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RESEARCH PAPERS

Response of Cut Snapdragon Flowers to Nanosilver and Recut during Postharvest Life

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Abstract—Preserving longevity in snapdragon (*Antirrhinum majus* L.) flowers, an important cut flower with a short vase life is an economic issue that can be improved with some postharvest treatments. The Nanosilver application in preservative solutions and recut is effective techniques that can potentially prolong the vase life of cut snapdragon flowers. Hence, in this study, the effects of Nanosilver and recut on some postharvest characteristics were investigated. The results indicated that cut flowers treated with Nanosilver + recut extended the vase life of the flower up to 18.71 days. The recut flowers kept in the solution containing Nanosilver revealed higher RWC, carbohydrate content, and pH. Soluble protein content also increased significantly on day 15 compared to other treatments. This treatment, by reducing the content of H_2O_2 and MDA, caused a decrease in the electrolyte leakage and maintained the function of the membrane. Examining the TEM micrographs of Nanosilver + recut treatment showed the activity of plasmodesmata on the 15th day. The results of the present study indicate that the presence of Nanosilver in preservative solutions, especially when the stem end is recut, can be a promising method for delaying the signs of senescence and maintaining the postharvest quality of cut flowers.

Keywords: *Antirrhinum majus*, ethylene, MDA, TEM, water balance

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INTRODUCTION

Snapdragon (*Antirrhinum majus* L.) is a high commercial value cut flower in the United States markets due to its wide range of colours, fragrance, and attractive spike-type inflorescence [1]. Snapdragon has also been considered a model plant in genetic, flower development, and programmed cell death studies [2]. This unique flower has indeterminate inflorescences with many flowers and buds and a relatively short vase life [3]. In recent years, cut snapdragon flower sales have risen 51.7% and reached $12.93 million, making snapdragons one of the top ten cut flowers in the US [4].

Genetic and environmental factors affect the longevity of the cut flowers, and an excessive 25% of the observed variability in postharvest longevity is attributable to the petal senescence signs [1]. The snapdragon flower senescence is distinguished by wilting, desiccation, abscission, incomplete flower opening, suppression of pigmentation, and bending of flower spikes [5, 6]. On the other hand, several environmental parameters can affect the maintenance of the postharvest quality of cut flowers including; water balance, carbohydrate supply, temperature, and susceptibility to ethylene [7]. Snapdragon flowers are extremely sensitive to ethylene because the corolla is abscised when exposed to ethylene [5]. Furthermore, studies have shown that this sensitivity leads to the bending of flower stems [8].

Silver thiosulfate complex (STS) and 1-methylcyclopropene (1-MCP), as inhibitors of ethylene action, are now routine commercial practices for ethylene-sensitive flowers. However, these treatments still have disadvantages, such as no ideal effect, poor stability, environmental pollution, and toxicity [9, 10]. Furthermore, studies have indicated the STS treatment has only slightly increased the vase life of cut snapdragons [5].

Recently, researchers have taken into consideration the Nanosilver (NS) application and its positive effects on improving the postharvest life of cut flowers. The NS has strong antibacterial activity due to nanometer-sized silver particles and intensive control of microorganisms. In addition, easy preparation, non-toxicity, and safety have extended its activity spectrum [1, 9]. In previous studies, the use of NP has prolonged successfully the vase life in many cut flowers such as cosmos [11], roses [12], gerbera [13], and snapdragon [1].

Abbreviations: TEM—transmission electron microscopy, NS—Nanosilver.

Fig. 1. (a) Developmental stage of cut snapdragon flowers. (b) The cut snapdragon in the experiment chamber after three days.

In addition, studies have revealed that the wilting of cut flowers occurs due to xylem occlusion at the end of the stem followed by poor water absorption [14]. This event disturbs water conduction through the stem and causes the stem bending problem in snapdragons, which is a major problem in the postharvest of cut snapdragon flowers [8]. Some studies have indicated that the recutting of the stem end has extended the cut flower vase life, as reported in H3O rose [14] and *Helianthus annuus* L. [15]. However, this treatment has not been investigated in many species like snapdragon flowers. Moreover, no studies have studied the combined effects of using NS with recut to improve postharvest quality and longevity of cut flowers. Therefore, the aim of the present experiment was to investigate the effects of Nanosilver and recut on the vase life of cut snapdragon flowers.

MATERIALS AND METHODS

Plant material. Snapdragon flowers (*Antirrhinum majus* 'Legend White') were grown in the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, and then harvested at the same developmental phase (Fig. 1a) without any disease and pest symptoms or mechanical defects. After that, cut snapdragon flowers were transferred to the postharvest lab and were maintained in a cold room (4°C) and then hauled to the experiment chamber. An experiment was conducted based on Completely Randomized Design (CRD) with seven replications. All cut flowers (120 cm) were trimmed to 45 cm, were removed excess leaves, placed in the bucket, and kept in an experiment chamber (experiment chamber condition was 20 ± 2°C, 60% RH, 20 μmol/m^{2}s irradiance from cool white florescent lamps, and 12 h photoperiod) for three days. After three days (Fig. 1b), the cut flowers were divided into two groups: non-recut and recut. For recut treatment, 5 cm of stem end was recut. Then half of the recut flowers and half of the non-recut flowers were placed into bottles containing water and water with 1 mg/L NS (each stem in one bottle). The solutions were not swapped during the experiment. However, they were refilled whenever necessary. The sampling was performed on days 0, 5, 10, and 15 after treatments for biochemical analyses.

Vase life. Vase life was recorded as the number of days after treatment (day 0) that 50% of the flowers in the spike were wilting or drying [1].

Relative water content (RWC). The RWC of upper and lower florets was calculated using the following equation [10] on days 5, 10, and 15 after treatment:

$$\mathrm{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fully turgid weight} - \text{dry weight}} \times 100,$$

fresh weight, the florets were freshly weighted; fully turgid weight, the florets were saturated in distilled water at 4°C for 24 h; dry weight, the florets were dried in an oven at 105°C for 72 h.

Soluble protein content. Soluble protein content was measured according to the Bradford assay [16]. Briefly, 100 mg of the petal was homogenized with

Table 1. Longevity of cut snapdragon flowers

Vase life, d			
non-recut	non-recut + NS	recut	recut + NS
11.43 ± 0.37d	14.71 ± 0.28c	15.71 ± 0.18b	18.71 ± 0.28a

Different letters indicate significant differences at $P \leq 0.05$ (Duncan's test). Data represent mean ± SE, $n = 7$.

phosphate buffer solution and then centrifuged at 10000 rpm for 10 min at 4°C. 5 mL of Coomassie brilliant blue solution was added to 100 μL of supernatant. The absorbance was read at 595 nm wavelength and total soluble protein was calculated using the BSA standard curve.

Carbohydrate content. Carbohydrate content was extracted and measured, as described by Nabipour Sanjbod et al [17]. The glucose standard curve was used for glucose equivalents calculation.

Electric conductivity. For electrolyte leakage from petal cells on day 15, small squares were cut from petals and placed in deionized water. After 30 min, initial conductivity was measured. Then, the samples were placed into a water bath of 96°C and boiled for 15 min, and then total conductivity was measured. Electric conductivity was expressed as the percentage of the initial conductivity versus the total conductivity.

pH of the cell sap. For cell sap pH on day 15, 1 g of corolla was ground in distilled water and stirred. The petal pH was read after 2 h with pH meter (pH-Meter 20 +, CRISON, SPAIN).

Lipid peroxidation. To measure the malondialdehyde (MDA) index on day 15, 1 gram of petal tissue was homogenized in 5 mL of trichloroacetic acid (TCA) buffer and then centrifuged at 10000× *g* for 20 min at 4°C. 2 mL of the supernatant was mixed with 4 mL of thiobarbituric acid buffer and placed for 20 min. Absorbance was read at 450, 532, and 600 nm. MDA concentration was calculated in μmol/ml from the following formula [18]:

$$\mathrm{MDA}\,(\mu\mathrm{mol/L}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}.$$

H_2O_2 content. The hydrogen peroxide (H_2O_2) content of the corolla (on day 15) was measured as described by [10]. 0.5 g of fresh petal was homogenized in 6 mL cold acetone and centrifuged at 10000× *g* for 10 min at 4°C. 100 μL of $Ti(SO_4)_2$ (5%) and 200 μL of NH_4OH solution was added to 1 μL of the extract and was centrifuged for 10 min. The resulting pellet was dissolved with 4 mL of H_2SO_4 (2 M) and expressed at 412 nm. Various concentrations of H_2O_2 were used as a standard curve, and the results were recorded as μmol/g FW.

Transmission electron microscopy (TEM). For TEM, the mesophyll cells of the first floret on day 15 were analyzed. The preparation stages of TEM samples were performed as described by Nabipour Sanjbod [2].

Statistical analysis of the data. Data were statistically analyzed by one-way analysis of variance using the SAS V9.2 software. Duncan's multiple range test at 5% probability levels was used to assess the significant differences between the means, and data were given as the mean ± SE.

RESULTS

Longevity of Cut Snapdragon Flowers

The average longevity of the non-recut flowers was 11.43 days. However, the application of Nanosilver prolonged the longevity to 14.71 days. The longevity of recut flowers without Nanosilver treatment (15.71) revealed a significant difference compared to non-recut flowers. Also, the longest longevity (18.71) was observed in Nanosilver treatment with recut (Table 1, Figs. 2–5).

Relative Water Content

On day 0, RWC was similar in all treatments and had no significant difference (data not shown). Difference between treatments started on day 5, when recut + NS treatment indicated higher RWC than other treatments. With the gradual senescence of flowers, RWC decreased, and the lowest was observed on day 15 and non-recut treatment. On the 15th day, the flowers of recut + NS treatment still had the highest RWC among the treatments (Fig. 6a).

Soluble Protein Content

The flowers of all treatments had similar protein content on day 0. After 5 days, the soluble protein content increased so that it was significant in recut + NS treatment (26% compared to the initial level on day 0). In the following, a decreasing trend was observed in the protein content. On the 15th day, the flowers under recut + NS treatment had a lower protein level than the other treatments. The highest amount of protein was recorded on day 15 in non-recut flowers (Fig. 6b).

Carbohydrate Content

The trend of changes in carbohydrate content was interesting. In non-recut flowers, carbohydrate content significantly decreased (77% compared to day 0) and reached 8.24 μg/mg FW. While the other treatments caused an increase in soluble carbohydrate until the 10th day. However, after the 10th day, the flowers

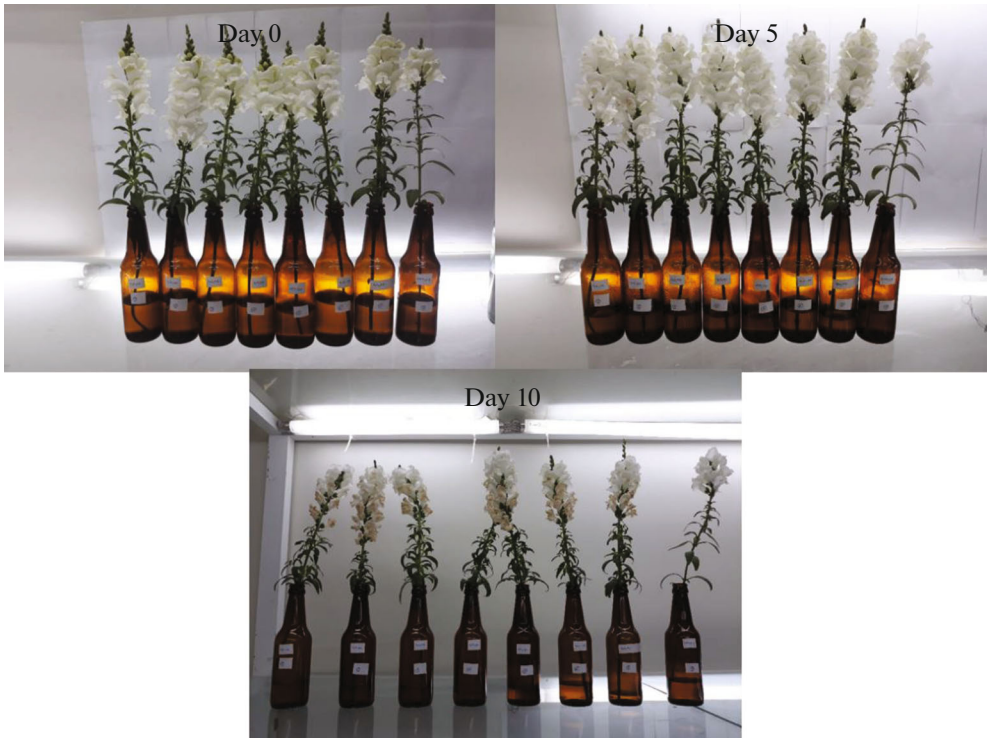

Fig. 2. The cut snapdragon flowers under non-recut treatment.

Fig. 3. The cut snapdragon flowers under non-recut + NS treatment.

Fig. 4. The cut snapdragon flowers under recut treatment.

treated with Nanosilver still had the highest carbohydrate content. However, in recut flowers, the carbohydrate content decreased by 15% (Fig. 6c).

Electric Conductivity

In this study, significant effects of treatments on cell sap EC were revealed. In flowers that were kept in solutions containing Nanosilver, the cell sap EC was lower during the vase life than in flowers that were not treated with Nanosilver. On the other hand, recut and non-recut flowers did not indicate any significant difference (Fig. 6d).

pH of the Cell Sap

Cell sap pH was also positively affected by Nanosilver. The value of this parameter was the highest in flowers placed in Nanosilver solutions. In the flowers that were not treated with Nanosilver, a noticeable decrease in the pH of the cell sap was observed on the 15th day. The lowest pH was recorded in non-recut flowers (Fig. 6e).

Lipid Peroxidation

MDA content was the highest in non-recut flowers on day 15. With recut, this index decreased. In addition, with the application of Nanosilver, lipid peroxidation decreased and the lowest value was observed in flowers treated with recut + NS (Fig. 6f).

H_2O_2 Content

Changes in H_2O_2 content similar to MDA on day 15 depended on the application of Nanosilver. The use of Nanosilver significantly reduced the H_2O_2 content. The highest H_2O_2 content was obtained in untreated flowers. Also, the use of recut for snapdragon cut flowers caused a significant difference in H_2O_2 content with untreated flowers (Fig. 6g).

Ultrastructural Changes

Figure 7 indicates the presence and absence of plasmodesmata in the tested treatments on the 15th day. In the flowers that were not treated with Nanosilver, plasmodesmata were either absent or were observed in limited numbers in some areas of the cell wall (Figs. 7a and 7b). In the flowers treated with Nanosilver, plasmodesmata with higher electron density and more numbers were observed in most areas of the cell wall (Figs. 7c and 7d).

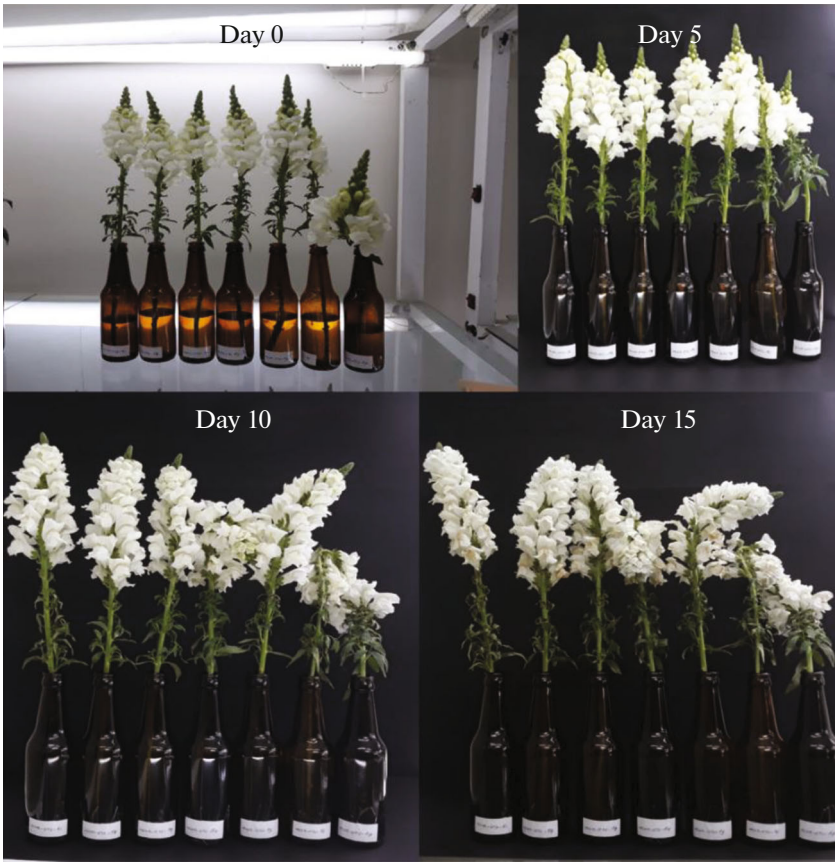

Fig. 5. The cut snapdragon flowers under recut + NS treatment.

DISCUSSION

Senescence, the last stage of flower development, is a vital factor in reducing the quality of cut flowers [19]. With the progress of senescence, a sequence of events occurs at different levels, which causes the degradation and remobilization of proteins, lipids, and nucleic acids [18, 19]. What is clear is that senescence is not inevitable. However, some postharvest treatments can delay it.

When a flower is harvested, due to being separated from the mother plant, it is severely under water stress [20]. In response to wounds and stress, the xylem vessels at the end of the stem are usually blocked (physiological response). In addition, xylem occlusion can be caused by microbial (bacterial activity) or physical (air emboli), which prevents water supply to the petals [14]. Reduction of water content along senescence results in premature wilting and reduced vase life of cut flowers. This case has also been reported in the cut snapdragon flowers when the end of the stem is clear and unobstructed, but the number of blocked xylem vessels increases gradually during vase life [1]. On the other hand, the decorative value and vase life decrease, which is an economic loss for producers and consumers [10]. Therefore, the postharvest maintenance of cut flowers requires special techniques to reduce the economic loss.

For many years, preservative solutions application has been an efficient method to increase the vase life of cut flowers [8]. These solutions mainly contain a biocide that prevents the growth of microbes and continues the water absorption [11]. Also, in some studies, the positive effects of re-cutting the stem end on water absorption have been indicated [15]. In general, these two treatments can maintain the turgor of petal cells.

In this study, cut snapdragon flowers under recut + NS treatment had a longer vase life (18.71 days). In addition, on the 15th day, RWC in flowers treated with recut + NS was higher than in other treatments. What is certain is that Nanosilver alone did not affect the vas life and RWC of flowers. A 5 cm cut at the stem end also had a positive role in increasing the vase's life. Recently, reports indicate that Nanosilver as a safe biocide can prolong the vase life of cut flowers [11]. Also, Rabiza-Świder et al. [1] reported that Nanosilver treatment during postharvest of the cut snapdragon flowers has extended the vase's life. In another study, re-cutting of the rose stem end caused the removal of the microbial community of the stem end and the blocked part of the stem, increased water absorption and fresh weight, maintained RWC, and as a result, extended vase life, which is consistent with our results. In addition, in some flower shops, the skins of the

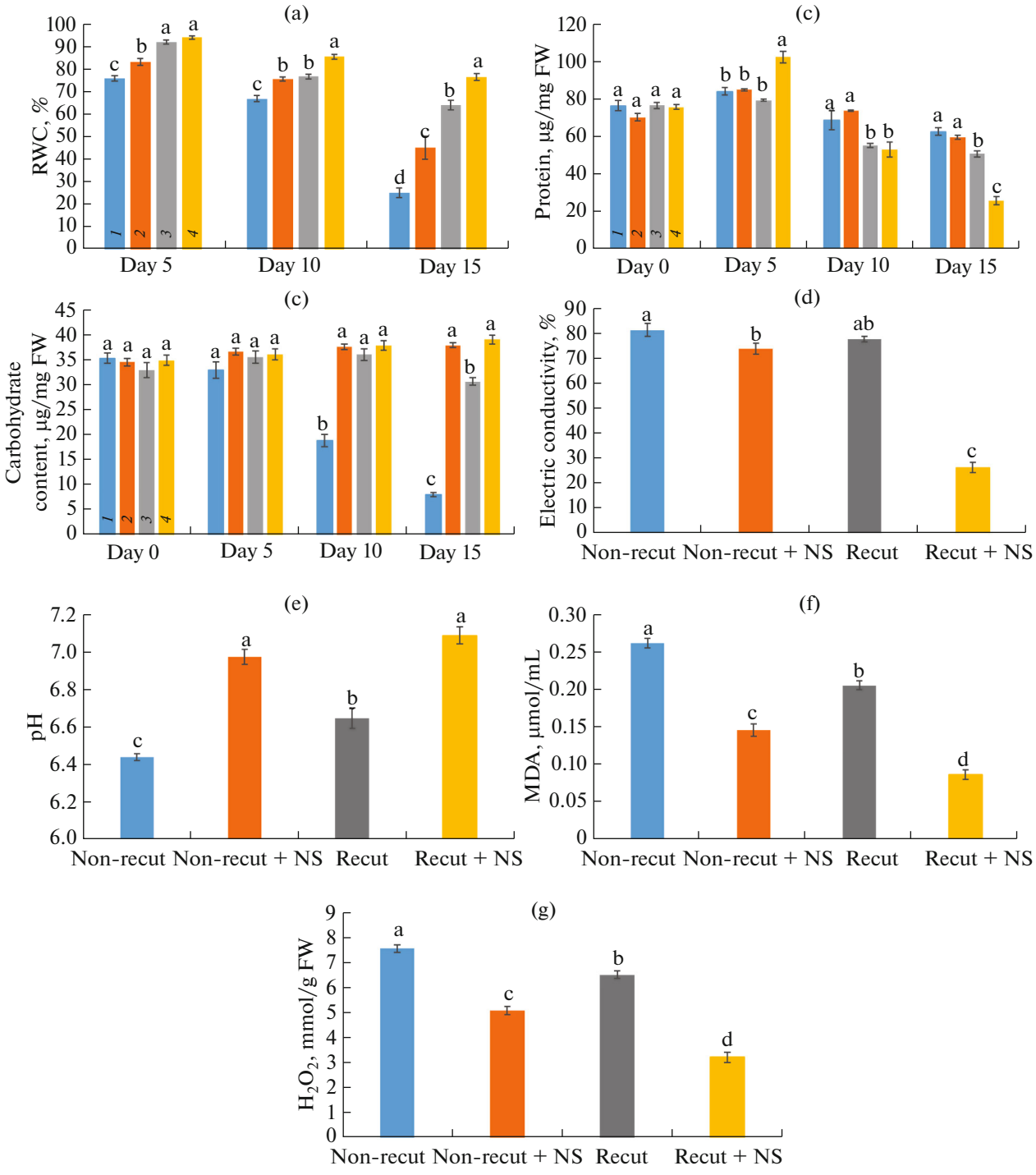

Fig. 6. Some senescence characteristic during vase life of cut snapdragon flowers. (a) RWC; (b) protein content; (c) carbohydrate content; (d) electric conductivity; (e) cell sap pH; (f) MDA content; (g) H_2O_2 content. Columns 1, 2, 3, and 4 are non-recut, non-recut + NS, recut, and recut NS treatments, respectively. Different letters indicate significant differences determined using wDuncan's multiple-range test ($P < 0.05$). Error bars represent mean ± SE, $n = 7$.

stem base of the cut flowers are removed to increase water absorption [14].

The increase in the activity of endoproteases and protein degradation during senescence of cut flowers has been reported in many species such as iris [21], petunia [22], *Lilium longiflorum* [23], and rose [24]. In this study, soluble protein content increased at the beginning of the vase life in all treatments and then decreased, and this increase was significant in the recut + NS treatment. This is similar to the pattern

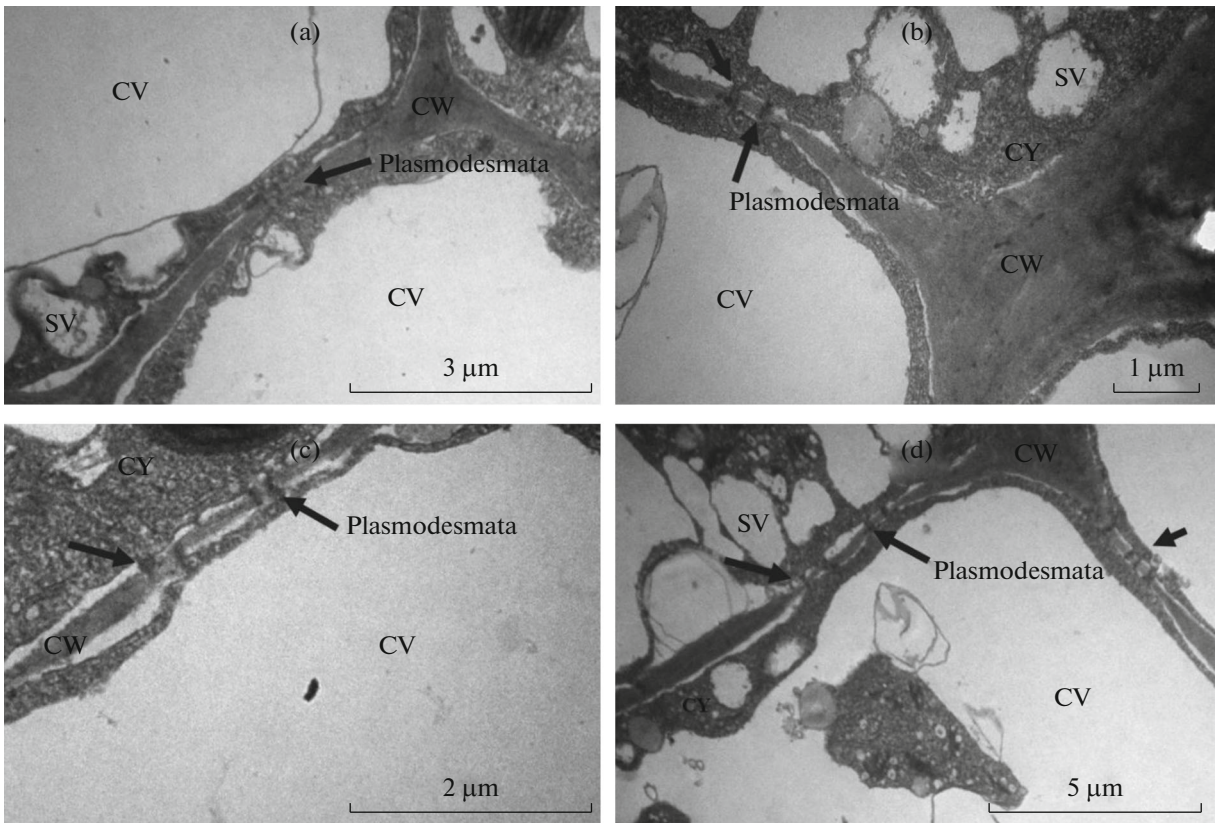

Fig. 7. TEM micrograph of mesophyll cells in cut snapdragon flowers. Arrows represent plasmodesmata. (a) Non-recut treatment; (b) non-recut + NS treatment; (c) recut treatment; (d) recut + NS treatment. CV, central vacuole, CW, cell wall, CY, cytoplasm, SV, small vacuole.

observed in Wagstaff [25] and Zhao's studies [26]. Carbohydrate accumulation in treated flowers was higher than in non-recut flowers. In Rabiza-Świder et al.'s study, sugar accumulation in snapdragon flowers under Nanosilver treatment has been reported. Studies have revealed that the presence of sugar in the cells provides respiratory substrate, improves water balance, and reduces sensitivity to ethylene, as a result, the vase life of cut flowers increases [19, 27].

During senescence, some distinct changes in the biochemical and biophysical properties of the cell membrane lead to the loss of membrane permeability. As a result, cell sap leaks and the cell dies [28]. In this study, the measurement of cell sap EC was considered as a measure of electrolyte leakage caused by changes in cell membrane permeability. Application of recut + NS significantly limited electrolyte leakage (increasing EC). Ion leakage is an efficient marker of senescence that may modify the pH and stability of cellular compounds [29]. The application of Nanosilver also inhibited the pH changes of the cell sap. On the contrary, flowers that were not treated with Nanosilver exhibited acidic pH. Reducing the pH of cell sap has increased the activity of degradative enzymes such as hydrolases, phospholipase, and RNases [30].

During senescence of cut flowers, some intermediate products of membrane lipid peroxidation accumulate, such as H_2O_2 and MDA [26]. The increase of reactive oxygen species and MDA levels raises the permeability of the cell membrane [31]. These indicators are considered to reveal the progress of senescence in petal cells [26]. Studies have indicated that Nanosilver treatment has reduced H_2O_2 and MDA levels in some flowers, such as roses [32], *Paeonia lactiflora* [26], and snapdragon [1]. In our experiment, H_2O_2 and MDA levels remained low in response to Nanosilver treatment. In the flowers that were not treated with Nanosilver, the content of H_2O_2 and MDA increased. Such a response has been reported in cut flowers of orchids that $AgNO_3$ application has reduced the H_2O_2 content [33].

In the TEM micrographs obtained from this experiment, Nanosilver treatment maintained the number and electron density of plasmodesmata in the cell membrane. In flowers that were not treated with Nanosilver, plasmodesmata were lost or were observed in limited numbers. Plasmodesmata plays the role of transferring sugars, phytohormones, and RNA between cells [34]. The change in the plasmodesmata state during petal senescence is the first morphological change in the direction of senescence and cell death that occurs at the level of symplasmic organization [35]. When plas-

modesmata are open, they stabilize the cytoplasmic connection of the developing tissue cells and safeguard their function as a metabolic unit. The plasmodesmata closure is an ultrastructural change that has been reported during the senescence of some cut flowers such as iris [35]. Although the significance of plasmodesmata closure has not been determined, this event prevents the transfer of sugar between cells, inhibits cell signalling, causes the depletion of ATP, and induces cell death [34].

What is certain is that silver nanoparticles show strong bactericidal, fungicidal, and viricidal properties [36]. Studies have indicated that the absence of two electrons in silver nanoparticles and the presence of a negative charge on the surface of the cell causes the absorption of silver nanoparticles into microorganisms. This can degrade the cell wall, sometimes even make a hole in the cytoplasm, and cause the degradation of proteins, the leakage of cytoplasmic substances, and the death of microorganisms [37]. Therefore, the release of silver ions from nanoparticles by inhibiting the growth of microorganisms causes the continuation of water absorption in the xylem (effect on microflow).

On the other hand, the adverse effects of ethylene can reduce the vase life of cut flowers postharvest. In previous studies, it has been reported that the expression of ACC, a key enzyme in ethylene biosynthesis, and RhAA and RhCG, two genes related to senescence, decreased in the presence of silver nanoparticles and delayed senescence [38]. Also, the production of ethylene by some microorganisms in preservative solutions can intensify the progress of senescence, which highlights the use of antimicrobial properties of silver nanoparticles and, as a result, the reduction of microbial load [39].

The results of this study revealed that despite the senescence program, some post-harvest treatments can significantly increase the flower's vase life. By examining all the measured indicators, it can be concluded that Nanosilver and recut application in cut snapdragon flowers can delay the signs of senescence. However, its mechanism of action is still unknown.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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