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Effect of Silicon and Acetylsalicylic Acid on Antioxidant Activity, Membrane Stability and ACC-oxidase Activity in Relation to Vase Life of Carnation Cut Flowers

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Abstract: To understand the factors that induce floral senescence in carnation, we have investigated the effects of various chemical agents on flower senescence. The study was conducted to investigate the effect of silicon and acetylsalicylic acid at different concentrations on carnation flower longevity, ACC-oxidase activity and bacterial population vase solution of cut flowers carnation in laboratory condition. The treatments were distilled water, silicon (0, 1.5, 2.5, 3.5 mM) and acetylsalicylic acid (0, 1.5, 3 mM). Results show that 2.5 mM silicon followed by 3 mM acetylsalicylic acid is more effective than 1.5 and 3 mM silicon alone in improving the vase life of cut carnation flowers. The percent of wilting was minimized as a result of using this combined treatment. However, the percent of wilting increased with the increase in concentrations of silicon and acetylsalicylic acid. Silicon at level 2.5 mM retarded the chlorophyll as well as carbohydrate degradation during the postharvest life. The results showed that silicon and acetylsalicylic acid in preservative mixture reduced ACC-oxidase activity number of bacterial on vase solution significantly of cut flower in compared to control.

Key words: Carnation, silicon, acetylsalicylic acid, vase life

INTRODUCTION

Carnation is one of the four famous cut flowers in the world. Senescence of carnation flowers (*Dianthus caryophyllus* L.) is accompanied by a marked increase in the synthesis of ethylene, during the climacteric, there is a coordinate increase in the activities of ACC-synthase and ACC-oxidase (Yang and Hoffman, 1984; Have and Woltering, 1997; Kader, 2003; Da Silva, 2003). Expression of the ACC synthase and ACC oxidase genes in carnation petals depends on the presence of ethylene (Savin *et al*., 1995). Ethylene promoted flower senescence, increased respiratory activity and loss of cell membrane fluidity. The effects of ethylene can be reduced by inhibitors of ethylene biosynthesis or action. Two of the preserving agents are ASA and Si that inhibits ethylene synthesis and reduces sensitivity of flowers to ethylene (Ansari and Misra, 2007; Mahdavian *et al*., 2007; Mba *et al*., 2007; Snyder *et al*., 2007; Canakci, 2008; Karlidag *et al*., 2009; Kazemi *et al*., 2011a-d). SA have been examined successfully in prolonging the vase life of cut flowers by inhibiting ethylene biosynthesis (Smith and Woodburn, 1984; Zheng *et al*., 2006; Fan *et al*., 2008) and its antimicrobial effects. Therefore, in this study, the preservative effects of silicon and acetylsalicylic acid and their interaction on the vase life of cut carnation flowers were studied.

MATERIALS AND METHODS

Plant material: The experiment was started on May 1, 2010. The factors were four levels of silicon (0, 1.5, 2.5, 3.5 mM) and three levels of acetylsalicylic acid (0, 1.5, 3 mM). The experiment was arranged in a factorial test with complete randomized design with 6 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.

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Chlorophyll index: Chlorophyll index was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan) which 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 (ACO) activity: ACC oxidase activity was assayed by measuring to the method described by Moya-Leon *et al.* (2004).

Assays of MDA (malondialdehyde) content (lipid peroxidation): Lipid peroxidation rates were determined by measuring the MDA 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.

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

Experimental design and statistical analysis: Experiment was arranged in a factorial test with complete randomized design with six 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 analysis in the same software ($p = 0.05$).

RESULTS AND DISCUSSION

Water uptake and fresh weight and microbe population: Fresh weight of cut flowers in all treatments increased initially and declined later. In solution containing 2.5 mM acetylsalicylic acid, water uptake decreased to 13 day after treatment ($p = 0.05$). Uptake rate decreased rapidly in distill water and solutions containing 3.5 mM acetylsalicylic acid while flowers in the solutions containing 2.5 mM silicon+1.5 mM acetylsalicylic acid showed the minimum decrease in water uptake rate from day 17 (Table 1). This indicates that with silicon+ acetylsalicylic acid concentration increased, the water uptake was decreased. Silicon+acetylsalicylic acid concentration affected on the microbial population in vase solution of carnation cut flowers significantly, the microbial population decreased with the increase in concentrations of silicon + acetylsalicylic acid (Table 1). Anjum *et al.* (2001) reported adding a suitable germicide in vase water can prevent the growth of microbes and increased water uptake. Kazemi *et al.* (2011a, b, c, d) showed that the treatment of salicylic acid reduced microbial population in vase solution of carnation cut flowers and increased water uptake in carnation cut flowers. Also silicon seems to act by germicide the decrease of microbial population.

Anthocyanin leakage and ACO activity: Anthocyanin leakage and ACO activity were affected by silicon combined with acetylsalicylic acid treatment ($p = 0.05$). Treatment with 2.5 mM silicon+1.5 mM acetylsalicylic acid higher delayed the climacteric ethylene production, anthocyanin leakage and extended vase life of the carnation (Table 1) ,While Treatment with silicon 3.5 and 3 mM acetylsalicylic acid increased anthocyanin leakage and ACO activity and senescence ($p = 0.05$). Highest means of anthocyanin leakage and ACO activity was found in cut flower treatment with 3.5 silicon, 3 mM acetylsalicylic acid and highest means of anthocyanin leakage and ACO activity inhibition was found with 2.5 mM silicon+1.5 mM acetylsalicylic acid (Table 1). Results showed adding silicon and acetylsalicylic acid were found to be positvely correlated with ACO activity and senescence of the carnation cut flower (Table 1). Similarly, Kazemi *et al.* (2011a) reported that treatment with salicylic acid significantly extends the vase life with reduced the anthocyanin leakage and ACO activity.

Chlorophyll index, MDA content and superoxide dismutase (SOD) activity: The treatment by 2.5 silicon+1.5 mM acetylsalicylic acid lead to a considerable delay in degradation of chlorophyll total compared to other concentrations and control ($p = 0.05$) (Table 1). Chlorophyll content decreased rapidly in present cut flower in solutions containing 3.5 silicon and 3 mM acetylsalicylic acid while flowers in the solutions containing 2.5 mM silicon showed the minimum decrease in chlorophyll content from day 17 (Table 1). Table 1 showed that under the effect of 2.5 mM silicon treatment increase SOD activity and decreased accumulation MDA significantly in compared to control. The results indicate that the treatment by 2.5 silicon+1.5 mM acetylsalicylic

Table 1: Mean comparisons of chlorophyll content, vase life, MDA, SOD activity, membrane stability and ACC-oxidase activity in Si, ASA and their combination treatment

Si (mM)	ASA (mM)	Vase life (day)	Total chlorophyll (SPAD reading)	ACC oxidase activity (nmol h mL^{-1})	Anthocyanin leakage (absorption at 525 nm)	MDA (µmol/ mg protein)	Water uptake (mL per flower)	Colony count (CFU mL^{-1})	SOD (U/g protein)
0	0.0	05	2.00	22.14	312.10	176.20	100	678	062
	1.5	08	6.30	12.14	130.11	131.87	110	200	121
	3.0	03	1.40	60.39	300.00	160.70	060	165	059
1.5	0.0	07	2.70	13.18	100.14	132.01	105	206	115
	1.5	10	3.30	12.00	94.00	120.00	100	152	120
	3.0	06	0.99	20.14	119.39	136.89	65.5	150	080
2.5	0.0	13	4.11	11.00	90.05	100.03	125	150	100
	1.5	17	7.05	07.11	64.11	52.11	165	100	189
	3.0	08	2.11	20.45	100.00	101.30	085	140	078
3.5	0.0	06	1.89	40.11	294.10	121.30	100	130	083
	1.5	06	1.00	29.11	117.00	122.00	100	160	090
	3.0	05	1.00	34.00	309.14	119.00	100	167	060
F-test probabilities									
	Si	0	0	0	0	0	0	0	0
	SA	0	0	0	0	0	0	0	0

acid improved membrane permeability by increasing SOD activity and decrease accumulation MDA in compared to control. This indicates that with silicon and acetylsalicylic acid concentration increased, the SOD activity was decreased. Similarity, Reezi *et al*. (2009) showed that Si could extend the vase life of Rose cut flowers by decreasing ROS, malondialdehyde content and ethylene.

Vase life: The vase life varied among cut flowers and ranged between 3 and 17 days (Table 1). The vase life was longer in 2.5 silicon+1.5 mM acetylsalicylic acid compared to other treatments and control ($p = 0.05$). The percent wilting increased with the increase in concentrations of silicon and acetylsalicylic acid. Fan *et al*. (2008) showed that the treatment of salicylic acid extended the vase life and improved flower quality with reduced respiration rate delay senescence and decrease Lipid per oxidation, MDA content. Jamali and Rahemi (2011) reported that treatment with silicon significantly extends the vase life carnation similarity, Kazemi *et al*. (2011a) reported that treatment with SA acid significantly extends the vase life.

CONCLUSIONS

In the present study, we found that use of disinfectants improve water conductance by preventing bacterial growth and producing occlusions. Result showed that treatment with Si and ASA extends the vase life of cut carnation flowers. Also, Si and ASA reduced chlorophyll total degradation and preserved chlorophyll total content. These findings are similar to previous results.

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