptake increased by two days and reduced after that (Fig. 1).

The highest vase solution uptakes were observed in 5 mg $L^{-1}$ NS (1.42 g stem.day$^{-1}$) and 5 mg $L^{-1}$ NS + 2% $CaCl_2$ (1.33 g stem.day$^{-1}$) treatments, with no significant differences between them. The lowest vase solution uptake was recorded in $CaCl_2$ 2%, which was not significantly different from the control treatment (Table 2).

**Table 1. Effects of different postharvest treatments on vase life of gerbera flowers.**

| Treatments | Vase life (days) |
|---|---|
| Control | 3.00 f |
| $CaCl_2$ 0.1% | 10.75 c |
| $CaCl_2$ 0.2% | 10.25 c |
| $CaCl_2$ 1% | 8.50 d |
| $CaCl_2$ 2% | 5.25 e |
| NS 5 mgl$^{-1}$ | 15.75 a |
| NS 5 mgl$^{-1}$+$CaCl_2$ 0.1% | 9.50 dc |
| NS 5 mgl$^{-1}$+$CaCl_2$ 0.2% | 10.00 c |
| NS 5 mgl$^{-1}$+$CaCl_2$ 1% | 13.00 b |
| NS 5 mgl$^{-1}$+$CaCl_2$ 2% | 6.00 e |

Means with similar letters were not significant ($P \leq 0.05$).

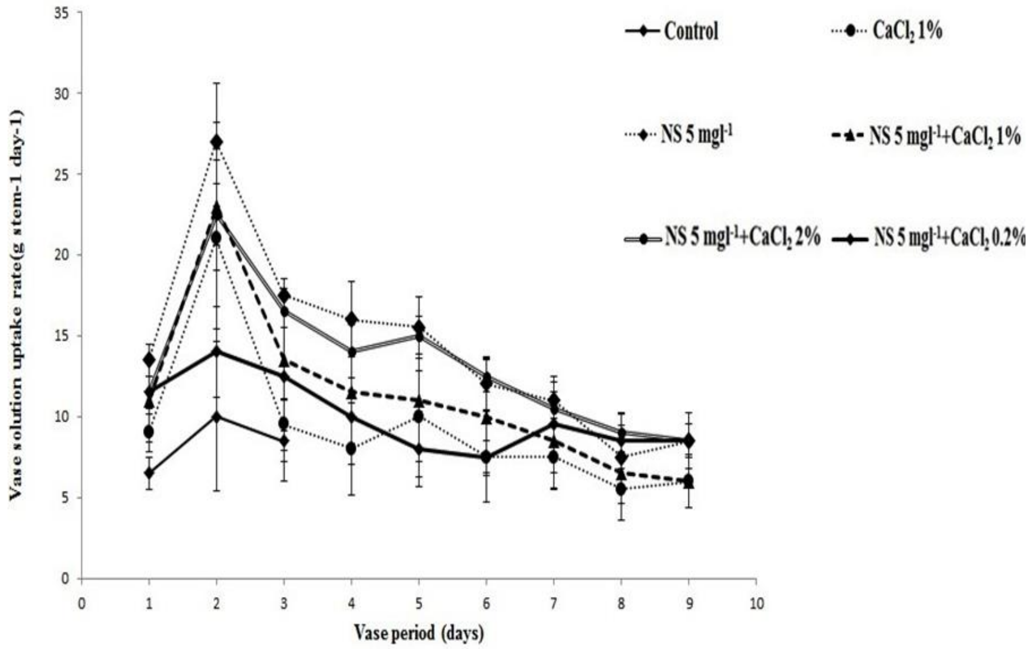


**Fig. 1. Effects of different postharvest treatments on the vase solution uptake of gerbera flowers.**

**Table 2. Effects of different postharvest treatments on relative fresh weight and vase solution uptake of gerbera flowers.**

| Treatments | Relative fresh weight (g) | Vase solution uptake (g stem.day$^{-1}$) |
|---|---|---|
| Control | 85.04 c | 0.85 d |
| $CaCl_2$ 0.1% | 86.02 c | 0.77 d |
| $CaCl_2$ 0.2% | 85.22 c | 0.82 d |
| $CaCl_2$ 1% | 87.85bc | 0.93 dc |
| $CaCl_2$ 2% | 86.22 c | 0.80 d |
| NS 5 mgl$^{-1}$ | 98.03 a | 1.42 a |
| NS 5 mgl$^{-1}$ +$CaCl_2$0.1% | 84.46 c | 0.88 dc |
| NS 5 mgl$^{-1}$+$CaCl_2$0.2% | 85.83 c | 1.00 dc |
| NS 5 mgl$^{-1}$+$CaCl_2$ 1% | 92.55 b | 1.12 bc |
| NS 5 mgl$^{-1}$ +$CaCl_2$ 2% | 99.59 a | 1.33 ab |

Means with similar letters are not significant ($P \leq 0.05$).

The results revealed that NS can positively accelerate water uptake, which may be due to lower bacterial infection in xylems. van Meeteren *et al*. (2001) demonstrated that Ag ions added to deionized water can have positive effects on the water status of *Bouvardia*. Furthermore, another study showed that ions in water, particularly cations, can enhance solution and water flow through xylem vessels (van Ieperen *et al*., 2000).

Although vase solution uptake reduced during the experiment (Fig. 1), their fresh weights reduced at a moderate pace, which was apparent in the 5 mg L$^{-1}$ NS and 5 mg L$^{-1}$ NS + 2% $CaCl_2$ solutions (Fig. 2).

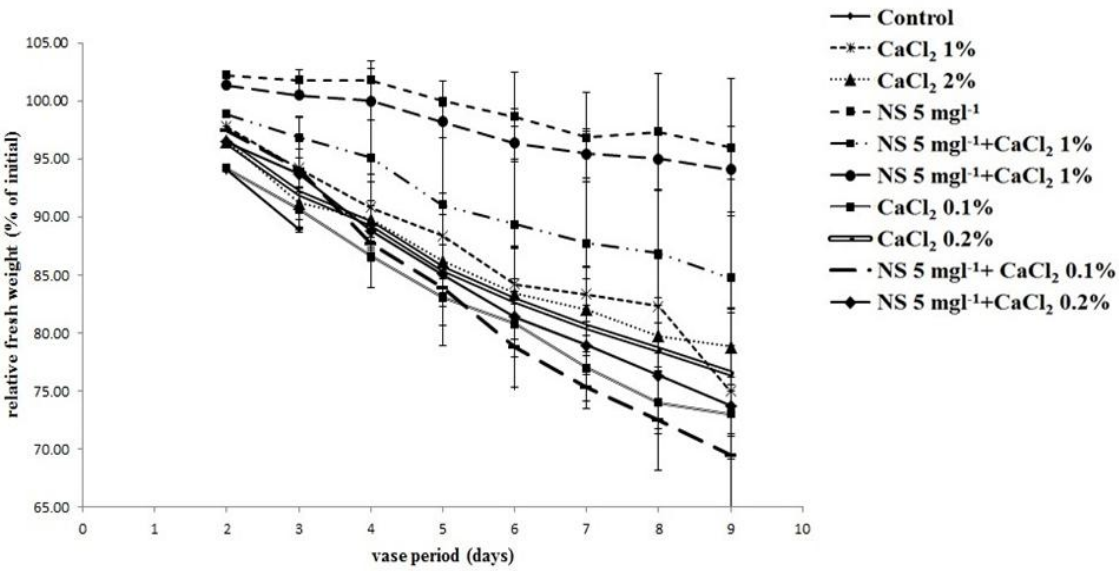


**Fig. 2. Effects of different postharvest treatments on the relative fresh weight of gerbera flowers.**

Additionally, the application of NS and $CaCl_2$ increased relative fresh weights on the first day. Flowers' fresh weight reduced for all treatments during the study, with the lowest loss rate being obtained for 5 mg $L^{-1}$ NS + 2% $CaCl_2$ and 5 mg $L^{-1}$ NS, both of which had the highest fresh weights of flowers (Table 2). The lowest fresh weights were observed in 5 mg $L^{-1}$ NS + 0.1% $CaCl_2$, the control, 0.2% $CaCl_2$, 5 mg $L^{-1}$ NS + 0.2% $CaCl_2$, 0.1% $CaCl_2$ and 2% $CaCl_2$; no significant differences were evident among these treatments (Table 2). Our findings were in accordance with results achieved by Emonger (2004), which indicated that antimicrobial substance treatments or the application of delayed fresh weight reduced and enhanced solution uptake of gerbera.

The application of $CaCl_2$ at 0.1 and 0.2% concentrations was able to increase postharvest life, relative solution uptake and the relative fresh weight of flowers; however, this increase was less than 5 mg $L^{-1}$ NS, 5 mg $L^{-1}$ NS + 1% $CaCl_2$ and 5 mg $L^{-1}$ NS + 2% $CaCl_2$ treatments (Fig. 2). It was revealed that $CaCl_2$ could not solely decrease bacterial contamination at stem ends of gerbera flowers. Calcium is generally seen as an agent for reducing the rate of plant senescence (Ferguson *et al*., 1985) and has long been associated with the regulation of the ripening of fruit and postharvest storage life (Ferguson, 1984). It has been reported that Ca is an important factor for providing stability and mechanical strength to cell walls (Poovaiah *et al*., 1988), and that it plays a key role in the maintenance of cell middle lamella (Siddiqui and Bangerth, 1996) and also in preventing scape firmness loss, thereby decreasing bent neck incidence and increasing cut flower longevity (Mayak *et al*., 1978). Gerasopoulos and Chebli (1999) reported that a 1.0% to 1.5% postharvest $CaCl_2$ dip increased vase life up to four days and decreased the bent neck disorder in gerbera flowers. They also showed that although the scape Ca content increased in higher $CaCl_2$ concentrations, this increase did not result in the better longevity of flowers, likely due to Ca toxicity. It was also shown that the application of 125 mg $L^{-1}$ $CaCl_2$ resulted in the higher fresh weight and water uptake of sunflowers, while these rates were lower for 250 and 500 mg $L^{-1}$ $CaCl_2$ (Sosa Nan, 2007).

In vitro cultivation and microscopic counting of microorganisms showed that using NS solely or in combination with

$CaCl_2$ restricted bacterial growth. The highest inhibition of bacterial growth at the stem end was observed in the 5 mg $L^{-1}$ NS treatment, while 1% $CaCl_2$ + 5 mg $L^{-1}$ NS and 2% $CaCl_2$ + 5 mg $L^{-1}$ NS treatments diminished bacterial infection with a 3.3 and 3.5 $log_{10}$ CFU $ml^{-1}$, respectively (Fig. 3).

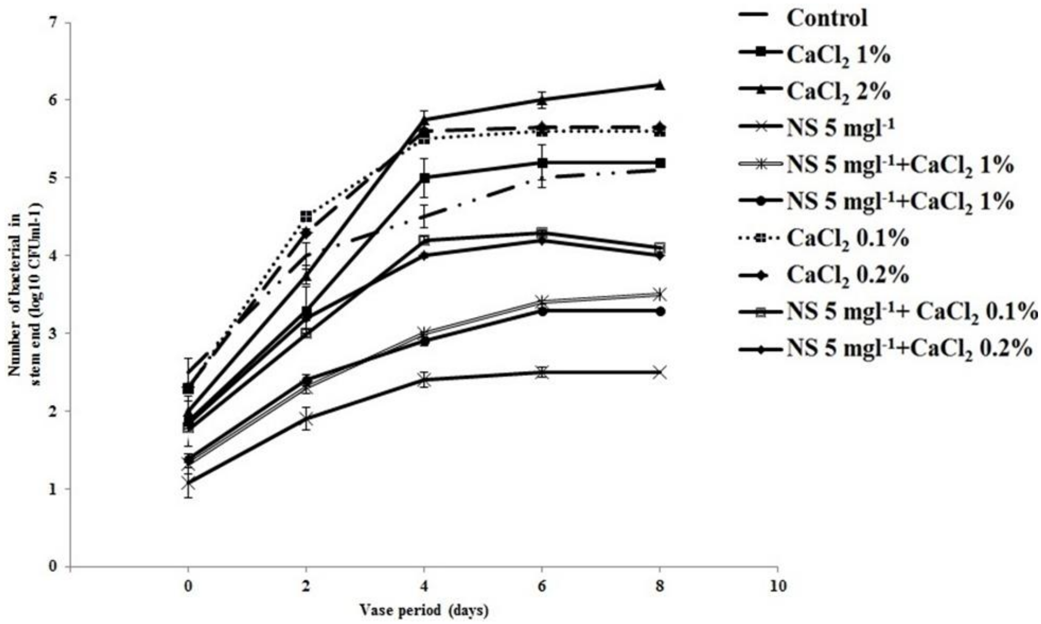


**Fig. 3. Effects of different postharvest treatments on the amount of bacteria at the stem end of Gerbera flowers (Log $_{10}$ CFU $ml^{-1}$).**

NS is a novel bactericide that can kill about 650 bacteria species in water (Furno *et al*., 2004). NS is thought to release monovalent $Ag^+$ ions that replace the $H^+$ of thiol groups (-SH) on the surface proteins of bacterial cell membranes, which decreases membrane permeability and eventually causes cell death (Feng *et al*., 2000). NS particles are commonly used in a range of fields for its anti-microbial properties, including the medical industry and for vegetable disinfestations (Jiang *et al*., 2004). These effects have been confirmed by Liu *et al*. (2009), i.e., that NS can profoundly inhibit bacterial growth in vase solutions, as well as at plant stem ends, which concomitantly results in the extended vase life of the flowers. Furthermore, $CaCl_2$, as a preservative reagent, can be used in different cultivars of gerbera to significantly prolong the vase life of these flowers. It can improve the value of water balance, reduced bent neck incidence, enhance stem conductance, increase fresh weight and delay the aging of cut gerbera flowers (Zhang *et al*., 2004).

## Conclusions

In conclusion, this study showed that a combination of nano-silver and calcium chloride treatments can provide a high preservative role that increases the vase life of gerbera cut flowers. Moreover, it was observed that these two treatments could effectively prevent bent neck disorder and stem blockage in this ornamental plant. Indeed, NS and $CaCl_2$ can promisingly increase and improve the vase life of cut gerbera flowers and can therefore be offered as a plant treatment to commercial gerbera producers. However, according to the results of this investigation, vase solution application of NS as an individual postharvest practice has a higher impact on gerbera flowers. Due to the low price of both $CaCl_2$ and NS, these elements can potentially play an important role in postharvest practices in future.

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