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Effect of Cobalt, Acetylsalicylic Acid and Glutamine to Extend the Vase-life of Carnation (*Dianthus caryophyllus* L.) Flowers

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ABSTRACT

The reduction of the ornamental value of cut flowers is mainly due to their short vase life. In this study we investigated the effect of cobalt, acetylsalicylic acid and Glutamine to extend the Vase-life of carnation (*Dianthus caryophyllus* L.) flowers. The treatments were distilled water, cobalt (0, 1.5, 3 mM), acetylsalicylic acid (0, 1.5, 3 mM) and glutamine (0, 1, 2 mM). Results show that solution containing 1.5 mM cobalt, 1.5 mM acetylsalicylic acid and 2 mM glutamine could increase flower longevity in compared to control. Vase life in solution containing 3 mM cobalt, 3 mM acetylsalicylic acid and their combination didn't have significantly difference than control and glutamine. The results showed that cobalt, acetylsalicylic acid and glutamine treatments increased cut flower water absorption, fresh weight and vase life, while decreasing MDA content, ACC-oxidase activity and membrane premeability together with total delay of senescence and peroxidation of lipids. Present results suggest the application of cobalt, acetylsalicylic acid and glutamine in preservative solutions for carnation flowers maintained the vase life of flowers for a longer period.

Key words: Cut flower, cobalt, acetylsalicylic acid, glutamine

INTRODUCTION

In many countries, carnation is one of the most popular cut flowers and of highest economic importance in the floriculture industry (Satoh *et al*., 2005). Carnation is highly sensitive to ethylene, postharvest quality of Carnation flowers reduced by ethylene (Mayak and Halevy, 1980; Pun *et al*., 1999; Nukui *et al*., 2004; Farokhzad *et al*., 2005; Hojjati *et al*., 2007; Kazemi *et al*., 2010). Ethylene promoted flower senescence, degradation of membrane lipids, increased production of oxygen free radicals (ROS), malondialdehyde (MDA) accumulation, respiratory activity and loss of cell membrane fluidity (Da Silva, 2003; Kazemi *et al*., 2010; Kazemi *et al*., 2011a-d). Ethylene antagonists inhibit the action of ethylene at the molecular level by blocking its receptor site (Khan *et al*., 2003; El-Tayeb *et al*., 2006; Shi and Zhu, 2008; Joseph *et al*., 2010). Salicylic acid used to increase plants tolerance against the adverse effects of biotic and abiotic stresses: Abdou *et al*. (2001), Shakirova *et al*. (2003), Sawada *et al*. (2006) and Kazemi *et al*. (2011b) showed that SA is inhibits ethylene synthesis and reduces sensitivity of flowers by decreasing ROS and ethylene. Glutamine is readily metabolized by plants and cells can metabolize glutamine for energy,

so, we considered using it as a possible substitute for sucrose (Kazemi *et al*., 2011b). Kazemi *et al*. (2011c, d) reported that treatment with SA acid reduces sensitivity of flowers by decreasing ROS and ethylene. Jamali and Rahemi (2011) reported that treatment with Co significantly extends the vase life carnation. Therefore, in this study, the preservative effects of Co, SA, Glu and their interaction on the vase life of cut carnation flowers were studied.

MATERIALS AND METHODS

Plant material: The experiment was started on July 1, 2010. The factors were three levels of cobalt ($COCl_2$) (0, 1.5, 3 mM), three levels of acetylsalicylic acid (0, 1.5, 3 mM) and three levels of glutamine (0, 1 and 2 mM). The experiment was arranged in a factorial test with complete randomized design with 4 replications. Carnations (*Dianthus caryophyllus* L. pink) were grown in the greenhouse standard production methods. (Pakdasht, Tehran, Iran). Cut flowers were selected to avoid malformations or damage. Flower stems were cut to 40 cm in length and after recording the fresh weight, each flower was placed in a 250 mL bottle containing distill water or chemical solutions. All experiments were performed in a postharvest room with a controlled environment at 19°C, 70% relative humidity.

Vase life: Vase life was determined as the number of days to wilting of flowers. The flowers were checked once a day for signs of deterioration.

Chlorophyll index: Chlorophyll index was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan); this is presented by SPAD value. Average of 3 measurements from different spots of a single leave was considered.

Determination of anthocyanin leakage: Anthocyanin leakage was measured based on the method of Poovaiah (1979).

Determination of ACC-oxidase activity: ACC oxidase (ACO) activity was assayed by measuring to the method described by Moya-Leon and John (1995).

Assays of MDA content (lipid peroxidation): Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according to Heath and Packer (1968).

Microbe population: Test Microbe population were isolated from vase solutions of carnation by measuring to the method described by Zagory and Reid (1986).

Water absorption by cut flowers: The water uptake was calculated by subtracting the mean volume of water evaporated from three control bottle without cut flowers, from the amount of water decreased in bottles containing flowers in experimental course.

Experimental design and statistical analysis: Experiment was arranged in a factorial test with complete randomized design with four replications. Analysis of variance was performed on the data collected using the General Linear Model (GLM) procedure of the SPSS software) Version 16, IBM Inc.). The mean separation was conducted by Tukey's analysis in the same software ($p = 0.05$).

Superoxide dismutase: The activity of superoxide dismutase was measured based on the method described by Beauchamp and Fridovich (1971).

RESULTS AND DISCUSSION

Anthocyanin leakage and ACO activity: During postharvest life, anthocyanin leakage and ACO activity of florets treated with CO and ASA decreased ($p \leq 0.05$). According to Table 1, treatment with 1.5 mM CO+1.5 mM ASA higher delayed the climacteric ethylene production, anthocyanin leakage and extended vase life of the carnation (Table 1), While treatment with cobalt 3 and 3 mM ASA increased anthocyanin leakage and ACO activity and senescence($p \leq 0.05$). Present result showed that he best treatment in this decrease anthocyanin leakage and ACO activity was 1.5 mM silicon+2 mM glutamine+1.5 mM SA. These findings are similar to previous results (Jamali and Rahemi, 2011; Kazemi *et al*., 2011a, b).

Chlorophyll index: The total chlorophyll content of the flower increased with ASA and GLU treated cut flowers (Table 1). ASA and GLU treatments significantly increased the total chlorophyll content to a larger extent when compared to control ($p \leq 0.05$). ASA and GLU treatments increased the total chlorophyll content (5.12) compared to control (0.84). Chlorophyll content was not affected

Table 1: Mean comparisons of chlorophyll content, vase life, MDA, SOD activity, Membrane stability and ACC oxidase activity in CO, SA and glutamine treatments and their interaction

CO (mM)	ASA (mM)	Glutamine (mM)	Vase life (day)	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h^{-1} mL^{-1})	Antocyanin leakage (absorption at 525 nm)	MDA (µmol/mg protein)	Water uptake (mL^{-1} flower)	Colony count (CFU mL^{-1})	SOD (U g^{-1} protein)
0	0.0	0	7	0.84	88.14	211.12	341.14	65.14	446	70.33
		1	8	1.08	64.80	154.36	211.00	75.00	246	75.14
		2	9	1.48	62.05	121.89	178.12	90.00	234	80.09
	1.5	0	10	2.09	29.16	40.09	55.12	110.00	84	159.63
		2	11	5.12	29.00	40.00	67.14	105.00	114	160.11
	3.0	0	5	0.55	100.00	210.89	401.00	30.00	86	33.14
1.5	0.0	0	9	1.09	33.45	66.38	70.21	95.00	91	155.14
		1	9	1.38	31.14	65.17	81.12	90.00	108	155.00
		2	10	2.00	31.00	66.00	80.78	95.00	110	155.69
	1.5	0	10	1.94	28.96	40.30	84.12	125.00	96	105.00
		1	13	3.14	29.00	40.98	85.14	100.00	121	100.00
		2	15	4.96	29.00	41.00	83.69	100.00	124	94.11
	3.0	0	6	1.00	59.14	103.48	94.00	55.00	98	70.00
3	0.0	0	6	1.41	66.18	165.11	354.12	65.00	94	38.74
		1	6	0.74	65.78	145.87	289.00	65.00	127	45.12
	1.5	0	7	0.83	59.60	145.11	210.54	60.00	92	59.60
		1	6	1.00	57.00	146.39	206.36	65.00	94	60.25
	3.0	0	5	0.61	94.18	220.06	406.30	40.00	90	30.70
		1	6	1.00	70.22	184.85	271.12	45.00	71	48.89
F-test probabilities										
CO			0.04	0.06	0.03	0.02	0.06	0.05	0	0.03
ASA			0	0.00	0.001	0.00	0.002	0.001	0	0.00
Glutamine			0.01	0.00	0.05	0.05	0.05	0.001	0	0.05

by CO treatment. These results are in agreement with those of (Kazemi *et al.*, 2011b, c) who found that adding SA and GLU in vase water increased chlorophyll content cut flowers. Similarity, Canakci (2008) reported that treatment with salicylic acid significantly extends the vase life with increases chlorophyll content.

MDA content and Superoxide dismutase activity: Significant decrease and increase in malondialdehyde (MDA) content and superoxide dismutase activity was observed after treated flower with ASA, CO and GLU (Table 1). Under the effect of 1.5 mM ASA treatment increase SOD activity and decreased accumulation MDA significantly in compared to control ($p<0.05$). The results indicate that the treatment by 1.5 mM ASA improved membrane permeability by increasing SOD activity and decrease accumulation MDA in compared to control. Superoxide dismutase activity was not affected by CO treatment. The protective function of ASA includes the regulation of ROS and antioxidant enzymes (Khan *et al.*, 2003; Shi and Zhu, 2008). Similarity, Yuping (2009) reported that treatment with salicylic acid significantly extends the vase life with increases the enzyme antioxidant activity and decreased ROS production. Similarly, Kazemi *et al.* (2011a) showed that pretreatment with SA decreased the level of lipid peroxidation induced by paraquat oxidative stress in cut flowers.

Water uptake, fresh weight and microbe population: ASA and CO treatments significantly increased the water uptake of the cut flowers when compared to control. Among the ASA and CO treatments 1.5 mM CO and 1.5 mM ASA increased water uptake compared to control. Increasing of ASA and CO levels caused decreases water uptake and fresh weight in vase solution of carnation cut flowers significantly, while the microbial population decreased with the increase in concentrations of ASA and CO (Table 1). The best treatment in this increased was 1.5 mM CO + 1.5 mM ASA. Canakci (2008) reported that treatment with salicylic acid significantly extends the vase life with increases water uptake. Kazemi *et al.* (2011a) showed that the treatment of salicylic acid reduced microbial population in vase solution of gerbera cut flowers and increased water uptake in carnation cut flowers.

Vase life: The result from Table 1 showed that flowers-exposed to ASA, CO and GLU (1.5 mM CO+1.5 mM ASA+2 mM GLU) regardless of exposure duration increased the carnation vase life by 8 days compared to control. Yuping (2009) reported that treatment with salicylic acid significantly extends the vase life with increases the enzyme antioxidant activity and decreased ROS production. Jamali and Rahemi (2011) reported that treatment with Co significantly extends the vase life carnation.

CONCLUSION

From the results of the present study, it can be concluded that salicylic acid and cobalt with glutamine treatments significantly decrease MDA and ACC-oxidase activity, bacterial populations in vase flower preservative solution, reduce the membrane permeability and peroxidation of lipids compared to the control.

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