



Research Article

Phytofabricated Silver Nanoparticles (AgNPs) Applied in Vase Solution as a Novel Anti-Microbial Agent for Enhancing the Vase-life of Cut-Rose Flower

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ABSTRACT

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Plant extract has been exploited for biogenic synthesis of metallic nanoparticles. This is considered as the promising alternative routes of chemical and physical synthesis methods owing to their abundancy in nature and ecofriendly synthesis protocol. Rose is the top-ranked and universally favorite cut flower. Poor post-harvest management and microbial proliferation of the stem base in the vase solution is a concern for these cut flowers because that deteriorates the quality as well as shortens their vase-life. To overcome this problem, a smart solution i.e. phytofabricated silver nanoparticles have been shown to act as anti-microbial agent. In this study, leaf extracts of *Camellia sinensis* were exploited to fabricate biogenic silver nanoparticles which showing UV-peak absorption ranging from 412-500 nm. This biogenic silver nanoparticles were applied @ 0.01, 0.05 and 0.1mM and compared with a control (without AgNPs) and silver nitrate (AgNO₃). Interestingly, AgNPs showed a strong antimicrobial activity in vase solution and cut roses extended their vase life up to 13 days compared to 8 days in control and 9 days in AgNO₃. Statistical differences in flower opening, bacterial growth (CFU mL⁻¹) in vase solutions, water uptake, relative fresh weight and vase life of cut roses were found among treatments. In vitro microbial analysis and microscopic investigation of vessels showed that the development of microorganisms was reduced by a high concentration of AgNPs (0.1mM) at the cut end of flower and improving water uptake that followed by extended flowers vase life. The unique phytofabricated AgNPs technology can serve as a promising preservative to increase the ornamental value of cut rose flowers. Taken all together, applied phytofabricated AgNPs in vase solution significantly enhance vase life of cut rose flowers over control.

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Introduction

Nanotechnology brought revolution in the field of applied science and technology. It integrates several disciplines, including physics, chemistry, biology, engineering and biotechnology (Nasimi and Haidari, 2013, Haider and Kang, 2015, Selvakumar et al., 2018,). In case of nanotechnology application, agriculture is a still marginal sector (Hossain et al., 2020) compared to other fields like medicine, IT, materials and energy in the globe. However, in the recent times, nanotechnology has been introduced into agriculture and it has established nanofertilizers, nanopesticides as sustainable methods for precision farming (Lv et al., 2018, Cheng et al., 2016, Prasad et al., 2017, Banerjee et al., 2021, Cuong et al., 2022). One of nanotechnology's most significant goal is to increase

production and decrease postharvest wastage (Solgi et al., 2009, Birla et al., 2009). In the field of nanotechnology, the main challenge is to manufacture large-scale nanoparticles with a negligible cost and in a non-polluting way across the world (Moriarty, 2001). The application of nanotechnology is the most important issues of our national agriculture policies for achieving agricultural related sustainable developmental goals (SDGs) by 2030 in Bangladesh (MoA, 2018).

Generally, nanoparticles are synthesized through three different kinds of methods: physical, chemical, and biological (Chandran et al., 2006, Birla et al., 2009). Physical and chemical methods are toxic, labor intensive and expensive. The idea of "green

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nanotechnology" evolved as a new consequence with the green synthesis of nanoparticles. Plant-mediated nanoparticles synthesis is preferred because it is a sustainable way of producing nanoparticles which is more easy, economic, rapid and ecofriendly method than the other synthesis processes (Kumar and Yadav, 2009, Akhtar et al., 2013). The potential of inorganic NPs has been explored worldwide, among inorganic NPs, AgNPs have emerged with leading contributions in diverse applications, such as nanomedicine (Carabineiro, 2017), agriculture (Hossain et al., 2019), antioxidants (Sharma et al., 2012), and antimicrobial agents (Zhang et al., 2017), chemical sensing (Roy et al., 2013), cell biology (Abdal et al., 2017), textiles (Bharathiet al., 2018), the food industry, photocatalytic organic dye-degradation activity (Fathima et al., 2018). Due to its novel chemical, physical, and biological properties as compared to their bulk form, AgNPs have attracted the attention of researchers from various academic laboratories (Sharma et al., 2009).

Cut flowers are flowers or flower buds (often with some stem and leaf) that have been cut from the plant bearing it and it is usually removed from the plant for decorative use (Solgi et al., 2009, Kumar and Yadav, 2009, Skutnik et al., 2020). Rose is a very popular and widely used as a decorative garden plant or as cut flowers. Rose (*Rosa hybrida*) cultivation is now a profitable enterprise to the farmers but there has been very little work done on the postharvest handling procedures for improving vase life. Cut flowers are economically important in Bangladesh. The demand of cut rose flowers is increasing day by day due to its uses in different special occasions such as marriage, valentine day, official programs etc. Hence, these cut flowers particularly rose give high economic returns.

Microbial contamination at the stem base or in the vase solution shortens the vase life of these cut flowers (Van Meeteren, 1978, Van Doorn and De Witte, 1994, Balestra et al., 2005). One of the most serious issues in postharvest flower physiology is a decrease in water uptake owing to vascular occlusion caused by air or bacterial development, which causes water stress in cut flowers (Elhindi, 2012). Xylem occlusion of fresh-cut flowers may occur due to physiological wound healing, microbial colonization (He et al., 2006) and air embolism. Xylem occlusion inhibits water transport to the petals of harvested flower resulting early wilt and senescence in cut flowers (Jedrzejuk et al., 2012). So, the cut flower's vase life mainly depends on microbial proliferation in vase solution and vascular occlusion. AgNPs mainly acts as an antimicrobial agent that inhibits bacterial growth in xylem vessels of cut flowers, leading to increased water uptake by the cut stem, and consequently, Bio-synthesized silver nanoparticles

(AgNPs) application to the vase solution improves the cut flower's vase life (Liu et al., 2009a, Li et al., 2012a, 2017, Rahman et al., 2019, Langroudi et al., 2019, Juthee et al., 2021). There is little study on the application of phytofabricated AgNPs in cut rose flowers for enhancing the vase life. The objective of this study was to extending the vase life of cut flowers by applying phytofabricated AgNPs to inhibit microbial blockage in the stem base. The current research work is most likely the first scientific investigation in Bangladesh for improving vase life of freshly cut-rose flowers using AgNPs.

Materials and Methods

Plant materials and chemicals

Tea leaf (*Camellia sinensis*), cut rose flower (*Rosa hybrida*), 'Red Ribbon' were collected from BAU botanical garden and horticulture field laboratory, Bangladesh. To prevent moisture loss during transit, buckets carrying flower stems were covered with a plastic film shroud. The experiment was started on the same day.

Chemicals like AgNO₃, Methanol, Agar and Nutrient broth for media of bacterial growth were obtained from Z.H. Scientific and Chemicals Mart Company, Bangladesh. The purity was at least 99.5%.

Preparation of AgNPs and treatments

The collected fresh leaves washed thoroughly in double distilled water. The leaves were then sliced into small pieces and 20 g was weighed out, crushed using a mortar and pestle after addition of 100 mL methanol. The mixture was filtered using filter paper followed by Whatman No. 1 filter paper and then stored at 4°C as Methanol Leaf Extract (MLE) for further use. Then 0.5 mL MLE was added to a 100 mL conical flask containing 50 mL aqueous solution of 1.75 mM (millimole) silver nitrate under stirring condition (Juthee et al., 2021).

The flower stems were then re-cut to a consistent length of 30 cm in deionized water. The stem end was recut to ensure there was no air blockage. Then, they were placed in preservative solutions containing an aqueous solution of nano-silver (0.01, 0.05 and 0.1 mM) and AgNO₃ (0.01mM) at room temperature, 16:8 h light/dark cycle, and 60±5% relative humidity (RH). The control flowers were kept in distilled water (DW). Each treatment was repeated three times.

Vase life evaluation

The rose flowers were considered senescent when showing 50% wilting of leaves or petals of flowers and neck bending. During the vase life period, cut roses were assessed daily.

Water Uptake and Relative fresh weight (RFW)

The volume of vase solution containing flowers and the weights of flowers were measured individually on a daily basis to track water absorption and relative fresh weight (RFW). Average daily water uptake was calculated as:

Water uptake ($\text{ml stem}^{-1} \text{d}^{-1}$) = $(st^1 - st)$, where st is volume of vase solution (ml) at $t =$ days 1, 2, 3, and so on, and st^1 is volume of vase solution (ml) on the preceding day.

The stems' RFW was calculated as follows:

RFW (%) = $(wt / wt-0) \times 100$; where, wt is weight of stem (g) at $t =$ days 1, 2, 3, and so on, and $wt-0$ is weight of the same stem (g) at $t =$ day 0 (He et al. 2006).

Destruction of the petal's carotenoids

The amount of carotenoids in petals was measured following the method described by Sultana et al., 2019. Carotenoids were then measured by spectrophotometer at the specified wavelengths using the following formula:

$$\text{Carotenoids (mg g}^{-1}\text{)} = 4.69 \times A_{660} - 0.268 \times 20.2 A_{645} + 8.02 A_{663}$$

Finally, the destruction of the carotenoids content was calculated by subtracting the concentration of carotenoid on the last day (the end of vase life) from that on the first day in case of each treatments.

Counting bacteria in the vase solution

To determine the number of bacteria in the vase solution, sampling was conducted at day 10 after keeping the flower in the solution.

Preparation of nutrient agar (NA) medium

Fourteen grams of prepared Nutrient Agar was suspended in 500 ml of distilled water in a conical flask. The mixture was boiled to dissolve the medium completely this method described by Sultana et al. 2018. After that, the flask was autoclaved for 15 minutes at 121°C and 15 PSI pressure. Then the medium was plated in the sterilized glass petri dishes and the petri dishes were placed in the laminar air flow cabinet to solidify for at least half an hour.

Bacterial colony count

1 mL liquid from each treatment was taken in falcon tube and diluted 3 times with sterile distilled water. 0.1 mL diluted samples were plated on nutrient agar medium in petri plates. Plates were incubated at 28°C for 48 hours, after which bacterial colonies were counted the method described by Chakraborty et al., 2020.

Then the CFU/mL was calculated by using the following formula:

$$\text{CFU/mL} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

Vascular occlusion

Vascular occlusion was determined according to Li, 1987. Segments of 3 cm length were excised for microscope observation from cut stem ends immediately after cutting and on the 10th day of the vase period. Explants were fixed initially in FAA (formaldehyde acetic acid ethanol). Sections were stained with eosin-haematoxylin solution and examined under ZEISS AxioCam ERc 5s5 megapixel microscope.

Experimental design and analysis

The collected data were statistically analyzed by using Minitab 17 statistical Computer Package Programmer in accordance using the Completely Randomized Design (CRD) principles with 3 replications ($n=3$). Duncan's Multiple Range Test (DMRT) was used to compare treatment differences.

Results and Discussion

Treatments of phytofabricated silver-nanoparticles (AgNPs) and vase life

Vase life was considered to have ended when the general appearance of flowers was no longer attractive due to un-opened buds, floret drop, petal discoloration, stem yellowing, petal wilting and flower epinasty resulting in abscission.

Different levels of AgNPs were applied into the vase solution and observed the vase life duration, decorative value, wilting of leaves and petals, color change of the petals (Figure 1) were noted.

The control flowers started to lose its decorative value and showing visible damage of leaves and the early abscission after 6th day whilst AgNPs (0.1 mM) treated did not lose its decorative value before at day 13. Stem bending (flower epinasty) started after 8th day and was evident at day 9 in AgNO_3 (0.01 mM) and AgNPs (0.01 mM) but after 10th days in AgNPs (0.05mM) treated vase solutions of the cut roses (Figure 1). The results revealed that AgNPs enhanced the duration of cut roses' vase life significantly by maintaining its decorative and aesthetic value. AgNPs are translocated into different floral parts and limit ethylene biosynthesis by decreasing the activity of the ethylene biosynthesis genes (ACO1 and ACS1), resulting in increased solution uptake, relative fresh weight, petal water content, and cut flowers' longevity (Naing and Kim, 2020). In the current investigation, duration of cut roses' vase life in the presence of AgNPs (Figure 1) could

be explained in the similar way though ethylene biosynthesis and its regulatory enzymes activities were not measured.

Different levels of AgNPs applied, the treatment with a concentration of 0.1 mM resulted in the longest vase

life (12.66 approximately equal to 13 days), while the control and the AgNO₃ concentration (0.01 mM) resulted in the shortest vase life. The treatment with concentration of AgNPs (0.05 mM) achieved 10 days of vase life. Generally, 0.1mM Ag concentration increased vase life (Table 1).



Figure 1. Effect of treatment concentration on cut roses' vase life from left to right (control, AgNO₃, 0.01, 0.05, 0.1 mM AgNPs conc. from left to right in each picture) at regular interval (0-13 days).

Table 1. Effect of biologically synthesized AgNPs concentration (mM) on vase life (days) of cut roses. Values marked with the same letters within the columns do not differ significantly at $P \leq 0.05$

Treatments (mM)	Vase life (days)
Control	8.33c
AgNO ₃ (0.01)	9.00bc
AgNPs (0.01)	9.33bc
AgNPs (0.05)	10.21ab
AgNPs (0.1)	12.66a

and increased water uptake rate in cut roses during vase life. The amount of water that is absorbed by all flowers increased up to 4 days except control and then decreased with time. Amount of water uptake in AgNPs treated flowers was greater than those treated with AgNO₃ and the control (Figure 2), although all treated and untreated flowers decreased their water uptake with progress in time. In our experiment, flower treated with AgNPs (0.1mM) significantly increase the vase life of the cut rose flowers in association with a relatively highwater content of leaves. Low water uptake induces flowers epinasty, petals wilting and finally the appearance of flowers was no longer attractive.

Water uptake and relative fresh weight (RFW)

Phytofabricated AgNPs treatments reduced the declining rate of relative freshness (eye observation)

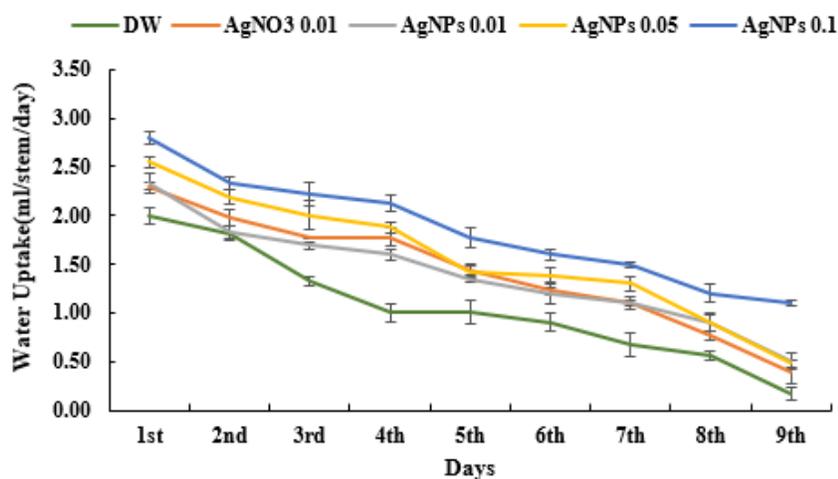


Figure 2. Effect of different treatments on daily water uptake ($\text{mLstem}^{-1}\text{day}^{-1}$) in cut roses. The mean ($\pm\text{SE}$) is shown by the vertical bars ($n=3$).

Relative fresh weight (RFW) variations of cut roses showed the similar trends for control and treatments, such that RFW increased (130% of initial) until day 4 except control after harvest and declined with time (Figure 3). In recent study, flower treated with AgNPs (0.1mM) had highest RFW compared to other treatments. Water deficit condition in stem reduces water uptake as well as RFW and that deficiency created by the microbes, which can infect the stem end of postharvest cuttings and develop a biofilm of microbial remnants, producing secretions that blocked

xylem vessels (Romero et al., 2014, Da Silva, 2003, Skutnik et al., 2020). The presence of nanosilver (Ag^0) had a profound effect on the water relations of cut rose flowers because AgNPs can act as both anti-microbial and anti-ethylene agent (Park et al., 2017). Therefore, AgNPs may have positively effects on water uptake because of antibacterial effects of Ag^0 in AgNPS may affect regulation of water channel activity as well as relative fresh weight and improve solution uptake.

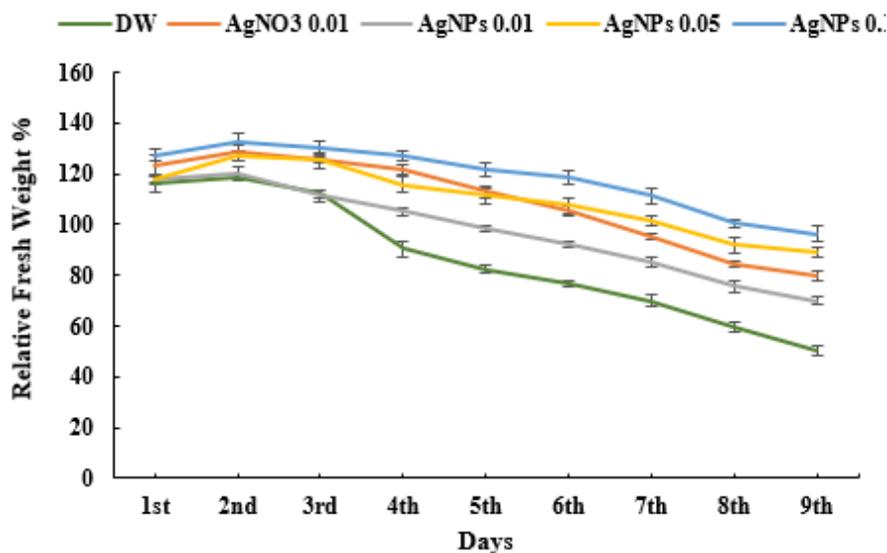


Figure 3. Effect of different treatments on relative fresh weight % in cut roses. The mean ($\pm\text{SE}$) is shown by the vertical bars ($n=3$).

Carotene content in the petals

In case of carotene, considerable difference was found due to the treatments @ 0.01, 0.05 and 0.1mM and compared with a control (without AgNPs) and silver

nitrate (AgNO_3). The highest amount of carotene was found in 0.1mM AgNPs is 0.56 mg g^{-1} compared to other treatments and control (Figure 4). Ag^0 containing AgNPs had significant roles in ethylene suppression and the

extension of flower longevity were reported in other cut flowers like rose, carnation and Lisianthus (Hasan et al., 2014, Kamiab et al., 2017, Xia et al., 2017). The flower treated with 0.1mM AgNPs having highest vase life also having highest pigments (carotene) content than other treatments and control. The results

indicated that AgNPs treated flowers were more attractive and loss their decorative value delayed than others that may due to high carotene content as well as vase life of the cut roses.

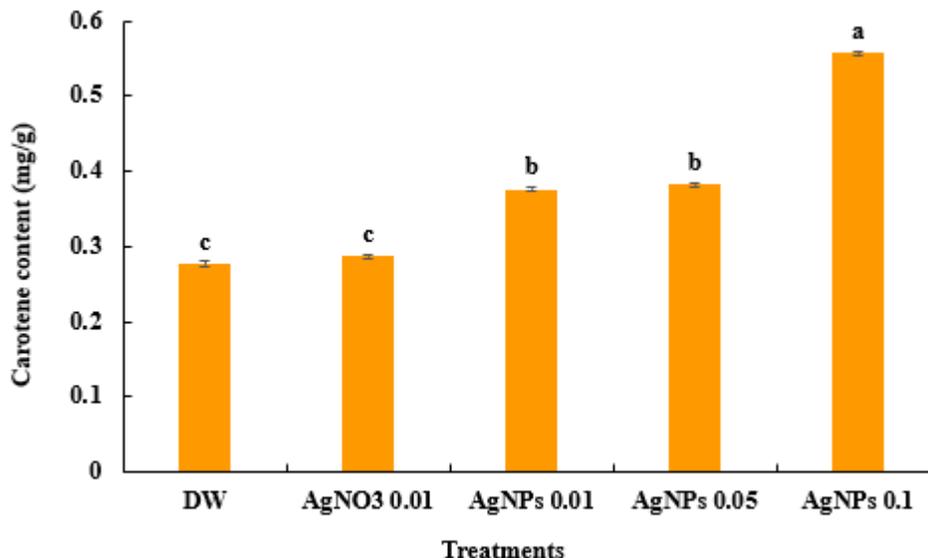


Figure 4. Carotene content of the rose petals due to different treatments. The mean (\pm SE) is shown by the vertical bars

Antibacterial effect of silver nanoparticles

The number of bacteria in the vase solution dropped when the concentration of AgNPs in the cut rose vases increased (Table 2). In the control, the bacterial colony was significantly different from that of treatments with non-nano AgNO₃ (0.01mM) and nano silver (0.01, 0.05, 0.1 mM AgNPs). When AgNPs were added to vase solutions, the quantity of bacterial colonies and, as a result, CFU mL⁻¹ reduced dramatically. The findings showed that silver ion (Ag⁺) efficiently reduced bacterial growth in the vase solution of cut roses and it was magnified by using AgNPs.

In the present study, Microscopic investigation showed that stem blockage due to microbial colonization (1.2×10^5 CFU mL⁻¹) was evident at the cut end of the control stem whilst AgNPs treated samples effectively inhibited the microbial growth (0 CFU mL⁻¹). The shortest vase life 8 days was observed in the control (Table 1) which could be explained by the presence of bacteria in the vase solution (Table 2) that prevents water transport towards the flower's petal. The opposite events were noticed in the flowers treated with AgNPs, especially at the concentration of 0.1 mM AgNPs. As a result, preventing microbiological blockage improves the quality and decorative value of cut flowers, and Nano-silver has been utilized to increase the vase life of cut

flowers. (Lu et al., 2010, Solgi et al., 2009, Amingad et al., 2017, Li et al., 2012, Zhao et al., 2018, Alekasir et al., 2017, Manzoor et al., 2020).

Table 2. Effect of Ag nanoparticles concentration (mM) on total bacterial count (CFU mL⁻¹) in cut roses' vase life of at 10th day

Treatments (mM)	Colony count	Total bacterial colonies (CFU mL ⁻¹)
Control (H ₂ O)	120	1.20×10^5
AgNO ₃ (0.01)	56	5.6×10^4
AgNPs (0.01)	24	2.4×10^4
AgNPs (0.05)	8	8.0×10^3
AgNPs (0.1)	0	0

Vascular Occlusion

Xylem occlusion is an important physiological phenomenon, appeared when stem or inflorescence is detached from the mother plant. Microbial proliferation and vascular occlusion at the cut end of the stem reduce water transport towards the flower buds and flowers causing premature wilting of the petals. In the present study, no blocked vessels were observed in cut stems kept in vase solutions with 0.1mM AgNPs (Figure 5). However, very clear blocked vessels were observed in cross-section of cut stem kept in vase solutions without AgNPs (control) on 10th day after harvest

(Figure 5). According to the findings, occluded xylem was not observed at the cut end of AgNPs (0.1mM) treated stem that may increase the cut roses' vase life by inhibiting bacterial growth. Microbes can grow and colonize quickly in the stem end of postharvest cuttings, generating microbial colonies that occluded xylem vessel (Romero et al., 2014, Da Silva, 2003, Skutnik et al., 2020). Thus, microbial blockage seriously inhibits the uptake and transport of water, causing water imbalances and the early wilt of cut flowers (de Witte et al., 2014, Lu et al., 2010a, He et al., 2018). Microbial contamination is thought to be the primary cause of stem blockage, which reduces the vase life of cut flowers (Lu et al., 2010). Inhibiting microbial growth is therefore an excellent technique to improve the aesthetic value of cut flowers, and Nano-silver has been successfully applied to increase the vase life of cut roses. (Lu et al., 2010, Solgi et al., 2009, Lu et al., 2010a, Amingad et al., 2017, Li et al., 2012, Zhao et al., 2018, Alekasir et al., 2017, Manzoor et al., 2020).

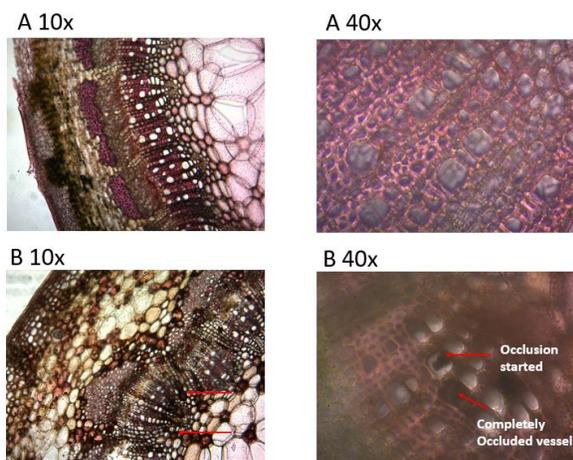


Figure 5. Cross sections (10x and 40x) of cut stems of rose showing microbial growth and vascular occlusion at the cut end of stem; (A) with AgNPs, (B) without AgNPs (right). ↖-blocked vessels

Conclusion

The phytofabricated AgNPs was synthesized using *camellia sinensis* leaf extract which exhibited strong antimicrobial activity when applied in the vase solution and vase life extended significantly by maintaining quality of cut roses. Application of treatment with 0.1mM AgNPs significantly increased the cut roses' vase life, along with suppression of microbial growth and vascular occlusion at the cut end of stems. Mobility of silver ion of 0.1mM AgNO₃ in stem of rose flowers is slow. Therefore, application of nano particle with strong antimicrobial effects would improve speed of nano-silver (Ag⁰) mobility due to its nano size and could prolong cut flower longevity. So, from the recent study, 0.1mM AgNPs is suggested to enhance the cut roses'

vase life. Finally, this study will provide useful information on the benefits of using AgNPs to control the postharvest damage of horticultural products, particularly with respect to improving the vase life of cut flowers.

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Competing interests

The authors have declared that no competing interests exist.

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