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The Effect of Malic Acid on the Bacteria Populations of Cut Flowers of Carnations Vase Solution

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Abstract: An experiment was conducted to study the effect of Malic acid on the bacteria populations in vase flower preservative solution, vase life and ACC-oxidase activity of cut flowers of carnations (*Dianthus caryophyllus L*). The treatments were distilled water (0, 100, 150 mg/l) Malic acid and sucrose (0.3%). The experimental results showed that Adding Malic acid limited ACC-oxidase activity and decreased the number of bacteria in vase solution. The best treatment involved 150 mg/l Malic acid. The vase solution having 150 mg/l Malic acid significantly decrease the number of bacteria on vase solution, Acc-oxidase activity and increased vase life compared to the control results showed that Malic acid prevent vascular blockage by reducing the number of bacteria on vase solution. In addition, the vase life was lower when sucrose was applied in combination with Malic acid. Results showed that only level of 150 mg/l Malic acid significantly prolonged the vase life and decreased the bacteria populations on vase solution in compare to control.

Key words: Carnation · Bacteria populations · ACC-oxidase activity · Vase life

INTRODUCTION

The length of vase life is one of the most important factors for quality of cut flowers. One of the important factors causing life reduction and cut flower deterioration is the disruption of cut flower water relation due to bacteria growth and proliferation in the vase solution [1-2]. The accumulation of bacteria in vase water is often associated with premature senescence in many cut flower species [3]. The major factor contributing to the rapid senescence of cut flowers appears to be the blockage of water conducting vessels of the xylem. In fact, bacteria in vase water may block vessels on the cut surface. Stem occlusion reduced the water uptake and increased the loss of turgidity [4]. Bacteria are a factor in vascular occlusion of cut flowers, but the mechanism by which bacteria cause the occlusion remained unknown [5]. Zagory and Reid found that some bacteria from vase water produced ethylene [6]. Exogenous ethylene has been found to induce vascular blockage in Ricinus communis [7]. The bactericide is one of the most important components in the preservative solutions to control harmful bacteria and help to prevent bacterial plugging of water conducting tissues [8-9]. It has been shown that antimicrobial compounds in the vase solution may prolong the vase life of several cut flowers [10-11]. Antimicrobial compounds in the vase solution reduced the number of bacteria in the vase solution.There is a correlation between decrease in hydraulic conductance and high number of bacteria per gram stem fresh weight.Malic acid is a well known organic acid that presumably can reduced the number of bacteria in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ethylene production and sensitivity. presumably Treatment MA can extends the vase life of cut carnation flowers.Sucrose alone have not been usually used because sugar treatment without germicides promotes bacterial proliferation, leading to shortening of the vase life. However, No evidence was found for the hypothesis that Malic acid cause low number of bacteria and fungi in the vase solution. In the present study, we test the hypothesis whether treatment with Malic acid, sucrose or their combination extended the vase life of cut flowers by reduce microbial populations of cut flowers vase solution.

MATERIALS AND METHODS

Plant Material: The experimental site was in horticulture laboratory of agriculture faculty of Azad University Karaj, Iran. Flower was harvested on February 15 2010. Flowers

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were weighed immediately after harvest and used for setting treatments. The experiment was arranged in a factorial test with complete randomized design with 4 replications. The factors were three levels of Malic Acid (0, 100, 150 mg/l) and two levels of Sucrose (0.3%).The flowers were individually placed in bottles containing 250 ml of preservative solution and were held at 19°C, 70% relative humidity. Results were analyzed by SPSS v16.0. All statistically significant differences were made with Tukey test at P<0.05levels.

Vase Life: Vase life was considered to be terminated when bent neck or wilting occurred.

Water Uptake and Fresh Weight: The volume of water uptake was calculated by subtracting the volume of water evaporated from a control bottle without cut flowers from the amount of water decreased in bottles containing flowers. The fresh weight of the cut flowers also measured in initial day and terminal day of experiment.

Chlorophyll(a+b)content Measurement: Chlorophyll total (a+b) content was evaluated by Chlorophyll meter SPAD-502, Minolta Co. Japan which represented by SPAD value. The petal was inserted into the meter and measured SPAD value was recorded as average of 3 measurements from different spots of a single petal.

ACC Oxidase Activity Measurement: ACC oxidase activity was measured according to the method described by Moya-Leon *et al.* [12]. one grams of frozen flesh was ground to a fine powder in liquid nitrogen in the presence of 5% (w/w) poly vinylpyrrolidone. The powder was transferred to a 50-mL centrifuge tube containing 20 ml of an extraction buffer consisting of 0.1 M Tris-HCl (pH 7.5), 10% (v/v) glycerol, 2 mm dithiothreitol and 30 mm sodium ascorbate. After the slurry had thawed completely, the tube was centrifuged at 20,000g for 20 min. The supernatant was passed through a membrane filter (Cellulose Nitrate, 0.45 μm, Toyo Roshi, Tokyo) and desalted by passage through Sephadex G-25 columns previously equilibrated with the extraction buffer. ACC oxidase activity was assayed by incubating 1 Mmol of the enzyme preparation with 1 ml of a reaction mixture consisting of 0.1 M Tricine (pH 7.5), 10% (v/v) glycerol, 1 Mmol ACC, 30 Mmol sodium ascorbate, 0.1 Mmol $FeSO_4$ and 20 mm $NaHCO_3$ at 30°C for 1 h and the ethylene produced was determined.

Microbe Population: Samples were isolated from vase solutions of carnations, When the flowers had senesced (about 11 days). Aliquots of the vase solutions were diluted 100-times and 25 μL aliquots of the diluted solution were spread on sterile Nutrient Agar,in sterile petri plates. The plates were allowed to incubate for 48 hr at room temperature and individual colonies of bacteria s, representing the most common colony morphology types, then were picked off the agar media with a sterile loop and streaked on EMB medium for purification. Purified Microbe population were maintained axenically on EMB medium and transferred daily to fresh medium.

RESULTS AND DISCUSSION

Vase Life: Carnation flowers treated by MA alone or together with sucrose had more vase life. MA alone increased vase life of carnation flowers more than sucrose alone or together with MA. The maximum vase-life (8.27 days) was recorded in 150 mg/l MA (Table 1). Statistically significant differences existed among 150 mg/l MA compared to other treatments and control. The minimum vase-life was noted in 100 mg/l MA and control (Table 1). Results suggest that adding MA in vase water can prevent the growth of microbes as well. Adding MA was found to be positively and significantly correlated with vase life of the carnation cut flower (Table 2). Bacteria, which grow in vase water harm cut flowers through their development in xylem and their consequent blockage at cut ends, preventing the water absorption. They also produce ethylene and toxins, which accelerate flower senescence and reduce vase-life. Adding a suitable germicide in vase water can prevent the growth of microbes [13]. These results are in agreement with previous workers who have reported increased vase life of cut flowers when placed in solutions of germicide [14]. Our results showed that the wilting percentage decreased with the increase in concentrations of MA. The data reported here provide good evidence that MA had an positive effect in prolonging vase life of cut carnation and helped to reduce the vascular blockage in the cut carnation (Table 1).

Water Uptake and Fresh Weight: Water uptake and fresh weight of cut carnation held in distilled water rapidly decreased after the first day, while water uptake and fresh weight of those held in MA decreased slightly for the first 8 days and rapidly thereafter. Water uptake of cut

Table 1: Mean comparisons of chlorophyll total content, Vase life, Water uptake, Microbe population and ACC Oxidase Activity in MA treatment

Treatment	Water uptake (ml)	Vase life (day)	Microbe population(cfu)	Chlorophyll total (a+b)content(spad reading)	ACC Oxidase Activity (nmol/gFW/h)
control	87.5b	6.17b	35.92c	1.48c	23.94b
MA100 mg/l	92.08b	6.17b	23.33b	2.42b	25.37b
MA150 mg/l	117.92a	8.27a	16a	4a	15.01a

Means in each column followed by similar letters are not significantly different at 5% level using Tukey.

Table 2: Simple correlation lines between the MA treatment with other variables

Treatment	Water uptake (ml)	Vase life (day)	Microbe population (cfu)	Chlorophyll total (a+b)content (spad reading)	ACC Oxidase Activity (nmol/gFW/h)
MA	.527**	.375*	-.590**	.232	-.367*

* and **: Significant different at 5% and 1% level, respectively.

Table 3: Mean comparisons of chlorophyll content, Vase life, Water uptake, Microbe population and ACC Oxidase Activity in MA*SU treatment

MA	SU	vase life (day)	Water uptake (ml)	Microbe population (cfu)	Chlorophyll total (a+b)content (spad reading)	ACC Oxidase Activity (nmol/gFW/h)
0	0	6.333b	92.5c	27.5c	2.826c	23.065c
	30	6b	82.5ab	44.333ab	2.014c	27.682c
100	0	5.833b	94.167c	16.667a	1.458ab	25.317c
	30	6.5b	90c	30	1.516ab	22.578c
150	0	8.208a	123.333a	12.167a	4.581a	14.037a
	30	7.333a	112.5b	19.833b	3.421b	16b

Means in each column followed by similar letters are not significantly different at 5% level using Tukey.

carnation held in MA alone was greater than those held in MA + sucrose.Results regarding the water uptake and fresh weight by the cut carnation show that maximum water was taken up by the cut flower kept in 150 mg/l MA, which differed significantly from other treatments (Table 1). When flowers are detached from the plant, water loss from these continues through transpiration. The ideal flower preservative is that which allows water absorption in flower tissues [15]. Water absorption from the preservative solution maintains a better water balance and flower freshness [16] and saves from early wilting resulting in enhanced vase life. Adding MA was found to be positively and significantly correlated with Water uptake and fresh weight of the carnation cut flower (Table 2). This indicates that with increased MA concentration, the Water uptake and fresh weight was increased as well. One of the greatest problems in postharvest flower physiology is the blockage of vascular system, due to air or bacterial growth [17],which reduces water uptake and this blocks xylem vessels leading to water stress. Our Result showed that Adding MA in vase water significantly reduced the microbial population.The improved Water balance brought about by MA resulted in increased Water uptake and fresh weight as well. Our data indicated that MA acts both by reducing the rate of water loss from the floret and by maintenance their fresh weight. It thus appears that MA itself may exert some effect on the aperture of the stomatates.

Chlorophyll total (a+b) content: The total Chlorophyll (a+b) concentration of flowers treated with 150 mg/l MA alone was the highest compared to other combinations and control. (Table 1). The differences of Chlorophyll concentration between treatments could be attributed to a different amount of MA taken up by flowers. The applied MA was found to be significantly and positively correlated with Chlorophyll (a+b) content of the carnation cut flowers (Table 2). This indicates that with increased MA concentration, the retained Chlorophyll concentration compared to the control increased. MA treatments lead to a considerable delay in degradation of total Chlorophyll (a+b) compared to control (Table 1). Chlorophyll contents were lower when sucrose alone at 3% was in vase solution.

ACC Oxidase Activity: The results show that MA effectively reduced ethylene production in carnation flower with 150 mg/l MA being the most effective. The ethylene is the major coordinator of senescence in many flowers including carnation. Results indicate that higher concentration of MA apparently reduced the formation of ethylene by inhibiting the action of ACC Oxidase. ACC oxidase activity of the control flower increased 6 days after harvest and remained almost at this level and ACC oxidase activity of flower treated with MA at 150 mg/l remained at a relatively low level until 8.27 days after harvest. Application of MA significantly decreased ACC Oxidase activity in carnation cut flowers compared to control., with the minimum ACC-oxidase activity noted in 150 mg/l MA treatment (Table 1). Zagory and Reid found that some bacteria from vase water produced ethylene and increase ACC-oxidase activity. We could attribute part of the observed decrease in ethylene production to the

preventive effect of MA on the growth of microbes and subsequently decrease in induced ACC-oxidase activity and ethylene production by micro-organisms. Results showed Adding MA being significantly and negatively correlated with ACC Oxidase activity of the carnation cut flower (Table 2).

Microbe Population: MA affected on the Microbial population in vase solution of carnation cut flowers significantly, the Microbial population decreased with the increase in concentrations of MA and the lowest microbial concentration was evident when cut flowers were treated with 150 mg/l MA which was significantly lower than control and other Combination of treatments (Table 1). Anjum *et al.* who reported Adding a suitable germicide in vase water can prevent the growth of microbes and increase vase life. While any direct biocidal activity is not reported for MA we could attribute the observes decrease in microbial population to selective effect of MA as being an easily consumable carbohydrate by plant tissue while most bacteria lack the necessary metabolic enzymes required for MA metabolism. In contrast, the sucrose in vase solution rapidly increased the Microbial population being a universal carbon source both for bacteria and the plant The observed and negative correlation between MA and the Microbial population of vase solutions supports the above hypothesis (Table 2).

CONCLUSION

Our result showed that it was possible to delay senescence, extend vase life and decrease ACC Oxidase activity and decrease Microbial population of vase solution of carnation cut flowers using 150 mg/l MA. Lower concentration of 100 mg/l MA was not effective on extension of vase life and reduction ACC Oxidase activity while still effective in reduction of Microbial population in vase solution of carnation cut flowers. The unique observed dual effect of MA in control of microbial population from one side, while serving as a carbon source to cut flower in the other side makes it a valuable tool in post harvest life extension of cut flowers.

REFERENCES

1. Jowkar, M.M., 2005. Effects of Different Compounds on the Microbial Population of Cut ´Shiraz Narcissus´ Vase Solution. Acta Hort. (ISHS) 682: 1705-1708.
2. Van Doorn, G. Wouter and A. Hoogerwerf, 1991. Numbers of bacteria in aqueous solutions used for postharvest handling of cut flowers. Postharv. Biol. Technol., 1: 161-168.
3 Van Doorn, W.G., H. Harkema and J.S. Song, 1995. Water relations and senescence of cut Iris flowers: effects of cyclohexamide. Postharvest Biol. Tech., 5: 345-351.
4. Van Meeteren, U., 1978a. Water relations and keeping quality of cut gerbera flowers. The cause of stem break. Scientia Hort., 8: 65-74.
5. Van Doorn, W.G., 1991. Effect of bacterial suspensions on vascular occlusion in stems of cut rose flowers. J. Applied Bacteriol., 71: 119-123.
6. Zagory, B. and M. Reid, 1986. Role of Vase Solution Microorganisms in the Life of Cut Flowers. J. AMI-R. Soc. HORT. Set. 1I1(I): 154-I58.
7. Vander Molegn, E. Labavitcjh, H. Strandl and E. Devayj, 1983. Pathogen-induced vascular gels: ethylene as a host intermediate. Physiologia Plantarum 59: 573-580.
8. Halevy, A.H. and S. Mayak, 1981. Senescence and postharvest physiology of cut flowers-Part 2. Hort. Rev., 1: 59-143.
9. Jones, R.B. and M. Hill, 1993. The effect of germicides on the longevity of cut flowers. J. Am. Soc. Hort. Sci., 118: 350-354.
10. AARTS, J., 1957. Over de houdbaarheid van snij-bloemen. Mededelingen Landbouwhogeschool Wageningen, 57(9): 1-62.
11. Marousky, F., 1976. Control of bacteria in vase water and quality of cut flowers as influenced by sodium dichloroisocyanurate, l,3-dichloro-5,5-dimethyl hydantoin and sucrose. USDA, Agricultural Research Service S-115.
12. Maye-Lean, M.A. and P. John, 1994. Activity of 1-Aminocyclo propane-1carboxylate (ACC) oxidase (ethylene-forming enzyme) in the pulp of.riepening bananase, J. Horst. Sci., 69: 243-250.
13. Akbar Anjum, M., F. Naveed, F. Shakeel and SH. Amin, 2001. Effect of Some Chemicals on Keeping Quality and Vase-life of Tuberose (Polianthes Tuberosal.) Cut Flowers. J. Res. Sci., 12(1): 01-07.
14. Saini, R.S., R. Yamdaqni and S. Sharma, 1994. South Indian Horticulture, 42: 376-378.
15. Salunkhe, D.K., N. Bhat and B. Desai, 1990. Post-Harvest Biotechnology of Flowers and Ornamental Plants, Springer-Verlag, Berlin.
16. Reddy, B.S. and K. Singh, 1996. J. Maharashtra Agricultural Universities, 21: 201-203.
17. Hardenburg, R.E., 1968. The commercial storage of fruits, vegetables and florist and nursery stock. In: Lutz, J.M. (Ed.), Agricultural Handbook No.66. United State. Department of Agriculture. Agricultural Research Sevice, pp: 130.