Nano silver application impact as vase solution biocide on postharvest microbial and physiological properties of ‘Cherry Brandy’ rose

Mohammad Mahdi Jowkar 1*, Ahmad Khalighi 1, Mohsen Kafi 2 and Nader Hassanzadeh 3
1 Department of Horticultural Sciences, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran. 2 Department of Horticultural Science, Faculty of Horticulture and Plant Protection, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. 3 Department of Plant Phytology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran. *e-mail: mjowk@yahoo.co.uk

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Abstract
The major cause of vase life reduction in cut flowers is water relation interruption which is mostly due to vase solution microbial proliferation and consequently vascular occlusion resulting in solution uptake reduction. In order to control microbial proliferation biocides are usually integrated in vase solution preservatives. Beside microbial proliferation control, biocides could affect cut flower’s quality and physiology in various aspects. In order to find an easy to use, non toxic and inexpensive compound for large scale application, cut ‘Cherry Brandy’ roses were treated with colloids of nano silver particles (1, 2.5 or 5 %) and sterilized distilled water (control). Effects of nano silver as vase solution biocide and its impact on vase life, water relation, vase solution microbial kind and population beside different physiological parameters such as chlorophyll degradation, chlorophyll fluorescence and membrane permeability were investigated during this study. Results indicated that nano silver treatment significantly increased vase life and did not result in any evident side effects. It also improved solution uptake and consequently retarded weight loss. Beside that nano silver application as vase solution efficiently disinfected vase solution and consequently prevented vase solution microbial proliferation. Membrane permeability was best maintained by 1% nano silver treatment. Besides that, 1% nano silver treatment resulted in the highest chlorophyll content. Nano silver application also resulted in chlorophyll fluorescence reduction and loss of photosynthetic activity during vase life.

Key words: Bacillus, chlorophyll content, chlorophyll fluorescence, ion leakage, microbial count, microbial kind.

Introduction
Cut flowers vase life is affected by several factors such as cell programmed death 1, ethylene induced senescence 18, 31, dehydration 16, 20, 39, 40, or loss of assimilates and substrates 9, 17. Among the above mentioned, water relation and balance play a major role in postharvest quality and longevity of cut flowers 20 and water stress during this period is often the reason of short vase life for cut flowers 40.

Water relation interruption is mostly due to microorganism proliferation in vase solution and occlusion in the basal end of the cut flower stem by microbes 1, 8, 19, 40. Stem blockage could take place by the bacteria 1, 8, 19, 40, or by extra cellular polysaccharides and degradation products of dead cells 1. Besides vessel blockage, bacteria secrete pectinases and toxic compounds and produce ethylene 42, thereby, accelerate senescence.

It has been shown that beside vase life reduction, disruption of water relation in rose flowers causes some physiological disorders such as bent neck 1, 6, 39, lack of flower opening 1, and wilting of the leaves accompanied by improper opening and wilting of flowers 1, 38. Therefore, controlling and reducing microbial proliferation is a prerequisite for extended quality and longevity of cut flowers, especially roses. On the other hand applied biocides could also affect other physiological properties of cut flowers specially their photosynthetic apparatus function and membrane permeability by their toxic compounds severally or moderately during aging and senescence.

In order to prevent microbial proliferation in vase solutions of cut flowers, various compounds and chemicals have been used, namely, silver nitrate 38, silver thiosulphate 38, 41, aluminium sulphate 39, hydroxyquinoline sulphate 18, hydroxyquinoline citrate 13, 23, 32, 39, 41 and sodium hypochlorite 23, 39, 41. Some of these compounds such as silver nitrate and silver thiosulphate have shown environmental risks and health hazards 3. Recently new efficient biocides with low toxicity have emerged. Nano silver is one which is more efficient compared to other Ag forms due to higher surface area to volume ratio 12. Formerly nano silver was broadly used as a biocide in different industrial products such as home appliances, cosmetics, textile, and pharmaceutics 12. Although it was initially introduced as a biocide into cut flower research 19, 32 on cut gerbera flowers and after that applied on cut ‘Movie Star’ roses 20, 21, still there is need of more evidence and knowledge on the application of nano silver in postharvest studies of cut flowers. This need is more required on physiological aspects such as chlorophyll degradation, chlorophyll fluorescence and membrane permeability which have not been studied before and on cut rose flowers which hold a very large portion of cut flower market and industry, and its cultivars benefit both from the biocidal and the ethylene antagonistic effect of silver.

Regarding the importance of cut rose flowers in the ornamental industry and the influence of vase solution microbes, and also, in order to found an easy to use, non-toxic and inexpensive compound
for large-scale application, in the present study we investigated the effects of nano silver particles application as vase solution biocidal preservative and its impact on vase life, water relation, vase solution microbial kind and population beside different physiological parameters such as chlorophyll degradation, chlorophyll fluorescence and membrane permeability of ‘Cherry Brandy’ rose.

Materials and Methods

Plant material: Rose (Rosa × hybrida) cv. ‘Cherry Brandy’ (licensed by Rosen Tantau, Germany) flowers were harvested at commercial maturity stage (i.e. outer petals starting to reflex and inner petals have become visible) from rose plants grown in hydroponic perlite in an automatic greenhouse. Flowers were harvested early in the morning and transferred to laboratory within 1 hour after harvest. Before treatment, all the leaves except the 5 most upper leaves of each flower stem were removed and then stems were recut slantly under water so that all flowers reach a height of 40 cm and probable air emboli gets removed.

Experimental design and treatments: Following recut, flowers were treated in a completely randomized design of 4 treatments and 9 replications. Treatments applied as vase solutions were colloid of nano silver particles (1, 2.5 and 5%) or sterilized distilled water (control). Vase solutions were not changed throughout the experiment and when needed, sterilized distilled water was added.

Experimental condition: Cut rose flowers were kept in a laboratory with a maximum and minimum temperature of 25 ±2°C and 21 ±2°C, respectively, relative humidity (RH) of 55± 5%, and light intensity of 14 µmol mm² s⁻¹ provided by white fluorescent lamps from 07.00 am to 20.00 pm.

Vase life and side effect evaluation: During vase life evaluation, cut rose flowers were daily checked and their appearance and condition were recorded to determine the vase life and if the applied chemicals had any side effects. Termination of vase life was recorded when wilting of the outer 5 petals occurred or bent neck was observed 1.

Solution uptake: Solution uptake of flowers was measured using a balance by weighing each vase containing its solution without subtracting the average of 4 evaporation data from solution uptake vases (vases which did not contain any flowers and were located a balance by weighing each vase containing its solution without subtracting the average of 4 evaporation data from solution uptake vases (vases which did not contain any flowers and were located within 1 hour after harvest). Before treatment, all the leaves except the 5 most upper leaves of each flower stem were removed and then stems were recut slantly under water so that all flowers reach a height of 40 cm and probable air emboli gets removed.

Vase solution samples during the first 6 days of the experiment, at 2 days intervals with 3 replications. From each sample 1 ml was diluted in 10-fold serial dilution. 0.1 ml from each concentration of diluted samples was plated on nutrient agar and all were incubated at 35°C for 48 hours. Microorganisms were counted by standard plate counting method (by counting the number of colonies formed after incubation) to generate the number of colony forming units ml⁻¹ (CFU ml⁻¹) 14.

Microbial counting: After plate counting, obtained colonies were studied and separated by their apparent morphological differences. This resulted in 3 bacterial isolates. The bacterial isolates were purified and differentiated according to their typical morphological and biochemical characteristics 11,30.

Bacterial morphological studies were motility, cell shape, and capsule presence. Bacterial bioassays were potato soft rot and hypersensitivity test on tobacco. The biochemical tests carried out on isolated bacterial colonies were Gram reaction using KOH, aerobic/anaerobic growth, acid production from glucose, gas production from D-glucose, fluorescent pigments production on KB, oxidase test, catalase test, gelatin hydrolysis, levan, growth at 50°C, growth at 5.7 pH, starch hydrolysis, Tween 80 hydrolysis, indol production, methyl red reaction, acetoin (VP), nitrate reduction, arginine dihydrolase and H₂S production from cysteine 11,30.

Ion leakage: Three 2.5 cm diameter discs were taken from leaf of each treatment’s flower stalk and placed into 50 ml centrifuge tubes containing 20 ml of 2 bar mannitol solution. Samples were kept at 25°C and dark for 24 h after which electric conductivity was measured and solution’s initial electric conductivity was subtracted in order to obtain electrolyte leakage.

Chlorophyll content: Total chlorophyll content was measured by non destructive method using chlorophyll meter (SPAD-502, Minolta Co. Japan) which provides a SPAD value 47. Measurement was conducted with 2 day intervals on 4 different flower stems (replications) in each treatment. For each flower stem, measurement was conducted on the marked spot of distal leaflet of 3 leaves.

Chlorophyll fluorescence: The quantum efficiency of open photo system II centers (Fv/Fm=ratio of variable to maximum fluorescence), was measured by a non-destructive method every 2 days with a Opti-Sciences OS-5P pulse amplitude fluorimeter (Opti-Sciences INC, Hudson, NH, USA) 24. Leaves were maintained in darkness for 20 min by a special clip before measurement of Fv/Fm. Minimal fluorescence (F0) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence (Fm) was obtained after a saturating pulse of 0.7 s at 8000 µmol m² s⁻¹. Fv is the difference between F0 and Fm 24,35.

Statistics: Data were analyzed by one-way ANOVA using MSTAT-C software and means were compared by the least significant difference (LSD) test at the 0.05 and 0.01 probability level (P < 0.05 and 0.01).

Results and Discussion

Vase life: As seen in Table 1, nano silver significantly increased vase life compared to control. This is in accordance with recent findings that silver application as nano particles increases vase life of some flowers such as roses 19,32 and gerberas 19,32. In
accordance to our findings, Liu et al. 19 found that application of nano silver either as pulse or vase solution treatment individually and/or in combination extends vase lives of *Rosa hybrida* flowers compared to deionized water. Lu et al. 20, 21 also saw that nano silver application as pulse treatments significantly extends vase life of cut ‘Movie Star’ roses. Within nano silver-treated flowers, there was no significant difference in vase life. Similarly Solgi et al. 32 also found that there was no significant difference between vase lives of different nano silver concentrations.

**Side effects:** Generally, effective concentrations of biocides can be toxic to many flowers 14, 16, 44, 46. Van Doorn et al. 46 concluded that at none toxic concentrations none of the applied compounds had constant and high anti-bacterial effect. This was while nano silver application on ‘Cherry Brandy’ roses did not result in vase life reduction. The only side effect of nano silver treatments was slight browning of the soaked skin part of treated stems. This did not result in any harm or injury to treated flowers. Controversially, flower leaves of these treatments were turgid and fresh even 2 days after vase life termination. Like our findings, Liu et al. 19 observed that beside vase life improvement, nano silver (as pulse or vase solution) also improves visual quality of cut roses. This is while Lu et al. 20 reported that nano silver pulse treatment at 250 mg l\(^{-1}\) caused visible damage to leaves of cut roses and their early abscission, thereby resulting in a similar vase life to control. High pulse application of this compound has been harmful for cut gerbera flowers. 19 Unlike our findings, Liu et al. 19 also found that continuous low level supply of nano silver may be toxic to cut gerberas.

**Microbial count:** Nano silver was completely effective in disinfecting and controlling vase solution microbial proliferation. All concentration of nano silver did not allow microbial proliferation until the last day of measurement (day 6) (Table 2). This was while vase solution microbial contamination of sterilized distilled water reached a relatively high count on day 2. Similar to our results, Liu et al. 19 also found that when nano silver is applied as vase solution, it strongly inhibits growth of vase solution microorganisms. This is while when nano silver is applied as pulse treatment, its biocidal benefits are transient 19, 20. It has been shown that nano silver pulse treatment inhibits bacteria growth in the vase solution and at cut stem ends during the first days of vase life. After that, numbers of vase solution bacteria increases during vase life 19, 20. In accordance to Liu et al. 19 and Lu et al. 20, our findings indicate that in order to have a long lasting anti-microbial effect, low-continues application of nano silver as vase solution should be applied.

**Microbial kind:** Although nano silver vase solutions did not contain any microbes, control (sterilized distilled water) was highly contaminated with bacteria. This is consistent with other published data 6, 14, 29. Although in vase water of cut roses, many different kinds of bacteria, yeasts and fungi have been identified 29, but in this study only bacteria were observed and the identified bacteria in control vase solution were only different species of *Bacillus* with white colonies. In other studies, *Bacillus* was also the most common vase solution microorganism 14, 15, 29.

Beside *Bacillus*, van Doorn et al. 42 found different bacterial strains such as pseudomonads, enterobacteria and some other genera such as *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Citrobacter* and *Flavobacterium* which occurred infrequently in rose stems. In another study there were Pseudomonads and Enterobacteria as the dominant bacterial strains in stems of cut ‘Sonia’ roses 43. Other isolated bacteria from rose vase solution were fluorescent pseudomonad and a nonfluorescent pseudomonad which reduced flower vase life of cut *Rosa hybrida* cv. ‘Cara Mia’ 46. In van Doorn and de Witte’s 43 study, tap water contained Pseudomonads and Enterobacter spp.

**Relative fresh weight (% of the initial):** Nano silver-treated flowers had a sharp increase in fresh weight on day 1 and consequently they reached their maximum fresh weight on day 3 except for 2.5% nano silver which had its maximum fresh weight on day 2 (Fig. 1). This group’s decrease in fresh weight had a slight slope, in which nano silver 1% reached its initial relative fresh weight on day 10, 5% nano silver reached its initial relative fresh weight on day 13, 2.5% nano silver did not reach its initial relative fresh weight even when its vase life terminated resulting in the best fresh weight changes and indicating the optimum concentration of nano silver for ‘Cherry Brandy’ roses from this aspect. In control flowers, weight change trend was similar to nano silver-treated flowers except that weight increment continued until day 4 after which there was a sharp decrease until end of vase life.

![Figure 1. Relative fresh weight changes trend of cut ‘Cherry Brandy’ rose flowers treated with nano silver](image)

There is a general sharp increase in relative fresh weight during the first days of the experiment. After reaching a maximum point there is a reduction until vase life termination.

### Table 1. Effect of nano silver on vase life of cut ‘Cherry Brandy’ rose.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vase life (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano Silver 1%</td>
<td>13.78 a</td>
</tr>
<tr>
<td>Nano Silver 2.5%</td>
<td>13.22 a</td>
</tr>
<tr>
<td>Nano Silver 5%</td>
<td>13.22 a</td>
</tr>
<tr>
<td>Sterilized Distilled Water (Control)</td>
<td>11.67 b</td>
</tr>
</tbody>
</table>

Means followed by the same lower-case letters are not significantly different at the 0.01 probability level using Least Significant Difference (LSD) test.

### Table 2. Effect of nano silver on cut ‘Cherry Brandy’ rose vase solution microbial count during days 2, 4 and 6.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbial count (log10 CFU ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
</tr>
<tr>
<td>Nano Silver 1%</td>
<td>0 b</td>
</tr>
<tr>
<td>Nano Silver 2.5%</td>
<td>0 b</td>
</tr>
<tr>
<td>Nano Silver 5%</td>
<td>0 b</td>
</tr>
<tr>
<td>Sterilized distilled water (Control)</td>
<td>4.477 a</td>
</tr>
</tbody>
</table>

\(N.S.\) means followed by the same lower-case letters are not significantly different at the 0.01 probability level using Least Significant Difference (LSD) test.

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Previous studies have indicated the beneficial effect of nano silver pulse treatment on relative fresh weight of different gerbera and rose cultivars. For ‘Movie Star’ roses, Lu et al. found that relative fresh weight of nano silver pulse-treated flowers was higher than control and all treatments showed similar trend for relative fresh weight change such that relative fresh weight increased until day 3 after harvest and decreased thereafter. Although in our study the relative fresh weight trend was similar to Lu et al., a different trend was observed for control flowers (Fig. 1).

**Solution uptake:** There was a high solution uptake in all treatments on day 1 (Fig. 2). After that, solution uptake trend in all treatments showed an initial decrease followed by 2 maximum absorption points (days 6 and 12) each having a decrease trend afterwards. Solution uptake trend and pattern by nano silver-treated flowers was very similar to sterilized distilled water (control). Until day 3 control flowers had the highest solution uptake after which nano silver 1% had the most uptake until the end of experiment. This was while there was not a significant difference between the mentioned. Although nano silver application increased solution uptake, at most days with nano silver concentration increment, solution uptake decreased.

Like our finding, Liu et al. found that nano silver pulse treatment increased vase solution uptake rates in cut ‘Rui Kou’ gerbera. In contrast, Lu et al. observed that in ‘Movie Star’ rose the amounts of water uptake decreased upon nano silver pulse treatment compared to control. Although solution uptake in previous studies tended to increase initially and then decreased with aging, throughout our experiment there were 3 critical points of maximum solution uptake which all treatments follow. This pattern was due to experimental environmental condition.

**Ion leakage:** Ion leakage trend in ‘Cherry Brandy’ roses showed a steady increase during vase life. Although nano silver treated flowers had a lower ion leakage, they showed a similar leakage trend as control flowers during the first week of experiment. As seen in Fig. 3, the most ion leakage was seen in control flowers indicating that nano silver treatment had retarded ion leakage or in other words, they have retained membrane permeability. Within nano silver treatments, ion leakage increased with concentration increment (Fig. 3). This caused nano silver 5% to have the most similar trend to control and being the least effective nano silver treatment too. The least ion leakage was seen in nano silver 1% which resulted in the best membrane permeability. Nano silver 1 and 2.5 % had a different ion leakage pattern compared to control and NS 5%. Until day 8 their leakage trend was similar to control showing an increase after which the increment was stopped.

Guiboileau et al. mentioned membrane lipids degradation as leaf senescence progress which results in ion leakage. Oren-Shamir et al. considered ion leakage as an index of membrane integrity and damage in plants during senescence. Our results confirm this issue and show that leaf ion leakage increases with aging. Like us, Sultan and Farooq have shown that senescence of cut flower is associated with ion leakage. Oren-Shamir et al. also found that in cut ‘Mercedes’ rose ion leakage increases during senescence progress. Sood et al. found same results for R. bourboniana and R. damascene flowers.

Although all reports agree on ion leakage increment during senescence, different trends have been reported for this issue. In cut ‘Mercedes’ roses ion leakage trend did not change until day-4 and after that it increased. Sood et al. observed that ion leakage trend in R. bourboniana was constant and suddenly increased upon vase life termination while in R. damascene it showed a slight increase during flower development and senescence. Our results indicate that ion leakage trend shows a steady increase during vase life and has been retarded significantly by nano silver application and consequently vase life has been increased.

**Chlorophyll content:** As seen in Fig. 4, there was an initial increase in chlorophyll content of nano silver-treated flowers compared to control causing a significant increase in all nano silver levels. After an increment on day-4, although chlorophyll content had fluctuation, it was almost steady until the end of vase life. The most chlorophyll content increment was observed in 1% nano silver treated flowers, this was while 5 and 2.5% nano silver had the highest chlorophyll content at the end of experiment. Results indicate the beneficial effect of nano silver application on chlorophyll content of cut ‘Cherry Brandy’ rose flowers.

Previously it has been shown that leaf chlorophyll content decreases during senescence. Senescence delay and chlorophyll preservation has been achieved by various compounds which mostly have growth regulatory behavior such as GA, benzyladenine and tidiazuron. As far as our knowledge, the present study is the first report on preservation solution biocidal effect on chlorophyll content and also the first
report on leaf chlorophyll increment and preservation by nano silver application. Bolla et al. 2 has shown that in ‘Euro Red’ rose even slight water stress reduces leaf chlorophyll content. We conclude that chlorophyll content retention in ‘Cherry Brandy’ rose might be to some extent due to water relation improvement by nano silver (Fig 2). Chlorophyll content increment in cut flowers by nano silver will have a great impact on commercialization and marketing especially on cut flowers which lose their green appearance of their leaves during vase life and senescence because of chlorophyll degradation.

Chlorophyll fluorescence: During vase life, leaf chlorophyll fluorescence of ‘Cherry Brandy’ rose decreased with aging and consequently reached its lowest level in all treatments at vase life termination. Control flowers had the least chlorophyll fluorescence reduction during vase life (Fig. 5). This was while in nano silver-treated flowers, chlorophyll fluorescence decreased with concentration increment. Within nano silver treatments, the highest chlorophyll fluorescence at the end of vase life was 0.803 in 1% nano silver treated flowers.

Similar to our findings, Tang et al. 26 have reported that with senescence initiation and progress, quantum yield of both photo system I and II decreases. Niewiadomska et al. 25 have also observed that during senescence quantum yield of photo system II reduces dramatically in tobacco leaves. Our findings on leaves of detached cut rose flower stems are in accordance with the mentioned reports on attached leaves. Controversially Pompodakis et al. 29 did not find a correlation between relative chlorophyll fluorescence reduction and vase life reduction of cold stored ‘First Red’ and ‘Akito’ rose flowers which seem to be due to low temperature injury of cold stored roses.

Chlorophyll fluorescence reduction indicated that quantum yield of photo system II reduces during vase life and reaches its lowest level at senescence. This fact and our results indicate a successive loss of photosynthetic activity during senescence and nano silver application in cut ‘Cherry Brandy’ rose. Considering the beneficial effect of nano silver treatment, increase in chlorophyll content of nano silver-treated flowers during the first days of experiment could be explained by this fact that flowers have increased their leaf chlorophyll level in order to overcome the loss of photosynthetic activity imposed by nano silver absorption.

Conclusions

For control flowers, as bacterial count in control vase solution reached above threshold on day 6 and vase life did not terminate until day 11, it seems that proliferated Bacillus bacteria do not reduce flower vase life by means of vascular blockage; pectinases or toxic compounds secretion. Solution uptake increment at the final vase life day of control flowers confirm the microbial findings regarding the fact that bacillus does not interrupt solution uptake. This fact confirms that not all microbes harm cut flowers and more research has to be carried out in order to determine harmful microbes.

On the other hand, while it has been concluded that none of the previously applied biocide compounds had a consistent or high anti-bacterial effects at concentrations that were not toxic to flowers, our findings suggest that low continues application of nano silver on ‘Cherry Brandy’ rose has consistent biocidal effects besides not being toxic to cut rose flowers.

The only physiological side effect of nano silver application was chlorophyll fluorescence reduction which was overcome by chlorophyll level increment. Therefore, we do not consider this as a toxic effect as it does not interrupt quality or biological function of this flower. From physiological point of view beside vase life improvement, nano silver application maintains membrane permeability and increases chlorophyll content and freshness of flowers and leaves.

As our report is the first report on physiological changes during vase life of cut rose flowers and that no other biocides have been studied in such physiological approach, it provides valuable information on different aspects of biocide application and shows the beneficial effects of nano silver application on cut ‘Cherry Brandy’ rose flowers.

References

6Florack, D. E. A., Stiekema, W. J. and Bosch, D. 1996. Toxicity of

\[ \text{Fv/Fm} \]

Figure 4. Leaf chlorophyll content changes trend of cut ‘Cherry Brandy’ rose flowers treated with nano silver.

While showing a fluctuation, nano silver has significantly increased leaf chlorophyll levels.

<table>
<thead>
<tr>
<th>Chlorophyll content (SPAD)</th>
<th>N.S. 1%</th>
<th>N.S. 2.5%</th>
<th>N.S. 5%</th>
<th>D.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>58.00</td>
<td>55.00</td>
<td>52.00</td>
<td>49.00</td>
</tr>
<tr>
<td>60</td>
<td>56.00</td>
<td>53.00</td>
<td>50.00</td>
<td>47.00</td>
</tr>
<tr>
<td>55</td>
<td>57.00</td>
<td>54.00</td>
<td>51.00</td>
<td>48.00</td>
</tr>
</tbody>
</table>

Figure 5. Leaf chlorophyll fluorescence changes trend of cut ‘Cherry Brandy’ rose flowers treated with nano silver.

Chlorophyll fluorescence reduces during vase life and with nano silver concentration increment, chlorophyll fluorescence declines.


