The effect of chemical treatments (silver thiosulfate and putrescine) on vase life and quality of cut Chrysanthemum morifolium (Ram.) flowers

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The effect of chemical treatments (silver thiosulfate and putrescine) on vase life and quality of cut *Chrysanthemum morifolium* (Ram.) flowers

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**Abstract:** The effect of silver thiosulfate (STS) and putrescine was studied on the vase life and quality of cut chrysanthemum in a factorial experiment based on a randomized complete block design. The experimental factors were composed of silver thiosulfate at four levels (0 or control, 0.02, 0.05 and 0.1 mM) and putrescine at three levels (0 or control, 150 and 300 ppm). The results showed that the application of STS at the rates of 0.05 and 0.1 mM improved vase life of cut chrysanthemum. Also, putrescine at the 150 and 300 ppm improved vase life by increasing relative weight and decreasing wilting percentage. The interaction of the two studied factors was insignificant for most traits including wilting percentage and vase life, which may imply that the application of either substance alone suffices to improve postharvest quality and it is not necessary to use them simultaneously in the preservative solution.

**Subjects:** Plant Biology; Horticultural Plants; Postharvest & Vase life

**Keywords:** *Chrysanthemum morifolium*; post-harvest life; putrescine; silver thiosulfate; Plant Biology; Horticultural Plants; Postharvest & Vase life

1. **Introduction**

Ornamental plants, especially cut flowers, are grown for local markets so that consumers can enjoy the freshness of flowers as much as possible. A US study published in 1970 reported that water had been the only material used to prolong the vase life of flowers for 70 percent of cut flowers (Nowak & Rudnicki, 1990). The appearance, quality, and vase life of plants depend on their...
growing conditions, correct harvesting time, and post-harvest cares. Plants grown under optimal conditions will have the highest quality. The present study aims to shed light on factors that influence the quality and vase life of cut flowers, especially chrysanthemums.

Chrysanthemum (Chrysanthemum morifolium Ram) is one of the most important flowers traded both in pots and as cut flowers at the world market. Chrysanthemum species belongs to family Asteraceae (Liu et al., 2012). The Asteraceae family consists of approximately 23000 species and 1535 genera (Ozturk & Çetin, 2013; Sevindik et al., 2018). It has been reported that a large number of species of the Asteraceae family show pharmacological activities. The plants in this family include sesquiterpene lactone metabolites having biological activities such as antibacterial, antifungal, and antitumor, in addition to diterpenes and flavonoids (Sevindik et al., 2018). Indeed, it has the second rank after roses in the economic perspective and in production. Since the vase life of cut flowers, or the so-called their vase life, is one of the most important qualitative factors, it has a significant impact on consumer demand and also on the value of cut flowers (Sedaghathoor, 2015). Post-harvest senescence is the major factor limiting the marketing of most cut flower species. Silver ion (applied in silver thiosulfate form) is the active ingredient widely used to postpone aging of ethylene-sensitive flowers. Silver inhibits the synthesis of ethylene (Ichimura & Niki, 2014; Sedaghathoor, 2015), thereby delaying the onset of wilting. However, concerns have been voiced about the application of silver because this is a heavy metal that may be toxic to the environment. Rezvanypour and Osfoor (2011) reported that the application of 0.5 mM silver thiosulfate along with sucrose was effective in the uptake of water by roses.

By controlling microbial activity in cut flowers (Chanasut et al., 2003), silver thiosulfate enhances water uptake. Similarly, Geng et al. (2009) reported that silver thiosulfate improved the vase life and quality of cut lilies by killing the microbes and improving water relations. According to Chikkasubbanna and Yogitha (2002), the treatment of silver thiosulfate reduced the loss of fresh weight of rose flowers. Hossini Davishani and Chamani (2013), also, reported the effect of applying 1 mM silver thiosulfate on reducing flower weight loss.

Unlike silver nitrate and silver acetate, which are mobilized slowly in plant tissues and decompose upon exposure to sunlight, silver thiosulfate is fluid within the branches of cut flowers and readily moves towards corolla, thereby prolonging the vase life of cut flowers (Macnish et al., 2004). Hadas et al. (1996) reported that the application of silver thiosulfate alone, without combining with other substances such as benzyl adenine, improve the vase life and quality of chrysanthemum.

Polyamines, including putrescine (diamine), spermidine (triamine), and spermine (tetraamine) are a new group of plant growth regulators that are involved in various processes such as increasing cell division, increasing enzyme biosynthesis, adjusting different development processes, differentiation, flowering, embryogenesis, rooting, fruit ripening, and aging (Anjum et al., 2001). Mahgoub et al. (2011) reported that the application of polyamines in cut flower preservative solutions improved flower fresh weight. Abbasi et al. (2017) also observed the positive effect of polyamines, such as putrescine, on inhibiting flower aging and prolonging post-harvest life. Mohammadi et al. (2014) obtained the highest relative fresh weight from gladiolus flowers treated with 100 mg L\(^{-1}\) putrescine + 100 mg L\(^{-1}\) salicylic acid. Mahgoub et al. (2011) reported that by delaying aging, polyamines improved the vase life of flowers by improving membrane stability. Kandil et al. (2011) found that putrescine increased the vase life of chrysanthemums significantly from 11–13 days in the untreated plants to 26–27 days in the treated plants. They attributed this enhancement to the increase in protein content of petals and ovaries, which can prevent the synthesis of internal ethylene.

2. Materials and methods
To study the effect of chemical treatments (silver thiosulfate and putrescine) on vase life and quality of cut chrysanthemum, a study was carried out as a factorial experiment based on a randomized complete block design with three replications. The experiment assessed the effect
of silver thiosulfate as the main plot (A) at four levels (0 mM as control, 0.02 mM, 0.05 mM, and 0.1 mM) and putrescine as the sub-plot at three levels (0 ppm as control, 150 ppm, and 300 ppm) in 12 treatments and 36 experimental plots. Each plot included three cut flowers. The experiment was carried out in the laboratory of Moqbeli Agricultural Research Center in Jiroft and Kohnuj, Iran.

The flowers were procured from Shahriar Commercial Greenhouse in Tehran province. They had been packaged in six batches, each containing 25 flowers. They were transferred to the laboratory as soon as possible. In the laboratory, they were placed in a cold room for a short time to get their initial heat. Then, they were homogenized in terms of stem length and leaf number to reduce their differences and minimize the experimental error. All stems were cut diagonally 40 cm in length. To hinder the penetration of air into the vessels, the cuttings were performed under water. Then, the end of the flowers was placed in Erlenm containing 500 ml of preservative solution.

In this experiment, 500-ml glass containers were used. After the preservative solution was prepared, the glass containers were filled with 500 mL of the solution and three flowers were placed in them. Then, they were kept at 22°C. The light at the test site was supplied by natural sunlight and fluorescent lamps as an artificial light source. The relative humidity was 65–75 percent. The base solution for all treatments including the control treatment was 300 mg L\(^{-1}\) citric acid + 2% sucrose. To prepare citric acid, 15 g of citric acid was adjusted to 500 mL in a volumetric flask and 10 mL of this solution was applied to each treatment. After 24 hours, the flowers were taken out of the solution and were placed in containers containing distilled water and were kept there until the end of the experiment. The treatments were applied for a short time. To facilitate the measurements, all three flower branches of the individual pots were weighed and placed in pots containing 500 mL of the relevant solution. Flower branch weights were separately weighed and pot weight + its solution was recorded by a 0.01 g precision scale every day. These weights were used to calculate the relative weight percentage and water absorption rate in subsequent steps. During the storage period traits such as solution uptake rate, relative flower weight, fresh flower weight, flower wilting percentage, TSS, vase life, electrolyte leakage percentage, and water content of petals were measured. TSS was measured with a refractometer.

To measure the relative flower weight, the flower fresh weight was measured every day, was divided by the flower fresh weight of the first day, and was multiplied by 100 to yield the relative flower weight. To measure the wilting percentage, the wilting of the individual flowers were checked as a major index of senescence in flowers.

\[
\text{Flower wilting percentage} = \frac{\text{Number of wilted flowers on the final day}}{\text{Total number of flowers}} \times 100
\]

Flower weight variations were read by a digital scale with an accuracy of 0.1 g. Ion leakage was also checked as an index of cell membrane stability. To this end, six days after the experiment was initiated, 0.5 g of petals were detached in 1 × 1 cm\(^2\) pieces from each treatment and was placed in a Petri dish containing distilled water to be rinsed for a few minutes. Then, they were transferred into tubes containing 22.5 mL of deionized water and were placed in a shaker at the temperature of 25°C and the speed of 150 rpm for 30 minutes. Finally, its EC was recorded as EC\(_1\). The same tubes were placed at 100°C boiling water for 15 minutes. Then, they were placed in cold water to be cooled down rapidly. After that, their EC was recorded as EC\(_T\). Then, the electrolyte leakage percentage was calculated as follows:

\[
\text{Ion leakage percentage} = \frac{\text{EC}_1}{\text{EC}_T} \times 100
\]

To measure the water content of the petals, they were weighed on the first and final day of the experiment and their fresh weight was recorded. Then, they were oven-dried at 60°C for 72 hours and their dry weight was recorded. The difference between the dry and fresh weight was placed in the following equation to yield the water content of the petals.
Water content (gg⁻¹ d.wt.) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}}

The following equation was employed to measure the rate of solution uptake.

Solution uptake rate = \frac{\text{Daily weight of flowers} - \text{weight on 1st day}}{\text{Weight on 1st day}} \times 100

Inflorescence life was recorded at the end of their lifespan. When the lower three rows of petals changed color, it was interpreted as the end of the inflorescence life. Data were analyzed by the MSTAT-C software package. Means were also compared by Duncan’s multiple range test (DMRT) at the p < 0.05 level.

3. Results and discussion

The results of analysis of variance (ANOVA) for the recorded traits (Table 1) showed that all traits except flower fresh weight and TSS percentage were influenced by at least one of the studied factors. The interactive effect was significant only on flower weight variations, but it was insignificant for other traits. This may imply that the consumption of each compound alone could suffice to improve post-harvest quality and it is not necessary to use them in the preservative solution simultaneously.

According to the results of ANOVA (Table 1), solution uptake percentage was influenced by different levels of silver thiosulfate significantly (p < 0.05), but the effect of putrescine and the interactive effect of the two factors were insignificant on this trait. Means comparison indicated that the application of silver thiosulfate at the rate of 0.1 mM had a positive effect on increasing solution uptake, but other rates did not differ from the control significantly. Solution uptake was 73.12 percent at the distilled water level, which was increased to 86.58 percent when 0.1 mM silver thiosulfate was applied (Figure 1). Rezvanypour and Osfoor (2011) reported the increase in water uptake by roses when they were treated with 0.5 mM silver thiosulfate + sucrose. The loss of solution uptake by flowers can be related to the microorganisms that grow inside flower stems and disrupt water uptake. This finally results in the wilting of the flowers. By controlling the microbial activity in cut flowers (Chanasut et al., 2003), silver thiosulfate enhances water uptake. Similarly, Geng et al. (2009) reported the desired effect of silver thiosulfate on the vase life and quality of lilies acting through killing the germs and improving water relations. Gandaby et al. (2008) reported the increase in solution uptake of lily cut flowers treated with 0.88 mM benzyl adenine and 0.6 mM silver thiosulfate. The insignificant influence of putrescine on solution uptake can be attributed to its low rates applied in the present experiment.

According to the results of ANOVA, relative weight percentage was influenced only by different rates of putrescine significantly (p < 0.05), but the effect of silver thiosulfate and its interaction with putrescine was insignificant on this trait (Table 1). Putrescine played a positive role in increasing flower relative weight so that its application at the rate of 150 or 300 ppm resulted in the highest increase in relative weight as compared to flowers not treated with it (Figure 2). Mohammadi et al. (2014) obtained the highest relative fresh weight from gladiolus plants treated with 100 mg L⁻¹ putrescine + 100 mg L⁻¹ salicylic acid.

The results of ANOVA revealed that petal water percentage was affected by different levels of silver thiosulfate and putrescine significantly (p < 0.01) but not by their interaction (Table 1). As the results of means comparison indicated, all levels of silver thiosulfate maintained water content of petals at the highest possible level. Among its different rates, 0.05 and 0.1 mM resulted in significant differences versus the control so that they increased petal water content from 3.52 to 4.93 and 4.84 percent, respectively. Also, 300 ppm putrescine improved petal water content significantly (Figure 3). The application of putrescine at the rate of 150 ppm did not show a significant difference from the control. Son et al. (2003) reported that silver thiosulfate reduced respiration, thereby maintaining petal water content.
| S.O.V.                  | df | Solution uptake % | Relative weight % | Petal water content | Flower fresh weight | Flower weight variations | Ion leakage | TSS % | Flower wilting % | Vase life |
|------------------------|----|-------------------|-------------------|--------------------|---------------------|-------------------------|------------|-------|-----------------|----------|
| Silver thiosulfate (S) | 3  | 337.04*           | 42.88**           | 4.21**             | 207.26**            | 44.25**                 | 17,261.79**| 0.20  | 549.16**        | 20.18**  |
| Putrescine (P)         | 2  | 253.19*           | 197.52*           | 7.70**             | 19.44**             | 22.83**                 | 6752.66**  | 0.19  | 217.32**        | 38.77**  |
| S × P                  | 6  | 139.56**          | 40.98**           | 0.76**             | 97.78**             | 6.01*                   | 2706.03**  | 0.37  | 22.86**         | 9.74**   |
| Experiment error       | 24 | 98.37             | 51.30             | 0.55               | 90.57               | 1.91                    | 2127.01    | 0.31  | 47.21           | 5.88     |
| C.V. (%)               |    | 12.79             | 7.98              | 17.30              | 13.47               | 14.63                   | 22.55      | 28.13 | 15.60           | 24.13    |

ns, * and ** show insignificance and significance at the $p < 0.05$ and $p < 0.01$ levels, respectively
Figure 1. Means comparison for the effect of different levels of silver thiosulfate on solution uptake rate of cut chrysanthemum flowers.

Figure 2. Means comparison for the effect of different levels of putrescine on relative weight percentage of cut chrysanthemum flowers.

Figure 3. Means comparison for the effect of different levels of silver thiosulfate and putrescine on petal water content of cut chrysanthemum flowers.
Flower weight variation was influenced by silver thiosulfate, putrescine, and their interactions significantly at the $p < 0.01$, $p < 0.01$, and $p < 0.05$ levels, respectively (Table 1). According to the results of means comparison for the effect of silver thiosulfate, its 0 and 0.1 mM levels were related to the highest variations in flower weight whilst the lowest variations were related to its 0.02 mM level. Silver thiosulfate at the rate of 0.02 mM changed flower weight by up to 50 percent versus the control. Putrescine at the rates 150 and 300 ppm reduced flower weight variations (Figure 4). Means comparison for the interactive effect revealed that the application of only silver thiosulfate at the rate of 0.02 mM or its application at the rates of 0.02 or 0.05 mM accompanied with putrescine at the rates of 150 or 300 ppm were related to the lowest variations of flower weight. However, putrescine at the two studied rates alone or in combination with 0.1 mM silver thiosulfate, as well as the application of silver thiosulfate at the rates of 0.05 or 0.1 mM alone, had negative effects on flower weight variations (Figure 5). As approaching their senescence phase, cut flowers lose their weight due to the loss of water uptake and their petals and

Figure 4. Means comparison for the effect of different levels of silver thiosulfate and putrescine on weight variations of cut chrysanthemum flowers.

Figure 5. Means comparison for the interactive effect of different levels of silver thiosulfate and putrescine on weight variations of cut chrysanthemum flowers.
leaves. Chikkasubbanna and Yogitha (2002) reported that the application of silver thiosulfate reduced flower fresh weight of roses. Hossini Darvishani and Chamani (2013) found that the application of silver thiosulfate at the rate of 1 mM was effective in reducing weight loss of the flowers. Mahgoub et al. (2011) stated that the application of polyamines to the preservative solution of cut flowers improved flower fresh weight.

The results of ANOVA revealed the significant effect of silver thiosulfate and putrescine on electrolyte leakage at the \( p < 0.01 \) and \( p < 0.05 \) levels, respectively, while their interaction was insignificant (Table 1). According to means comparison, the lowest electrolyte leakage was obtained from silver thiosulfate rates of 0.05 and 0.01 mM so that silver thiosulfate at these two rates reduced electrolyte leakage by about 30 percent. The application of putrescine at the rate of 300 ppm in flower preservative solution reduced electrolyte leakage by 20 percent significantly (Figure 6). Zhang et al. (2003) reported an increase in the post-harvest synthesis of ethylene and the activity of membrane and cell wall-decomposing enzymes such as polygalacturonase (PG), lipoxygenase (LOX), cellulose, and pectinmethylesterase (PME). The inhibiting effect of silver thiosulfate on electrolyte leakage is related to its effect on hindering ethylene synthesis (Nowak & Rudnicki, 1990) and its antibacterial property and the inhibiting effect of putrescine is associated with its antimicrobial effect that reduces enzymatic activities.

The results of ANOVA indicated that wilting percentage was affected by silver thiosulfate and putrescine significantly (\( p < 0.01 \) and \( p < 0.05 \), respectively), but not by their interactive effects (Table 1). Different rates of silver thiosulfate reduced wilting percentage significantly so that its 0.05 and 0.1 mM rates were the most effective in this respect. The plants treated with silver thiosulfate at the rates of 0.05 and 0.1 mM showed 35.94 and 39.6 percent of wilting versus 53.41 percent for the control plants (Figure 7). According to Van Doom and Woltering (1992), silver reduces the capacity of ethylene binding and stops internal ethylene synthesis. Therefore, phenomena like early wilting are delayed.

The effect of putrescine was significant on the reduction of wilting percentage. Means comparison revealed that the application of 150 and 300 ppm of putrescine reduced wilting percentage by 43.45 and 40.11 percent, respectively, but when only 300 ppm of putrescine was applied, no significant differences were entailed versus the control (Figure 7). Abbasi et al. (2017) reported the positive effect of polyamines including putrescine on inhibiting flower aging and the extension of post-harvest life. According to the results of ANOVA, vase life was significantly influenced by silver thiosulfate and putrescine at the \( p < 0.05 \) and \( p < 0.01 \) levels, respectively, but the interactive effect of these factors was not significant for this trait (Table 1). Compared to the plants not treated with silver thiosulfate, the application of silver thiosulfate at the rates of 0.05 and 0.1 mM prolonged vase life significantly so that it was increased from 8.55 days to

Figure 6. Means comparison for the effect of different levels of silver thiosulfate and putrescine on electrolyte leakage in cut chrysanthemum flowers.
11.56 and 11.11 days, respectively. As well, the application of putrescine at the rates of 150 and 300 ppm increased vase life by, on average, (Figure 8). The ability of silver to block the action of ethylene was due to silver replacement instead copper, a receptor site for ethylene. Silver thiosulfate (STS) completely blocked the ethylene production normally preceding the onset of senescence in carnation. The silver inhibited the ACC content and rise in respiration of flowers, by blocking the ethylene action. STS appears to be having also additional profits than as a biocide (Sharma & Bhardwaj, 2015). Mahgoub et al. (2011) reported that polyamines improved vase life through retarding senescence and improving membrane stability. Mohammadi et al. (2014) reported the increase in vase life of gladiolus plants treated with salicylic acid and putrescine at the rate of 100 ppm. They attributed this increase in vase life to bacteria control by these two substances. Hadas et al. (1996) found that the application of silver thiosulfate alone and without its mixture with other compounds including benzyl adenine increased vase life and quality of chrysanthemum cut flowers. Likewise, Liao et al. (2000) reported the desirable effect of silver thiosulfate on increasing the vase life of cut flowers. Karimi et al. (2017) revealed that the addition of polyamines such as, putrescine and spermidine to the preservative solution of carnations retarded their senescence and inhibited the synthesis of ethylene. Macnish et al. (2000) found that the treatment of silver thiosulfate was effective in hindering ethylene synthesis.

4. Conclusions
The results indicated that the application of silver thiosulfate at the rates of 0.05 and 0.1 mM extended the vase life of cut chrysanthemum flowers through increasing solution uptake, increasing petal water content, suppressing electrolyte leakage, and reducing wilting percentage. As well,
putrescine at the rates of 150 and 300 ppm contributed to extending vase life by increasing relative weight, reducing electrolyte leakage, and decreasing wilting percentage. The results, also, showed that the interaction of the two studied factors was insignificant for most traits including wilting percentage and vase life, which may imply that the application of either substance alone suffices to improve post-harvest quality and it is not necessary to use them simultaneously in the preservative solution. According to the results, the application of 0.05 mM silver thiosulfate or 150 ppm putrescine in the preservative solution is recommended to improve the post-harvest quality of chrysanthemum. However, putrescine at the rate of 150 ppm can be replaced for silver thiosulfate which tends to be a pollutant.

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