ISPEC Tarım Bilimleri Dergisi, 9(2): 434-446, 2025
ISPEC Journal of Agricultural Sciences, 9(2): 434-446, 2025
DOI: https://doi.org/10.5281/zenodo.15010913

Silver Nanoparticle and Cold Storage Improves Postharvest Quality of Cut Gerbera (*Gerbera jamesonii* L.)

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Abstract

Gerbera is one of the world's five most important cut flower crops. The most important factor in the cut flower industry is vase life, which significantly affects both producer and consumer preference. In this study, the effects of different concentrations of silver nanoparticles (0 (control), 10 ppm, 20 ppm, 30 ppm, and 40 ppm) on post-harvest quality criteria and vase life of gerbera plants were investigated. In addition, this study was carried out in 3 different environments (room conditions, room conditions after 48 hours in 2 °C cold storage and 2 °C cold storage until vase life expires). As a result of the study, the 3rd environment gave the best results in all parameters compared to the other two environments. In particular, it was determined that the applications made in cold storage had 40 days more vase life than the other environments. In the 3rd environment, the application with the highest vase life was determined in 20 ppm AgNPs application (54.17 days), and it was observed that these environments provided an 18.61% increase in vase life compared to the control (45.67 days). The best results in relative fresh weight, daily vase solution uptake, and total vase solution uptake were determined in 40 ppm AgNPs treatment. Bacterial densities between media and treatments were close to each other. This study showed that the products can be preserved in cold storage and then released to the market, and 20 ppm AgNPs application can play a protective role, especially in extending the vase life.

Research Article

Article History
Received :16.11.2024
Accepted :29.12.2024

Keywords
AgNP
cut flower
vase life
nanoparticles
relative fresh weight

1. Introduction

In the world, Gerbera (*Gerbera jamesonii* L.) ranks fourth among the ten most popular commercial cut flowers (Atefepour et al., 2021), with economic importance in the international market (García-González et al., 2022). Türkiye is among the important countries, producing 103 million pieces of gerbera on 1173 decares of greenhouse area in 2023 (TurkStat, 2023). Gerbera is a popular due to its attractive appearance, a wide variety of colors, and the ability to thrive in different environmental conditions (Hema et al., 2018).

Cut flowers generally have short vase life due to genetic and environmental factors, which limits the development of the cut flower industry (Kumar et al., 2014; Van Meeteren and Aliniaeifard, 2016; Aalifar et al., 2020). Vase life, which is also critical in determining the commercial value of gerbera, is a highly sensitive species to stem bending (neck bending or shape bending) (Muraleedharan et al., 2019; Shabanian et al., 2019). The different vase solutions are being trialed to reduce post-harvest problems of cut flowers. These vase solutions reduce ethylene production in sensitive flowers and prevent the growth of microorganisms that cause dehydration by preventing water passage on the stem (Hema et al., 2018; Muraleedharan et al., 2019). The active ingredients used in vase solutions are classified according to their functions. These are ethylene inhibitors (such as silver thiosulfate), antibacterial agents (such as 8-hydroxyquinoline, silver nitrate, and silver nanoparticles), and antioxidants (such as salicylic and ascorbic acids) (Li et al., 2018).

A common way to extend the economic feasibility of cut flowers is to expose the flowers to a low temperature after harvest. Low temperatures can control metabolism, reduce consumption of stored compounds and water loss through respiration, and limit the development of pathogens (Jahnke et al., 2020). When considering cold storage, cut flowers have three temperature classes. Tropical flowers such as anthurium (*Anthurium*), orchids (Orchidaceae), and poinsettia (*Euphorbia pulcherrima*) are sensitive to cold and should be stored between 12 and 18 °C. Subtropical flowers such as Protea (Proteaceae) are stored between 2 and 8 °C. Finally, most cut flowers such as gerberas, roses, carnations, chrysanthemums, dutch irises, and tulips are stored between 0 and 2 °C (Jahnke et al., 2020). In the industrial production of cut flowers, fungicides are often added to prevent or minimize the growth of microorganisms, thus delaying aging and increasing the appreciation of cut flowers (Sun et al., 2022). For over half a century, 8-hydroxyquinoline esters have been used as potent biocides for cut flowers in mixtures with sucrose (Skutnik et al., 2020). Its effects on cut flowers have been demonstrated in *D. caryophyllus* (Edrisi et al., 2015), *H. macrophylla* (Kazaz et al., 2019), rose (*Rosa hybrid*) (Lama et al., 2015) and *E. grandiflorum* (Sharifzadeh et al., 2014). Another such biocide is nanosilver (NS). As a new nano-material, NS is recognized as a safe inorganic antibacterial material and has been widely used in conservation (Rai et al., 2009). Furthermore, the effect of NS as a novel fungicide on cut flowers has been reported in *R. hybrida* (Hassan et al., 2014), *G. jamesonii* (Safa et al., 2015), lily (*Lilium* spp.) (Li et al., 2012) and *D. caryophyllus* (Naing et al., 2017). Studies also indicate that AgNPs synthesized by microalgae effectively control pathogens in agricultural applications (Terra et al., 2019). Nanosilver (NG) has strong antibacterial activity due to its small particle size (Lok et al., 2006; Rai et al., 2009). NG is widely used as a preservative due to its advantages, such as ease of preparation, non-toxicity, and the absence of environmental threats (Rai et al., 2009). Nanometre-sized silver particles have a high surface area/volume ratio and are considered to control microorganisms more intensively than other forms of Ag (Furno et al., 2004). Silver nanoparticles (AgNPs) act as antibacterial agents to extend the vase life of gerbera flowers (Atefepour et al., 2020) In recent studies, biosynthesized AgNPs and purified AgNPs using plant extracts have exhibited numerous biological activities such as antibacterial (Nahar et al., 2020), anticancer (Wang et al., 2020), and antioxidant (Rajoka et al., 2020). Preservative solutions with NG have been successfully used for cut flowers such as lily (Kim et al., 2005) and gerbera (Liu et al., 2009; Kazemi and Ameri, 2012; Liu et al., 2012), rose (Liu et al., 2009b; Lü et al., 2010; Kader, 2012; Rafi and Ramezanian, 2013), tuberose (Bahremand et al., 2014), common lilac (Jędrzejuk et al., 2016), carnation (Naing et al., 2017), peony (Zhao et al., 2018) and snapdragon (Rabiza-Świder et al., 2020).

This study aims to determine the vase life and post-harvest quality criteria of Gerbera (*Gerbera jamesonii* L.) flowers kept in pre-cooling and cold storage with silver nanoparticle applications with different concentrations.

2. Material and Methods

2.1. Plant material

The gerbera seedlings (*Gerbera jamesonii* L.) used in the study were obtained from a commercial gerbera greenhouse in Tokat province (40°40'21 "N, 36°36'27 "E, altitude 236m) and grown in a polythene greenhouse with soil 60 cm soil between rows and 30 cm soil above rows. 60 cm between rows, 30 cm above rows. In the early morning, two rows of male organs with healthy plants and homogeneous quality criteria were harvested.

2.2. Experimental plan and treatments

Harvested flowers were transported to the laboratory in buckets filled with silver thiosulphate. For preliminary dehydration, all plants were treated with 0.2 mM silver thiosulphate for 6 h under room conditions (Reid et al., 1980). The three-media study plan was as follows:

- In the 1st environment, at the end of the 6th hour, the plants were kept in room conditions, and the quality parameters were analyzed.

- In the 2nd environment, after silver thiosulphate treatment, the plants were kept in cold storage for 48 hours at 2 ºC, 12/12 light/dark photoperiod, and 1000 lux light intensity. After 48 hours, the plants were kept in room conditions, and then quality parameters were analyzed.

-In the 3rd environment, after silver thiosulphate treatment, the plants were stored in cold storage at 2 ºC under 12/12 light/dark photoperiod and 1000 lux light intensity until the end of the vase life. Quality parameters were monitored throughout the storage period. All plants were cut into 40 cm lengths and placed in 4 vase solutions containing silver nanoparticles (Nanografi, Türkiye) (Table 1). The content of all vase solutions was determined as 500 mL.

Table 1. Vase solutions and concentrations

Application	Concentration
(U1) Distilled Water (Control) (mL)	-
(U2) Silver nanoparticles (AgNPs) (ppm)	10
(U3) Silver nanoparticles (ppm)	20
(U4) Silver nanoparticles (ppm)	30
(U5) Silver nanoparticles (ppm)	40

During the vase life study, the room temperature was 25±2 °C, relative humidity was 50±5% (Hobo Data Logger U12-012), and photoperiod was 12 hours in environments 1 and 2.

2.3. Vase life (days)

Vase life was determined as the number of days from the day the flowers were placed in the vase (start) until the day the flowers wilted and/or the flower stem was bent more than 90º (Mohammadi et al., 2021).

2.4. Relative fresh weight (RFW)

In the 1st and 2nd environments, the relative fresh weight was measured on day 0 (establishment day) and the 2nd, 4th, 6th, and 8th days following the start of the experiment (Alkaç et al., 2020). In the 3rd environment, the data taken on the 8th, 16th, 24th, 32nd, and 40th days were used in the calculations.

Calculations were made according to the formula below:
RFW (%) = (At / At = 0) x100
At: branch weight at day t (e.g. 2, 4, 6, etc.)
At=0: Initial (day 0) weight of the branch (He et al., 2006).

2.5. Daily vase solution uptake (DVSU)

Daily vase solution uptake was calculated according to the formula below:
DVSU= (St-1)-(St)
St-1= Weight of the previous day's vase solution
St= vase solution weight at day t (e.g. 2, 4, 6, etc.) (He et al., 2006).

2.6. Total vase solution uptake (TVSU)

Total vase solution uptake was calculated according to the formula below:
TVSU=A-B
A: Vase solution weight measured at initial installation.
B: Vase solution weight measured at the end of the plant's vase life (He et al., 2006).

2.7. Number of bacteria in vase solution and its diagnosis by MALDI-TOF MS technique

Samples were taken from the solution on the last day of the vase's life to determine the number of bacteria in the vase solution. The bacterial density in the samples was determined using the dilution series method. A dilution series was prepared by taking 1 ml of the vase solution samples, placing them in tubes containing 9 ml of physiological saline (0.85% NaCl solution-saline buffer), and diluting them six times. From the last two tubes of the dilution series, 100 µl each was taken and spread in 90 mm diameter petri dishes containing Nutrient Agar (NA) medium with the help of a sterile glass baguette. Petri dishes were incubated at 37 °C for 24 hours. At the end of the incubation period, bacterial colonies were counted, and the bacterial density in the vase solution was determined (Liu et al., 2009a). The colonies were then purified by selection from the bacterial colonies growing on a Nutrient Agar medium. The MALDI-TOF MS method identified the selected bacteria (Mustafa Kemal University, Plant Health Clinic Application, and Research Centre).

2.8. Statistical analysis

The study was established according to the Coincidence Plots experimental design with three replications and three plants per replicate. Vase life was measured daily, and other traits were measured at intervals of two days. The data obtained were calculated according to the analysis of variance (One-way ANOVA) in the SPSS 17.0 (IBM) program. Duncan multiple tests ($p<0.05$) were used to compare means.

3. Results

3.1. Vase life

The study aimed to determine the effects of different media on the vase life of Gerbera plants. Statistical differences ($p<0.001$) between the media were found to be significant. The 3rd environment (47.50 days) was found to have the best vase life compared to the other media. The lowest vase life was determined in the 1st environment (7.82 days). The effects of 5 different treatments used in the study on Gerbera plants in 3 environments were not statistically significant ($p>0.05$). In the 1st environment, the longest vase life was determined in 40 ppm AgNPs application (9.55 days), and in the 2nd environment and 3rd environments in 20 ppm AgNPs application (10.33 days - 54.17 days). The lowest vase life was determined in 10 ppm AgNPs application (6.22 days - 7.00 days - 43.67 days) in all environments (Figure 1).

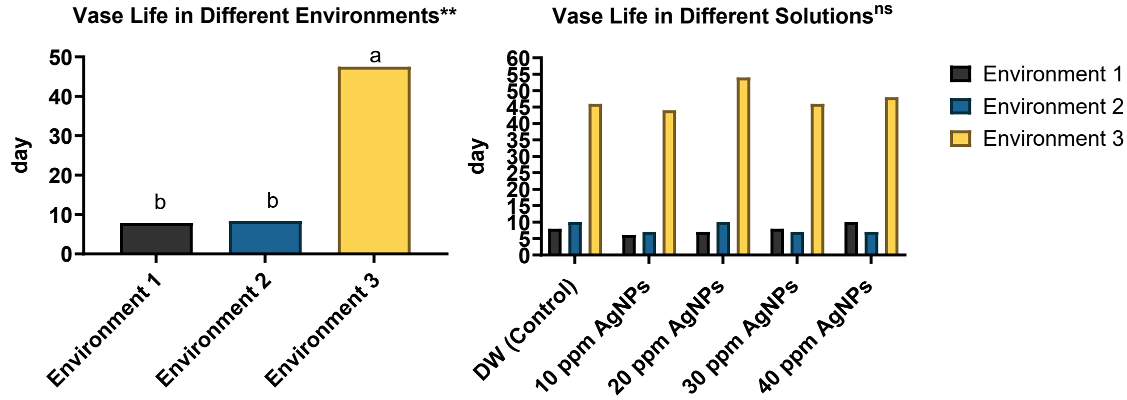

Figure 1. The effect of different environments and different solutions on *Gerbera* flower vase life (day), **: $p<0.01$, ns: non-significant ($p>0.05$).

3.2. Relative fresh weight

The study aimed to determine the effect of different media on the relative fresh weight of Gerbera plants at 48-hour intervals. On the 2nd day, the effects of different media were not found to be significant ($p>0.05$), while statistical differences ($p<0.001$) were found to be significant on the 4th, 6th, and 8th days. The highest relative fresh weight in all environments was determined as the 3rd environment on the 2nd day (117.08%). The lowest relative fresh weight was determined in the 2nd environment on the 8th day (75.07%) (Figure 2).

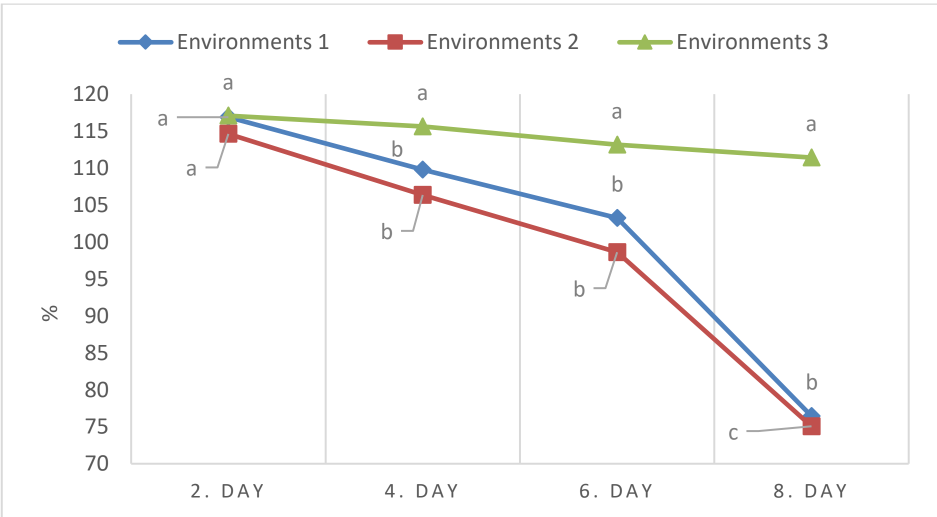

Figure 2. The effect of different environments on relative fresh weight in Gerbera (*Gerbera jamesonii*) flower (%), *: $p<0.05$.

The effect of 5 different treatments used in the 1st environment on the relative fresh weight percentage was found to be statistically significant ($p<0.001$) on the 2nd and 4th days. On day 2, the highest relative fresh weight was 40 ppm AgNPs (118.13%) and on day 4, the highest relative fresh weight was 30 ppm AgNPs (119.33%). On the 6th and 8th days, the percentage of relative fresh weight was not statistically significant ($p>0.05$). The lowest relative fresh weight percentage of 10 ppm AgNPs was found on the 8th day (30.60%). The effect of 5 different treatments used in the 2nd environment on the relative fresh weight percentage was found to be statistically significant ($p<0.001$) on the 6th day. The highest relative fresh weight was determined as control (117.58%) on the 2nd day and the lowest relative fresh weight was determined as DW (61.06%) on the 8th day. There was no statistically significant difference ($p>0.05$) between treatments for the 2nd, 4th, and 8th days. The effect of 5 different treatments used in the 3rd environment on the relative fresh weight percentage was found to be statistically significant ($p<0.001$) on the 8th day. In the 3rd environment, the highest relative fresh weight percentage was measured in 40 ppm AgNP application (119.59%), and the lowest relative fresh weight percentage was measured in 20 ppm AgNPs (114.61%) on the 8th day. As of the 32nd day, the highest relative fresh weight was found in 40 ppm AgNPs treatment (114.11%) and the lowest relative fresh weight was found in 20 ppm AgNPs treatment (110.11%). In these treatments, 16th, 24th and 32nd days were not statistically significant ($p>0.05$) (Figure 3).

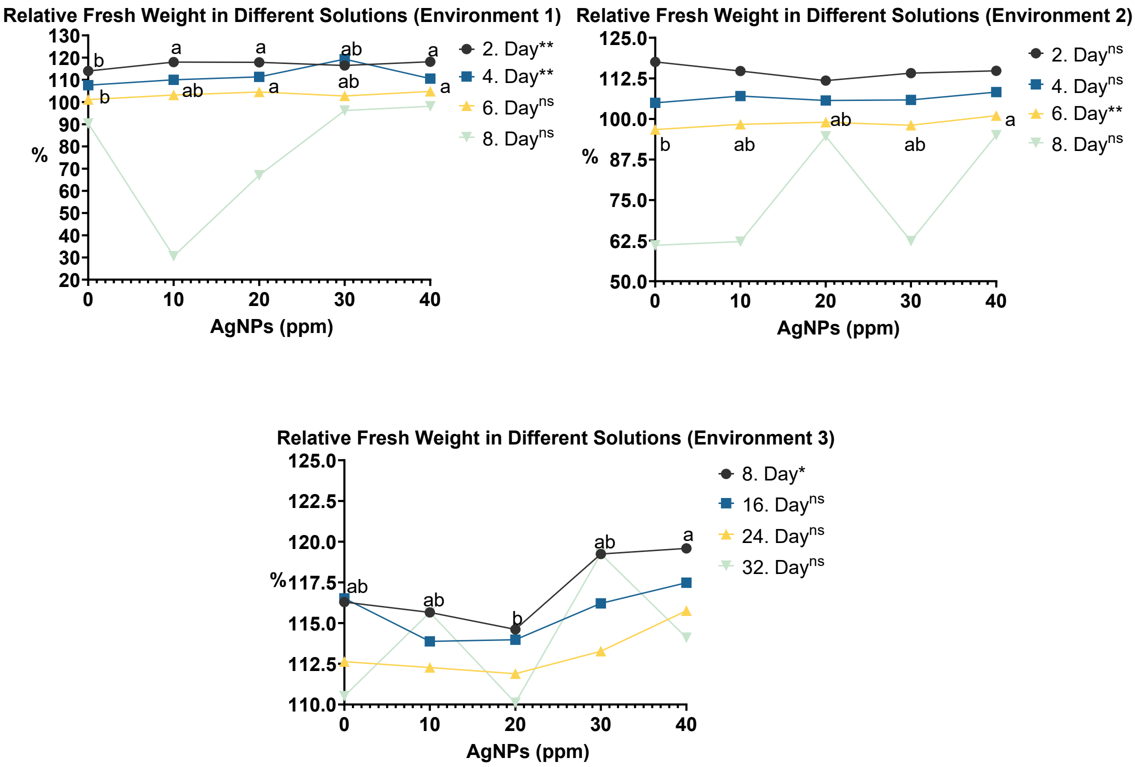

Figure 3. The effect of different vase solutions on relative fresh weight in Gerbera flower, *: $p<0.05$, **: $p<0.001$, ns: non-significant ($p>0.05$).

3.3. Daily vase solution uptake

In this study, the effects of different media on total vase solution uptake of Gerbera plants were aimed. Statistical differences ($p<0.001$) between the media were found to be significant. On days 0-2, the highest vase solution uptake was measured in the 3rd environment (2.44 g), and the lowest vase solution uptake was measured in the 2nd environment (2.10 g). As of days 8-10, the highest vase solution loss was determined in the 2nd environment (-9.20 g), while the lowest loss was determined in the 3rd environment (-0.62 g). The effect of 5 different treatments used in the 1st environment on daily vase solution uptake in Gerbera plants was found to be statistically significant ($p<0.001$). The highest vase solution uptake was realized on the 2nd day with 10 ppm AgNPs (2.95 g), and the lowest vase solution uptake was realized in control (1.76 g). It was determined that daily vase solution uptake decreased from day 0-2, and the highest loss was determined in the control treatment (-7.74 g), and the lowest loss was determined in the 40 ppm AgNPs treatment (-4.73 g) as of day 8-10. The effect of 5 different treatments used in the 2nd environment on the daily vase solution uptake in Gerbera plants was found to be statistically significant ($p<0.001$) on the 2nd day. The highest daily vase solution uptake on days 0-2 was determined in 30 ppm AgNPs treatment (2.34 g), while the lowest daily vase solution uptake was determined in 20 ppm AgNPs treatment (1.70 g). Daily vase solution uptake decreased from day 0-2, and the highest loss was found in the 40 ppm AgNPs treatment (-13.27 g), and the lowest loss was found in the control treatment (-4.62 g) as of day 8-10. The effect of 5 different treatments used in the 3rd environment on daily vase solution uptake in Gerbera plants was found to be statistically significant ($p<0.001$) between the 8th and 24th days. The highest vase solution uptake was measured on day 0-8 with 30 ppm AgNPs (2.92 g). From day 0-8, daily vase solution uptake decreased, and the highest loss was found in the control AgNPs treatment (-0.93 g) and the lowest loss was found in the 10 ppm AgNPs treatment (-0.46 g) as of day 32-40. From the 24th-32nd day onwards, no significant difference ($p>0.05$) was found in terms of statistical data (Figure 4).

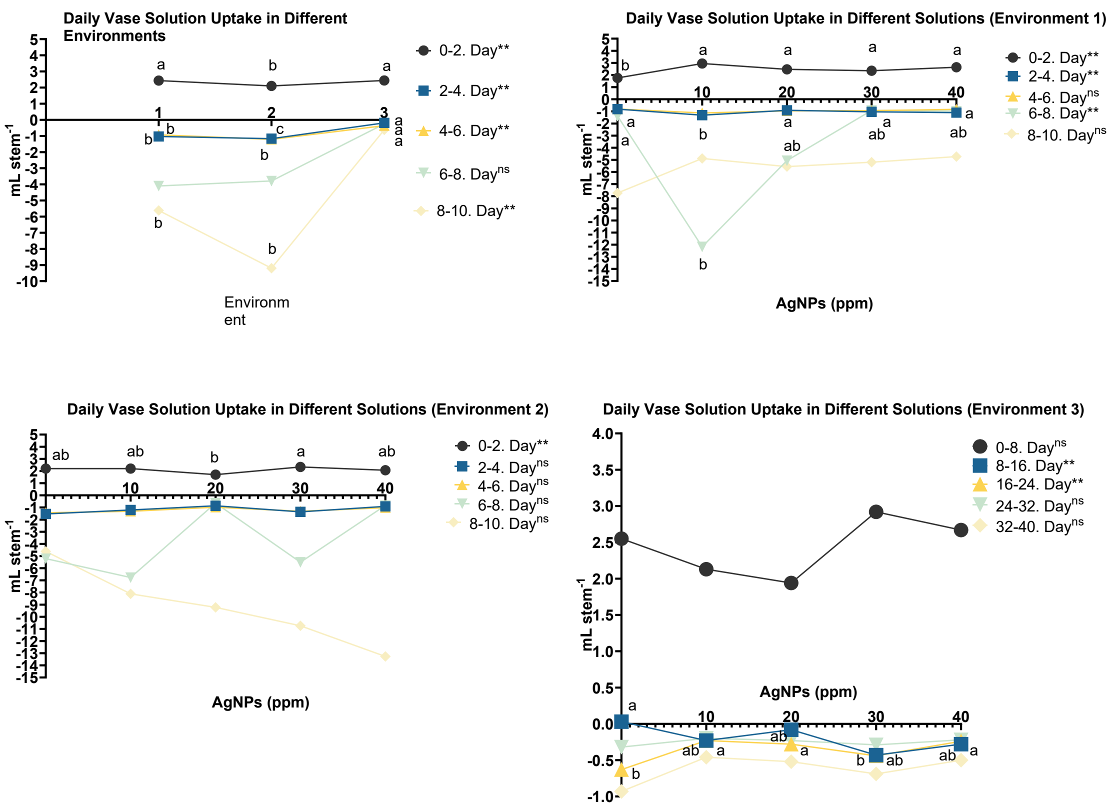

Figure 4. The effect of different environments and different solutions on daily vase solution uptake in *Gerbera* flower (*Gerbera jamesonii* L.), **: $p<0.001$. ns: non-significant ($p>0.05$).

3.4. Total vase solution uptake

The aim of the study was to determine the effects of different media on the total vase solution uptake of Gerbera plants. Statistical differences ($p<0.001$) between the media were found to be significant. The 3rd environment (129.96 ml) was found to have the best vase solution uptake compared to the other media. The lowest vase solution uptake was determined in the 2nd environment (91.94 mL). According to the treatments, the highest average solution uptake was determined in 40 ppm AgNPs (121.79 mL). Again, the highest solution uptake (128.56-145.77 mL) was measured at 40 ppm AgNPs in the 1st and 3rd environments. The lowest mean solution uptake was found at 10 ppm AgNPs (98.91 mL) and the lowest solution uptake was found at 10 ppm AgNPs (86.54 ml) in the 2nd environment (Figure 5).

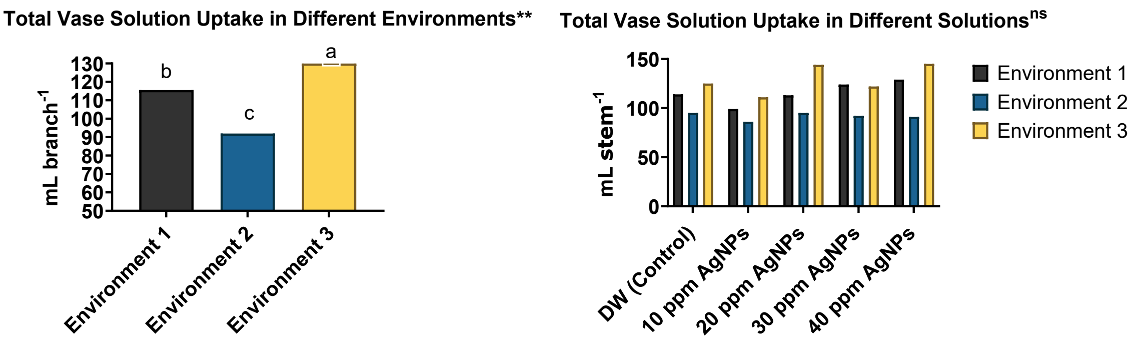

Figure 5. The effect of different environments and different solutions on total vase solution uptake in Gerbera flower (*Gerbera jamesonii* L.), **: p<0.001. ns: non-significant (p>0.05).

3.5. Counting bacteria in the vase solution

When the bacterial densities in different vase solutions were analyzed, it was observed that bacterial densities were close to each other between treatments and media. In the 1st medium, the lowest bacterial density was determined in the T3 treatment (1.3×10^6 CFU mL^{-1}), while the highest bacterial density was measured in the T2 treatment (4.9×10^8 CFU mL-1). In the 2nd medium, the lowest density was calculated in the T1 treatment and the highest bacterial density in the T2 treatment. In the 3rd medium, the highest bacterial density was determined in the T2 treatment (Table 2). In addition, *Microbacterium paraoxydans, Pseudomonas rhodesiae,* and *Serratia ficaria* bacteria species were detected in different vase solutions using the MALDITOF-MS technique.

Table 2. The effect of different solutions on bacteria population in vase solution

Treatments	**Bacteria population (CFU mL^{-1})**		
	Environment 1	Environment 2	Environment 3
T1	1.2×10^8	8.5×10^5	1.8×10^7
T2	4.9×10^8	4.6×10^7	1.4×10^8
T3	1.3×10^6	5.4×10^7	5.4×10^7
T4	4.1×10^8	9.2×10^7	0
T5	6.5×10^7	1.0×10^8	1.2×10^7

4. Discussion

When the silver nanoparticle study was analyzed, it was found that the treatments with low silver nanoparticle concentration had lower vase life compared to the control, but 40 ppm silver nanoparticle had more vase life. In a study conducted on rose (*Rosa hybrida*), carnation (*Dianthus caryophyllus*), and gerbera (*Gerbera jamesonii*), it was reported that the vase life increased with increasing silver nanoparticle doses, and the highest doses had the highest vase life (Liu et al., 2008). Solgi et al. (2009) increased the vase life of silver nanoparticle applications from 8.3 days to 16 days in their study on Gerbera (*Gerbera jamesonii* cv. 'Dune'). Hajizadeh (2015) reported that using silver nanoparticles and sugar in Freesia increased the vase life, and the 10 ppm dose had the highest vase life. Recent studies show that using nanosilver particles shows antimicrobial effects (due to their high area/volume ratio) and has been proven useful in the preservative solutions of various cut flowers (Hajizadeh, 2015).

When the effect of the environments on the relative fresh weight was analyzed, it was found that this ratio was quite low in the 3rd environment as the number of days progressed. Ethylene synthesis is one of the most important criteria for affecting the quality of the product after harvest. Therefore, it is also known that in excess of ethylene synthesis, wilting and aging increase in plants. It is also stated in the research that the respiration rate increases as the storage temperature increases, thus shortening the vase life and causing weight loss (Kazaz, 2015). In the 3rd environment, the main effect of the low rate is estimated to be since the study was carried out at 2 ºC and the respiration rate was slow in this environment. As a result, ethylene synthesis is reported to be low (Skutnik et al., 2020). Considering the effect of applications, it was noted that with the increase in silver nanoparticle concentrations, the relative fresh weight increased, and losses decreased. In the study conducted in freesia, it was also reported that the highest silver nanoparticle dose directly affected the relative fresh weight and provided an increase (Hajizadeh, 2015). In the same way, it was determined that silver nanoparticle applications in cut carnation directly affected the relative fresh weight change and gave positive results (Hamed Chaman et al., 2012). The study conducted in Lisianthus determined that silver nanoparticles increased the relative fresh weight and had a greater effect than the control (Skutnik et al., 2021). The study also reported that the lowest relative fresh weight losses were in silver nanoparticle treatments (Safa et al., 2015).

In the daily vase solution uptake, when the effect of the environments is examined, it is seen that there are less losses in the 3rd environment compared to the other environments. The main reason for this is due to low temperature application. Studies on low-temperature applications reported that storage between 0 and 1 °C is the most effective method to maintain quality in most cut flower species (Reid, 1992). It was noted that similar results were found in plants stored at 2 ºC, quality was preserved and minimum losses were experienced in daily vase solution uptake. Especially in the treatments in the 3rd environment, these losses were considerably lower than in the other environments. Among the treatments, the treatments with high silver nanoparticle concentration were found to have the least losses in daily vase solution uptake. Hamed Chaman et al. (2012) and Skutnik et al. (2021) reported that silver nanoparticle applications were higher in daily vase solution uptake than others. In total vase solution uptake, it was determined that the study in the 3rd environment had the highest solution uptake compared to the other environments. It is estimated that, especially at low temperatures, bacterial growth slows down and clogging decreases, which is related to the increase in solution uptake. Considering the effect of the treatments, it was noted that more vase solution uptake was realized in the treatments with the highest concentration of silver nanoparticles in the 1st and 3rd environments. Thanks to the anti-microbial properties of silver nanoparticles, it prevents the clogging of the plant's xylem, thus directly affecting the vase life, relative fresh weight, and solution uptake (Li et al., 2017). In addition, nanometre-sized silver (Ag^+) particles (NS) have been reported to act as anti-microbial and ethylene inhibitors in various applications (Liu et al., 2009a; Solgi, 2014; Safa et al., 2015; Liu et al., 2018). In addition, nanotechnology-based formulations have been found to provide many ways to inhibit the growth and development of microorganisms (Upadhyay et al., 2022). It was concluded that similar results were obtained with this study and that increasing the concentration of silver nanoparticles increased vase life, relative fresh weight, daily and total vase solution uptake.

5. Conclusion

As a result, it was determined that the 3rd environment gave the best results in all parameters compared to the other two environments. In particular, it was determined that the treatments carried out in cold storage had 40 days more vase life compared to the other environments. In the 3rd medium, the application with the highest vase life was determined in 20 ppm AgNPs application (54.17 days), and it was observed that these environments provided an 18.61% increase in vase life compared to the control (45.67 days). The best results in relative fresh weight, daily vase solution uptake, and total vase solution uptake were determined in 40 ppm AgNP treatment. Bacterial densities between the media and treatments were close to each other. This study showed that the products can be released to the market later by keeping them in cold storage, and the 20 ppm AgNP application can especially play a protective role in extending the vase's life. In addition, it was observed that keeping it in cold storage and applying silver nanoparticles can effectively prevent neck bending disorder and clogging of xylem tissue in gerbera. Therefore, it is concluded that silver nanoparticle application and cold storage at 2 °C can be offered to commercial gerbera producers to improve post-harvest quality criteria and increase vase life.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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To Cite	Alkaç, O.S., Tuncel, M.E., Saraçoğlu, O., Belgüzar, S., 2025. Silver Nanoparticle and Cold Storage Improves Postharvest Quality of Cut Gerbera (*Gerbera jamesonii* L.). *ISPEC Journal of Agricultural Sciences*, 9(2): 434-446. DOI: https://doi.org/10.5281/zenodo.15010913.