

Vase Life Extension of Rose Cut Flowers (*Rosa Hybrida*) as Influenced by Silver Nitrate and Sucrose Pulsing

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Abstract: Problem statement: This experiment was carried out in the laboratory of the Department of the Biology, Taif University, Saudi Arabia. **Approach:** The study was conducted to investigate the silver nitrate (AgNO_3) and sucrose at different concentrations on rose cut flowers longevity, which determined on the basis of wilting, chlorophyll retention and carbohydrate degradation. The treatments were, water control, 20, 30 or 50 ppm AgNO_3 and sucrose at 1,2 or 3% w/v. **Results:** The results showed, flower vase life was prolonged by all AgNO_3 treatments. The best concentration was 30 ppm. The effect was further improved when AgNO_3 was combined with 3% sucrose, which recorded the best vase life compared to other concentrations of sucrose. The per cent of wilting was minimized as a result of using this combined treatment. However, the per cent of wilting increased with the increase in concentrations of AgNO_3 and complete wilting occurred after 10, 8 and 7 days when treated with 20, 30 and 50 ppm of AgNO_3 , respectively, while sucrose shortened the period to reach wilting. Also AgNO_3 at 30 ppm retarded the chlorophyll as well as carbohydrate degradation during the postharvest life. The experiments were repeated three times with three replicates and a completely randomized design had been used. The experiments extended from February, 2006-September, 2008. The difference between means was performed using Duncan multiple range test at 0.05 levels. **Conclusion:** A significant improvement in vase life of rose cut flowers was occurred when treated with 30 ppm (AgNO_3) more effects when combined with 3% (w/v) sucrose, so we recommended that to use the previous treatments with these concentrations for a long vase life and commercial productions.

Key words: Vase life, rose cut flowers, sucrose pulsing, carbohydrate degradation, randomized design, silver nitrate, bacterial proliferation, *Rosa hybrida*, chlorophyll determination

INTRODUCTION

Rose, a universally celebrated flower, has been used as a garden plant since the dawn of civilization. Rose is a symbol of perfection, elegance, romance and love. It was called "The Queen of Flowers" firstly by Greek poetess in her "Ode to the Rose" (Muhammad *et al.*, 1996). Roses (*Rosa hybrida*) belong to family Rosaceae and Genus *Rosa* which contains more than 150 species and 1400 cultivars (Synge, 1971). Rose enjoys superiority over all other flowers being extensively used for decorative purposes and is prized for its delicate nature, beauty, charm and aroma. Rose is recognized for their high economic value, which are used in agro-based industry especially in cosmetics and perfumes. Additionally, roses play a vital role in the manufacturing of various products of medicinal and nutritional importance. However, the main idea of rose plant cultivation is to get the cut flowers, which greatly deals with the floricultural business (Butt, 2003).

Vase life of cut rose flowers is usually short. Cut flowers wilt and floral axis become bent (bent-neck) just below the flower head (Van Doorn *et al.*, 1997). The development of such symptoms is considered to be

caused by vascular occlusion, which inhibits water supply to the flowers (Loub and Van Doorn, 2004).

Several methods to increase the vase life of cut flowers and keep their freshness for longer periods have been reported. Cut flowers should be free of any deterioration, as this is one of the principal entry points for decay organisms (Hardenburg, 1968). A major form of deterioration in cut flowers is the blockage of xylem vessels by air and microorganisms that cause xylem occlusion (Hardenburg, 1968).

Silver nitrate (AgNO_3) is one of the most common forms of silver salts used in commercial flower preservatives solutions and mostly used as ethylene binding inhibitor. Pulsing with (AgNO_3) strikingly enhanced vase life and solution uptake in rose cut flowers (Singh and Tiwari, 2002). Also Darras *et al.* (2010) reported that, pulsing with 20 or 40 mg L^{-1} AgNO_3 for 24h extended vase life by 1.6 and 1.9 days, respectively, compared to the control.

Furthermore, treatment with germicides, such as silver nitrate (Ohkawa *et al.*, 1999) or 8-hydroxyquinoline sulfate (Gilman and Steponkus, 1972; Ichimura, 2003), inhibited bacterial proliferation and maintained the hydraulic conductance of stem. These

findings suggest that bacterial proliferation is responsible for vascular occlusion, which shortens the vase life of cut rose flowers. Pulsing of cut roses for 10-20 mint with AgNO_3 improved the vase life up to 6.0 and 5.3 days, respectively (Reddy *et al.*, 1988).

On other hand, sucrose pulsing increased the vase life of different cut flowers. Different concentrations of sucrose had been investigated by Butt (2005) on two cultivars of *Rosa hybrida* and results showed that sucrose at 25 gmL^{-1} extended the vase life by 8.2 days in var. Whisk Mc and 7.5 days in var. Trika as compared to 5.3 days in control (Butt, 2005). Also Pun *et al.* (2005) treated cut spray carnation by different concentrations of sucrose ranging from 0-7.5% and found that 5.0% sucrose recorded the best vase life and delayed the climacteric ethylene in petals. The vase life was more extended when added sucrose to the AgNO_3 . Furthermore, Cho and Lee (1980) study the effect of combination between these two chemical on cut rose cv, Mary Devor and result in the vase life was doubled when treated with 3% sucrose+50 ppm AgNO_3 compared to control. Also, pulsing with AgNO_3 and sucrose + citric acid solution for 16 h was extended the vase life of rose cut flowers due to preventing the bent-neck of flower stem (Halevy *et al.*, 1978). Also addition of bactericides and fungicides in solution of AgNO_3 and sucrose improved the flower size and largest buds opened in three days instead of five days; whereas, their vase life was four days greater than control (Nikolova and Koneazak, 1986).

MATERIALS AND METHODS

Site: The experiment site was University of Taif, laboratory of the Department of Biology, Saudi Arabia.

Plant materials: Cut flowers of roses were obtained from a commercial grower in Taif, Saudi Arabia. Flower stems were trimmed to 30 cm underwater to avoid air embolisms (Van Leperen *et al.*, 2001). All leaves on the lower section of the stem were removed.

Treatment setting: Treatments were set following completely randomized design. Each treatment replicated by 3 replication.

Experiment (1): Silver nitrate (AgNO_3) was applied at concentrations of 0, 20, 30 or 50 ppm. Sucrose was used at concentrations of 0, 1, 2 or 3% w/v. The two compounds were dissolved in sterilized distilled water in 250 mL bottle glass (Miyamoto *et al.*, 1999). The sample had been divided into seven groups with three replications containing three flowers each. The flowers were kept at room temperature ($23 \pm 1^\circ\text{C}$) at normal day light and natural ventilation. Visual rating of flowers

was carried out on the basis of a scale from 1-4 according to Hassan (2005) where: 1= entirely fresh flowers and 4= wilting in 50-100% of the petals.

Experiment (2): AgNO_3 at 30 ppm and sucrose at concentration 3% (w/v) gave the best results in experiment (1). Hence, the effect of both concentrations of AgNO_3 and sucrose on chlorophyll retention and carbohydrate degradation was further investigated.

Chlorophyll determination: Chlorophyll was extracted by methanol and absorbance was determined by a spectrophotometer on day 1, 3 and 5, according to the method of Harborne (Nowak and Rudnicki, 1990). Chl a and Chl b were then calculated using the following equation:

$$\text{Chl a (mg l}^{-1}\text{)} = 12.21 A_{663} - 2.81 A_{646} \quad \text{Chl b (mg l}^{-1}\text{)} = 20.13 A_{646} - 5.03 A_{663}$$

Carbohydrates determination: Carbohydrates were determined on the stems and petals of the best treatment of the two compounds. Samples were taken on day 1, 3 and 5 and separated by a High Performance Liquid Chromatography (HPLC) fitted with differential refractometer to detect fructose, glucose and sucrose in the different samples (Hassan, 2005).

RESULTS

Effect of AgNO_3 and sucrose on vase life of rose cut flowers: The vase life of rose cut flowers was extended by the different concentrations of AgNO_3 used in Table 1 and Fig. 1. The vase life was longer in AgNO_3 at 30 ppm which resulted in 8.97 days compared to other concentrations (Table 1). Sucrose resulted in the lowest vase life compared to AgNO_3 at the different concentrations used. The longest vase life was attained when sucrose was applied at 3% w/v, which gave 6 days in comparison to 5 days for control. However, the two compounds used significantly extended the vase life of rose cut flowers compared to control.

Table 2 showed that the per cent wilting increased with the increase in concentrations of AgNO_3 . The vase life was terminated on day 11, 10 and 9, when cut flowers were treated with 20, 30 or 50 ppm 8-HQS, respectively compared to 6 days in control. Sucrose resulted in the lowest period to reach wilting per cent. Thus, wilting occurred on the 8th day after treatment with sucrose at different concentrations compared to 6 days in control (Table 2).

Table 1: Effect of AgNO₃ and sucrose on vase life of rose cut flowers (*Rosa hybrida*)

| Treatment | Vase life (days) ¹ |
|--------------------------|-------------------------------|
| AgNO ₃ 20 ppm | 9.30b |
| AgNO ₃ 30 ppm | 10.97a |
| AgNO ₃ 50ppm | 7.97c |
| Sucrose 1% | 5.77ef |
| Sucrose 2% | 5.97e |
| Sucrose 3% | 7.07cd |
| Control | 4 f |

¹: Different letters indicate the significant differences between means, according to Duncan multiple range p = 0

Table 2: Calculation of per cent wilting in rose cut flowers treated with different concentrations of AgNO₃ and sucrose compared to control

| Days after treatments | Per cent of wilting in the different concentrations of 8-HQS and sucrose ¹ | | | | | | Control |
|-----------------------|---|------|------|-----------------------|------|------|---------|
| | AgNO ₃ conc. (ppm) | | | Sucrose conc. % (w/v) | | | |
| | 20 | 30 | 50 | 1 | 2 | 3 | 0 |
| 2 | 1.0 | 0.1 | 2.9 | 3.9 | 3.6 | 2.4 | 3.6 |
| 4 | 14.5 | 11.3 | 15.5 | 26.2 | 25.3 | 22.6 | 54.9 |
| 6 | 25.9 | 25.5 | 38.3 | 74.3 | 56.6 | 61.9 | 93.2 |
| 8 | 91.6 | 50.6 | 79.7 | 93.3 | 94.8 | 92.3 | - |
| 10 | 98.0 | 82.3 | 93.8 | - | - | 97.5 | - |
| 11 | - | 96.3 | - | - | - | - | - |
| 12 | - | 96.5 | - | - | - | - | - |

¹: Reading of per cent wilting was done every two days after treatments

Table 3: Effect of the best treatment of AgNO₃ and sucrose on vase life and postharvest quality of rose cut flowers

| Treatments | Vase life values (day) |
|-------------------------------------|------------------------|
| AgNO ₃ 30 ppm | 12.5 b |
| AgNO ₃ 30ppm+ 3% sucrose | 14.5 a |
| Sucrose 3% | 7 c |
| Control treatment | 5 d |

Letters explain the significant differences between means, according to Duncan multiple range p = 0.05

Effect of the best treatment for AgNO₃ and sucrose on vase life and postharvest quality of rose cut flowers:

Results of Table 3 showed that treatment by AgNO₃ at 30 ppm prolonged the vase life of rose cut flowers with or without sucrose compared to control. When sucrose was added to 30 ppm AgNO₃ the vase life was extended to 12 days compared to 9 days without sucrose. However, sucrose at 3% extended the vase life by 7 days compared to 6 days only in control (Table 3).

Chlorophyll content: The previous treatments lead to a considerable delay in degradation of Chl a and Chl b compared to control (Table 4). The concentration of chlorophyll a was higher than chlorophyll b at any point of time throughout the vase life. When flowers were treated with AgNO₃ 30 ppm chlorophyll content on the 1st day was 0.672, 0.330 mg 1-1 weight for chl. a and chl. b, respectively (Table 4).

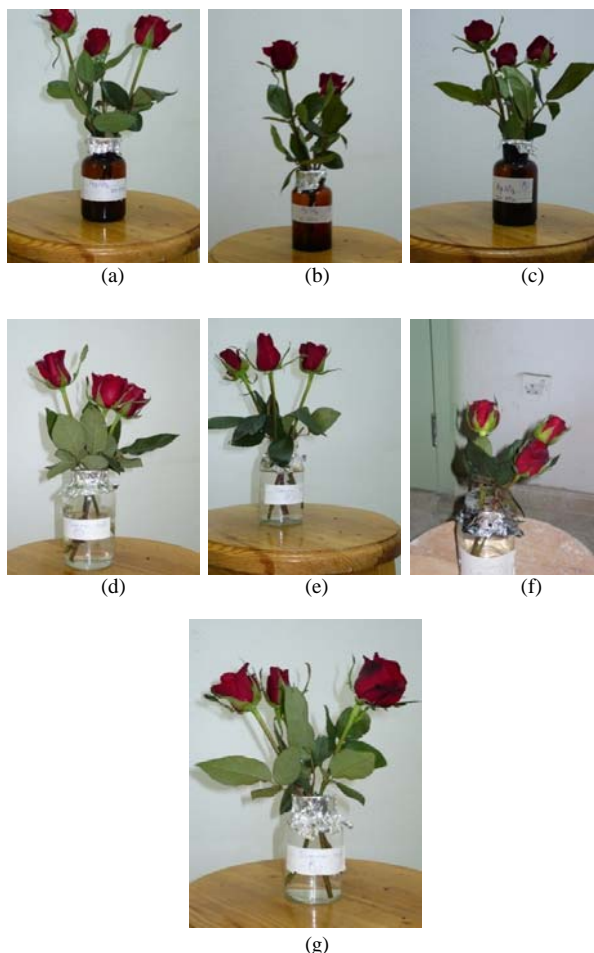


Fig. 1: Effect of AgNO₃ and sucrose on vase life of rose cut flowers (*Rosa hybrida*) (a) AgNO₃ 20 ppm (b) AgNO₃ 30 ppm (c) AgNO₃ 50 ppm (d) Sucrose 1% (e) sucrose 2% (f) sucrose 3% (g) control

When sucrose at 3% was added chlorophyll content increased. Thus, at the end of the experiment the accumulated chl. A and chl. b, were 1.762, 0.391 mg l⁻¹, respectively (Table 4).

Carbohydrate content: Data of Table 5 and 6 show that fructose, glucose and sucrose were the main soluble carbohydrates in petals and stems of cut roses. Fructose was the major component in the petals as well as in stems but, generally, its value was higher than in stems. Sucrose contents in petals and stems were lower than those of glucose.

The carbohydrate content significantly increased as a result of using 30 ppm AgNO₃+3% sucrose till the 3rd day then sharply decreased on the 5th day at which the vase life of control was terminated.

Table 4: Effect of AgNO₃ with or without sucrose and sucrose alone on chlorophyll content for rose cut flowers.

| Treatments | Days of determination of chl. a ¹ and chl. b ¹ | | | | | |
|--------------------------------------|--|--------|---------|--------|---------|--------|
| | 1st day | | 3rd day | | 5th day | |
| | Chl. a | Chl. b | Chl. a | Chl. b | Chl. a | Chl. b |
| AgNO ₃ 30ppm | 0.727 | 0.384 | 2.402 | 0.541 | 1.757 | 0.521 |
| AgNO ₃ 30ppm + sucrose 3% | 0.743 | 0.400 | 1.912 | 0.482 | 2.299 | 0.407 |
| Sucrose 3% | 0.733 | 0.410 | 1.421 | 0.743 | 0.687 | 0.186 |
| Control treatment | 0.727 | 0.384 | 2.940 | 0.406 | 0.596 | 0.070 |

¹: mg l⁻¹ fresh weight

Table 5: Effect of AgNO₃ with or without sucrose and sucrose alone on carbohydrate content (in mg⁻¹ dry weight) for petals of rose cut flowers

| Treatment | Days of determination of carbohydrate content | | | | | | | | |
|--------------------------------------|---|---------|---------|----------|---------|---------|----------|---------|---------|
| | 1st day | | | 3rd day | | | 5th day | | |
| | Fructose | Glucose | Sucrose | Fructose | Glucose | Sucrose | Fructose | Glucose | Sucrose |
| AgNO ₃ 30 ppm | 1.48 | 1.22 | 0.04 | 1.58 | 1.24 | 0.07 | 2.73 | 1.91 | 0.76 |
| AgNO ₃ 30ppm + sucrose 3% | 2.99 | 2.01 | 0.89 | 2.27 | 1.79 | 1.03 | 3.71 | 2.03 | 1.93 |
| Sucrose 3% | 3.35 | 1.97 | 0.93 | 2.99 | 1.55 | 0.22 | 1.37 | 1.08 | 0.32 |
| Control | 0.33 | 0.09 | 0.16 | 1.11 | 0.30 | 0.18 | 3.71 | 1.97 | 0.91 |

Table 6: Effect of AgNO₃ with or without sucrose and sucrose alone on carbohydrate content (in mg⁻¹ dry weight) for stems of rose cut flowers

| Treatment | Days of determination of carbohydrate content | | | | | | | | |
|---------------------------------------|---|---------|---------|----------|---------|---------|----------|---------|---------|
| | 1st day | | | 3rd day | | | 5th day | | |
| | Fructose | Glucose | Sucrose | Fructose | Glucose | Sucrose | Fructose | Glucose | Sucrose |
| AgNO ₃ 30 ppm | 4.00 | 0.31 | 1.40 | 4.09 | 0.33 | 1.46 | 5.17 | 0.62 | 1.76 |
| AgNO ₃ 30 ppm + sucrose 3% | 1.33 | 1.55 | 0.03 | 1.44 | 1.67 | 0.05 | 1.77 | 4.58 | 0.09 |
| Sucrose 3% | 1.37 | 1.08 | 0.32 | 1.66 | 1.23 | 0.44 | 2.00 | 1.87 | 0.61 |
| Control | 0.91 | 0.30 | 0.08 | 0.99 | 0.60 | 0.11 | 1.01 | 0.18 | 0.24 |

The concentrations of fructose, glucose and sucrose in rose petals were 0.65, 0.18 and 0.26 mg g⁻¹ dry weight in controls at the end of the experiments (Table 5). At the same time values of these sugars in mg g⁻¹ dry weight when petals were treated with 30 ppm AgNO₃ and 30 ppm AgNO₃+ sucrose 3% and sucrose 3% alone were 1.96, 1.26 and 0.17, respectively (Table 5).

While stem contents of the previous sugars increased at the beginning of the experiment, then decreased towards the end of the experiment compared to control (Table 6).

DISCUSSION

Silver nitrate (AgNO₃) is very potent inhibitors of ethylene action in plant tissues. The treatment of AgNO₃ may be decreased the ethylene production by rose cut flowers tested in comparison to control. It is also provides some antimicrobial activity inside the plant tissues, thus its beneficial for ethylene sensitive flowers such as carnation (Nowak and Rudnicki, 1990). This might explain the effective role of AgNO₃ in prolonging the vase life of rose cut flowers (Table 1) In addition, under AgNO₃ treatment the percentage of wilting, chlorophyll and carbohydrate degradation was

minimized as a consequence, the vase life was extended (Table 2). These results are in harmony with the result of Singh and Tiwari (2002); Harode *et al.* (1993) and Reddy *et al.* (1988).

It is well known that sucrose supply increases the longevity of many cut flowers, since sucrose can act as a source of nutrition for tissues approaching carbohydrate starvation, flower opening and subsequent water relations (Kuiper *et al.*, 1995), similar findings were obtained by Lalonde *et al.* (1999); Nichols (1973); Ichimura (Ichimura, 1998) and Downs (1988).

Concerning the role of sucrose with AgNO₃ in the previous results show that adding sucrose extended the vase life and improved the quality of rose cut flowers. The data on chlorophyll and carbohydrate contents show the positive role of AgNO₃ with or without sucrose and sucrose individually on preserving the leaves in good condition by lowering the per cent of wilting and inhibiting the chlorophyll and carbohydrate degradation, similar results were obtained by Serek *et al.* (1996). Even in absence of exogenous ethylene, the life of the flowers was significantly increased by inhibiting ethylene action. As a similar tendency, Bartoli *et al.* (1997) and WeiMing *et al.* (1997) reported that.

CONCLUSION

A significant improvement in vase life of rose cut flowers was occurred when treated with 30 ppm silver nitrate and the effect was further improved when silver nitrate at 30 ppm combined with 3% (w/v) sucrose which attained the best result compared to other concentrations of sucrose. Also the per cent of wilting was minimized, beside chlorophyll as well as carbohydrate degradation had been retarded during the postharvest life as the result of using this combination treatment.

REFERENCES

- Bartoli, C.G., G. Juan and M. Edrdo, 1997. Ethylene production and responses to exogenous ethylene in senescing petals of *Chrysanthemum morifolium* RAM cv. Unsei. *Plant Sci.*, 124: 15-21. DOI: 10.1016/S0168-9452(97)04582-2
- Butt, S.J., 2003. A Review on prolonging the vase life of Roses. *Pakistan Rose Annual*. Published by Pakistan National Rose Society, pp: 49-53.
- Butt, S.J., 2005. Extending the vase life of roses (*Rosa hybrida*) with different preservatives. *Int. J. Agricul. Biology*, 7: 91-99.
- Cho, H.K. and J.M. Lee, 1980. Studies on extending the vase life of cut flowers of rose and carnation with various chemical preservatives. *J.KSHS*, 20: 106-110.
- Darras, A., A. Loannidou and N. Pompodakis, 2010. Evaluation and improvement of post-harvest performance of cut *Viburnum tinus* inflorescence. *Sci. Hort.*, 124: 376-380. DOI: 10.1016/j.scienta.2010.01.018
- Downs, C.G., 1988. Bud-opening treatments to improve *Gypsophila* quality after transport. *Sci. Hort.*, 34: 301-310. DOI: 10.1016/0304-4238(88)90103-3
- Syngé, P.M., 1971. *The dictionary of rose in color*. 1st Edn., Madison Square Press, New York, ISBN-10: 0448025043, pp: 191.
- Gilman, K.F. and P.L. Steponkus, 1972. Vascular blockage in cut roses. *J. Am. Soc. Hort. Sci.*, 97: 662-667.
- Halevy, A.H., T.G. Byrne, A.M. Kofranek, D.S. Famham and J.F.B. Thompson *et al.*, 1978. Evaluation of postharvest handling methods for transcontinental truck shipment of cut carnations, chrysanthemum and roses. *J. ASHC*, 103: 151-155.
- Hardenburg, R.E., 1968. *The commercial storage of fruits, vegetables and florist and nursery stock*. Intrafinity Corporation. http://www.msue.msu.edu/workspaces/one.cfm?workspace_id=213590&object_id=284535&tab=object_view
- Harode, S.M., V.G. Kotake and J.P. Zend, 1993. Effects of selected treatments on longevity and freshness of selected flowers in home decoration. *J. Maharashtra Agricul. Univ.*, 18: 448-450.
- Hassan, F., 2005. The Forgotten Museums of Egypt. *Mesuem Int.*, 57: 42-48. DOI: 10.1111/j.1468-0033.2005.00509.x
- Ichimura, K. *et al.*, 2003. Shortage of soluble carbohydrates is largely responsible for short vase life of cut 'Sonia' rose flowers. *J. Japan. Soc. Hort. Sci.*, 72: 292-298.
- Ichimura, K., 1998. Improvement of postharvest life in several cut flowers by the addition of sucrose. *Japan Agric. Res. Q.*, 32: 275-280.
- Kuiper, D., S. Ribots, H.S. Van Reen and N. Marissenn, 1995. The effect of sucrose on the flower bud ripening of "Madelon: Cut roses. *Sci. Hort.*, 60: 325-336.
- Lalonde, S., E. Boles. H. Hellmann, L. Barker and J.W. Patrick *et al.*, 1999. The dual function of sugar carriers: Transport and sugar sensing. *Plant Cell*, 11: 707-726.
- Loub, M. and G. Van Doorn, 2004. Wound-induced and bacteria-induced xylem blockage in roses, *Astible*, and *Viburnum*. *Hort. Sci.*, 32: 281-288.
- Miyamoto, M., K. Inoue and Y. Gu, 1999. Effectiveness of acidic oxidative potential water in preventing bacterial infection in islet transplantation. *Cell Transpl.* 8: 405-411. PMID: 10478721 <http://www.ncbi.nlm.nih.gov/pubmed/10478721>
- Muhammad, S.M., S. Hiroyasu and N. Shahzad, 1996. Diversity in roses. *Nat. Agric. Res. Center*, Islamabad, pp: 1-2.
- Nichols, R., 1973. Senescence of cut carnation flowers: respiration and sugar status. *J. Hort. Sci.*, 8: 111-121.
- Nikolova, N. and I. Konezjak, 1986. Development of tight greenhouse rose buds into flowers on an artificial medium. *Acta Hort*, 167: 435-440.
- Nowak, J. and R.M. Rudnicki, 1990. *Postharvest Handling and Storage of Cut Flowers, Florist, Greens and Potted Plants*. 1st Edn., Timber Press, Inc., ISBN-10: 0881921564, pp: 210.
- Ohkawa, K., Y. Kushhara and J.N. Suh, 1999. Mobility and effects on vase life of silver-containing compounds in cut rose flowers. *Hortscience*, 34: 112-113.
- Pun, U.K., H. Shimizu, K. Tanase and K. Ichimura, 2005. Effect of sucrose on ethylene biosynthesis in cut spray carnation flowers. *Acta Hort.*, 669: 171-174.
- Reddy, T., C. Nagarajaiah and B. Raju, 1988. Impregnating cut rose stem with nickel increases in vase life. *Hort. Abst.*, 59: 2360.

- Serek, M., E. Sisler and M. Reid, 1996. Ethylene and the postharvest performance of miniature roses. *Acta Hort.*, 424: 145-150.
- Singh, A.K. and A.K. Tiwari, 2002. Effect of pulsing on postharvest life of Rose v. Doris Tystermann. *South-Indian-Hort.*, 50: 140-144.
- Van Doorn, W.G., Y. Witte, W.G. Van Doorn and Y. De-Witte, 1997. Sources of the bacteria involved in vascular occlusion of cut rose flowers. *J. Am. Soc. Hort. Sci.*, 122: 263-266.
- Van Leperen, W., J. Nijssse, C.J. Keijzer and U. Van Meetern, 2001. Induction of air embolism in xylem conduits of pre-defined diameter. *J. Experimental Botany*, 52: 981-991. DOI: 10.1093/jexbot/52.358.981
- WeiMing, G., W. Zeng and F. Chen, 1997. Regulation of ethylene on senescence of cut chrysanthemum flower. *J. Nanjing Agric. Univ.*, 20: 24-29.