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Research Article

Biosynthesized Silver Nanoparticles Used in Preservative Solutions for *Chrysanthemum* cv. Puma

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The use of pulse solutions containing antimicrobials has been reported, but more research is necessary. To increase vase life and to study their effect on opening inflorescences, silver nanoparticles were used in vase solutions for cv. Puma *Chrysanthemum* stems. The nanoparticles were synthesized biologically using *Chenopodium ambrosioides* L. applied at concentrations of 0.01, 0.05, 0.1, 0.5, 1, and 5 mM and compared with a control. Treatments were replicated five times. The stems were cut to 50 cm and observed until the end of their vase life. Low concentrations of silver nanoparticles promoted inflorescence opening and leaf yellowing, while the control leaves remained green, but there was a lower degree of inflorescence opening. High concentrations of silver nanoparticles (0.5, 1, and 5 mM) caused senescence due to low water uptake through the stems. Statistical differences in inflorescence opening and diameter, bacterial growth (CFU mL^{-1}) in vase solutions, fresh weight, water uptake, and vase life were found among treatments. Longer vase life and less weight loss were observed in the stems exposed to low concentrations of silver nanoparticles. Low concentrations of silver nanoparticles promoted inflorescence opening and increased vase life of *Chrysanthemum* cv. Puma.

1. Introduction

Chrysanthemum (*Dendranthema grandiflora* Tzeleu.) is one of the most popular ornamental crops in the world [1]. It is a member of Asteraceae family; today, it is the second most economically important flower in the world after roses [2]. Introduced in Mexico in 1960 [3], this species has since been produced in soil, although it is reported that when grown in a system of recirculated nutrient solution with sand substrate, higher quality stems are produced [4]. *Chrysanthemum* is one of the most important plant species in Mexico, planted on an area of 2,564 ha with a production of 9,529,819 stems [5]. The state of Mexico has the largest production area, 2,377 ha. Although Texcoco produces less than 1% of the national production of *Chrysanthemum*, the area is highly important because of its traditional production methods and the destination of its production. More than 30 years ago, the construction of rustic greenhouses began. They had cinder block and plastic walls. Growers used organic fertilizers for plant nutrition and homemade preparations for pest and disease control. Over the years, infrastructure and production techniques have modernized. Today, shade cloth, steel structure greenhouses, chemical fertilizers, and pesticides are used. However, it should be noted that empirical knowledge is part of the idiosyncrasy of Texcoco growers [6]. Knowledge passed down from generation to generation still predominates in areas such as the production of cuttings from mother plants and the use of handmade metal mesh for tutoring the flower stems. The target market for the production of *Chrysanthemum* in Texcoco is the wholesale market in the center of Iztapalapa, Mexico City, the most important market in Mexico. However, there is no control of postharvest stem physiology and technology, nor is there control of optimal cutting time to ensure good flower opening [6].

In cut flowers, the most common causes of early senescence are inhibition of water uptake, excessive water loss

caused by poor management, low supply of carbohydrates for respiration, ethylene production, attack by microorganisms, and vascular system blockage [7]. Ag^+ ions are known to be effective when used as antimicrobial because they reduce cytoplasmic membrane thickness, loosen the cell wall, and condense DNA molecules [8]. One of the most important *Chrysanthemum* postharvest problems is yellowing leaves and the inability to absorb water, leading to premature leaf wilting [9]. Generally, water shortages cause obstruction in stem vessels and the formation of air bubbles (vascular obstruction), reducing flower quality [10]. Methods of eliminating vascular obstruction include a pulsing treatment, which can contain sugars (sucrose), acids (citric acid or 4-aminooxyacetic acid), growth regulators, such as benzyladenine and gibberellic acid, and antimicrobials [11]. Because microorganisms can also obstruct vessels and reduce water uptake, it is necessary to use antimicrobials, such as 8-hydroxyquinoline sulfate [9], silver nitrate [12], silver thiosulfate [11], and, recently added to the list, silver nanoparticles. Other chemical substances (antimicrobials) in preservative solutions to improve postharvest characteristics include silver thiosulfate, silver nanoparticles, aminooxyacetic acid, and 8-hydroxyquinoline sulfate [12]. A few studies have investigated the effect of Ag nanoparticles synthesized with plant extracts on vase life, but either most do not report the origin of the particles or their synthesis is chemical [13, 14]. Liu et al. [15] evaluated the effect of pulse treatment (24 h) with Ag nanoparticles (5, 10, and 20 mg L^{-1}) on the vase life of *Gerbera*. They concluded that pulse treatment with 5 mg L^{-1} Ag nanoparticles increased vase life more than the double, relative to the control, because it reduced the bacterial population and prevented blockage of xylem vessels.

Beni et al. [16] reported that the use of Ag nanoparticles increased water absorption and fresh weight in tuberous flowers. There was also a reduction in lipid peroxidation relative to the control. Solgi et al. [17] increased vase life of *Gerbera* cv. Dune using 5 or 10 mg L^{-1} Ag nanoparticles. Similar results led Ghaleshakhani et al. [18] to conclude that 10 mg L^{-1} Ag nanoparticles, in combination with humic acid, increased vase life in *Alstroemeria*. However, most of this research fails to report the source of Ag nanoparticles used in the solutions. A few mention the use of commercial Ag nanoparticles (Sigma-Aldrich®) synthesized chemically and polydisperse nanoparticles of different sizes depending on the solvent in which they are found (ethylene glycol, aqueous buffer, and isopropyl alcohol), making handling difficult in vase solutions.

The biosynthesis of Ag nanoparticles from plant extracts has been reported to be clean, economically efficient, and nontoxic to the environment, with the advantage that it can be synthesized on a large scale [19]. Using silver nanoparticles has advantages over silver nitrate. Less silver nitrate is needed to produce an effect on vase life because silver nanoparticles have proportionally more surface area. It has been reported that mobility of silver ions in flower stems is very low. Therefore, application of nanoparticles with antimicrobial effects can improve mobility and prolong longevity [14]. Another advantage of silver nanoparticles synthesized environmentally is their ease of handling because they are dispersed in deionized water and can be easily added to the water in the vase. On the other hand, silver nitrate may be environmentally risky. Restricted distribution may explain why silver nitrate is rarely used as a commercial treatment for cut roses [20]. For these reasons, in this research, Ag nanoparticles were synthesized in aqueous extracts, according to Carrillo-López et al. [21], for use in vase solutions, and survival and stem quality were assessed in order to choose the treatment that most increases vase life and improves quality of *Chrysanthemum* cv. Puma for the regional flower market.

2. Materials and Methods

2.1. Biosynthesis and Characterization of Ag Nanoparticles. The procedure for synthesis of Ag nanoparticles was according to Carrillo-López et al. [21]. Mature leaves of *Chenopodium ambrosioides*, a Mexican herb that grows wild in the *Chrysanthemum* cv. Puma beds, were collected in Texcoco, Mexico. The leaves were washed three times with deionized water and dried in an oven at 60°C and ground with a mortar; 2 g of *Chenopodium ambrosioides* dry plant material was weighed and added to 100 mL of boiling deionized water. It was boiled for 5 min and, once cooled, filtered with Whatman 4 paper. This constituted the reducing agent. An aqueous solution of 10 mM $AgNO_3$ (Sigma-Aldrich) was prepared. The aqueous solution of Ag nanoparticles (AgNPs) was prepared with 5 mL of plant extract, 5 mL of 10 mM $AgNO_3$, and 5 mL of deionized water. Evaluation of AgNPs was done with UV-Vis spectrophotometry (Perkin Elmer Lambda 40 operated at a resolution of 1 nm). A scan between 350 and 700 nm was performed to evaluate the surface plasmon resonance in time. The shape and size of AgNPs were characterized by transmission electron microscopy (TEM Jeol 2010 operated at 200 kV).

2.2. Plant Material. In November 2014, stems of *Chrysanthemum* cv. Puma were harvested at commercial cutting maturity; this cutoff point is 1, as seen on the scale of floral opening, Figure 1. Stems were harvested at 7:00 h directly from a greenhouse in Texcoco, Mexico. Environmental conditions were 14°C and 60% relative humidity. The *Chrysanthemum* growing beds are 1 m wide by 30 m long. The flowers were cut randomly using pruning shears 10 cm above the soil. Transport time to the storage of the stems was 30 min. The stems were trimmed back under water (to avoid embolism) to a height of 40 cm, taking care to cut diagonally to increase the area of water uptake. The basal leaves were removed leaving only five leaves per stem. The experiment was conducted under a completely randomized design using the software SAS System 9.1, with five replications. Evaluated Ag nanoparticle concentrations were prepared from 10 mM AgNPs: 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, and 5 mM; control was deionized water. A commercial solution Floralife Crystal Clear® was included. One floral stalk was placed in each of the 40 vases.

2.3. Vase Life Assessment Space. The conditions in the vase life room were 24°C and 65% relative humidity. Twelve h of light per day was provided by 1100 lux fluorescent lights.

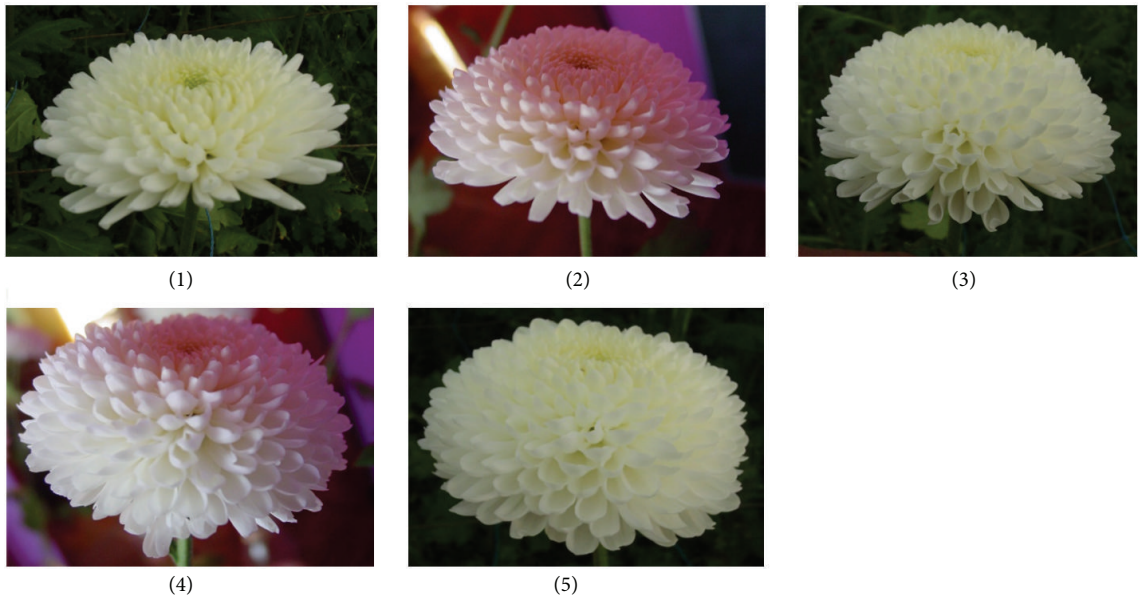


Figure 1: Five-point hedonic scale to evaluate cv. Puma *Chrysanthemum* flower opening.

2.4. Vase Life and Fresh Weight. Vase life, expressed in days, was defined as the time from onset of treatment with silver nanoparticles to stem senescence, which was determined when 50% of the petals withered and/or leaves changed color (yellowing).

2.5. Fresh Weight Loss. Based on initial fresh weight and daily fresh weight during vase life, percentage of fresh weight loss was calculated with the following expression:

$$\%\ \text{Loss of fresh weight} = \left(\frac{(\text{initial fresh weight} - \text{end fresh weight})}{(\text{initial fresh weight})}\right) * 100. \quad (1)$$

Fresh weight was determined with Ohaus® digital scale with 0.1 g precision.

2.6. Total Bacterial Count. After 8 days, an aliquot of 1 mL of the vase solution was taken and placed in a 3M® Petrifilm® Aerobic Count plate. The results are expressed in CFU mL^{-1}.

2.7. Floral Opening. Floral opening was determined with a 5-point hedonic scale (Figure 1). For *Chrysanthemum* variety Snow Eleonora, a flower opening scale is not known.

2.8. Water Uptake. Water uptake volume in mL was measured daily using a graduated glass cylinder.

2.9. Maximum Diameter of Inflorescences. The inflorescence diameter was measured with a graduated Vernier (accuracy of 1 mm) at the end of vase life.

2.10. Scanning Electron Microscopy. Cross sections measuring 0.5 cm were cut under a stereoscope microscope (Carl Zeiss®) and placed in 2.5% glutaraldehyde with Sorensen's phosphate buffer at pH 7.2 for 4 h, in periods of 5 min vacuum during the first hour of fixing. The samples were rinsed twice in Sorensen's phosphate buffer at pH 7.0 for 10 min. The samples were then dehydrated with an ethanol series starting at 30% and increasing for 45 min to 100%. The samples were then dried to the critical point with CO_2 (Tousimis Samdri® 780A) and coated with gold for 10 min in an ionizer (Jeol Fine Coat Ion Sputter JFC-1100) for observation in a scanning electron microscope (Jeol JSM-6390) operated at 20 kV.

3. Results and Discussion

3.1. Evaluation and Characterization of AgNPs. UV-Vis spectra are a qualitative indicator of the amount, size, and shape of silver nanoparticles in aqueous suspensions. After adding the extract to the solution of silver nitrate, there was a change in color of the aqueous solution, from yellow to yellowish brown, which deepened over time (Figure 2, inserts a and b). This color change is due to the excitation of surface plasmon vibrations [22]. Figure 1 shows the absorption spectrum of the nanoparticles produced with 5 mL of *Chenopodium ambrosioides* extract and 10 mM $AgNO_3$. Over time, absorbance increased, obtaining maximum absorbance at 438 nm. In Figure 2, high absorbance values are observed in a volume of 5 mL and a molar concentration of 10 mM $AgNO_3$, which corresponds to intense color in the aqueous solution of nanoparticles and a larger number of particles formed.

The system used for biosynthesis is remarkably good because it produces very high absorbance (and therefore is more effective in reducing to obtain more particles) and system stability (because it does not change the symmetry of the spectra; i.e., there is no spreading over time or change

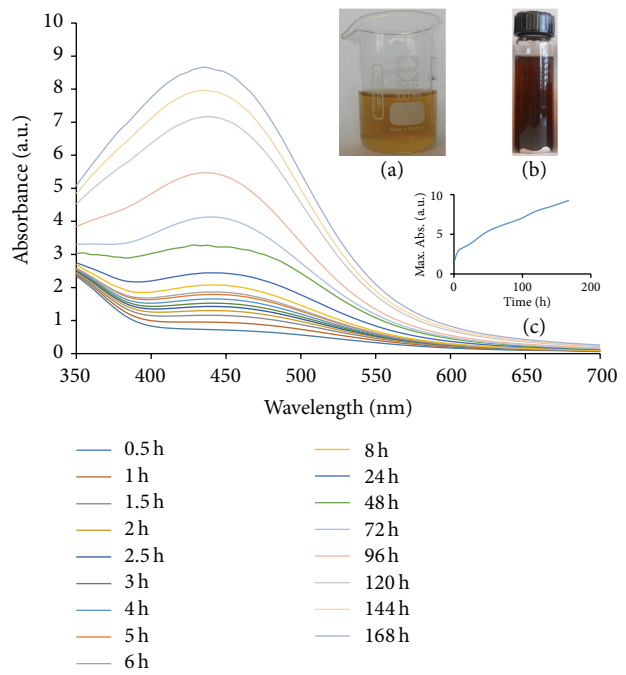


Figure 2: UV-Vis spectrum of Ag nanoparticles synthesized with 5 mL *C. ambrosioides* extra and 10 mM $AgNO_3$. Inserts (a) and (b) show the extract before and after reduction, respectively, and (c) shows system setting time.

in the values of maximum absorbance). Other biosynthetic studies with plant extracts show low absorbance values [23, 24]. Insufficient biomolecules for reducing silver ions lead to the formation of low particulate and low maximum absorbance values [25]. The movement of the plasmon band at longer wavelengths may be because the nanoparticles are relatively large, polydisperse, and anisotropic [22].

Figure 3 shows representative micrographs of transmission electron microscopy (TEM): two magnifications of particles resulting from the reaction of silver ions and 5 mL *C. ambrosioides* extract. The silver nanoparticles produced exhibit little uniformity in size since they are polydisperse. Similar results were obtained by evaluating different amounts of *Cinnamomum camphora* biomass, where the anisotropic nanostructures, such as nanoparticle, nanotriangles, or irregular contours, are obtained with large quantities of biomass [25]. Carrillo-López et al. [21] concluded that the lower the volume of extract (1 mL), polydispersity, and concentration of silver nitrate (1 mM), the smaller the particles obtained.

Figure 4 shows the particle size distribution of 5 mL *C. ambrosioides* extract and 10 mM $AgNO_3$. Particle size is 10.2 nm ± 4.3.

3.2. Vase Life. Analysis of variance showed statistically significant differences between 0.01 mM AgNP concentration and the other treatments for prolonging vase life of *Chrysanthemum* cv. Puma. Of the different levels of AgNPs applied, the treatment with a concentration of 0.01 mM resulted in the longest vase life (21 days), while the control and the highest AgNP concentration (5 mM) resulted in the shortest

Table 1: Effect of Ag nanoparticle concentration (mM) on vase life (days) of *Chrysanthemum* cv. Puma.

AgNP concentration (mM)	Vase life (days)
Control	12.00[c]
0.01	21.00[a]
0.05	19.33[b]
0.10	19.33[b]
0.50	17.33[b]
1.00	17.00[b]
5.00	15.66[b]
Floralife Crystal Clear	18.00[b]

Vase life data are the average of five experiments. Values in columns with different letters are statistically different ($P < 0.05$).

vase life (16 and 12 days, resp.). The treatment with Floralife Crystal Clear achieved 18 days of vase life. Generally, a low Ag concentration favored vase life (Table 1). For cut flowers to fully open and stay fresh, they need a continuous free-flowing supply of nutrients and water inside stem vessels that easily transport them. Ranwala [26] found that treatment with Floralife Crystal Clear extended longevity of *Chrysanthemum* by 47%; in *Chrysanthemum* "Galliaro," the vase life in the flower food was 15 days, while vase life in water was 10.2 days. Other types of *Chrysanthemum* had 61% and 88% longer vase lives. Safa et al. [27] revealed that silver nanoparticles and chlorophenol have the potential to extend vase life and enhance the postharvest quality of cut *Gerbera* cv. "Balance" flowers. On the other hand, studies revealed that when nanosilver concentration rose by 6 to 8 and 10 mg L^{-1}, a toxic effect was detected with no increase in antibacterial efficacy [28]. According to Hatami et al. [14], pulse treatments with AgNPs plus sucrose significantly extended the vase life of cut roses. This was associated with a relatively high leaf water content, attributed to increased hydraulic conductance as well as inhibition of transpiration.

3.3. Relative Fresh Weight and Water Uptake. At the beginning of vase life, flowers increased in weight, and, over time, fresh weight decreased (Figure 5). High AgNP concentrations inhibited water uptake, resulting in greater relative fresh weight loss. The highest fresh weight, reflected in increased vase life, was obtained with the AgNP concentrations 0.01, 0.05, and 0.1 mM. Statistically significant differences in fresh weight were found among treatments at end of vase life (Table 2). The heaviest weight was obtained using low AgNP concentrations and Floralife Crystal Clear due to increased water uptake.

Initially, cut flower fresh weight generally increases due to water uptake and subsequently decreases. Nemati et al. [29] found that treatment with nanosilver extended longevity of *Lilium orientalis* flowers; 30 ppm concentration of nanosilver enhanced absorption of nanosilver vase solution and thus increased initial fresh weight, as well as lowering bacterial colonization during the first two days of vase life. Solgi et al. [17] showed that 1 or 2 mg L^{-1} AgNPs effectively increased relative fresh weight and solution uptake of *Gerbera*.

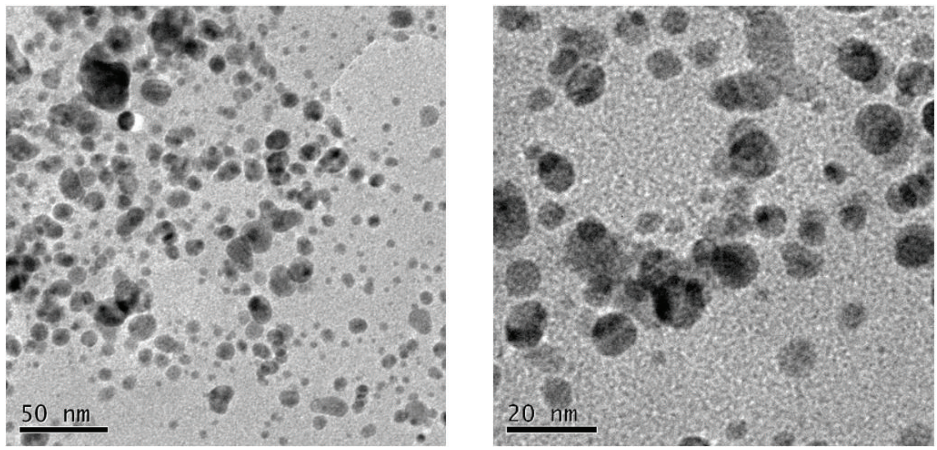


Figure 3: TEM micrographs of Ag nanoparticles obtained with 5 mL of *C. ambrosioides* extract and 10 mM $AgNO_3$.

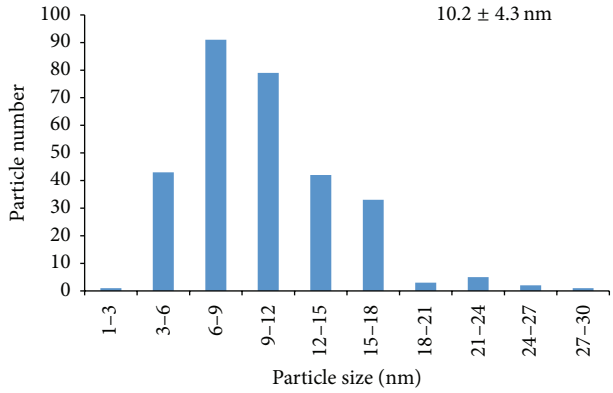


Figure 4: Histograms distribution of particle size with *C. ambrosioides* AgNPs synthesized at 25°C. Extract volume of 5 mL and 10 mL $AgNO_3$. The insert corresponds to the mean ± standard deviation (n = 300).

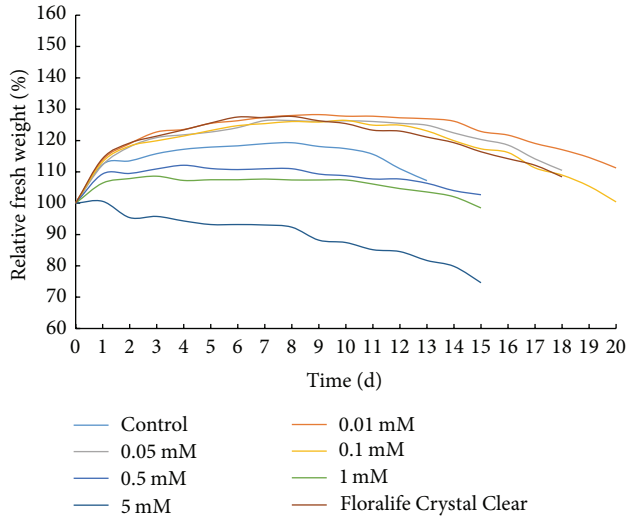


Figure 5: Changes in relative fresh weight during the vase life of *Chrysanthemum* stems cv. Puma exposed to AgNPs.

From day 3, relative fresh weight remained stable in all concentrations assessed, changing a little during stem vase life. However, with 5 mM AgNPs, a clear drop occurred in fresh weight from the first day in vase because the stems did not take up water. At low AgNP concentrations (0.01, 0.05, and 0.1 mM) and using Floralife Crystal Clear, evolution of weight was similar: it increased strongly in the first three days in vase and had slight increases thereafter until day 13, after which it decreased until the end of vase life. The concentrations of 0.5 and 1 mM had performance similar to the untreated control (no AgNPs), in which the slight increase in weight in the first day lasted until the end of vase life. However, the control (deionized water) had a sharp decline at the end of vase life. Safa et al. [27] found that the positive effect of silver nanoparticles is due to its antimicrobial effect and inhibition of stem-tip blockage in cut flowers. Generally, in control plants, water content decreased more rapidly during vase life period, but the lowest fresh weight occurred in the treatment with 20 mg L^{-1} silver nanoparticles. Treatments that had the lowest fresh weight loss had the highest vase life [9].

The results showed a direct relationship between the increase in fresh weight of *Chrysanthemum* stems and water uptake (Figure 6). This is evident because the heaviest fresh weight obtained at low AgNP concentrations was due to increased water uptake by stems (treatments using 0.01, 0.05, and 0.1 mM). These results are similar to Kazemipour et al. [9], who found that silver nanoparticles at a concentration of 10 mg L^{-1} had the lowest fresh weight loss, while 20 mg L^{-1} silver nanoparticles and the control had the highest fresh weight loss. The Floralife Crystal Clear treatment had performance similar to low AgNP concentrations. However, water uptake in the control treatment was as low as in treatments with high concentrations of AgNPs. The highest fresh weights at the end of vase life were obtained with low concentrations of silver nanoparticles (Table 2). Although the treatment with the commercial solution had a vase life similar to the treatments with low concentrations of AgNPs, final fresh weight of the stems was lower.

Table 2: Effect of Ag nanoparticle concentration (mM) on final fresh weight (g) of *Chrysanthemum* cv. Puma stems.

AgNP concentration (mM)	Fresh weight stems (g)
0.00	101.3^{ab}
0.01	122.9^{a}
0.05	120.0^{a}
0.10	117.3^{a}
0.50	102.7^{ab}
1.00	98.5^{ab}
5.00	74.6^{b}
Floralife Crystal Clear	115.8^{a}

Fresh weight data are averages of five experiments. Values in columns with different letters are statistically different ($P < 0.05$).

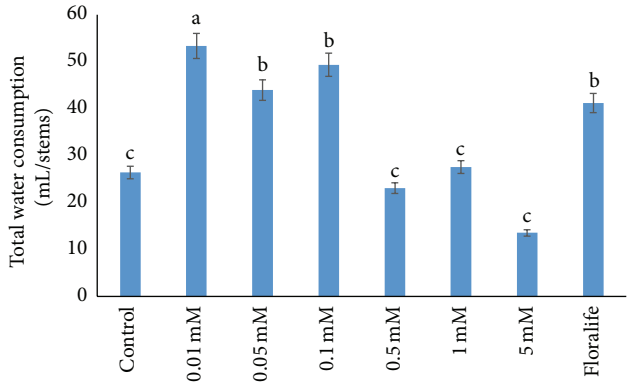


Figure 6: Total water uptake (mL) in *Chrysanthemum* stems exposed to AgNP solutions. Vertical bars show the standard error of the mean ($n = 5$).

3.4. Bacterial Count. The number of bacteria in the vase solution increased as the concentration of silver nanoparticles in vases decreased (Table 3). There was a positive relationship between the number of bacteria in the preservative solution and the vase life of *Chrysanthemum* cv. Puma, except in the control. The addition of silver nanoparticles to vase solutions significantly lowered the number of bacteria. However, vase life decreased at high AgNP concentrations. In the control, the bacterial count was significantly different from that of treatments with silver, and water uptake was low due to vascular occlusion in the xylem vessels because of the proliferation of bacteria. The treatment with the commercial solution Floralife Crystal Clear had higher bacterial growth than the solutions with the lowest concentrations of silver nanoparticles.

Bacteria presumably lead to physical occlusion of xylem, resulting in reduced water uptake and low water potential [30]. Bacteria also indirectly induce physiological obstruction by releasing toxic and/or enzymatic metabolites retained in the water [31]. It has also been reported that some bacteria produce ethylene, which causes senescence of cut flowers [32].

Silver nanoparticles applied in vase solutions exhibit much more efficient antimicrobial properties than mass salts (silver nitrate) because their greater surface area provides more contact with the microorganisms. In many research papers, silver nanoparticles are not considered toxic, but the nanosize suggests that they are dangerous for the environment. In our research, toxic effects were observed when using high concentrations of silver nanoparticles. Liu et al. [15] observed toxic effects of silver nanoparticles at high concentrations. Toxic effects usually manifest as necrosis in leaves or petals.

Table 3: Effect of Ag nanoparticle concentration (mM) on total bacterial count in vase solutions with *Chrysanthemum* cv. Puma stems.

AgNP concentration (mM)	Bacterial count (CFU mL^{-1})
Control	10^{5a}
0.01	2^{c}
0.05	1^{c}
0.10	0^{c}
0.50	0^{c}
1.00	0^{c}
5.00	0^{c}
Floralife Crystal Clear	10^{2b}

Bacterial count data are the average of five experiments. Values in columns with different letters are statistically different ($P < 0.05$).

There is much controversy over the mechanism of action of silver nanoparticles on bacteria. Liu et al. [15] mention that when silver nanoparticles are introduced into the bacterial cell, they form a region of low molecular weight in the center of the bacteria, inducing them to conglomerate to protect their DNA from the silver ions. Morones et al. [33] proposed that the silver ions interact strongly with vital thiol groups in enzymes and bases containing phosphorus. Thus, the damage is caused by interactions of silver nanoparticles with DNA; these interactions prevent cell division and DNA replication and thus cause cell death.

Silver nanoparticles alter cell function by merging with the surface of the cell membrane. They penetrate the bacteria and release silver ions. They are effective agents for killing a broad spectrum of Gram negative bacteria, such as *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, and *Vibrio*, and Gram positive bacteria, such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus*, as well as antibiotic resistant bacteria and those that form biofilms. Biofilms are secretions of a matrix of extracellular polysaccharides; they act as efficient barriers against antimicrobial agents, an immune system that protects the bacterial colony. Silver nanoparticles inhibit the formation of these biofilms [34].

3.5. Inflorescence Opening and Diameter. Figure 7 shows statistically significant differences in the diameter of inflorescences by effect of the AgNP concentrations evaluated. Low concentrations of AgNPs favored inflorescence opening to a degree of 5. This was evidenced by yellowing of leaves because of translocation of more photosynthates from leaves to inflorescence. However, at high concentrations of silver (and in the control), the leaves remained green due to low translocation of sugars to inflorescences (Figure 8).

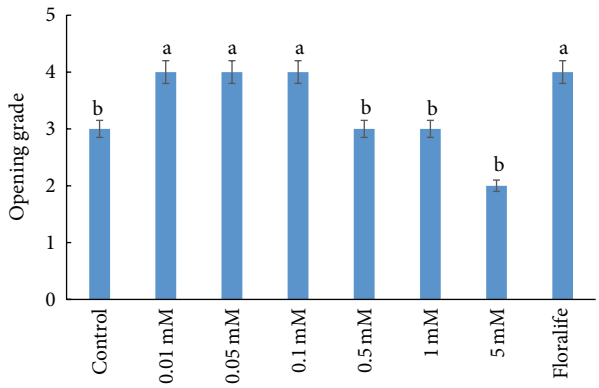


Figure 7: Degree of inflorescence opening of *Chrysanthemum* stems exposed to AgNP solutions. Vertical bars show the standard error of the mean ($n = 5$).

Table 4: Effect of Ag nanoparticle concentration (mM) on final diameter of *Chrysanthemum* cv. Puma inflorescences.

AgNP concentration (mM)	Diameter of inflorescences (cm)
Control	6.23[c]
0.01	6.86[b]
0.05	6.96[a]
0.10	7.03[a]
0.50	6.16[b]
1.00	5.83[b]
5.00	6.16[b]
Floralife Crystal Clear	6.89[a]

Diameters of inflorescences are averages of five experiments. Values in columns with different letters are statistically different ($P < 0.05$).

Leaves on stems exposed to high concentrations of AgNPs (5 and 10 mM) exhibited senescence due to low water uptake. Necrosis, indicating toxicity, was also observed on edges of leaves on stems placed in high AgNP concentrations.

Many flowers are harvested before full development to minimize mechanical damage that occurs during transport, storage, and marketing and to increase postharvest longevity of stems in vase. Flowers experience physiological changes leading to early senescence. Development of cut flowers and maintenance of their metabolic activities require adequate availability of sugars. In addition, production of ethylene is a major adverse effect on cut flowers [35]. The accumulation of microorganisms, especially bacteria, in the vase solution, air embolisms, and physiological injury caused by stem blockage lead to reduced vase life. The reduction in soluble carbohydrates, the imbalance of water, and the presence of ethylene are the main causes of short vase life of cut flowers. Ansari et al. [36] found that preservative solution supplemented with 5 mg L^{-1} AgNPs and 4% sucrose effectively increased flower diameter of cut *Gerbera* flower. In addition, sucrose acts mainly as a food source or to maintain water balance, and it prevents blockage of xylem vessels [29].

Similar results were obtained in the final diameter of inflorescences *Chrysanthemum* (Table 4). The largest inflorescence diameters were found on stems exposed to low concentrations of AgNPg and using Floralife Crystal Clear. Again, small inflorescence diameters are due to low water uptake, which occurred in the stems exposed to high concentrations of AgNPs.

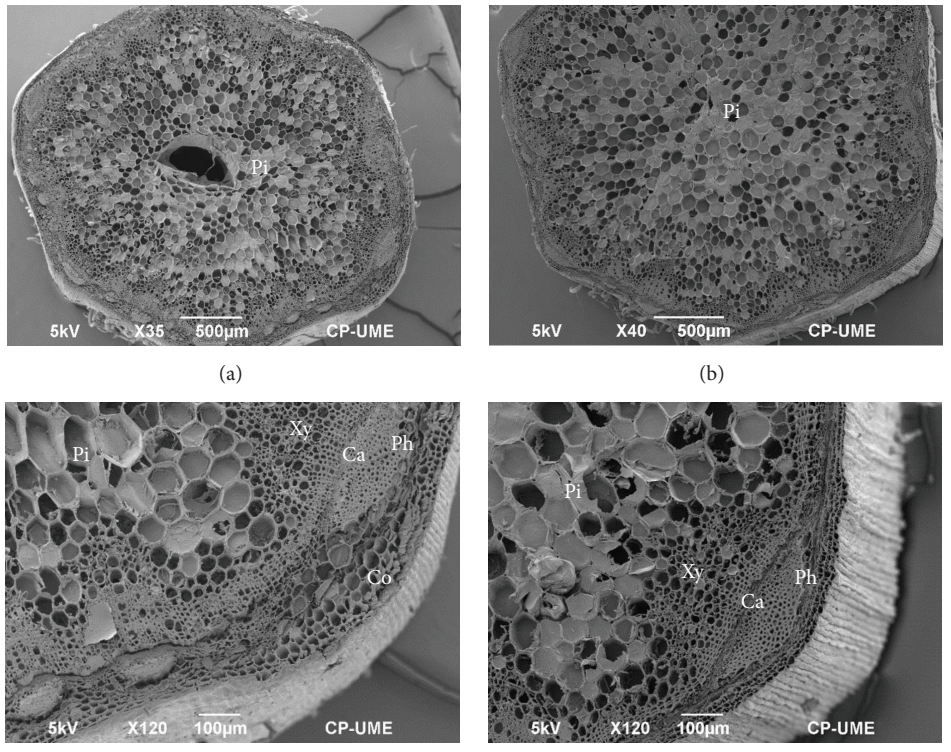
Mortazavi et al. [37] concluded that the application of nanosilver or sucrose increased the opening of cut roses, but the combination of sucrose (4%) and nanosilver (5 ppm) was more effective. We believe that silver nanoparticles kill the bacteria that obstruct the xylem vessels and thus favor greater water uptake. Safa et al. [38] achieved optimum flower diameter when cut *Gerbera* flowers were treated with 10 mg L^{-1} AgNPs. However, promotion of floral opening evidenced by yellowing of leaves suggests increased transport of soluble carbohydrates from the leaves to the tubular and radial flowers, allowing a higher degree of opening. The commercial solution Floralife Crystal Clear promoted inflorescence opening because it is composed of soluble carbohydrates that are transported to the flowers, allowing the leaves on the floral stems to remain green. This did not occur in the treatments with low AgNP concentrations. Here, the leaves yellowed due to sugar transport from leaves to the flowers.

3.6. Electron Microscopy and Toxicity. The treatment with 5 mM silver nanoparticles caused damage to the pith parenchyma (Figure 9), contrasting with the treatment with 0.01 mM silver nanoparticles, which did not alter stem anatomy. No changes in the cells of the vascular bundle (xylem, phloem, and cambium) were observed in any of the evaluated treatments. Therefore, high concentrations of AgNPs are harmful for parenchyma cells. Studies on the effect of heavy metals, such as lead, in stems of *Pisum sativum* L. [39] have found abnormal lignification in the pith parenchyma. They also observed development of metaxylem in the pith region, including cell rupture and elongation. We believe that the presence of Ag^+ ions in vase solutions with high concentrations of Ag nanoparticles caused abnormal cell elongation and induced abnormal cell division. The toxic effect of Ag was localized in the pith parenchyma and caused cell death. Accumulation of heavy metals varies depending on the type of metal and type of plant tissue. Vollenweider et al. [40] found that accumulation of cadmium in willow leaves was observed in the cell wall of the collenchyma > pith > cortical parenchyma > xylem. Changes in the diameter of xylem vessels, cell area of the parenchyma, pith, root cortex, stem dimensions, vascular bundles, number of xylem vessels in root, stoma frequency, and leaf abaxial surface, and a reduction in grain yield due to stress caused by heavy metals have been observed [41].

Studies on AgNP toxicity in plants have been controversial, centered on whether the cause of toxicity is nanosize and shape of the particles or their release as ionic Ag^+. However, all studies agree that silver nanotoxicity is positively concentration-dependent and negatively size-dependent. Geisler-Lee et al. [42] found that AgNPs accumulated progressively in this sequence in the root: border cells, root cap, columella, and columella initials; AgNPs were apoplastically transported in the cell wall and found aggregated at plasmodesmata. Stampoulis et al. [43] found

(a) (b) (c) (d) (e) (f) (g) (h)

Figure 8: Yellowing of leaves on stems of *Chrysanthemum* cv. Puma exposed to silver nanoparticles; (a) Floralife Crystal Clear, (b) 0.01 mM, (c) 0.05 mM, (d) 0.1 mM, (e) deionized water, (f) 0.5 mM, (g) 1 mM, and (h) 5 mM.



(a) (b) (c) (d)

Figure 9: Morphological properties in the stem of *Chrysanthemum* cv. Puma. Anatomy of stem: (a) and (c) 5 mM AgNPs treatment and (b) and (d) 0.01 mM AgNPs. Typical stem anatomy consists of cortex (Co), the pith parenchyma (Pi), and ring of vessels with phloem (Ph), cambium (Ca), and xylem (Xy) cells. Bars are 500 and 100 μM.

that exposure to Ag nanoparticles at 500 and 100 mg L^{-1} resulted in 57% and 41% decreases in plant biomass and transpiration, respectively, and zucchini shoots exposed to Ag nanoparticles contained 4.7 greater Ag concentration than the plants from the corresponding bulk solutions. Thwala et al. [44] observed induction of oxidative stress in *Spirodela punctata* exposed to AgNPs. Oukarroum et al. [45] demonstrated that intracellular uptake of Ag directly from AgNPs triggered cellular oxidative stress possibly due to the release of free Ag inside plant cells. This is the first report in which damage to the pith parenchyma was observed, while vessels remained unharmed.

4. Conclusions

The use of silver nanoparticles in vase solutions is an easy and economically viable technique for promoting flower opening and increasing vase life of *Chrysanthemum* cv. Puma stems. The main advantage of using silver nanoparticles, compared with other silver salts, is its large surface area that comes into contact with bacteria. Thus, the amount of product needed in vase solutions decreases, and, therefore, toxicity for flower stalks and the environment is lowered. Production of silver nanoparticles from plant extracts also constitutes a simple, cheap, and environment-friendly technique. Current protocols for the production of nanoparticles using plant extracts aim to obtain monodisperse particles that are stable over time. Hence, use of low concentrations (0.01 mM and 0.05 mM) of silver nanoparticles (10.2 nm ± 4.3) synthesized environmentally with *Chenopodium ambrosioides* extract is suggested for extending vase life of *Chrysanthemum* flower stems. The effectiveness of the commercial solution Floralife Crystal Clear in prolonging vase life and promoting opening of *Chrysanthemum* flowers is comparable to low nanoparticle concentrations. An advantage of the commercial solution is that the leaves on the stems remain green. The use of *C. ambrosioides* for synthesis of silver nanoparticles provides particles that are stable for over 15 months at room temperature. Thus, the particles can be used during handling, storage, and marketing of *Chrysanthemum* cv. Puma. High AgNP concentrations in the vase solution damage the pith parenchyma of *Chrysanthemum* cv. Puma stems.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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