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Salicylic and Citric Acid Treatments Improve the Vase Life of Cut Chrysanthemum Flowers

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ABSTRACT

Chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura cv. Pattriot) is one of the most important and marketable cut flowers in the world. However, a relatively limited vase life reduces its marketability. The aim of this study was to evaluate the efficacy of salicylic acid (SA) and citric acid (CA) in extending the vase life of chrysanthemum flowers. Therefore, a factorial experiment based on completely randomized design with SA at (0, 100, 200, 300 ppm) and CA at (0, 100, 200 ppm) with 3 replicates and 3 samples (individual flowers) for each replicate, was conducted. Applying SA and CA increased vase life, petal water content (%), initial fresh weight (%) and marketability, significantly. SA treatments increased leaf relative water content (RWC), petal water content (%) and initial fresh weight (%) by 49, 73 and 23 %, compared to the controls, respectively. The highest vase life (21.77 days) was observed for the treatments of SA (300 ppm). The significant increase (300%) in vase life is considered to be due to plant regulating and anti-stress properties of SA and CA. According to the results of this experiment, SA and CA as natural, cheap, safe and biodegradable compounds are suitable alternatives for conventional chemical treatments in order to prolong vase life of cut flowers of chrysanthemum. Commercialization of these compounds for optimum formulations needs further experiments.

Keywords: Bio compounds (chemicals), CA, Chrysanthemum cut flowers, Preservation effect, SA, Vase life,

INTRODUCTION

Cut flowers are precious products of horticulture. Maintaining good quality of cut flowers and extending the vase life, are considered important and practical for having acceptable products for the markets. For this reason, a considerable number of studies have been undertaken for this purpose. (Redman, *et al*, 2002; Macnish *et al*, 2008 and Solgi *et al*, 2009, Zencirkiran, 2005; Zencirkiran, 2010). Vase life of cut flowers is mainly affected by two main factors, namely ethylene which accelerates the senescence of many flowers and microorganisms which cause vascular blockage and thus reduce the vase life of cut flowers (Van Doorn, 1994, Zencirkiran, 2005; Zencirkiran, 2010). A floral preservative usually is a complex mixture of sucrose (sugar), acidifier, an inhibitor of microorganisms and also an ethylene action or synthesis inhibitor like STS and SA (Marry, 2000).

Chrysanthemum is ranked as the second most economically important cut flower in the world, after rose (Kafi and Ghahsareh, 2009). However, it has a relatively short vase life and finding methods to increase flower longevity is of great importance. Using vase preservatives in vase solutions is one of the most common methods for prolonging cut flowers' vase life.

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SA is considered to be a potent plant hormone because of its diverse regulatory roles in plant metabolism (Popova *et al*., 1997). It was first extracted from willow trees, and named after the Latin word "Salix" by Rafacle Piria in 1938. SA has been found to play a key role in the regulation of plant growth, development and in responses to environmental stresses (Hayat *et., al*, 2009). Further, its role is evident in ion uptake and transport (Harper and Balke, 1981), photosynthetic rate, stomatal conductance and transpiration (Khan *et al*., 2003). SA is considered to be an important signaling molecule which is involved in local and endemic disease resistance in plants in response to various pathogenic attacks (Enyedi *et al*., 1992; Alverez, 2000). Besides providing disease resistance to the plants, SA can modulate plant responses to a wide range of oxidative stresses (Shirasu *et al*., 1997). SA was also found to suppress ACC synthase and ACC oxidase activities and biosynthesis of ethylene, and hence retarded the climacteric rise in ethylene production, in kiwi fruit (Zheng, 2002). SA has been shown to interfere with the biosynthesis and/ or action of ethylene, abscisic acid and cytokinins in plants (Hayat *et., al*, 2009). SA and its derivative, acetyl salicylic acid (ASA) have been reported to inhibit ethylene production in pear (Leslie and Romani, 1988), banana (Srivastava, 2000) and carrot cell suspension cultures, suggesting the role of SA as an antagonist to ethylene action. In another experiment, the effects of SA and sucrose on cut roses were investigated and the results showed a significant decrease in respiration rate, alleviation of the moisture stress and improved decorative quality of cut flowers, improving the vase life (Li *et al*., 2004). Also, the upward gravitropic bending of snapdragon was inhibited using SA (Friedman *et al*, 2003). SA reduced pH of water and consequently, the proliferation of bacteria was reduced (Guy *et al*., 2003).

CA is a widespread organic acid in the plant kingdom and makes a weak acid in water. CA is used to adjust water pH and to control the growth of microorganisms. CA is commercially advised for a number of cut flowers like chrysanthemum (Dole and Wilkins, 1998). Also, CA reduces the risk of vascular blockage in cut flowers through its anti- embolism trait. (Bhattacharjee *et al.*, 1993).

This experiment was designed to investigate the effects of SA and CA as components of the vase solution on the vase life of cut chrysanthemum flowers.

MATERIALS AND METHODS

Plant Material

Chrysanthemum (*D. grandiflorum* (Ramat.) Kitamura cv. 'Pattriot') flowers were supplied from a local grower. Plants were grown in under standard greenhouse conditions with 22 and 16 °C day and night temperatures, respectively. They were harvested in the early morning when outer petals were fully extended (Kafi and Ghahsareh, 2009). Cut flowers were trimmed to 30 cm and were placed in prepared solutions.

SA, CA and GCF (Giant Cut Flower liquid food) Commercial Preservative Treatments

SA and CA are soluble in distilled water and are easily dissolved at lower concentrations. Four levels of SA namely; 0, 100, 200, 300 ppm and three levels of CA at concentrations of 0, 100, 200 ppm were used in combination. A 300 ml preservative solution was used for each replication and cut flowers were placed in the solutions after cutting to 30 cm lengths. Sucrose at 4% was added in all treatments as a base solution.

GCF is one of the common preservative liquids commercially used in Iran and is available in flower markets. This product is made in the Netherlands and is exported to Iran in 60 ml dark bottles with unknown constituents. According to the instructions

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on the bottle, 35-30 drops were added per ½ liter water. GCF solution was also used beside SA and CA treatments in this experiment.

Leaf Relative Water Content (RWC), Petal Water Content (%WP) and Water Content of Cut Flower (%LWC)

Leaf relative water content (RWC) from each sample was determined according to the method described by Barros and Virtly (1962). To measure the RWC, two excised leaves per plant were weighed (fresh weight, FW) and placed in water in the dark with their petioles plunged in distilled water for 6 hours to allow them to reach full turgidity and, hence, to determine their turgid weight (TW). These leaves were then dried at 70 °C for 24 h and their dry weight (DW) was recorded. Finally, RWC was calculated using the below equation:

% RWC= (FW-DW)/ (TW-DW) × 100

In the next stage and on the 12th day from the start of the experiment, 1g of petals from all replications and each sample was taken as FW and then dried at 70 °C for 24 h and the DW was recorded. Petal water content (% WP) was then determined with the below equation (Kalate jari *et., al,* 2008):

%WP= FW-DW/DW × 100

Span and Stomatal Conductance

Leaf chlorophyll related SPAD units and leaf stomatal conductance (L. poro.) were determined using a SPAD-502 Chlorophyll Meter (Konica, Minolta, Tokyo) and a steady state diffusion porometer (SC-1) on day 12 of the experiment, respectively.

Solution Uptake

The opening of each vase was covered in order to limit vase solution evaporative loss and to allow determination of the uptake of the different preservatives used by stems. Weight reduction or in other words water uptake of vases and relative fresh weight of cut flowers were measured every day through the experiment from day 1 to 10 (Karimi *et., al*. 2008).

Vase Life and Marketability

Vase life of cut flowers was assessed in a controlled environment maintained at 21±1°C, 60% RH, and under continuous cool white fluorescent lighting with an intensity of 47 μ mol m^{-2} s^{-1}. The vase life of flowers was terminated when moderate wilting of flower head was observed (Macnish *et., al,* 2008). Marketability is a qualitative trait for cut flowers and is defined to say how many days cut flowers can be kept and are suitable for consumers. The marketability of cut flowers is limited by their short display life and frequent failure to open fully and in this experiment it was determined according to Macnish *et., al,* 2010.

Weight of Initial (%)

Fresh weights of cut flowers were measured every day. In order to determine the weight of initial (%), fresh weight (flowers+ leafy stems) determinations were made just before the immersion in the test solutions and were repeated to day 9 (Petridou *et., al,* 2001). By this time some flowers like control treatments were out of vase life. FW10/FW1 ratio was also measured and calculated.

Experiment Design and Data Analysis

This experiment was conducted as a factorial experiment based on completely randomized design with SA at (0, 100, 200, 300 ppm) and CA at (0, 100, 200 ppm) with 3 replicates and 3 samples (individual flowers) for each replication. Data were analyzed as factorial ANOVAs using JMP4.

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Where significant ($P \leq 0.05$) treatment effects were determined by ANOVA, data means were separated by the LSD test.

RESULTS

Vase Life and Marketability

According to the results shown in Table 1, using SA and/or CA and their interactions as preservatives, significantly increased the vase life of chrysanthemum cut flowers, over control (P≤1% and P≤5% respectively). The highest and lowest vase lives (21.77 and 6.88 days) was observed in solutions containing SA 300 ppm and the control, respectively (Figure 1). Marketability (display life and frequent failure to open fully) showed a similar response (Table 1).

Weight of Initial (%) and FW10/FW1

Effect of SA was highly significant on this trait (P≤1%) and the SA 300 ppm which had the highest effect among other treatments, even increased the fresh weight by (4.19%) in day 9 of the experiment, while a noticeable reduction (-19.71%) was observed in the control (Table 1 and Figure 2-a). Effect of CA was also significant (P≤5%), but the interaction levels were not significant (Table 1). The greatest increase (1.4%) in CA treatments was observed at 200 ppm (Figure 2-b). The rate of weight reduction between days 1 to 10 was significantly different in treatments used compared to the control (Figure 2a and b). FW10/FW1 was also significant for SA application (P≤1%) (Table 1).

Petal Water Content (%)

Results from petal water content (%) measurement show a significant difference in SA treatment over the control (P≤5%). SA treatments with 34% increase on average compared to the control and no significant difference between its levels, showed the highest petal water content (%) in applied

Table 1. Effects of different concentrations of salicylic and citric acid on measured traits.

	W. of ini (%) [a]	VL (Day) [b]	SPD [c]	WP (%) [d]	FW_{10}: FW_1 [e]	RWC [f]	L.poro ($mmol.m^2/s$) [g]	M. ability (Day) [h]
SA								
0 ppm	-19.71c	10.32c	44.82a	43.29b	0.79b	23c	9.75a	8c
100 ppm	3.27a	17.92b	45.34a	58.74a	1.03a	37.38bc	7.24a	15.44b
200 ppm	0.15b	18.55b	48.59a	58.17a	1a	46.42bc	7.63a	16.1b
300 ppm	4.19a	21.59a	46.2a	58.02a	1.04a	34.7c	9.83a	19.14a
CA								
0 ppm	-8.44c	15.27c	47.37a	53.04a	0.92b	32.27a	6.54a	12.97b
100 ppm	-2.03b	17.05b	47.57a	53.42a	0.96ab	41.08a	9.49a	14.55b
200 ppm	1.4a	18.97a	45.78a	57.2a	1.01a	32.77a	9.8a	16.5a
SA	**	**	ns	*	**	**	ns	**
CA	*	**	ns	ns	ns	ns	ns	**
SA*CA	ns	*	ns	ns	ns	ns	ns	*
GCF	-5.15d	11.11d	42.26b	40.36b	0.72c	28.55b	9b	14.06c

ns, not significant; *, ** indicate significance at P < 0.05, 0.01, respectively

[a] Weight of initial (%), [b] Vase life, [c] PAD, [d] Petal water content (%), [e] fresh weight on day 10 fresh weight on day 1 ratio, [f] Leaf relative water content, [g] Stomatal conductance and [h] Marketability.

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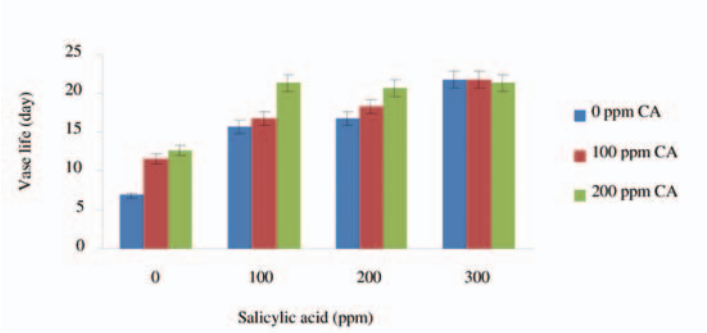

Figure 1. Interaction effect of salicylic and citric acid on extending vase life of cut chrysanthemum flowers. Bars indicate ± standard error.

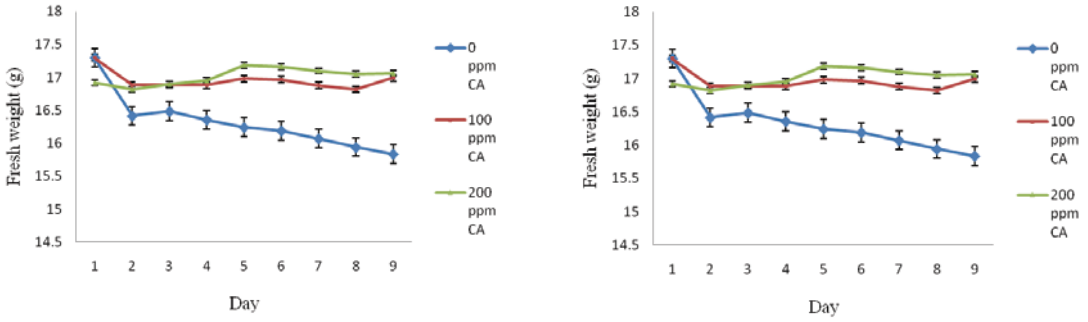

Figure 2. Cut flowers fresh weights in salicylic acid (a) and citric acid (b) treatments on the first 9 days of the experiment. Bars indicate ± standard error.

treatments. CA and interaction treatments were not significant. The highest petal water content (%) in CA (57.2%), was observed at 200 ppm which was not significantly different compared to the control (53.04%) (Table 1).

Figure 3. Comparison of SA 300 ppm and control treatments (right), SA 300 ppm and GCF commercial preservation solution (left) on the 12th day from the start of experiment (in each picture the right one is the SA treated flowers).

Leaf Relative Water Content

According to the results in Table 1, placement of chrysanthemum cut flowers in vase water containing SA, significantly increased RWC by day 10 of the experiment (P≤1%), whereas CA and the interaction treatments were not significant (Table 1). The highest (46.42%) and lowest (23%) values of this trait were observed at 200 ppm SA and control treatments, respectively, which indicate a 201% increase. The highest amount (41.08%) in case of CA was observed at 100 ppm (Table 1) whereas it was 58.7% at 200 ppm SA plus 100 ppm CA in case of interaction treatments.

Effects of GCF Commercial Preservative Solution

It seems that according to the results of this experiment, this commercial preservative solution has no significant effect on visual quality and vase life of chrysanthemum cut flowers and only increases the vase life by a few days compared to the control. Results showed that maximum vase life (11.11 days) of this solution is a few days more than the control, whereas, SA and CA treatments used extended the vase life by 15 and 7

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Figure 3. Comparison of SA 300 ppm and control treatments (right), SA 300 ppm and GCF commercial preservation solution (left) on the 12th day from the start of the experiment (in each picture the right one is the SA treated flowers).

days compared to the control, respectively (Figure 1). On the other hand, in other quality traits such as petal water content, relative water content and fresh weight in day one and nine, flowers in GCF solution had a very poor quality (Figure 3). Petals were completely destroyed in 8 days and leaves all wilted. In salicylic acid treatments these qualities were held in an acceptable manner even until day 20 of the experiment. In general it seems that cut flowers placed in this solution are capable of absorbing water like other treatments but the major amount of the absorbed water is lost through transpiration. Therefore, significant weight and visual quality loss is obviously observed in this treatment.

Other measured traits such as, SPAD, L. poro. and solution uptakes were not significant at any statistical level (Table 1).

DISCUSSION

A large number of factors such as pre-harvest conditions, packaging and post harvest handling and storage, interfere with the vase life. Salicylic acid has been found to play a key role in the regulation of plant growth and in the responses to environmental stresses (Raskin, 1992; Yalpani *et al*., 1994; Senaratna *et al*., 2000). SA and CA treatments extended vase life in association with inhibition of ethylene production (Srivastava, 2000).

Pathogens also affect vase life due to vascular blockage (Van Dome, 1998). In free state, SA has a pH of 2.4 and the acidic solution inhibits bacteria growth and proliferation (Raskin, 1992). CA is also known as an acidifier which inhibits the growth of microorganisms and is commercially advised for a number of cut flowers including chrysanthemum (Dole and Wilkins, 1998). CA can alleviate water uptake and extend vase life due to its anti-embolism trait (Bhattacharjee *et al*., 1993). The addition of SA to vase water has previously been shown to extend the longevity of cut *Rosa* flowers (Lee *et al.*, 2004 and Guy *et al*., 2003).

Water constitutes a large proportion of horticultural products weight. In addition to water, carbohydrates are the other major constituent of these products. These products commonly take water and other materials from the mother plant, but when cut off, they rapidly move into senescence and death which take place of water loss and weight reduction. This reduction is much higher in stress conditions. SA can modulate plant responses to a wide range of oxidative stresses and prevents cell wall degradation (Shirasu *et al*., 1997). As an apparent result in this experiment, in SA treatments after 10 days, no fresh weight reduction was observed and in fact a small increase is seen.

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Petals of a cut flower are the main ornamental parts and turgidity of these parts is important for a good looking product. Petal turgidity depends largely on water uptake and maintenance in treatments used. Results of this experiment show a significantly higher water uptake and water maintenance in cut flowers, which subsequently increase the cut flower fresh weight.. The increases in water uptake and subsequently cut flower fresh weight, are apparently due to the acidifying and stress alleviating properties of SA (Lee *et al*, 2004). According to our results, we can generally discuss that the major part of the absorbed water is gathered in the petals which in fact helps to have a better visual quality in SA treated cut flower samples (Figure 3).

Relative water content (RWC) is an index representing the amount of water in the plant organs and shows the ability of a plant in maintaining water under stress conditions (Abbaszadeh *et al.*, 2008). Therefore, in a controlled environment for an experiment, the measured RWC shows the response of a plant and the higher the measured amount, the greater the ability of the treatment for keeping water (Abbaszadeh *et al*, 2008). Thus, according to our results, it seems that at day 10 of the experiment (end of the control vase life), samples placed in control treatments were under sever stress and could not take up and keep water properly, whereas SA treatments in comparison, at the same time were in normal non-stress conditions. Mean measured RWC in leaves of SA treatments was 50% higher compared to that of the control. CA had no significant effect on this trait. In case of solution uptake, our results did not agree with Karimi *et., al*. 2008.

In conclusion, all substances tested extended the vase life of cut chrysanthemum flowers. Adding salicylic and citric acid to cut flower preservation solutions, increases vase life and preserves cut flowers for a longer period. SA showed a greater effect between treatments and a significant difference in vase life of chrysanthemum cut flowers, compared to CA used. In terms of overall performance, SA was the most effective followed by CA. According to the results of this experiment we can generally say that SA and CA as natural, cheap, safe and biodegradable compounds can be suitable alternative chemical treatments in order to prolong vase life of (*Dendranthema grandiflorum* (Ramat.) Kitamura, cv. Pattriot) which is a fact that would be much appreciated by the growers and handlers of cut flowers. Commercialization of these compounds for optimum formulations needs further experiments.

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تیمارهای سالیسیلیک و سیتریک اسید عمر گلجایی گل بریدنی داودی را افزایش می‌دهند

ن. وحدتی مشهدیان، ع. تهرانی‌فر، ح. بیات و ی. سلاح‌ورزی

چکیده

گل داودی یکی از مهمترین و بازار پسندترین گل‌های بریدنی در سطح جهان است. اما با این وجود عمر گلجایی نسبتا کوتاه یکی از مشکلات پیش روی این گل است. هدف از انجام این تحقیق بررسی اثر سالیسیلیک و سیتریک اسید در افزایش عمر گلجایی گل بریدنی داودی بود. برای این منظور آزمایشی به صورت فاکتوریل بر پایه طرح کاملا تصادفی شامل ۴ سطح سالیسیلیک اسید (۰ ppm، ۱۰۰، ۲۰۰ و ۳۰۰) و ۳ سطح سیتریک اسید (۰ ppm، ۱۰۰ و ۲۰۰) با ۳ تکرار و ۳ نمونه آزمایشی در هر تکرار، انجام پذیرفت. کاربرد سالیسیلیک و سیتریک اسید سبب افزایش عمر گلجایی، محتوی آب گلبرگ (٪)، وزن نمونه‌ها نسبت به روز اول و بازار پسندی به طرز معنی‌داری شد. محتوی نسبی آب برگ، محتوی آب گلبرگ (٪) و وزن نمونه‌ها نسبت به روز اول در اثر استفاده از سالیسیلیک اسید به ترتیب به میزان ۴۹، ۷۳ و ۲۳ ٪ نسبت به تیمار شاهد، افزایش نشان داد. بالاترین عمر گلجایی (۲۱/۷۷ روز) در تیمار ۳۰۰ پی‌پی‌ام سالیسیلیک اسید مشاهده گردید. به نظر می‌رسد، افزایش نزدیک به ۳ برابری (۳۰۰ درصد) عمر گلجایی در ارتباط با ویژگی‌های ضد تنشی و خصوصیات تنظیم کنندگی رشد سالیسیلیک و سیتریک اسید، باشد. با توجه به نتایج حاصل از این آزمایش می‌توان بیان نمود، سالیسیلیک و سیتریک اسید با منشا طبیعی، ترکیباتی ایمن، ارزان و زیست تجزیه پذیر بوده و جایگزین مناسبی برای مواد شیمیایی متداول مورد استفاده در افزایش عمر گلجایی گل‌های داودی می‌باشند. تجاری سازی این محصول و رسیدن به فرمولاسیون مطلوب نیازمند بررسی‌های دقیق تر در آینده است.

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