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Effect of Some Chemical Treatments on Keeping Quality and Vase Life of Chrysanthemum Cut Flowers

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Abstract: The experiment was carried out to investigate the effect of different concentrations of salicylic acid (SA), malic acid (MA), citric acid (CA) and sucrose (Suc) on keeping quality and vase life of Chrysanthemum cut flowers. In this study three levels of malic acid (0, 100 and 150 mg l^{-}) and salicylic acid (0, 1.5 and 3 mM) and two levels of sucrose (0 and 3% w/v) and of citric acid (0 and 150 mg l^{-1}) were applied in a factorial arrangement, carried out in a complete randomized design on 144 Chrysanthemum cut flowers in horticulture laboratory of agriculture faculty of Islamic Azad University, Karaj branch. The recorded traits included Vase life, total chlorophyll content (SPAD reading), anthocyanin leakage, malondialdehyde (MDA) content, ACC-Oxidase activity and water absorption. The results showed that malic acid and salicylic acid treatments increased cut flower water absorption, fresh weight and vase life, while descreased the MDA content, ACC-oxidase activity and membrane premeability together with total delay of senescence and peroxidation of lipids. Maximum flower vase life was recorded in 150 mg l^{-1} MA +1.5 mM SA + 3% Suc treatments. A direct relationship between vase life and, increasing of fresh weight and water uptake was observed as well.

Abbreviations: SA, Salicylic acid; MA, malic acid; MDA, malondialdehyde; ROS, reactive oxygene species; ACO, ACC-Oxidase activity

Key words: Vase life · Chrysanthemum · Chemical treatments · Cut flowers

INTRODUCTION

Flowers are extremely perishable; maintaining their physiological functions vary actively even after harvest and the beginning of their senescence very often depends on ethylene. Longevity of vase life is an important factor in consumer preference and considerable research has been carried out on the causes of cut flowers senescence [1,2]. Senescence of cut flowers is induced by several factors, e.g., water stress [3], carbohydrate depletion [4], micro-organisms [5], and ethylene effects. Ethylene enhanced flower senescence and wilting, increased permeability of petal cells and accelerated the decrease in cell membrane fluidity. Ethylene production causes a sharp increase in production of oxygen free radicals (ROS) which is responsible for stress dependent peroxidation of membrane lipids [6]. One effect of ROS accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage [7,8]. To scavenge ROS, plants posses specific mechanisms, which include activation of antioxidant enzymes [9] and non enzymatic antioxidants such as, carotenoids, phenols component and ascorbic acid [10]. SA is a well known phenol that can prevent ACC-oxidase activity that is the direct precursor of ethylene and decrease ROS with increase enzyme antioxidant activity. SA is considered as a hormone-like substance, which plays an important role in regulating a number of physiological processes and provide protection against biotic and abiotic stresses in plant. The protective function of SA includes the regulation of ROS and antioxidant enzymes [11,12]. MA is a well known organic acid that can reduced the number of bacterial in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components and sensitivity [13]. Citric acid seems to act

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by reducing the pH of water and, consequently, the proliferation of bacterial, which block the xylem vessels in the cut region and interfere with the normal flux of water through the stem [14]. Other important factor in the deterioration of cut flowers involves the diminishing of respiration substrates, the speed of these changes depend, at least in part, on the amount of reserves that are present in the flower when they are cut [15]. Therefore, an exogenous carbohydrate supplementation would be enough to delay the senescence, considering that the main effect would be to maintain the structure and activity of the mitochondria [16,17]. The aim of this work was to study the responses Chrysanthemum to the interactive effects of salicylic acid, malic acid, citric acid and sucrose.

Plant Material and Storage Conditions: Chrysanthemum (*Chrysanthemum morifolium* L. cv. yellow) were obtained from local commercial greenhouses (Pakdasht, Tehran, Iran). Following harvest and transport to the laboratory, the stems were recut to 40 cm length. In this study three levels of malic acid [MA (0,100 and 150 mg l^{-1})] and Salicylic acid [SA (0, 1.5 and 3 mM)] and two levels of sucrose [Suc (0 and 3% w/v)] and citric acid [CA (0 and 150 mg l^{-1})] were applied on 144 Chrysanthemum cut flowers at horticulture laboratory of agriculture faculty of Islamic Azad University, Karaj Branch. After recording the fresh weight, each flower was placed in a 250 ml bottle containing preservative solutions. The flowers were held at ambient temperature (19 ±5 ? and 85 % RH). The experiment was started on February 15 /2010 and chlorophyll content, membrane stability, MDA content and ACC-oxidase activity (ACO) were measured at 11th day of vase life.

Vase Life: Vase life was determined as the number of days to wilting of flowers.

Chlorophyll Content Measurement: Total chlorophyll content 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 leaves was considered.

Determination of Anthocyanin Leakage: Anthocyanin leakage was measured based on the method of Poovaiah [18].

Determination of Acc-oxidase Activity: ACC-oxidase activity was assayed by measuring to the method described by Moya-Leòn and John [19].

Assays of MDA Content (Lipid Peroxidation): Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according to Heath and Packer [20].

Microbe Population: Test microbe population were isolated from vase solutions of Chrysanthemums. 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 microorganisms, 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.

Water Uptake and Weight Fresh: 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.

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 analysis in the same software (p= 0.05).

RESULTS AND DISCUSSION

Vase Life: Vase life of Chrysanthemum flowers treated with CA was not different from the control while those treated with MA+ SA + Suc had longer vase life, particularly the solution containing 150 mg^{-1} MA+ 1.5 mM SA + 3% Suc gave the longest vase life of 11 days (Table 1). The minimum vase life was noted in 3 mM SA and 150 mg^{-1} MA+ 3 mM SA treatments compared to control. Adding SA was found to be negativly correlated with vase life of the Chrysanthemum cut flower (Table 2). This indicates that with SA concentration increased, the vase life was decreased. Kazemi *et al.,* [13] showed that the treatment MA treatment extended the vase life and improved flower quality with reduced ACC-oxidase ativity

Table 1: Mean comparisons of chlorophyll content, Vase life, MDA, SOD activity, Microbe population, Membrane stability and ACC Oxidase activity in MA, SA, Citric acid, Sucrose and Interaction between their

MA (mg^{-1})	SA (mM)	Suc (w/v)	Vase life (day)	Total chlorophyll content	ACO (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Microbe population (cfu)	Water uptake(ml)
0	0	0	7.08C	2.07B	27.1C	320.33AB	100.73C	30.83AB	20C
	1.5	0	9.72B	3.13A	20.25B	152.33B	73.47B	24.83B	33B
	3	0	5.66AC	1.74C	38.88AC	346.83AC	127.02C	21.83B	15AB
100	0	0	7.667C	2.11B	24.2B	267.41C	99.86B	27.16C	30B
	1.5	0	8.75C	2.413B	24.63B	211C	83.45B	26.5C	31B
		3%	9B	2.25B	31.235AB	150.5B	78.5B	32AC	40B
	3	0	6.25AB	1.862C	31.202AB	300.5	119.88C	24.25B	25C
		3%	6AB	2B	31.655AB	239.5C	144.46AC	22B	29B
150	0	0	8.81C	3.44A	17.55A	111.5A	93.8B	22.58B	50A
	1.5	0	10.5A	4.058A	17.71A	107.75A	66.645A	19A	50A
		3%	11.188A	3.58A	18.23A	111.5A	66.265A	22B	55A
	3	0	5.75AC	2.014B	32.04AB	251.25C	125.135C	19.5A	25C
		3%	6.5AB	1.59C	35.63AB	293AB	134.82AB	22B	24C

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

* Data recorded on day 11of experiment

Table 2: Simple correlation lines between the MA, SA, Citric acid and sucrose treatments with other variables

treatment	Vase life(day)	Total chlorophyll content	ACC Oxidase Activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	Microbe population (cfu)	Water uptake (ml)
MA	0.336*	0.390*	-0.162	-.460**	-0.083	-0.288	0.783**
SA	-.513**	-0.321	.576**	0.285	.443**	-.435**	-0.055
Citric acid	0.017	-0.134	-0.081	-0.045	0.079	-0.071	0.279
sucrose	-0.122	-.384*	0.229	-0.047	.333*	.552**	-0.245

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

(ACO) and microbial population in vase solution of carnation cut flowers. Also, Mei-hua *et al.,* [21] showed that the SA and Suc treatmens extended the vase life and improved flower quality with reduced respiration rate delay senescence, decrease lipid per oxidation and MDA content. Our results showed that adding SA, MA and Suc extended the vase life and improved flower quality with reduced respiration rate, delay senescence, decrease lipid per oxidation and decrease the MDA content of the Chrysanthemum cut flower *Water uptake, fresh weight and microbe population.*

Results regarding the water uptake by the Chrysanthemum flowers show that maximum water uptake and fresh weight were in Chrysanthemum flowers that treated by 150 mg^{-1} MA+ 1.5 mM SA + 3% Suc and 150 mg^{-1} MA+ 1.5 mM SA, with no differences among these treatments (Table 1). The minimum water uptake and fresh weight was noted with 3 mM SA treatment compared to control. Adding MA was found to be positivly correlated with water uptake of the Chrysanthemum cut flower (Table 2).This indicates that with MA concentration increased the water uptake and fresh weight were increased. MA and SA affected on the microbial population in vase solution of Chrysanthemum cut flowers significantly, the microbial population decreased with the increase in concentrations of MA and SA. However, the lowest microbial concentration was evident when cut flowers were treated with 150mg^{-1} MA+ 1.5 mM SA (Table 1). Anjum *et al.,* [22] reported adding a suitable germicide in vase water can prevent the growth of microbes and increase water uptake. Kazemi *et al.,*[13] showed that the MA treatment reduced microbial population in vase solution and increased water uptake of carnation cut flowers. Also, the SA seems to act by germicide the decrease of microbial population. In contrast, the Suc in vase solution rapidly increased microbial population in vase solution of Chrysanthemum cut flowers.

Total Chlorophyll and MDA Content: MA, SA and Suc treatments significantly increased the total chlorophyll content to a larger extent when compared to control.

The maximum total chlorophyll content was noted with 150 mg^{-1} MA+ 1.5 mM SA + 3% Suc and 150 mg^{-1} MA+ 1.5 mM SA treatments compared to control. The lowest values of total chlorophyll content were noted with 3 mM SA and 150 mg^{-1} MA+ 3 mM SA + 3% Suc treatments as compared to the control. The MDA content was reduced by MA and significantly lower in preservative mixtures containing just MA when compared to the control and those containing Suc (Table1). The results also, indicate that the SA (3 mM) increased the lipid per oxidation, while, addition of 1.5 mM SA to 3% Suc and 150 mg l^{-1} MA significant decreased the MDA content as compared to control (Table 1). Also, our results showed that CA alone or plus the other chemicals did not cause an decrease in the MDA content of cut flower petals. A significant negatively correlation was observed between MA concentration and the MDA content of Chrysanthemum cut flowers (Table1).

Anthocyanin leakage and ACC-oxidase *activity(ACO)":* The results in Table (1) indicated that 150 mg l^{-1} MA caused a significant decrease in anthocyanin leakage and ACO compared to control. Addition of citric acid and sucrose alone either caused no positive effect or had negative effect as increasing anthocyanin leakage and ACO (Table 1). Addition of 3% sucrose and 1.5 mM SA to 150 mg l^{-1} MA significant decreased in anthocyanin leakage and ACO compared to control (Table 1). The results indicated that (150 mg l^{-1} MA plus 1.5 mM SA and 150 mg l^{-1} MA + 1.5 mM SA + 3 % Suc treatments improved membrane permeability by decrease ACO (Table 1). Highest means of ACO was found in cut flowers treated with 3 mM SA (Table 1). SA is a well known phenol that can prevent ACO that is the direct precursor of ethylene and decrease ROS with increase enzyme antioxidant activity. Mei-hua *et al.,* [21] showed that SA can extending the vase life of cut flowers with decrease ROS and ACO. SA acid with increases the enzyme antioxidant activity cause delay the onset of hydrolysis of structural cell components, decrease ROS production, ACO and sensitivity. SA acid decreased the permeability of plasma membrane of floret cells and improved the structure of chloroplasts which were badly damaged by ethylene. Also, Kazemi *et al.,*[13] showed that the treatment of MA reduced ACO in of carnation cut flowers and increased vase life in carnation cut flowers.This research was designed to investigate the role of SA and MA in alleviating membrane lipid per oxidation and MDA content in cut flowers of Chrysanthemum.

CONCLUSION

From the results of the present study, it can be concluded that SA and MA treatments significantly decrease bacterial populations in vase flower preservative solution, reduced MDA content, ACO and the membrane permeability and per oxidation of lipids. However, our results showed that SA, MA and Suc treatments maintained the vase life of flowers for a longer period.

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