

Comparative postharvest responses of carnation and chrysanthemum to synthesized silver nanoparticles (AgNPs)

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All relevant data are within the paper and its Supporting Information files.

Competing Interests:

The authors declare no competing interests.

Abstract: Carnation (*Dianthus caryophyllus* L.) and chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitam.] cut flowers are among the most important commodities that dominate flower markets throughout the world. Two major problems in the transportation and marketing of these flowers are their relatively short vase life and the rapid decline of their aesthetic value. In this respect, the current study investigates the effects of silver nanoparticles (AgNPs) on ethylene-sensitive (carnation) and ethylene-insensitive (chrysanthemum) cut flowers. Specifically, this research examines their morpho-physico-chemical characteristics, antioxidant enzyme activities and vase life. Here, the AgNPs were synthesized by chemical methods and then applied on both flowers by a pulsing method. The treatments involved two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water), and the duration of exposure lasted for 24 h. Then, the flower stems were placed in an aqueous sucrose solution (4%) until the end of the experiment. All traits, except the vase life, were evaluated after 0, 3, 6 and 9 d following the treatments during the vase period. During this time, the control groups of both flowers showed considerable amounts of decrease in the relative fresh weight (RFW), vase solution uptake (VSU), flower diameter, membrane stability index (MSI) and total soluble carbohydrate (TSC). Meanwhile, there were increases in hydrogen peroxide content (H₂O₂) and peroxidase (POD) activity. The bacterial population of the stem end and total soluble protein (TSP) increased in carnation petals, but decreased in chrysanthemum petals. The activity of superoxide dismutase (SOD) dropped in carnation petals, whereas it rose in chrysanthemum petals. Using AgNPs at concentrations of 0.08 and 0.04 g L⁻¹ can optimally extend the vase life of carnation and chrysanthemum, respectively.

1. Introduction

Nowadays, the floriculture industry is one of the most profitable sectors in horticulture. Its financial turnover in all respects amounts to over 300 billion dollars, and one-third of which is related to cut flowers (Chandler and Sanchez, 2012). Consumer demand for cut flowers and

ornamental plants are increasing, and the value of production is inevitably becoming higher. In this regard, flower quality is one of the most important indices in the sale and marketing of cut flowers. This makes flower quality an important factor in attracting customers over and over again (Scariot *et al.*, 2014). Therefore, the advancement of post-harvest programs is a definite requirement within the cut flower market.

Even after their separation from mother plants, cut flowers continue to be metabolically active and proceed with all of their vital processes by consuming the available food in their tissues. Carbohydrates, proteins and fat can be used by cut flowers to meet their metabolic demands. The increase in vase life and the delay in flower senescence can be achieved by upholding the normal rate of water uptake, preventing the depletion of carbohydrate storage and limiting the exposure of flowers to ethylene (Halevy and Mayak, 1981).

Water balance is an essential factor in determining the quality and vase life of flowers. It should be maintained between water uptake and transpiration (Lu *et al.*, 2010). Water uptake can be reduced due to the occlusion phenomenon generated by bacterial accumulation, physiological factors or air embolism (Damunupola and Joyce, 2008). Post-harvest senescence occurs within a few days, and is a major limitation in the marketing of cut carnation and chrysanthemum flowers. Stem end blockage is one of the important factors in early wilting of leaves and inflorescences in some cut flowers. van Doorn and Vaslier (2002) reported stem blockage is a major factor causing severe leaf wilting of chrysanthemum.

The in-rolling of petal margin and wilting of whole petals in senescence process in some cut flowers (e.g. carnation), is associated with the self-regulation of ethylene production (Yang and Hoffman, 1984), as a hormone, implying that the flowers are sensitive to ethylene. On the other hand, there are flowers such as chrysanthemum, pericallis and narcissus that initiate senescence without being influenced by ethylene, and thus are considered insensitive to ethylene (Dole and Wilkins, 2005; Jones 2013). Therefore, the short vase life and early wilting of inflorescences of carnation and chrysanthemum may be due to sensitivity of ethylene and stem end blockage, respectively. In fact, in ethylene-sensitive flowers, ethylene is a necessary requirement for the initiation and sustenance of senescence-based processes. The mechanisms by which ethylene can stimulate senescence involve changing the cell structure and increasing the

concentration of reactive oxygen species (ROSs) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (Abeles *et al.*, 1992).

Studies have also shown that physiological changes occur during senescence. These include chlorophyll decomposition, decreased activity of antioxidant enzymes, increased productions of ethylene and ROSs, along with membrane damage to the cells of cut flowers (Prochazkova and Wilhelmova, 2007).

Advancements in nanotechnology, especially the development of silver nanoparticles (AgNPs), have led to a wide range of nanocomposites with antimicrobial properties. The high surface-to-volume ratio of these particles makes them biologically more active and increases their contact with fungi and bacteria (Chau *et al.*, 2007). AgNPs are becoming popular in the flower industry for their ability to inhibit the synthesis of ethylene and delay the senescence in climacteric flowers (Kim *et al.*, 2005). In addition, AgNPs tend to regulate the stomatal aperture, maintain the chlorophyll content, preserve the relative fresh weight (RFW) and membrane stability index (MSI), reduce the transpiration rate, weight loss, hydrogen peroxide (H₂O₂), Malondialdehyde (MDA) and ROSs, and increase the activity of antioxidant enzymes along with their effects on the vase life of different cut flowers such as gerbera (Liu *et al.*, 2009; Solgi *et al.*, 2009; Nazari and Koushesh Saba, 2017), rose (Lu *et al.*, 2010; Nazemi Rafi and Ramezani, 2013; Hassan *et al.*, 2014), chrysanthemum (Carrillo-López *et al.*, 2016), carnation (Naing *et al.*, 2017; Lin *et al.*, 2019 b), gladiolus (Li *et al.*, 2017), peony (Zhao *et al.*, 2018) and cut gardenia foliage (Lin *et al.*, 2019 a). The application of AgNPs in vase solution of carnation alleviated vascular occlusion by inhibiting bacterial colonisation and biofilm formation on stem-end cut surfaces and in the xylem vessels (Lin *et al.*, 2019 b). Maity *et al.* (2019) also showed that the *Piper betle* silver nanoparticles (PbSNPs) in vase solution of *Gladiolus* have played an important role for scavenging ROSs by enhancing antioxidant enzyme activities that led to decrease in MDA and increased the MSI.

Nanosponges are new nano-sized colloidal carriers synthesized from β-cyclodextrins that have been prepared for delivering preservative and anti-ethylene compounds (Devecchi *et al.*, 2009). Devecchi *et al.* (2009) evaluated the effect of nanosponges including anti-ethylene molecules, such as 1-methylcyclopropene (1-MCP), 1-methylcyclopentene (1-MCPt), 2,5-norbornadiene and AgNO₃ on vase life of carnation flowers. They concluded that, 1-MCP-

nanosponge complex outperformed the other treatments in extending the vase life. In addition, Seglie *et al.* (2011) reported that 1-MCP in cyclodextrin-based nanosponges improved the vase life of carnation cut flowers.

Nonetheless, no report has so far described comparisons between the responses of ethylene-insensitive and ethylene-sensitive flowers to AgNPs. Therefore, the current research is aimed at evaluating how the cut flowers of carnation (highly sensitive to ethylene) and chrysanthemum (insensitive or slightly sensitive) respond to the application of AgNPs by the pulsing method. Comparisons are made between the two types of cut flowers by measuring their morphological, physiological and biochemical properties, as well as their enzymatic activities.

2. Materials and Methods

Plant materials and application of treatments

The cut flowers of carnation (*Dianthus caryophyllus* cv. Yellow Viana) and chrysanthemum (*Dendranthema grandiflorum* cv. Boris Becker Sunny) were harvested at their commercial harvesting stage from a soilless-cultured greenhouse. They were taken to the laboratory and the basal ends of the stems were immediately cut to reduce the stem length to 40 cm. Apart from 3-4 leaves on the top of each stem, all other leaves were removed. Then, AgNPs were applied at two concentrations (0.04 and 0.08 g L⁻¹) by the pulsing method for 24 h. The flowering stems were individually put in a bottle vase containing 300 mL deionized water. This also contained 4% (w/v) sucrose until the end of the experiment. To prevent the evaporation and contamination of the

vase solution, the vase opening was covered with aluminum foil. All traits, except for vase life, were evaluated after 0, 3, 6 and 9 d following the application of treatments. The pH of vase solution at the first day (d 0) was 7.82 and 8.76 for carnation and chrysanthemum, respectively. On d 9 of the vase period the pH of vase solution was 3.95 for carnation and 4.06 for chrysanthemum. The pulsing treatments and vase life were evaluated at 23±2°C, 50%±10% RH and 12 h of 15-20 μmol m⁻²s⁻¹ irradiance from cool-white fluorescence lamps.

Synthesis of AgNPs

To a solution of 0.265 mM (0.045 g) of silver nitrate in 100 mL distilled water, 10 mL of a trisodium citrate aqueous solution (1%) was added slowly at room temperature. After 10 minutes, 0.2 mL of ascorbic acid (0.005 M) was added to the mixture of reaction and stirred for 1 h until a yellow-green AgNPs colloid was formed (Tavallali and Pouresmaeil, 2012). The surface morphology of AgNPs was indicated by scanning electron microscopy (SEM) (TSCAN, Czech Republic) and showed in figure 1, it is exhibited that AgNPs have a size of about 8-80 nm.

Measurement of flower and stem diameter

The flower diameter was measured in two directions, and the stem diameter was measured in three parts (i.e. under peduncle, middle, and end of the stem). These measurements were repeated every three days and the average of values were reported.

Measurement of relative fresh weight

The relative fresh weight (RFW) of flowering stems in both types of cut flower was calculated every three days by the following formula:

$$\text{RFW (\%)} = (\text{Wt}/\text{Wt}_0) \times 100$$

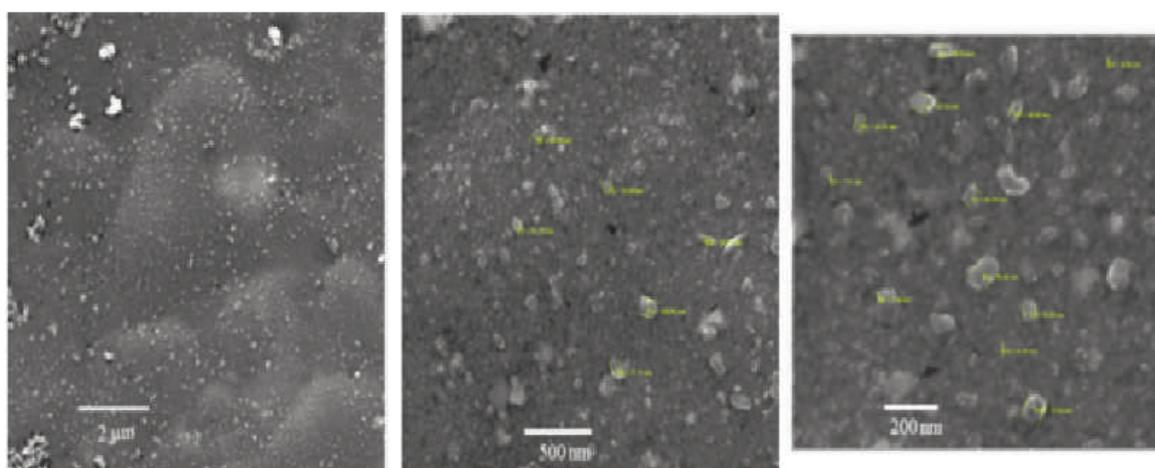


Fig. 1 - The SEM image of AgNPs.

where W_t is the weight of the stem (g) at $t = d$ 0, 3, 6, and 9, while the W_{t_0} is the weight of the same stem (g) at $t = d$ 0 (He *et al.*, 2006).

Measurement of vase life

The vase life of both types of cut flower was measured by counting the number of days from the beginning of the experiment until 50% of the flower had wilted. In carnations, the occurrence of in-rolling and browning petals by more than one-third was considered as the end of the vase life (Naing *et al.*, 2017). In chrysanthemum, however, the vase life ended when 50% of petals had wilted and the leaves had yellowed (Carrillo-López *et al.*, 2016).

Measurement of vase solution uptake

The weight of the vase with and without the flower shoots was recorded daily, and the following formula was used for calculating the vase solution uptake (VSU): VSU ($mg\ g^{-1}$ stem f. w.) = $(S_{t-1} - S_t)$; where S_t is the weight of vase solution (g) at $t = d$ 0, 3, 6 and 9, S_{t-1} is the weight of vase solution (g) on the previous day (He *et al.*, 2006; Lu *et al.*, 2010).

Measurement of membrane stability index

To measure the membrane stability index (MSI) of petals, this method involved the preparation of petal discs (measuring 1 cm in diameter) which were placed in falcons containing 20 mL distilled water. After this, a series of falcons were placed in a warm bath (40°C) for 30 min and their electrical conductivity (EC) was read by a conductivity meter (C1) after the falcons had cooled down to 25°C. Then, the second series of falcons was placed in a warm bath of 100°C for 20 min and their EC was read after having cooled down to 25°C (C2). In the end, the MSI was calculated using the following equation (Sairam *et al.*, 2002):

$$MSI = [1 - (C_1/C_2)] 100$$

C_1 = EC after exposure to 40°C and C_2 = EC after exposure to 100°C.

Measurement of bacterial population of stem end

To measure the bacterial population on the stem end, one gram of the stem end was homogenized and diluted with peptone water until a concentration of 10^{-3} was reached. Subsequently, 1 mL of this solution was transferred to Petri dishes and then a volume of 10 mL sterilized plate count agar medium was added to each Petri dish. These were slowly mixed for 5 to 10 s. The cultured Petri dishes were then kept in an incubator at 32°C for 2 d and, after counting the bacterial colonies, the results were reported

as log CFU g^{-1} (Balestra *et al.*, 2005; Liu *et al.*, 2009).

Measurement of total soluble protein

According to the Bradford method (1976), 0.5 g of petal tissue was powdered with liquid nitrogen, and then 0.25 g of polyvinylpyrrolidone (PVP) was added to the solution when stirring the 1.5 mL of potassium phosphate buffer containing sodium metabisulfite (0.019 g per 100 mL buffer). The homogenized samples were centrifuged (HETTCH, Germany) at 4°C for 20 min at 15,000 g. Then, 50 μ L of supernatant was mixed with 950 μ L of Bradford solution and, after 15 min, the light absorption was read at 595 nm by a spectrophotometer (UNICO 2100, USA).

Measurement of peroxidase activity

To measure the peroxidase (POD) enzyme activity, according to a method reported by Hemeda and Klein (1990), first 400 μ L of 50 mM potassium phosphate buffer (pH 7) was mixed with 40 μ L of 1% glycol and 40 μ L of 0.3% hydrogen peroxide in an ice bed. Then immediately, 65 μ L of protein extract was added to the mentioned composition. The changes in light absorption were read at 120 nm by a spectrophotometer within a wavelength of 470 nm.

Measurement of superoxide dismutase activity

The activity of superoxide dismutase enzyme (SOD) was measured by the Beyer and Fridovich (1987) method. According to this method, the solution used for the reaction was prepared by mixing 25 mL of 50 mM phosphate buffer (pH 7) with 0.0035 g L-methionine (9.9 mM), 0.004 g NBT (57 μ M) and 7.5 μ L Triton X-100. One mL of the reaction mixture was blended with 10 μ M riboflavin and 20 μ L of protein extract. The mixture was placed on a shaker at a distance of 30 cm from a 20-watt fluorescent lamp for 10 min. Then, the light absorption was measured at 560 nm by a spectrophotometer.

Measurement of total soluble carbohydrate

To measure total soluble carbohydrates (TSC), 0.5 g of petal tissue was crushed using liquid nitrogen along with 5 mL of 95% ethanol which helped obtain uniform extracts. The supernatant extract was centrifuged for 10 min at 3,500 g. Then, 1 mL of this extract was combined with 3 mL of Anthrone before being transferred to a warm bath of 100°C. The heat caused the appearance of a colored phase after 10 min. Subsequently, the samples were removed from the warm bath and were allowed to cool down at room temperature. Their light absorption was read at 625 nm. The TSC content of petals was determined by creating a standard curve using standard glucose.

The results were expressed as mg g^{-1} f.w. (Irigoyen et al., 1992).

Measurement of hydrogen peroxide

In order to measure the amount of hydrogen peroxide (H_2O_2), the method was similar to the one used by Alexieva et al. (2001). Accordingly, 0.2 g of petal tissue was completely ground with 5 mL trichloroacetic acid (TCA). The extract was obtained and centrifuged at 10,000 g for 5 min. Then, 250 μL of the supernatant was mixed with 250 μL of 100 mM phosphate-potassium buffer (pH7) and 500 μL of 1 M of potassium iodide (KI). The absorbance of each sample was read by a spectrophotometer within a wavelength of 390 nm.

Statistical analysis

This research was conducted as a factorial based on a completely randomized design (CRD), it had three factors that the first factor was the type of flower at 2 levels (carnation and chrysanthemum), second factor was AgNPs at 3 levels (0.04 and 0.08 g L^{-1} along with deionized water) and the third factor was the sampling time at 4 levels (0, 3, 6 and 9 d of the vase period) with 3 replications, each of which included 8 cut flower stems. For measuring the vase life, two factors of the type of flower and the sampling time were not considered and its design was as a CRD with three treatments (AgNPs at 0.04 and 0.08 g L^{-1} along with deionized water). Data were analyzed using SAS software and a comparison of mean values was made by the LSD test at 5% probability level.

3. Results

Relative fresh weight

Based on the comparison of mean values, the relative fresh weight (RFW) of both cut flowers gradually decreased in the control treatment during the vase period. A faster rate of this decline was observed in carnations, as compared to the chrysanthemum. From the initial days to the ninth day, the decrease of RFW in carnations was about twice as much as the decrease in chrysanthemum. The application of 0.04 and 0.08 g L^{-1} AgNPs caused the percentage of RFW to remain relatively constant in both cut flowers until the third days, but then the RFW gradually decreased. Furthermore, treating the cut flowers with AgNPs caused a better preservation of their RFW from d 3 to d 9 of the vase period, as compared to the control, but the difference between the two concentrations was no significant. On d 9 of the vase

period, the RFW of chrysanthemum was heavier than that of carnations, and the differences were significant (Fig. 2).

Vase solution uptake

There was an increase in the rate of vase solution uptake (VSU) by both flowers until the third day of the vase period, by which time the VSU in carnations was almost twice as much as that in chrysanthemum. From the third day onward, the VSU in both flowers decreased. The application of AgNPs caused an increase in the VSU in both flowers compared to the control. On d 9 of the vase period, the VSU in carnations had been significantly affected by both concentrations of AgNPs, whereas the chrysanthemum was only affected by 0.08 g L^{-1} AgNPs to a substantial degree (Fig. 3).

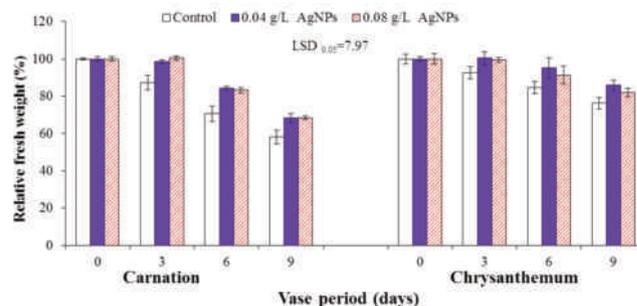


Fig. 2 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L^{-1}) along with the control (deionized water) on RFW of two cut flowers during the vase period. Values represent means \pm standard error ($n=3$). Least significant difference (LSD) at $P \leq 0.05$ was used for means comparison.

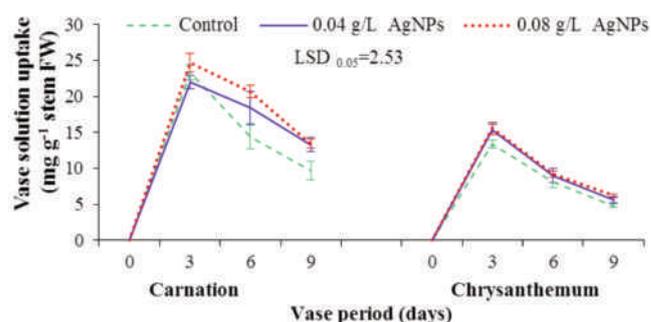


Fig. 3 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L^{-1}) along with the control (deionized water) on VSU of two cut flowers during the vase period. Values represent means \pm standard error ($n=3$). LSD at $P \leq 0.05$ was used for means comparison.

Bacterial population of stem end

The bacterial population of the stem end in carnations increased throughout the vase period. In chrysanthemum, however, the same trend continued until the third days before declining. Both concentrations of AgNPs reduced the bacterial population of the stem end in chrysanthemum during the vase period, making it significantly different when compared to the control on d 9. On the other hand, this trait in carnations was only affected by 0.08 g L⁻¹ AgNPs to make a significant difference compared to the control. In general, in both flowers, 0.08 g L⁻¹ AgNPs was more effective than the 0.04 g L⁻¹ in reducing the bacterial population of the stem end. The highest of this trait among both cut flowers was observed in the control group of chrysanthemum on d 3, whereas the lowest of this value was caused by 0.08 g L⁻¹ AgNPs and measured on d 9 (Fig. 4).

Changes in the stem and flower diameter

The stem diameter was one of the traits which was not significantly affected by AgNPs in both flowers. Even a comparison between d 0 and 9 of the control groups showed no significant difference. Despite the fact that 0.08 g L⁻¹ AgNPs in both flowers caused the stem diameter to become thicker in comparison with the stems of plants treated by 0.04 g L⁻¹ and the control, there were no significant differences between these groups (data not shown).

From d 3 onward, the diameter of both flowers decreased. However, it occurred more dramatically in carnations which shrank twice as much as the chrysanthemum. The effect of AgNPs on changing the flower diameter was significant only in carnations, during their vase period. Although AgNPs increased the diameter of flowers in both species, as compared

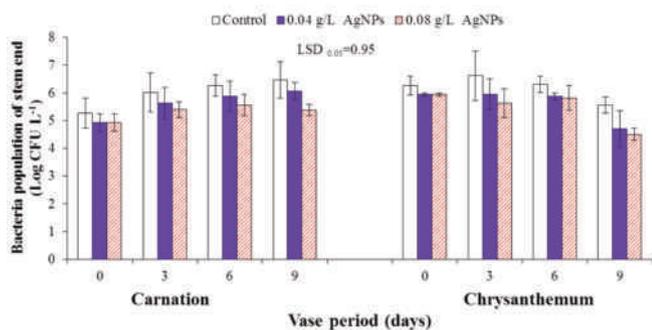


Fig. 4 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water) on bacterial population of the stem end of two cut flowers during the vase period. Values represent means ± standard error (n=3). LSD at P≤0.05 was used for means comparison.

with their respective control groups, the diameters had finally decreased by d 9 of the vase period. On the mentioned day, these treatments had made a significant difference in carnations only, as compared to the carnations control group. In general, the biggest flower diameter was observed in chrysanthemum on d 0 after being treated with 0.04 g L⁻¹ AgNPs, and the smallest of all diameters was obtained in the control group of carnations on d 9 (data not shown).

Membrane stability index

The decline in values of the membrane stability index (MSI) occurred in both flowers during the vase period, and no significant differences were observed between the two flowers in this respect. In the beginning of the vase period (d 0), the MSI in carnations was higher than in chrysanthemum, but the value of this trait decreased in both flowers through time. On d 9, this decrease was twice as much in carnation when compared to chrysanthemum. In both flowers, AgNPs caused the MSI to increase during the vase period, as compared to the control, but chrysanthemum responded more strongly to the treatment than the extent to which carnations did. In response to AgNPs, chrysanthemum showed a gradual increase in the MSI value - so much so that it became slightly ⁻¹ AgNPs can be a successful treatment for increasing the MSI in carnations. In contrast, however, the concentration of 0.04 g L⁻¹ worked optimally on chrysanthemum. In general, the highest value of MSI was observed in carnations on d 0 when treated with 0.08 g L⁻¹ AgNPs, whereas the lowest value occurred in the control on d 9 (Fig. 5).

Total soluble protein

A comparison of the mean values showed that the

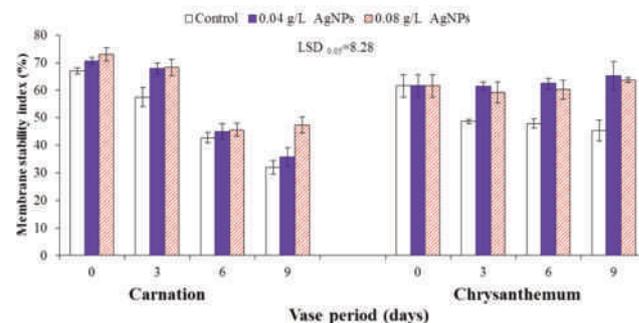


Fig. 5 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water) on MSI of two cut flowers during the vase period. Values represent means ± standard error (n=3). LSD at P≤0.05 was used for means comparison.

content of total soluble protein (TSP) in chrysanthemum petals was about 4 times higher than that of carnation petals on d 0. In carnations, the TSP content of the petals increased during the vase period. Meanwhile, chrysanthemum underwent a different pattern of change, whereby the content of TSP increased from d 0 to d 3, but then decreased sharply until d 6, and continued to decline at a gradual rate until d 9. The AgNPs increased the TSP in both flowers during the vase period, as compared to the control. Generally, the highest value of TSP was observed in chrysanthemum on d 3 in the treatment group of 0.04 g L^{-1} AgNPs, whereas the lowest value was observed in the control on d 9 (Fig. 6).

Total soluble carbohydrate

The total soluble carbohydrate (TSC) content in carnation petals was more than the content in chrysanthemum petals. In the control groups of both flowers, the content of TSC decreased. The use of AgNPs in both flowers did not cause a significant difference in TSC on d 0, as compared with the control, but thereafter the difference gradually became significant until d9. Applying the AgNP at 0.08 g L^{-1} on carnations and at 0.04 g L^{-1} on chrysanthemum significantly increased the TSC in comparison with their respective control groups. In general, carnations responded more strongly to the use of AgNPs, and the increase in their TSC content was much greater than in the case of chrysanthemum. The highest TSC content was measured in carnations on d 3 of the vase period after being treated with 0.08 g L^{-1} AgNPs. The lowest content was measured in the control group of chrysanthemum on d 9 (Fig. 7).

Hydrogen peroxide content

The petals of both flowers initially contained simi-

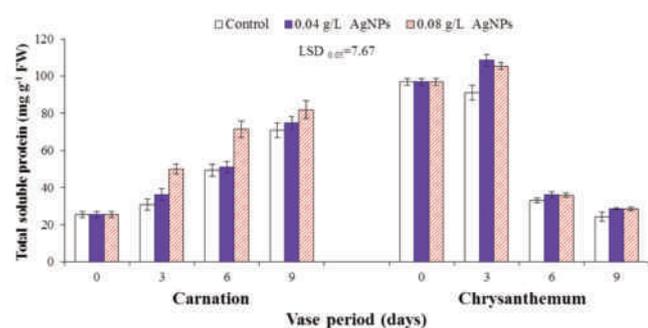


Fig. 6 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L^{-1}) along with the control (deionized water) on TSP of two cut flowers during the vase period. Values represent means \pm standard error ($n=3$). LSD at $P \leq 0.05$ was used for means comparison.

lar amounts of hydrogen peroxide (H_2O_2) which gradually increased during the vase period. The rate of this increase was higher in carnations compared to the chrysanthemum. When comparing the H_2O_2 content between d 0 and d 9, its increase in carnations was about three times more than the increase measured in chrysanthemum. The application of AgNPs on both flowers reduced the H_2O_2 content in their petals, as compared with the control, but the increase was not prevented completely. On the last day of the vase period, the application of AgNPs on carnations led to a significant difference in H_2O_2 content when compared with the control. However, this was not the case in chrysanthemum. In general, the highest amount of H_2O_2 was measured in the control group of carnations on d 9 of the vase period. Its lowest amount was obtained in chrysanthemum on d 6 by 0.08 g L^{-1} AgNPs (Fig. 8).

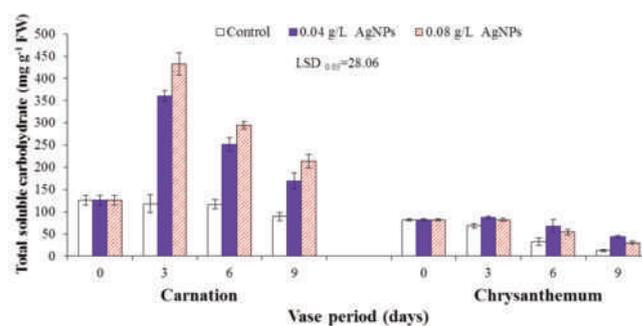


Fig. 7 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L^{-1}) along with the control (deionized water) on TSC of two cut flowers during the vase period. Values represent means \pm standard error ($n=3$). LSD at $P \leq 0.05$ was used for means comparison.

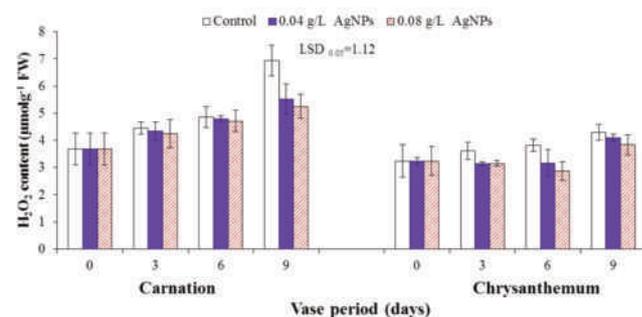


Fig. 8 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L^{-1}) along with the control (deionized water) on H_2O_2 content of two cut flowers during the vase period. Values represent means \pm standard error ($n=3$). LSD at $P \leq 0.05$ was used for means comparison.

Peroxidase activity

Based on the results, peroxidase (POD) activity in both flowers increased until d 3 of the vase period and then decreased until d 6 before increasing again thereafter. Using AgNPs on chrysanthemum, unlike carnation, significantly increased the activity of POD in comparison with the control. When treated with 0.04 g L⁻¹ AgNPs, the chrysanthemum showed a level of POD activity on d 9 that was about 2.5 times greater than the activity in carnations on the same day. In general, the highest level of POD activity was measured in chrysanthemum on d 9 after the treatment with 0.04 g L⁻¹ AgNPs, whereas the lowest level of activity was measured on d 6 in the control (Fig. 9).

Superoxide dismutase activity

Based on the results, that the superoxide dismutase (SOD) activity in carnation petals was about 2.5 times higher than that of chrysanthemum petals on d 0, but on d 9 it was completely different, and the activity of this enzyme in chrysanthemum was more than 4.5 times that of carnation. Generally the activity of SOD dropped in carnation petals, whereas it rose in chrysanthemum petals. The AgNPs decreased the SOD in both flowers during the vase period, as compared to the control. Generally, the highest value of SOD was observed in chrysanthemum on d 9 in the control treatment, whereas the lowest value was observed in carnation on this day of vase period in the treatment group of 0.08 g L⁻¹ AgNPs (Fig. 10).

Vase life

Clearly, AgNPs caused the vase life of both flowers to increase. Both concentrations had a significant effect on carnations, while chrysanthemum was significantly affected by the 0.04 g L⁻¹ only. In carnations, the vase life increased parallel to the increase in applied concentrations of AgNPs from 0.04 to 0.08 g L⁻¹, the effects of which were significantly different compared to each other. Using 0.08 g L⁻¹ AgNPs yielded more appropriate results in carnations. On the other hand, the vase life of chrysanthemum was slightly affected in a negative manner as the concentration of AgNPs rose from 0.04 to 0.08 g L⁻¹, but the difference between the two concentrations was insignificant. Nonetheless, both caused significant differences in comparison with the control (Fig. 11A and B).

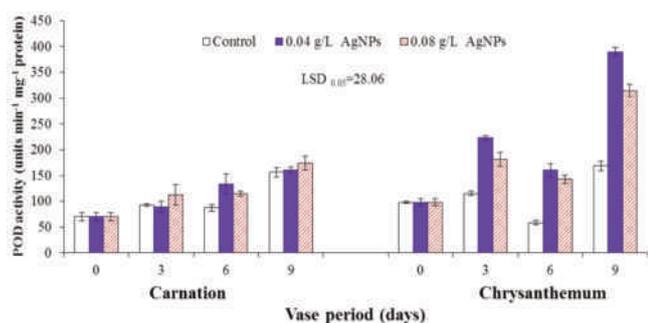


Fig. 9 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water) on POD activity of two cut flowers during the vase period. Values represent means ± standard error (n=3). LSD at P≤0.05 was used for means comparison.

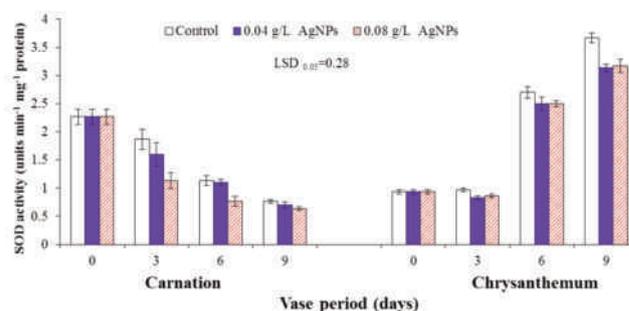


Fig. 10 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water) on SOD activity of two cut flowers during the vase period. Values represent means ± standard error (n=3). LSD at P≤0.05 was used for means comparison.

On the other hand, the vase life of chrysanthemum was slightly affected in a negative manner as the concentration of AgNPs rose from 0.04 to 0.08 g L⁻¹, but the difference between the two concentrations was insignificant. Nonetheless, both caused significant differences in comparison with the control (Fig. 11A and B).

4. Discussion and Conclusions

In this study, the cut flowers of carnation and chrysanthemum showed various levels of decrease in RFW (Fig. 2), VSU (Fig. 3) and flower diameter throughout the vase period. Similar results have been reported after assessing the RFW and VSU of gerbera cut flowers (Liu *et al.*, 2009), rose (Chamani *et al.*, 2005) and cut gardenia foliage (Lin *et al.*, 2019 a) during the vase period. The current study showed that using the AgNPs reduced the bacterial population of the stem end in both flowers, while the RFW was improved as compared to the control. Previous studies confirm such findings on the use of AgNPs in cut flowers such as *Gerbera* (Liu *et al.*, 2009; Solgi *et al.*, 2009; Nazari and Koushesh Saba, 2017) and rose (Lu *et al.*, 2010; Nazemi Rafi and Ramezani, 2013; Hassan *et al.*, 2014). These studies showed that the vase life improves when the bacterial population of the stem end decreases, besides when the VSU and RFW increase.

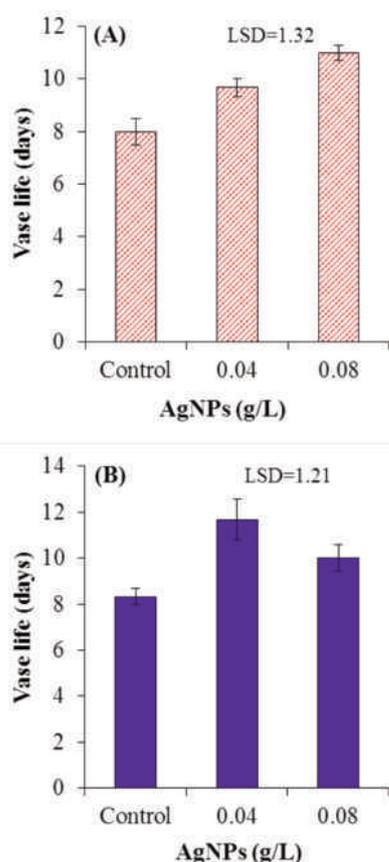


Fig. 11 - Effect of two concentrations of AgNPs (0.04 and 0.08 g L⁻¹) along with the control (deionized water) on vase life of carnation (A) and chrysanthemum (B). Values represent means \pm standard error (n=3). LSD at P \leq 0.05 was used for means comparison.

The decrease in the RFW of cut flowers marks the beginning of senescence in flowers, and the more the flowers become closer to the process of senescence, their ability to take up water from the vase is reduced. The imbalance between water uptake and transpiration distorts cell turgor, thereby causing the flowers to wither (Reid and Jiang, 2012). The survival of cut flowers depends largely on a positive water balance: an increase in water uptake and a decrease in water loss (Halevy and Mayak, 1981; Van Doorn, 1997). After being placed in the vase, the flower loses RFW in part because of vascular occlusion and the growth of microorganisms which grow in the vase solution. They reduce the water uptake by blocking the stem end and vessels, thereby causing water stress which is a main factor in the reduction of vase life (van Doorn, 1997; Macnish *et al.*, 2005). Adding a germicide to the vase solution may control the activity of microbes. It has been reported that adding antimicrobial compounds containing Ag⁺ ions to the vase solution improves the vase life of cut flowers (Hassan *et al.*, 2014). AgNPs nanoparticles function

effectively because of their high surface-to-volume ratio and their crystallographic surface structure (Rai *et al.*, 2009). Furthermore, Ag⁺ ions cause bacterial cell death by affecting membrane structure and permeability, inhibiting DNA transcription, disrupting transport activity and changing cellular content and ATP (Feng *et al.*, 2000; Sondi and Salopek-Sondi, 2004; Rai *et al.*, 2009; Dakal *et al.*, 2016).

This difference in the bacterial population of the stem end of the two cut flowers may depend on the genotype of the plant, as well as the extract and metabolites that are secreted from the stem end and are released into the vase solution, thereby affecting the growth of bacteria. Phenolic compounds are secondary metabolites that are involved in plant defense against pathogens (Naczka and Shahidi, 2004). In traditional Chinese medicine, the flowering heads of *Chrysanthemum indicum* are used as a source of bactericide, with additional antifungal and antiviral properties (Shunying *et al.*, 2005). In agreement with our results, Shunying *et al.* (2005) showed that the chemical composition and secondary metabolites of *Ch. indicum* possess antimicrobial activity.

Flower diameters in this study were significantly influenced by AgNPs in carnation flowers, but the effect on chrysanthemum was not significant. Dar *et al.* (2014) reported that the flower diameter in *Dianthus chinensis* first increased and then finally decreased during post-harvest development and senescence, respectively. The current research showed that the flower diameter of carnations tends to increase after being treated with AgNPs. This accords closely with a previous report on *Polianthes tuberosa* which was treated with 0.015 g L⁻¹ AgNPs and led to a significant increase in the diameter of flowers (Bahrehmand *et al.*, 2014).

It is well known that the MSI gradually decreases from the time when the flowers open to the time of their senescence. Such a trend occurs evidently in certain flowers such as *Lilium* (Bieleski and Reid, 1992), rose (Hassan *et al.*, 2014) and Iris (Ahmad and Tahir, 2016). The senescence process is mainly associated with protein loss, increased lipid peroxidation, membrane leakage, cell wall component degradation and cellular membrane disruption (Buchanan-Wollaston, 1997). AgNPs may preserve the membrane stability by curbing the peroxidation of lipids (Hatami and Ghorbanpour, 2013). There was a lower percentage of MSI in carnation petals, compared to chrysanthemum, which may be due to the fact that carnations are highly sensitive to ethylene. The reason becomes clear when knowing that ethylene

degrades the cell membrane and increases its leakage (van Doorn and Woltering, 2008), thereby resulting in a lower value of MSI. However, AgNPs acts against the production of ethylene (Hassan *et al.*, 2014). In fact, AgNPs induce an efficient cellular electron exchange mechanism which reduces electron leakage and, subsequently, limits the creation of ROSs. AgNPs also dys regulate lipid peroxidation and have a propensity to maintain the MSI (Lu *et al.*, 2010; Hassan *et al.*, 2014).

Previous studies have reported a decrease in TSP content and an increase in protease activity during the vase life of cut flowers (Wagstaff *et al.*, 2005; Dar *et al.*, 2014; Zhao *et al.*, 2018). Quite differently, however, we concluded that changes in the TSP content may vary depending on the type of flower, species and cultivar. Still, contrary to our results, Dar *et al.* (2014) showed that protein degradation in *Dianthus chinensis* is a key factor in regulating the senescence process of flowers. Shahri *et al.* (2011) also reported that TSP decreased during the senescence process of *Helleborus orientalis* cv. 'Olympicus', while there was an increase in its low-molecular-weight proteins. Accordingly, realizing an increase of TSP content in carnation petals could be a result of these low-molecular-weight proteins.

It has been shown that in flowers such as *Helleborus orientalis* and *Dianthus chinensis*, the TSC is reduced during the senescence process (Shahri *et al.*, 2011; Dar *et al.*, 2014). There was a lower content of TSC in chrysanthemum petals, as compared with carnations, which may be due to the genetic differences between the two plants, as well as the difference in the rate of polysaccharide decomposition during the opening and development of flowers and petals. In agreement with our results, it has been found that the starch and fructan polysaccharides are degraded and reduced during the development of flowers and the expansion of petals in chrysanthemum (Trustyl and Miller, 1991). Therefore, in this study, AgNPs in both flowers may have benefited the vase life by maintaining the content of TSC in petals and by promoting the mechanisms through which carbohydrates stabilize the cell membrane (Ashraf *et al.*, 2010). Furthermore, AgNPs regulate and protect the cellular osmotic potential, inhibit the formation of free radicals (Parida and Das, 2005) and regulate the expression of genes (Rahdari *et al.*, 2012).

Usually, the amount of H₂O₂ increases in plant cells during senescence (Ezhilmathi *et al.*, 2007; Saeed *et al.*, 2014). It can be suggested that high levels of H₂O₂ in carnation petals occur because of the

plants sensitivity to ethylene. This can be compared with chrysanthemum which is not sensitive to the hormone. Our results showed that the use of AgNPs on both flowers reduced the production of H₂O₂ in petals, as compared with the control. These results accord closely with a previous report by Hassan *et al.* (2014) where the production of H₂O₂ became significantly limited in roses because AgNPs was used.

Senescence is an oxidation process in which ROS and antioxidant systems are involved (Buchanan-Wollaston, 1997). Plant cells have developed a series of antioxidant mechanisms for defense to prevent the production of ROSs and to limit their destructive effects on proteins, fats and nucleic acids (Arora *et al.*, 2002). The enzymatic part of this system consists of antioxidant enzymes such as SOD, POD, catalase (CAT) and ascorbate peroxidase (APX) which degrade all types of ROSs (Balakhnina and Borkowska, 2013). Depending on the species or type of cultivars, the POD activity and SOD enzymes can exhibit different patterns of change in cut flowers during the senescence process (Hassan *et al.*, 2014). As the production of H₂O₂ increased in the petals of both flowers during the vase period, the POD enzyme likewise increased its activity to scavenge the higher amounts of H₂O₂. Furthermore, Hassan *et al.* (2014) reported similar results which indicate that petals of the rose cut flower cv. 'First Red' - which is sensitive to ethylene (Chamani *et al.*, 2005) - showed declining levels of SOD activity during the vase period. However, contrary to our results on the incremental trend of POD in carnations and chrysanthemum during the vase period, Hassan *et al.* (2014) reported that the POD activity in the rose cultivar decreases. It has also been reported that SOD activity in ethylene-sensitive flowers such as carnation is relatively lower compared to ethylene-insensitive flowers such as chrysanthemum. This comprises a major factor in accelerating the senescence (Bartoli *et al.*, 1995).

In addition, it may be suggested that in ethylene-insensitive flowers such as chrysanthemum, ROS cause the greatest amount of damage to the cell components, thereby leading to a shorter vase life. For this reason, the SOD activity has to increase so as to create a parallel level of scavenging. In certain flowers such as carnations, which are sensitive to ethylene, the level of SOD activity may not be pronounced as much. As previously mentioned, AgNPs cause the plants to acquire an efficient cellular electron exchange mechanism, whereby the electron leakage and ROS production are reduced (Hassan *et al.*, 2014). Perhaps, the activity of SOD does not

increase in carnations even when AgNPs are applied.

AgNPs increased the vase life of chrysanthemum and carnation (Figs. 11A and B) by increasing the RFW (Fig. 2) and reducing the bacterial population of the stem end (Fig. 4). The scientific literature contains several reports that mention the efficiency of AgNPs in increasing the vase life of gerbera (Liu *et al.*, 2009; Solgi *et al.*, 2009; Nazari and Koushesh Saba, 2017), rose (Lu *et al.*, 2010; Nazemi Rafi and Ramezani, 2013; Hassan *et al.*, 2014), chrysanthemum (Carrillo-López *et al.*, 2016), carnation (Naing *et al.*, 2017) and gladiolus (Li *et al.*, 2017).

In conclusion, AgNPs improved the vase life of both cut flowers by contributing to the values of their RFW, VSU, flower diameter, MSI, TSC, TSP and POD activity and by limiting their bacterial population of the stem end and H₂O₂ levels, as compared to the control. It is highly probable that AgNPs are capable of reducing the expression of genes responsible for the production of ethylene, as well as limiting the rate of transpiration and the opening of stoma. Furthermore, AgNPs have roles in regulating the aperture of the stoma and in reducing MDA production, thereby prolonging the vase life of cut flowers. Ultimately, this research revealed that the use of AgNPs at 0.04 g L⁻¹ and 0.08 g L⁻¹ can extend the vase life of at least two cultivars belonging to chrysanthemum and carnation, respectively.

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