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Regulation of chrysanthemum cut flower senescence using 5-sulfosalicylic acid and aluminium sulphate

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Abstract

Vase-life is a key factor for evaluating the post-harvest quality of cut flowers that determines their marketability. Chrysanthemum is one of the top most cut flowers sold in international flower markets. In the present study, trials were conducted to improve the post-harvest life of chrysanthemum cut flowers using preservative solutions such as 5-sulfosalicylic acid (5-SSA) and aluminium sulphate ($Al_2(SO_4)_3$) alone or in combination with 1.5 % sucrose. Treatments using 5-SSA (100 ppm and 150 ppm) or 200 ppm $Al_2(SO_4)_3$ along with 1.5 % sucrose showed a significant increase in vase-life, fresh weight of the cut stems, vase solution uptake, membrane stability index of the petals and leaf chlorophyll as compared to other treatments. Among different vase solutions evaluated, T10 (200 ppm $Al_2(SO_4)_3$ +1.5 % sucrose) gave maximum vase life of22.3 days, followed by T8 (5-SSA 150 ppm + 1.5 % sucrose) and T6 (100 ppm 5-SSA+ 1.5 % sucrose) treatments with 20.85 and 19.85 days respectively as compared to 17.84 days in control. High concentrations of both the chemicals (5-SSA and $Al_2(SO_4)_3$) without sucrose showed toxicity symptoms.

Key words: Chrysanthemum, senescence, vase life, aluminium sulphate, 5-sulfosalicylic acid

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the important cut flower crops probably next to rose in the world flower market. Cut flower longevity or vase-life is an important factor for evaluation of cut flower quality in both domestic as well as international markets. Addition of chemical preservatives to the holding solutions is recommended to improve the vase-life in several cut flowers. Most of these preservatives consist of carbohydrates, germicides, ethylene inhibitors, growth regulators and mineral compounds (Nowak and Rudniciki, 1990). These preservatives were used in pulsing or holding solutions to improve the longevity of the cut stems.

In cut stems there is a problem of depleting carbohydrate levels due to cessation of photosynthesis which can be alleviated by exogenous application of sucrose. Added sucrose acts as an additional respiratory substrate that helps in prolonging the vase-life of flowers. Sucrose also reduces the moisture stress by maintaining the water balance in cut flowers (Gowda and Gowda, 1990). Merwe *et al.* (1986) reported increase in vase-life and visual quality of gladiolus cut flowers with the addition of sucrose to vase solution. Vase solution containing 4 % sucrose increased the water uptake and fresh weight of the gladiolus spikes when compared to control (Murali *et al.*, 1991). Application of 25 ppm sucrose enhanced the longevity of cut roses by 8.2 and 7.5 days respectively in 'Whish Mc' and 'Trika' rose cultivars compared to 5.3 days in control (Butt, 2005). In case of cut spray carnations, 5 % sucrose was found to be effective in increasing vase-life and detaining the climacteric ethylene production in petals (Pun *et al.*, 2005).

5-Sulfosalysilic acid (5-SSA) serves as a donor compound for salicylic acid (SA) production, which is a signalling molecule and considered as a hormone like substance in plants. SA plays a vital role in regulating number of physiological processes and provides protection against biotic and abiotic stresses (Promyou *et al.*, 2012). 5-SSA acts as an effective anti-microbial agent that reduces the ACC-oxidase activity, anthocyanin leakage and increased vase-life in carnations (Leslie and Romani, 1986; Kazemi and Ameri, 2012). Vase solution containing 5-SSA enhanced the tube rose flower longevity by reducing lipid peroxidation and electrolyte leakage from the cut stems (Nasibi *et al.*, 2014).

In vase solutions, aluminium sulphate ($Al_2(SO_4)_3$) has been recommended for maintaining the longevity of several cut flowers as well as foliage (De Stigter, 1981; Van Doorn, 1997; Ichimura and Ueyama, 1998). Aluminium sulphate acts as an anti-microbial agent in vase solutions (Halevy and Mayak, 1981; Jowkar *et al.*, 2012) and helps to retain moisture content in the cut stems by reducing the transpirational losses through stomatal regulation (Ichimura and Ueyama, 1998). $Al_2(SO_4)_3$ treated tuberose flowers attained a better quality than controlled ones (De Stigter, 1981). Reid (1989) noticed the bactericidal properties of aluminium sulphate in *Lisianthus* cut flowers. Application of $Al_2(SO_4)_3$ helps to retain the fresh weight of *Eustoma* cut flowers even after 8th day of its vase-life treatment. Inhibition of transpirational losses in cut flowers was also reported with aluminium sulphate (Liao *et al.*, 2001). In roses, $Al_2(SO_4)_3$ treatment enhanced the vase-life and improved the post-harvest visual quality of cut stems by retaining freshness in leaves (Jowkar *et al.*, 2012). The aim of the present work was to study the response of cut chrysanthemums

to vase solutions containing 5-sulphosalicylic acid, aluminium sulphate and sucrose.

Materials and Methods

Plant material and storage conditions: Fresh cut flowers (*Chrysanthemum morifolium* Ramat *cv*. 'Thai Chen Queen') were harvested in the morning from naturally ventilated polyhouse, Division of Floriculture and Landscaping, IARI, New Delhi. Harvested flowers were immediately transported to laboratory in a bucket containing water. Before placing the flowers in vase solution, stems were cut in a slanting manner to a uniform length of 25 cm. All the leaves were removed except a few below the inflorescence. Flowers were placed in 250 mL conical flasks containing 200 mL vase solution. The conical flasks were maintained in the laboratory at a room temperature of 18±2 ℃, relative humidity 70±5 % under continuous illumination of florescent light.

Vase-life treatments: Total of 14 treatments were planned using 1.5 % sucrose, three levels each of 5-sulpho salicylic acid (75 ppm, 100 ppm and 150 ppm) and aluminium sulphate (200 ppm, 300 ppm and 400 ppm).

Details of chemical treatments are as follows:

T1= Control (Distilled water),
T2= Sucrose (1.5 %),
T3= 5-Sulphosalicylic acid (5-SSA) (75 ppm),
T4= 5-SSA (75 ppm) +Sucrose (1.5 %),
T5=5-SSA (100 ppm),
T6=5-SSA (100 ppm) +Sucrose (1.5 %),
T7=5-SSA (150 ppm),
T8=5-SSA (150 ppm) +Sucrose (1.5 %),
T9=$Al_2(SO_4)_3 \cdot 16H_2O$ (200 ppm),
T10=$Al_2(SO_4)_3 \cdot 16H_2O$ (200 ppm) +Sucrose (1.5 %),
T11=$Al_2(SO_4)_3 \cdot 16H_2O$ (300 ppm),
T12=$Al_2(SO_4)_3 \cdot 16H_2O$ (300 ppm) +Sucrose (1.5 %),
T13=$Al_2(SO_4)_3 \cdot 16H_2O$ (400 ppm),
T14=$Al_2(SO_4)_3 \cdot 16H_2O$ (400 ppm) +Sucrose (1.5 %).

Vase-life determination (d): Wilting of flower petals and leaves were used as the measure for determining chrysanthemum cut flower vase-life. Visual ratings of leaf and flower senescence were assessed periodically during the vase-life period. Chrysanthemum senescence evaluation was based on a scale ranging from one to four when,

I = completely green leaves and good flowers,
II = initiation of wilting in 25 % of leaves and petals,
III = wilting in 25-50 % of leaves and petals,
IV = wilting in 50-100 % of leaves as well as inflorescence.

The longevity of chrysanthemum cut flowers was determined finally as the number of vase-life days required for 50 % of the flowers to reach stage two or later stages (El-Rahman, 2005).

Vase solution uptake (mL) and fresh weight of the flowers (g): Flower stems were placed in 250 mL conical flask containing 200 mL of vase solution. The amount of solution absorbed by cut flowers was calculated by finding the difference in the amount of water evaporated from a control conical flask without cut flowers and that containing flowers. Fresh weight of the cut flowers was measured on 1st, 3rd, 7th, 14th and 20th days of vase-life.

Membrane stability index (MSI): Leakage of ions from the chrysanthemum petals was measured based on the method standardized by Bailey *et al.* (1996). MSI was measured on 1st, 7th and 14th days of vase-life period.

Estimation of leaf chlorophyll (mgg^{-1} FW): Total leaf chlorophyll content was measured by dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979). Samples were collected from the upper most leaves of flowering stems.

Statistical analysis: Experiment was arranged in completely randomized design (CRD) with four replications. Analysis of variance (ANOVA) was calculated using OPSTAT software.

Results and discussion

Vase-Life: Vase-life of chrysanthemum flowers treated with a combination of $Al_2(SO_4)_3$ (200 ppm) or 5-sulphosalicylic acid (75 ppm, 100 ppm, 150 ppm) along with 1.5 % sucrose exhibited better vase-life when compared to sole use of these chemicals. Vase solution uptake, stem fresh weight, flower diameter, membrane integrity and cut stem vase-life were found to be better in treatments which contained both sucrose and preservative chemicals. In the present study sucrose might have synergized the effect of preservative chemicals by increasing their osmotic concentration, thereby improving water absorbing ability and turgidity levels in cut stems. Sucrose also maintains the extra carbohydrate levels and helps to sustain high respiratory rate in cut stems (Kuiper *et al.*, 1995). The treatment (T10) with 200 ppm ($Al_2(SO_4)_3$ and 1.5 % sucrose displayed a maximum vase-life of 22.3 days followed by treatment T8 (5-SSA 150 ppm +1.5 % sucrose) and T6 (5-SSA 100 ppm +1.5 % sucrose) with 20.85 days and 19.85 days respectively in comparison with 17.84 days in control. In a similar way 4 % sucrose along with 200 ppm $Al_2(SO_4)_3$ gave better result in *Eustoma* cut flowers than using $Al_2(SO_4)_3$ alone in vase solutions (Liao *et al.*, 2001). Increased water uptake and shelf life period of *Schefflera arboricola* was observed with 200 ppm $Al_2(SO_4)_3$ + 4 % sucrose (El-Quesni *et al.*, 2012). Similarly, 5-SSA (2 mM) was also effective in extending vase-life for lilies with the addition of 2.5 % sucrose (Kazemi *et al.*, 2012). Lio *et al.* (2001) and Singh *et al.* (2007) reported maximum vase-life in *Eustoma* cut flowers and standard carnations with the application of 200 ppm and 150 ppm $Al_2(SO_4)_3$. Higher concentrations of $Al_2(SO_4)_3$ (300 ppm and 400 ppm) and 5-SSA (100 ppm and 150 ppm) in vase solutions resulted in development of toxicity symptoms on leaves and flowers resulting in decreased vase-life. As the chemical concentration increases, sudden decrease in chlorophyll, carotenoid contents and fresh weight of the cut stems were observed along with more leakage of ions from the membrane. This might be a reason for expression of rapid senescence in flowering stems with higher concentrations of $Al_2(SO_4)_3$ and 5-SSA. Similar kind of negative results were observed by Zamani *et al.* (2011) with increasing concentrations of SA in cut chrysanthemums. Higher concentrations of $Al_2(SO_4)_3$ decreased chlorophyll content, fresh weight of the stems and vase-life in rose cultivar 'Cherry Brandy' (Jowkar *et al.*, 2012).

Total vase solution uptake and changes in fresh weight of cut stems: Higher vase solution uptake and fresh weight retention of the stems were observed in treatments T10 and T8. Minimum vase solution uptake and fresh weight retention was noticed with treatments T7, T11 and T13 compared to control (Table1). In most of the treatments fresh weights of the stems increased up to 7th day followed by slight decrease from 14th day of vase-life contrary to T10 and T12 treatments where increase in fresh weight was noticed even up to 14th day. Positive correlation was

observed between sucrose addition and vase solution uptake in cut stems. Sucrose helped to improve the vase-life of flowers by maintaining water balance in cut stems (Gowda and Gowda, 1990). Increased fresh weight and water uptake in gladiolus spikes were observed with the addition of 4 % sucrose to the vase solution (Murali *et al.*, 1991). Treatments (T10, T12 and T14) which contained $Al_2(SO_4)_3$ showed more moisture retention rates as compared to treatments with 5-SSA (Table1). $Al_2(SO_4)_3$ helped to retain the moisture content in the cut flowers by reducing the transpirational losses through stomatal regulation (Ichimura and Ueyama, 1998). Ichimura *et al.* (2006) also reported an increasing fresh weight of the *Eustoma* cut flowers even after 8th day of its vase treatment. Jowkar *et al.* (2012) observed that treatment with $Al_2(SO4)_3$ improved the visual quality of cut roses and the freshness of the leaves were retained even at the end of its vase-life.

Final flower diameter: Chrysanthemum flowers were initially collected and kept in vase solution at petal unfurling stage. Diameter of the flowers increased as further whorls opened up during the vase-life. Flower opening in most of the cut flowers depends upon the presence of food reserves and type of vase solution used (Van Doorn *et al.*, 1991). In our experiment, flower opening was measured by taking flower diameter reading on 14th day of its vase-life. Maximum diameter of 10.52 cm was observed in T10 (($Al_2(SO_4)_3$ 200 ppm +Sucrose 1.5 %) followed by T8 (5-SSA 150 ppm +Sucrose 1.5 %) and T6 (5-SSA 100 ppm +Sucrose 1.5 %) with 9.72 cm and 8.93 cm respectively as compared with 8.69 cm in control. Addition of sucrose to the vase-solutions positively correlated with the flower opening in chrysanthemums. Sucrose plays a major role in flower bud and inflorescence development as well as in improving the post-harvest quality of *Liatris* cut flowers (Han, 1992). Terek *et al.* (2010) reported a greater increase in diameter of the carnations with the addition of sucrose to vase solution. External addition of carbohydrate to the vase solution improved the petal colour, helped in bud opening and strengthening of pedicels as well as increasing overall inflorescence longevity up to 8 days in *Eustoma grandiflorum* cut flowers (Cho *et al.*, 2001). Maximum flower diameter and vase-life was observed in chrysanthemum flowers kept in vase solution containing $Al_2(SO_4)_3$ in combinations with sucrose and citric acid (Amin, 2017). From the above study we can infer that, respiratory substrate and water balance are main factors responsible for inflorescence opening in chrysanthemum cut flowers.

Membrane stability index (MSI): MSI values of the chrysanthemum flower petals were observed on 1st, 7th and 14th day of its vase-life. MSI values decreased with increasing vase-life of chrysanthemums (Table 1). In both the treatments T10 and T8, MSI value decreased by 11.4 % over initial value followed by T6 and T9

Table 1. Treatments and their effect on Chrysanthemum cut flowers vase life parameters

Treatments	Vase life (Days)	Vase solution uptake (ml)	Final flower diameter (cm)	Membrane Stability Index MSI (µs)			Chlorophyll (mg/g FW)			Changes in mean fresh weight of cut stems (g)				
				Day 1	Day 7	Day 14	Day1	Day7	Day14	Day1	Day3	Day7	Day14	Day20
T_1	17.84	38.95	8.69	68.74	67.64	57.93	1.538	1.412	1.259	21.11	23.58	23.84	23.74	16.74
T_2	18.76	40.02	8.73	68.23	65.14	57.58	1.559	1.364	1.33	19.22	21.17	22.61	21.83	17.63
T_3	18.50	39.4	8.61	69.21	68.14	58.35	1.513	1.351	1.273	19.98	20.87	22.5	21.68	17.63
T_4	18.90	42.49	8.83	71.59	68.44	60.88	1.351	1.614	1.203	17.41	19.47	21.47	20.81	16.73
T_5	17.18	34.94	8.51	67.75	64.88	55.29	1.647	1.533	1.179	19.75	20.43	21.69	19.66	14.91
T_6	19.85	43.17	8.93	69.08	66.46	60.42	1.453	1.483	1.399	18.61	20.06	21.9	22.17	18.7
T_8	20.85	49.2	9.72	68.01	66.11	60.05	1.342	1.633	1.319	19.47	21.74	24.24	23.96	19.95
T_9	19.53	40.5	8.87	67.16	66.74	57.66	1.425	1.457	1.334	21.84	23.97	25.59	24.11	21.15
T_{10}	22.30	44.49	10.52	72.54	70.65	64.24	1.364	1.425	1.395	21.97	24.25	25.39	25.49	24.52
T_{11}	16.15	34.45	7.89	68.91	65.87	56.02	1.363	1.575	0.741	25.67	26.73	26.68	24.6	18.95
T_{12}	17.77	38.86	8.51	69.3	68.18	58.21	1.546	1.605	1.241	20.84	23.65	23.8	23.83	22.01
T_{13}	15.61	33.08	7.83	70.51	65.95	54.19	1.63	1.579	0.406	19.53	21.17	21.89	19.28	13.48
$T1_4$	17.45	35.2	8.37	68.39	64.78	56.3	1.571	1.365	1.246	18.64	20.42	21.3	20.8	19.22
SEm±	2.08	0.72	0.33	2.87	1.39	0.99	0.046	0.044	0.043	0.3	0.35	0.37	0.25	0.29
CD at 5 %	0.714	2.09	0.971	NS	NS	2.88	0.134	0.128	0.124	0.87	1.02	1.06	0.73	0.85

Treatments: T_1 = Control (distilled water), T_2 = Sucrose (1.5 %), T_3 = 5-Sulphosalicylic acid (75 ppm), T_4 = 5-Sulphosalicylic acid (75 ppm) +sucrose(1.5 %), T_5 = 5-Sulphosalicylic acid (100 ppm), T_6 = 5-Sulphosalicylic acid (100 ppm) +Sucrose (1.5 %), T_7 = 5-Sulphosalicylic acid (150 ppm), T_8 = 5-Sulphosalicylic acid (150 ppm) + Sucrose (1.5 %), T_9 = $Al_2(SO_4)_3$ 16 H_2O (200 ppm), T_{10} = $Al_2(SO_4)_3$ 16 H_2O (200 ppm) + Sucrose (1.5 %) , T_{11} = $Al_2(SO_4)_3$ 16 H_2O (300 ppm), T_{12} = $Al_2(SO_4)_3$ 16 H_2O (300 ppm) +Sucrose (1.5 %), T_{13} = $Al_2(SO_4)_3$ 16 H_2O (400 ppm) , T_{14} = $Al_2(SO_4)_3$ 16 H_2O (400 ppm) + Sucrose (1.5 %).

with 11.7 % and 12.53 % when compared to 15.72 % in control. A high MSI value of the petal tissue indicates, delayed petal senescence and extended longevity of cut flowers. Singh *et al.* (2007) reported higher MSI of gladiolus florets treated with 300 ppm hydroquinone + 5 % sucrose. Menon *et al.* (2012) observed higher membrane integrity of gladiolus spikes in vase solution containing 50 mgL^{-1} malic acid + 5 gL^{-1} sucrose. The vase solution containing GA_3 or BA along with sucrose significantly increased the MSI and improved the vase-life in gladiolus (Singh *et al.*, 2008). In the present study, addition of sucrose to the preservative chemicals might be helping in delaying senescence and retaining the membrane integrity in chrysanthemum cv. 'Thai Chen Queen'.

Changes in leaf chlorophyll: Total chlorophyll content was measured from leaf tissues during 1st, 7th and 14th days of cut flower vase-life. Minor fluctuations in the chlorophyll values were observed in different vase-life treatments of chrysanthemum cv. 'Thai Chen Queen'. In most of the treatments chlorophyll content increased up to 7th day followed by decrease in the value from 7th day to 14th day (Table 1). In treatment T10 chlorophyll levels remained high (2.2 %) over the initial value up to 4th day of its vase-life. Treatments T8 and T6 exhibited very less decline in the chlorophyll with 1.74 % and 3.85 % respectively as compared to control (22.16 %). Treatments which contain sucrose along with preservative chemicals showed minor decline in the chlorophyll content over initial values. A rapid decline in chlorophyll content was noticed at higher concentrations of $Al_2(SO_4)_3$ (300 ppm and 400 ppm) and 5-SSA (100 ppm and 150 ppm). In the previous studies it had been shown that leaf chlorophyll content decreased with the initiation of senescence (Tang *et al.*, 2005; Ferrante *et al.*, 2009; Guiboileau *et al.*, 2010). Senescence delay and chlorophyll retention had been achieved by using growth regulators as well as some preservative chemicals (Petridou *et al.*, 2001; Khan *et al.*, 2007; Ferrante *et al.*, 2009; Tiwari *et al.*, 2010). Jowker *et al.* (2012) reported an increase in chlorophyll content in cut rose flowers treated with $Al_2(SO_4)_3$. Degradation of chlorophyll content in the cut flowers was reduced by using $Al_2(SO_4)_3$ (Amin, 2017). 5-SSA was also effective in minimizing the loss of chlorophyll content and improving the post harvest life of flowers (Kazemi and Ameri, 2012). This could be attributed to its role in lowering the pH of petals and stabilizing the anthocyanins. From this study we could infer that use of vase chemicals, 5-SSA and $Al_2(SO_4)_3$ together with higher water retention induced by sucrose might be the reason for retention of chlorophyll levels in treated chrysanthemum cut flowers.

Among different treatments used for enhancing vase-life of chrysanthemum, T10 (($Al_2(SO_4)_3$ 200 ppm+ sucrose 1.5 %) was best in all the aforementioned parameters. Other treatments T8 (5-SSA 150 ppm + Sucrose 1.5 %) and T6 (5-SSA 100 ppm + Sucrose 1.5 %) were also effective in controlling the chrysanthemum cut flowers senescence.

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