1-MCP and STS as ethylene inhibitors for prolonging the vase life of carnation and rose cut flowers

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Summary: The effect of STS and 1-MCP on the postharvest quality of carnation and rose cut flowers was studied. Cut flowers of *Dianthus caryophyllus* L. cv. Asso and *Rosa hybrida* cv. Baroness were treated with silver thiosulfate (STS) at 0.4 mM with sucrose at 50 g l^{-1} and 1-methylcyclopropene (1-MCP) at 0.5 g m^{-3} for 6h.

Pretreatment with STS and 1-MCP significantly extended the vase life and minimized the % loss of initial weight of carnation and rose cut flowers comparing to the untreated control. The two chemicals applied inhibited the chlorophyll degradation and carbohydrate loss and hence, significantly improved the postharvest quality of carnation and rose cut flowers comparing to the control. Ethylene production by cut flowers was inhibited as a result of using these chemicals. In general, there were no differences between STS and 1-MCP but the later does not have the heavy metal implications of STS treatment, and hence, using 1-MCP pretreatment for extending the vase life of carnation and rose cut flowers was recommended.

Key words: carbohydrates, carnation, chlorophyll, ethylene, rose, 1-MCP, STS.

Introduction

Carnation and rose are very important cut flowers in flower industry and the market of these cut flowers in various countries has been good (AIPH 2003). Understanding the variable postharvest performance of these cut flowers is important not only for ensuring maximum life of those that presently are of commercial importance, but also for developing new selections with improved postharvest quality.

The longevity and quality of cut flowers depend also on the composition of the ambient atmosphere. The most adverse effects on cut flowers are caused by ethylene. Ethylene responses can be derived from either internal synthesis within plants and fruit (endogenous) or through exposure to external sources such as engine exhausts heaters, fungi or ripening fruit (exogenous). Cut flowers produce small amounts of ethylene just after harvest. Meanwhile, there is sharp increase in ethylene production few days after harvest. Some deleterious effects of ethylene exposure include leaf yellowing, flower (or petal) drop, irregular opening and premature death. (*Nowak* & *Rudnicki* 1990).

Because of the diverse effects of ethylene on a wide range of plant species, many of which are harmful, it would be highly beneficial to manage the effect of ethylene during the postharvest life of cut flowers potted plants. The most common chemical used in floral industry against ethylene is STS (silver thiosulfate) which can at least double the vase life of cut flowers. *Menguc* & *Usta* (1994) reported that STS + sucrose pretreatment had positive effect on the vase life and petiole size of carnation cut flowers. *Celikel* & *Karacaly* (1995) found that STS pulsing prolonged the vase life of cut carnation flowers to 15.5 days compared to 6.8 days in control. *Altman* & *Solomos* (1995) mentioned that flowers continuously treated with 0.2 mM STS exhibited no morphological or respiratory responses to any concentration of exogenous ethylene, whereas both a respiratory increase and irreversible petal wilting were observed in flowers pulsed with 0.5mM STS. They also suggested that the interaction between silver ions and ethylene is competitive. Pulsing with AgNO3 (300 ppm) strikingly enhanced vase life and solution uptake of rose cut flowers (*Singh* & *Tiwari* 2002). There was an increasing trend in the fresh weight and vase life of rose cut flowers when STS was used (*Chikkasubbanna* & *Yogitha* 2002, *Tiwari* et al. 2002). Also, *Hassan* & *Schmidt* (2003 and 2004) found that STS increased the vase life and minimized the % loss of different cut flowers.

Because STS contains silver, which recently considered a potential environmental pollutant, there has been some restriction on its commercial use (*Serek* & *Reid* 1993, *Cross* 1996). Researchers have therefore been seeking alternative strategies, including the use of inhibitors of ethylene biosynthesis and inhibitors of ethylene binding for preventing the undesirable postharvest effects of ethylene. A new tool, 1-methylcyclopropene (1-MCP), has been added to the list of options for extending the vase life of cut flowers. 1-MCP marketed under the trade name EthylBloc, has been discovered by Prof. E.C. Sisler, North Carolina State

University, Raleigh, North Carolina, United States. 1-MCP is a gas in its natural state (as is ethylene), which provides opportunities and challenges in commercial use. EthylBloc comies in powder form, which is added to water to release the gas. 1-MCP is a non-toxic inhibitor of ethylene action which acts as a competitive and irreversible inhibitor of binding of ethylene to its receptor (*Sisler* et al. 1996).

Even at very low concentrations 1-MCP has been shown to eliminate the effects of ethylene on abscission and wilting of many ornamental crops such as carnation and rose (*Serek* et al. 1995, *Sisler* et al. 1996). The longevity of cut carnations pre-treated with 1-MCP increased and 1-MCP led to protect carnation flowers for several days against ethylene and extended the vase life of carnation cut flowers (*Serek* et al. 1995, *Sisler & Serek* 2001, *Hassan & Schmidt* 2003). The treatment of 1-MCP extended the vase life of different cut flowers (*Sisler & Serek* 2001, *Skog* et al. 2001 and *Hassan & Gerzson* 2002, *Tar & Hassan* 2003).

There is a limited information in the literature about the effect of 1-MCP on rose cut flowers but there is some information about potted roses. The treatment of 1-MCP led to extend the display life of potted roses (*Serek* et al. 1994 & *Muller* et al. 2000).

The aim of this research was to study the postharvest quality of carnation and rose cut flowers as affected by STS with sucrose and 1-MCP and further probe their role in chlorophyll content, carbohydrate content and ethylene production which is connected to longevity and quality of cut flowers. In addition, to make a comparison between STS and 1-MCP in this respect in order to see is it a good alternative to STS or not under the condition of the experiment.

Material and method

Plant material

Cut flowers used in the experiment were *Dianthus caryophyllus* cv. Asso, which produce white, large, and standard flowers and *Rosa hybrida* cv. Baroness. The flowers were obtained from a commercial grower in Hungary at commercial maturities (half-open flowers for carnation and open bud stage for rose). Lower leaves were removed and the flowering stems were trimmed to an uniform length of 50 cm for carnation and 45 cm for rose, respectively.

This experiment was carried out at seasons of 2002 and 2003 in the laboratory of BKÁE, Department of Floriculture and Dendrology, Budapest. Three replications of five flowers each were used per treatment in this experiment. The flowers were arranged in complete randomize block design and the treatments were as follows:

- Control
- 0.4 mM STS + 50 g l^{-1} sucrose
- 0.5 g m^{-3} 1-MCP for 6h.

Chemical treatments

STS treatment

STS was prepared as described by Gorin et al. (1985). Cut flowers were treated with STS for 6h at concentration of 0.4 mM with sucrose at 50 g l^{-1}. After pulsing treatments the flowers were placed in glass vials containing 500 ml tap water till the end of experiment

1-MCP treatment

1-MCP (as EthylBloc) was obtained from AgroFresh Inc. Rohm and Haas company. The flowers, which were treated with EthylBloc were lied inside a tight box. The box was sealed well with greenhouse plastic cover and the concentrations of 1-MCP were calculated as g m^{-3} (EthylBloc per cubic meters). Soon, the EthylBloc powder was weighed and placed in a test tube fixed with a self adhesive tape taped to the inside wall of the box. Since a significant percentage of 1-MCP is released immediately after addition of hot water, the box was first sealed, and then, hot water was injected into the test tube (just enough to cover the powder for each treatment). 1-MCP was used at 0.5 g m^{-3} for 6h. The 1-MCP treatment was conducted at 15 °C. After the treatment the flowers were aerated and then were placed into glass vials containing 500 ml tap water.

Control and treated flowers with STS were put in such boxes of 1-MCP treatment during the treatment of 1-MCP. Control flowers were put into glass vials containing 500 ml tap water during the whole period of the experiment.

Vase life determination

The longevity of cut flowers was determined in a vase life evaluation room at normal day light at 20 ± 1°C and 80–90% RH.

Carnation cut flowers

Visual rating of flowers was evaluated on a scale from 1 to 4 when: 1 = entirely white flowers, 2 = initiation of darkening (wilting) in 20% of petals, 3 = darkening in 20–50% of petals, 4 = darkening in 50–100% of petals. The longevity of carnation cut flowers was defined by the number of days in vase life required for 50% of the flowers to reach stage 2 or more advanced stages.

Rose cut flowers

Visual rating of rose flowers was evaluated on a scale from 1 to 4 when: 1 = entirely fresh flowers, 2 = initiation of wilting in 20% of petals and beginning of bent neck, 3 = wilting in 20–50% of petals and increasing the bent neck, 4 = wilting in 50–100% of petals. The longevity of rose cut flowers was defined as the number of days in vase life

required for 50% of the flowers to reach stage 2 or more advanced stages.

Fresh weight measurements

Fresh weight determinations of the carnation and rose cut flowers were made just before the immersion of the flowers into the glasses of water and were repeated on the day when the vase life of the control flowers was terminated. The flowers were taken out of solutions for as short time as possible (20–30s). The fresh weight of each flower was expressed relative to the initial weight to represent the % loss of initial weight.

Chlorophyll determination

Chlorophyll content of carnation and rose leaves was extracted by acetone as previously described by (*Dawood* 1993) from samples of cut leave segments (0.5 g) on day 0 (at the beginning of the experiment), day 3 and on the day when the vase life of the control flowers was terminated. The samples were taken from the upper part of stems. The chlorophyll content was calculated as mg g^{-1}.

Determination of carbohydrates

The soluble carbohydrate concentration was determined in petals of cut flowers tested in this study. The dried samples were ground into fine powder and a 0.5 g sub-sample was used for extracting the soluble carbohydrate. The carbohydrates separated using a high performance liquid chromatography (HPLC) system. The instrument used was a Waters 510 isocratic solvent delivery system with a Rheodyne 7125 (Berkeley, CA) injector. A 20 µl stainless-steel injector loop was employed. Differential refractometer (Type: RIDK-2, Praha, Czech Republic) was used to detect of fructose, glucose and sucrose. The stacionary (stationary) phase was a Hypersil 5 APS column (250 x 4 mm) (BST Budapest, Hungary). The mobile phase consisted of mixture of acetonitrile-water (80:20 v/v %).

Peak identity was confirmed using authentic carbohydrates. Peak area was determined by an integrator and the percent of each carbohydrate in the sample was computerize calculated. The carbohydrate content was calculated as mg g^{-1} dry weight.

Determination of ethylene production

Flower stems of carnation and rose cut flowers were cut to 2 cm and individually weighed and placed in 700 ml air tight glass vessels containing 30 ml tap water fit with gas sampling ports. The vessels were kept at 22 °C and 70–80 % R.H. with 16-h-light and 8-h-dark periods. 1 ml gas samples were withdrawn from the headspace for ethylene determination at different time points. Ethylene content of the samples was quantitatively analyzed by gas chromatography using a Packard 427 GC, which was equipped with an aluminum oxide column (1/8 inch x 1 m) and a flame ionization detector. The injector, column, and detector temperatures were 80, 100 and 220 °C, respectively (*Heiser* et al. 1998). Ethylene values were indicated in arbitrary units and each treatment comprised four vessels.

Analysis of results

Results were analyzed by using SPSS program Base 9, SPSS Inc., USA. The analysis of variance (ANOVA) as well as differences between means were performed by using Duncan multiple range test at 0.05 level.

Results

Vase life and % loss

Cut carnations

The treatments of STS and 1-MCP extended the vase life and minimized the % loss of initial weight of carnation cut flowers comparing to the control *(Table 1)*. There were no significant differences between the two chemicals in this concern. The vase life was 21.7 days for STS and 20.3 days for 1-MCP, respectively comparing to 8.3 days for the untreated control *(Table 1)*.

Table 1: Effect of STS and 1-MCP on the vase life and % loss of initial weight of *Dianthusaryophyllus* cv. Asso. and *Rosa hybrida* cv. Baroness cut flowers.

Treatments	Carnation		Rose	
	Vase life (Days)	% loss	Vase life (Days)	% loss
Control	8.3 a	11.8 a	5.33 a	24.05 a
0.4 mM STS+50 g l^{-1} sucrose	21.7 b	4.0 b	12.33 b	3.43 b
1-MCP 0.5 g m^{-3} 6h	20.3 b	3.9 b	10.66 c	3.12 b

– Means followed by different letters differ significantly for each other according to Duncan multiple range test at $P = 0.05$.

Cut roses

The vase life of rose cut flowers was significantly prolonged and the % loss of initial weight was kept in minimum as a result of applying STS and 1-MCP treatments. The STS treatment was more effective than 1-MCP treatment (Table 1).The best results in this respect were obtained by using STS at .04 mM + 50 g l^{-1} sucrose which gave 12.33 days vase life comparing with 5.33 for the control *(Table 1)*

Chlorophyll content

Cut carnations

Using STS and 1-MCP treatments led to a significant delay in chlorophyll loss (chl. *a* and chl. *b*) of carnation cut flowers comparing with untreated control. *(Fig. 1)*. There were no differences between the STS and 1-MCP treatments for carnation cut flowers *(Fig. 1)*.

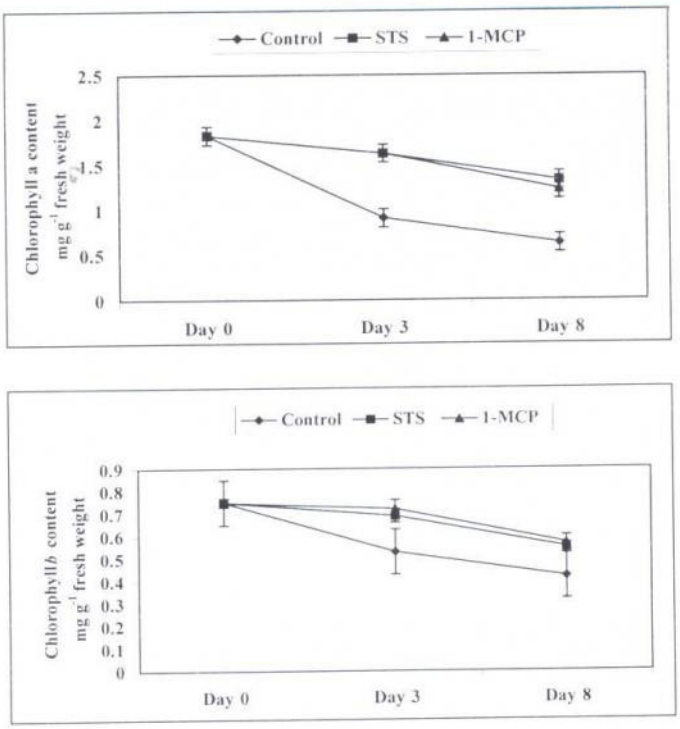


Fig. 1: Effect of STS at 0.4 mM + 50 mg l^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the concentrations of chlorophyll *a* and chlorophyll *b* (mg g^{-1} fresh weight) of carnation leaves as compared to the control. The determination was done at the beginning of experiment (Day 0), day 3 and on the day when the vase life of control was determined (Day 8). The values are means of 3 replicates and each point represents means ± SD.

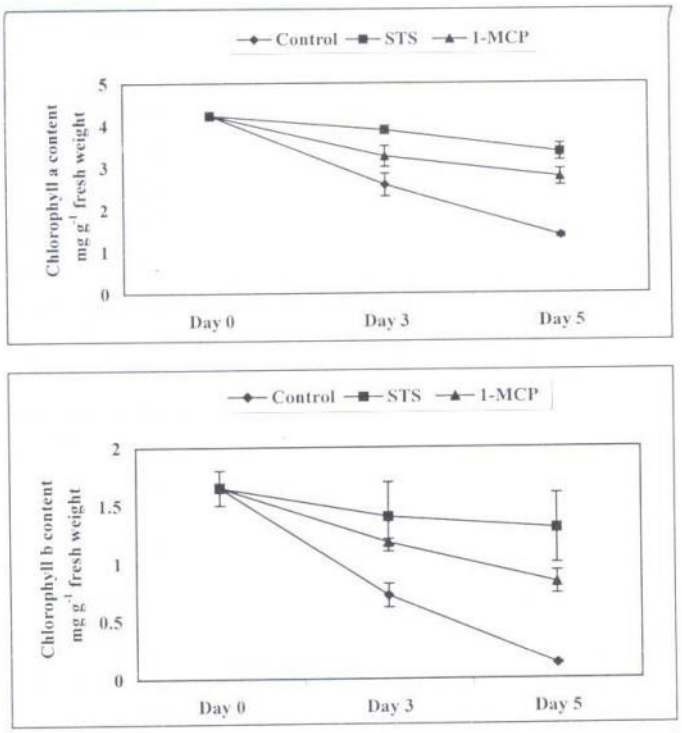


Fig. 2: Effect of STS at 0.4 mM + 50 g l^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the concentrations of chlorophyll *a* and chlorophyll *b* (mg g^{-1} fresh weight) of rose leaves as compared to the control. The determination was done at the beginning of experiment (Day 0), day 3 and on the day when the vase life of control was determined (Day 5). The values are means of 3 replicates and each point represents means ± SD.

Cut roses

Although the two chemicals significantly inhibited the chlorophyll degradation of rose leaves comparing to the control, there were significant differences between STS and 1-MCP in this respect. The best results were obtained by pretreatment with STS at 0.4 mM + 50 g l^{-1} sucrose. Under this treatment, the chl.*a* and chl. *b* at the end of vase life of control (Day 5) were 3.36 and 1.3 mg g^{-1} fresh weight respectively while, the control in the same time resulted in 1.36 and 0.13 mg g^{-1} fresh weight for chl. *a* and chl. *b*, respectively *(Fig. 2)*.

Carbohydrate content

Cut carnations

Fructose, glucose and sucrose were the main soluble carbohydrates in petals of carnation cut flowers *(Fig. 3)*. The carbohydrate content of carnation petals of the control increased from the beginning of the experiment till day 3 and sharply decreased till day 8, which the last day in vase life of control flowers while treated flowers maintained most of its carbohydrate content in the same time. Generally, there were no differences between STS and 1-MCP treatments in this respect *(Fig 3)*.

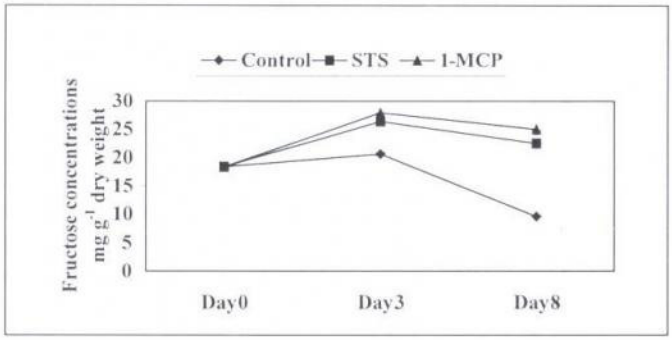
Cut roses

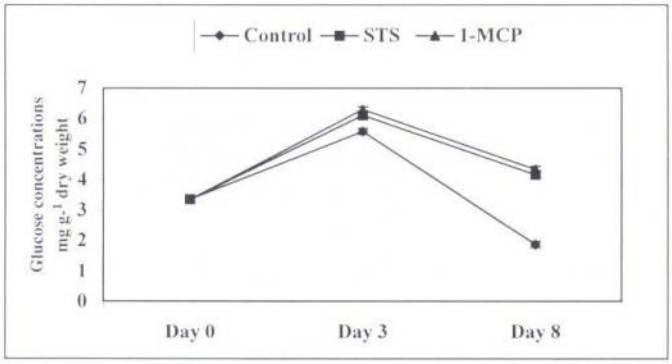
Fructose was the main component of carbohydrates in rose petals. The pre-treatments of STS and 1-MCP significantly increased the carbohydrate content compared to the control at day 3 and suppressed the decrease of fructose, glucose and sucrose concentrations till the end of the vase life of control (Day 5). The concentrations of fructose, glucose and sucrose in untreated rose petals decreased from day 3 and thereafter *(Fig. 4)*. STS and 1-MCP treatments improved the carbohydrate content till day 3 and significantly inhibited the loss of carbohydrate components during the vase life period than the control *(Fig. 4)*.

Ethylene production

Cut carnations

Pre-treatment of carnation flowers with the two ethylene inhibitors significantly decreased the ethylene production *(Fig. 5)*. The maximum ethylene production of untreated carnation cut flowers was recorded at 50 hours after treatments. In the same time, the treatment of 1-MCP and STS significantly minimized the production of ethylene *(Fig. 5)*. There were no significant differences between the two ethylen inhibitors until 200 hours after treatments. On the other hand, after this time the 1-MCP treatment significantly decreased the production of ethylene as camapared to STS.





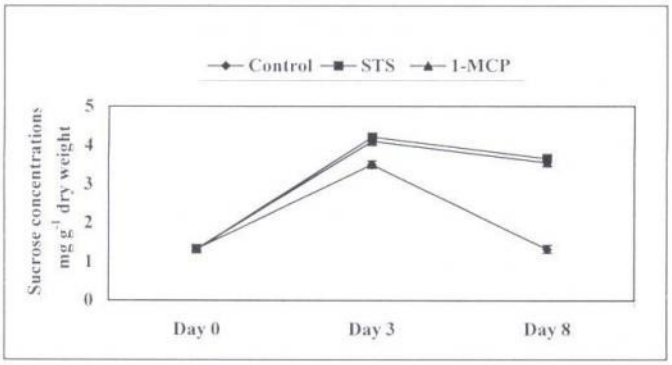
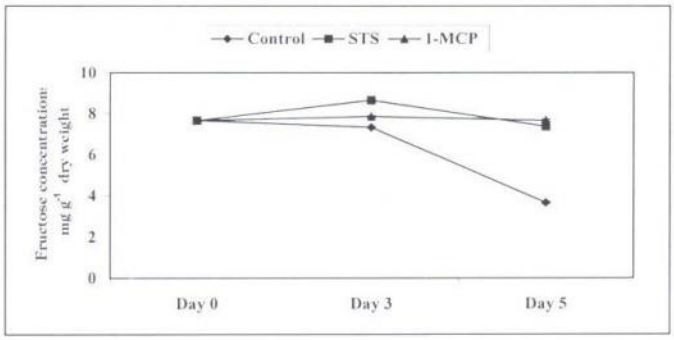
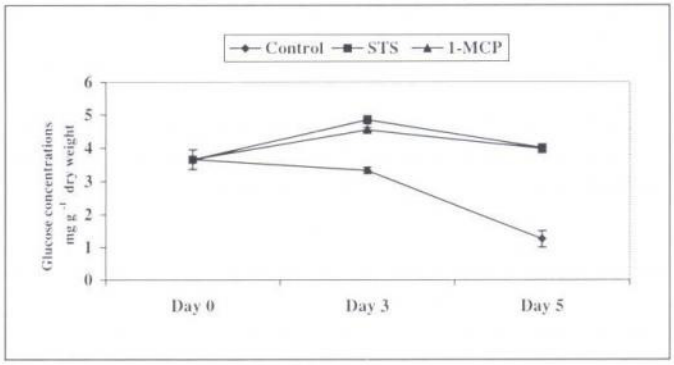


Fig. 3: Effect of STS at 0.4 mM + 50 gl^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the concentrations of fructose, glucose and sucrose (mg g^{-1} dry weight) of carnation leaves as compared to the control. The determination was done at the beginning of experiment (Day 0), day 3 and on the day when the vase life of control was determined (Day 8). The values are means of 3 replicates and each point represents means ± SD





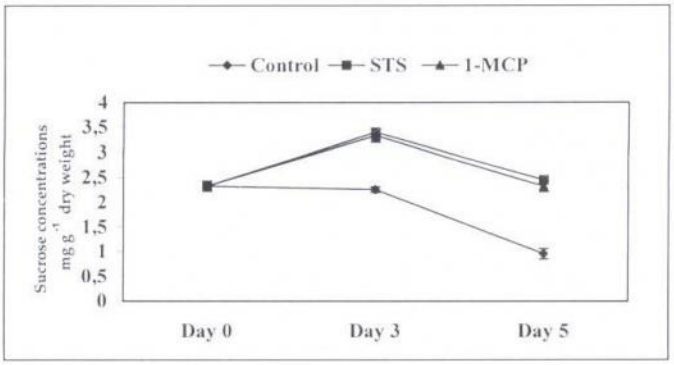


Fig. 4: Effect of STS at 0.4 mM + 50 gl^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the concentrations of fructose, glucose and sucrose (mg g^{-1} dry weight) of rose leaves as compared to the control. The determination was done at the beginning of experiment (Day 0), day 3 and on the day when the vase life of control was determined (Day 5). The values are means of 3 replicates and each point represents means ± SD

In addition, the ethylene production was decreased at the end of vase life in the untreated and treated flowers *(Fig. 5)*.

Cut roses

1-MCP and STS treatments significantly decreased the production of ethylene by rose cut flowers as compared to the untreated control. The maximum values in this concern were recorded by control flowers *(Fig. 6.)*. There were no significant differences between 1-MCP and STS treatments until 250 hours after treatments. After 300 hours, the differences between the two ethylene inhibitors appeared and STS treatment was better than 1-MCP treatment. The production of ethylene was decreased in both untreated and treated flowers at the end of flower life *(Fig. 6)*.

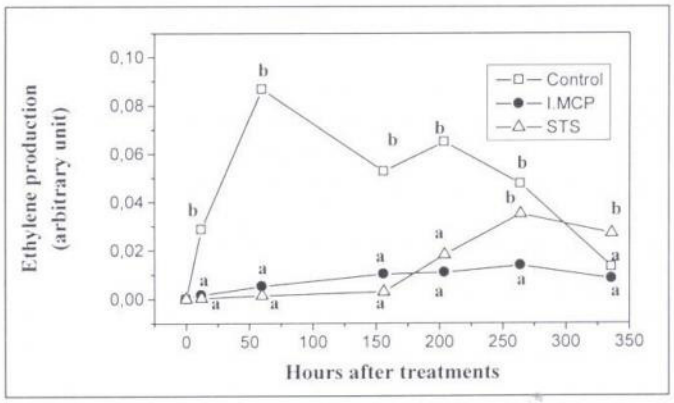


Fig. 5: Effect of STS at 0.4 mM + 50 gl^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the ethylene production (Arbitrary units) of carnation cut flowers as compared to the control during the period of post harvest life. Means followed by different letters differ significantly for each other according to Duncan multiple range test at $P = 0.05$.

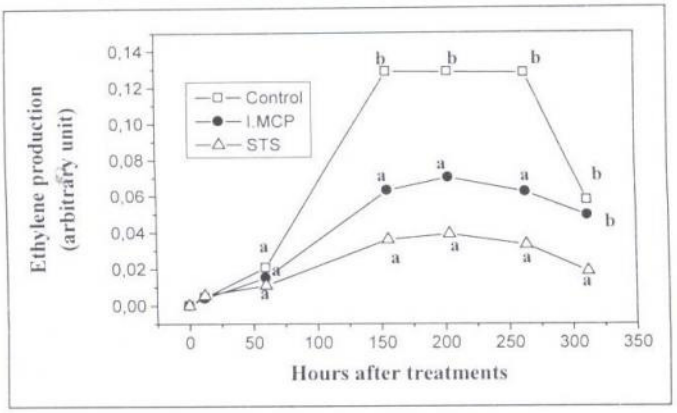


Fig. 6: Effect of STS at 0.4 mM + 50 g^{-1} sucrose and 1-MCP at 0.5 g m^{-3} 6h on the ethylene production (Arbitrary units) of rose cut flowers as compared to the control during the period of post harvest life. Means followed by different letters differ significantly for each other according to Duncan multiple range test at $P = 0.05$.

Discussion

The data obtained show that STS with sucrose treatment was very effective in extending the vase life and enhancing the postharvest quality of carnation and rose cut flowers. These results could be explained through the role of STS in keeping the quality of leaves and retarding the loss of fresh weight. In addition, this treatment improved the chlorophyll and carbohydrate contents and hence, the vase life of cut flowers was extended. The STS plus sucrose pretreatment has also decreased the ethylene production by cut flowers (*Fig. 5* and *6*) and, by preventing the deleterious effects of ethylene the vase life was increased. These results are in harmony with the findings of *Nowak* & *Rudnicki* (1990) who reported that STS was a very potent inhibitor of ethylene action in plant tissues. It also provides some antimicrobial activity inside the plant tissues, thus it is beneficial for ethylene-sensitive flowers such as carnation. Similar results were obtained by *Menguc* & *Usta* (1994), *Celikel* & *Karacaly* (1995), *Hassan* & *Schmidt* (2003) on carnation cut flowers and Bhattacharjee and De (1998), *Chikkasubbanna* & *Yogitha* (2002), *Tiwari* et al. (2002) on rose cut flowers.

Concerning the role of sucrose, it is well known that sugar supply increases the longevity of many cut flowers. While sucrose can act as a source of nutrition for tissues approaching carbohydrate starvation, it may also act as an osmotically active molecule thereby having a role in flower opening and subsequent water relations (*Kuiper* et al. 1995). Similar findings were obtained by *Erin* et al. (2002) who found that vase solutions containing sugar can improve the vase life of many cut flower crops.

Extending the vase life of carnation and rose cut flowers by using 1-MCP could be attributed to the role of 1-MCP as an inhibitor of ethylene biosynthesis as well as ethylene binding and consequently preventing the undesirable postharvest effects of ethylene as reported by Serek et al. (1995), and as shown in *Fig. 5* and *6*. Also, keeping the leaves in a good state by lowering the percent of weight loss, retarding the chlorophyll degradation and inhibiting the carbohydrate loss may be led to increase the vase life. In the same direction, *Celikel* & *Reid* (2002) reported that, even in the absence of exogenous ethylene, the life of the flowers was significantly increased by inhibiting ethylene action using pretreatment with 1-MCP. Similar results were obtained by *Sisler* & *Serek* (2001) who reported that the treatment of 1-MCP led to protect carnation flowers for several days against ethylene and consequently, the vase life were extended. Also, the treatment of 1-MCP at 0.5 g m^{-3} for 6h extended the vase life and improved the quality of different cut flowers (*Hassan* & *Gerzson* 2002, *Hassan* and *Schmidt* 2003, *Hassan* & *Schmidt* 2004). In addition, *Serek* et al. (1994) & *Muller* et al. (2000) obtained similar results on potted roses.

Although STS had the same or better effect than 1-MCP, the later does not have the heavy metal implications of STS treatment. The use of STS, a possible environment pollutant, has been banned in several countries. 1-MCP is effective and safe alternative to STS (*Cross* 1996, *Sisler* & *Serek* 2001, *Celikel* & *Reid* 2002). Consequently, using 1-MCP pretreatment for extending the vase life and improving the postharvest quality of carnation and rose cut flowers is recommended.

Conclusion

The treatments of STS and 1-MCP used extended the vase life and improved the postharvest quality of carnation and rose cut flowers and based on our results it could be concluded that 1-MCP is an effective blocker of ethylene perception in the these cut flowers. Furthermore, its non-toxic character makes the material an excellent replacement for the environmentally unsafe silver ion. It should, however be noted that these results are valid for an ethylene-free atmosphere and would be different if the flowers would be exposed to ethylene.

Acknowledgement

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