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EFFECT OF PULSING IN PRESERVATIVE SOLUTIONS, GROWTH REGULATOR BA AND COLD STORAGE ON THE LONGEVITY OF CHRYSANTHEMUM CUT FLOWERS CV. ROYAL ACCENT

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ABSTRACT

The interaction of pulsing in preservatives, growth regulators and cold storage on vase life and quality of Chrysanthemum (*Dendranthema grandiflora*) cut flowers was investigated. Chrysanthemum cut flowers were treated with benzyl adenine (BA), silver nitrate ($AgNO_3$), cobalt sulfate ($CoSO_4$) and sodium hypochlorite (NaOCl) and then were placed in cold storage at 5ºC for periods of 1, 2 and 3 weeks. Vase life, fresh weight, total chlorophyll and total carbohydrate were determined. Cold storage for one week and pulsing with BA and $AgNO_3$ showed longest vase life, the lowest chlorophyll decrease and highest total carbohydrate content.

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum*), family Asteraceae, is one of the most important cut flowers in the world. Addition of floral preservatives and also cold storage can extend vase life and improve flower quality in addition to cold storage for *D. grandiflora* var. Royal Accent **(Carlson and Dole 2013)**.

Different chemicals with different roles were used, silver nitrate ($AgNO_3$) considered as an inhibitor on growth of fungus and bacteria in the vase solution **(Gupta et al 2006)**. Cobalt sulfate ($CoSO_4$) is used to encourage absorption of solution by flowers and cobalt works to increase the production of amino acid methionine a starter for a series of protein **(Nabigol et al 2006)**. Sodium hypochlorite (NaOCl) is considered as a microbial pesticide **(Belle et al 2004)**. Eight - hydroxy quinoline sulfate (8 - HQS) is considered as a germicide **(Witte et al 2014)**. Benzyl adenine (BA) is used to reduce the sensitivity of the flower for leaf yellowing, and relieves wilt and delays foliar chlorosis during storage and transport, and also has activity as an antioxidant enzyme such as catalase **(Shimizu-Yumoto and Ichimura 2013)**. Citric acid (C.A.) is known also as an organic acid and a decreases pH solution **(Macnish et al 2008)**. Sucrose also has the preservation of cell respiration, very important as a source of energy for the performance of biological processes inside the flower and has an organizer role for osmotic adjustment in vase solution **(Doi and Reid 1995)**.

The obtained results were as follows: BA or $AgNO_3$ + C. A. + sucrose 3 % with cold storage for one week increased vase life, fresh weight, total chlorophylls and total carbohydrates of *D. grandiflora* cut flowers **(Devecchi et al 2009 and Rabiza-Swider et al 2012)**. The best treatment gave the longest life flower, less decrease in the fresh weight and the largest fresh weight and the highest content of total chlorophyll and total carbohydrates in the leaves are citric acid 75 ppm + sucrose 3 %

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+ benzyl adenine 0.5 ml mol and the treatment of citric acid 75 ppm + sucrose 3 % + silver nitrate 25 ppm with cold storage in the refrigerator for only one week compared with the other treatments.

MATERIALS AND METHODS

This study was carried out in the Lab. of Hort. Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt, for the two successive seasons of 2012 and 2013 to investigate the effect of pulsing in preservative solutions, growth regulators and cold storage periods on the vase life, fresh weight, changes in total chlorophyll and total carbohydrate contents of *Dendranthema grandiflora* cv. Royal Accent cut flowers (purple ray petals).

Cut Chrysanthemum flowers were obtained from a private farm on 28th of March 2012 and 2013. The flowers were cut at the commercial maturity and half opening of the petals when they began to open. Lower leaves were removed up to about 15 cm from the cut stem base. The flowers were then cut to 50 cm long and placed in vases containing a recently prepared (1000 cm^3) of the following solutions before cold storage:

1- Citric acid at 75 ppm + sucrose at 3 % + benzyl adenine at 0.5 mm.
2- Citric acid at 75 ppm + sucrose at 3 % + silver nitrate at 25 ppm.
3- Citric acid at 75 ppm + sucrose at 3 % + cobalt sulphate at 50 ppm.
4- Citric acid at 75 ppm + sucrose at 3 % + sodium hypochlorite at 50 ppm.
5- Control (distilled water).

The cut flowers were placed in these treatments for 24 h except for BA for 20 min as they were placed for 20 minute only and wrapped in wax paper then placed inside cartoon boxes and put were in the refrigerator at 5°C for 1, 2 and 3 weeks and after exited from the refrigerator were dipped in one solution consisted of 8 - hydroxyquinoline sulfate at 300 ppm + citric acid at 50 ppm + sucrose at 2 %, for all treatments.

The following data were recorded (on flowers after the cold storage periods):

- Vase life (days): vase life was terminated when the edges of the petals curled, wilted and yellowing of the lower green leaves.
- Minimum decreasing of fresh weight (FW) of flower (as %).
- Maximum increasing of FW of flowers (as %).
- Total chlorophylls reading in leaves (SPAD) after 7 and 10 days of the beginning of experiment and before and after the different cold storage periods.
- Total carbohydrates in the leaves (mg / g dry weight) before and after the different cold storage periods **(Dubois et al 1956 and A.O.A.C. 2005)**.

The experiments were performed in the a laboratory as its temperature averaged 21 ± 2 ºC, and relative humidity 50 – 60 % and under light intensity of 1000 lux from cool white fluorescent lamps for 12 h during the day.

Total chlorophylls assay were measured by using a Chlorophyll Meter Minolta device SPAD – 502 **(Wood et al 1992)**. The results of total chlorophylls were expressed as SPAD. It measures the relative amount of total chlorophyll present by measuring the transmittance of the leaf in two wave bands 600 - 700 and 400 - 500 nm and the youngest fully expanded mature leaves were used. The design of the experiment was a factorial in randomized complete blocks in 5 treatments of solutions and 3 cold storage periods with 3 replicates and every replicate had 3 flowers as periods of storage occupied the main plot and preservative occupied the sub plot. The differences between treatments were determined by using L.S.D. test according to **(Snedecor and Cochran 1989)**. Data of vase life, increase of fresh weight and decrease of fresh weight were statistically analyzed using SAS procedures **(SAS Institute Inc. 1996)**.

RESULTS AND DISCUSSION

1- Minimum decrease of FW of flower (as %)

Data presented in **Table (1)** show that the minimum decrease of FW was noticed from treatments C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm and C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm by values of 12.1 % and 10.8 %, while the maximum decrease of FW was obtained from treatment C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm by values of 14.7 and 15.8 % and the differences between them were significant. Also, the minimum decrease of FW was found with one week storage 8.7 % and 9%, while the maximum was recorded with 3 weeks storage by values of 16.5 and 15.9 % and the differences were significant. The minimum decrease of FW occurred with treatment C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm and 1 week storage which gave 6.9 and 7.5 %, but the maximum decrease of FW was reported with treatment C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol and

Table 1. Effect of pulsing in preservative solutions, growth regulator and cold storage periods on minimum decreasing of fresh weight percentage (%) of chrysanthemum cv. Royal Accent during the two seasons of 2012 and 2013

Treatments / Cold storage	First season			
	1 Week	2 Weeks	3 Weeks	Mean
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	9.1 FG	12.3 DE	17.7 A	13 B
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	9.3 FG	17.4 AB	17.5 AB	14.7 A
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	6.9 G	13.2 CD	16.1 AB	12.1 B
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	8.1 FG	13.2 CD	15.7 ABC	12.3 B
5- Distilled water (control)	10.1 EF	14.8 BCD	15.3 ABC	13.4 AB
Mean	**8.7 C**	**14.2 B**	**16.5 A**	
Treatments / Cold storage	**Second season**			
	1 Week	**2 Weeks**	**3 Weeks**	**Mean**
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	9.4 FGH	11.5 DEF	17.2 B	12.7 B
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	9 FGH	17.3 B	21.1 A	15.8 A
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	7.5 H	13.2 CDE	14.8 BC	11.8 BC
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	8.5 GH	11.2 DEFG	12.7 CDE	10.8 C
5- Distilled water (control)	10.4 EFG	12.3 CDE	13.4 CD	12.1 BC
Mean	**9 C**	**13.1 B**	**15.9 A**	

* C. A.: citric acid.

** Means with the same letter are not significantly different at $P < 0.05$ level according to Duncan's Multiple Range Test.

storage for 3 weeks and treatment C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm with storage for 3 weeks gave 17.7 and 21.1 % and the differences were significant. These results were in agreement with **(Meeteren et al 2000)**, who found that deionized water gave a sharp decrease after 1 - 3 days on chrysanthemum cv. Cassa cut flowers. However, **(Talukdar et al 2004b)**, found that there is a positive effect of the holding solution on vase life of cut Chrysanthemum (*Dendranthema grandiflorum* Tzvelev) flowers, whereas the fresh weight loss during senescence was lowest by 33.65 %. Concerning postharvest quality, **(Talukdar et al 2004a)**, in his work on Chrysanthemum (*Dendranthema grandiflora* Tzvelve) cut flowers found that the minimum loss in fresh weight occurred with distilled water.

2- Maximum increase of FW of flowers (as %)

Data presented in **Table (2)** show that the maximum FW occurred with treatment C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm which gave 29.2 and 25.4 %, while the minimum FW was reported with treatment distilled water (control) which gave 17.4 & 19.3 % and the differences were significant. The maximum fresh weight was obtained from cold storage for one week by value of 31.5 & 30.8 %, but the minimum FW recorded with cold storage for three weeks was valued at 17.6 & 15.5 % and the differences were significant. The maximum FW showed in treatment C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm with 1 week of cold storage and treatment C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol with one week cold storage valued at 35.5 and 35.2 %, also the minimum FW was noticed with treatment distilled water (control) with 3 weeks of cold storage was estimated at 13.1 & 14.3 % and the differences were significant. The data were in agreement with **(Meeteren et al 2000)** who found that deionized water as vase water and a control solution treatment enhanced fresh weight probably by reducing bacterial growth in the cut open vessels. Also, **(Ichimura et al 1999)** stated that in cut Roses (*Rosa hybrida* cv. Sonia) flowers treated with sucrose + HQS was the most effective treatment in fresh weight that markedly increased it 20°C. In addition, **(Elanchezhian and Srivastava 2001)** in their work on chrysanthemum found that the fresh weight was greatest in flowers kept in GA or control solution.

Table 2. Effect of pulsing with preservative solutions, growth regulators and cold storage periods on maximum increase of fresh weight percentage (%) of chrysanthemum cv. Royal Accent during the two seasons of 2012 and 2013

Treatments / Cold storage	First season			
	1 Week	**2 Weeks**	**3 Weeks**	**Mean**
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	35.2 A	25.9 BCD	18.1 DEF	26.4 AB
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	35.5 A	32.6 AB	19.5 CDEF	29.2 A
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	33.1 AB	27.2 BC	18.5 DEF	26.3 AB
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	31.9 AB	20.2 CDEF	18.5 DEF	23.5 B
5- Distilled water (control)	21.9 CDE	17.1 EF	13.1 F	17.4 C
Mean	**31.5 A**	**24.6 B**	**17.6 C**	
Treatments / Cold storage	**Second season**			
	1 Week	**2 Weeks**	**3 Weeks**	Mean
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	35.2 A	21 CDEFG	14.9 G	23.7 AB
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	34 A	26.2 ABCDE	15.9 FG	25.4 A
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	32.3 AB	21.5 CDEFG	15.2 G	23 AB
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	27.8 ABCD	30.1 ABC	17.4 EFG	25.1 A
5- Distilled water (control)	24.5 BCDEF	19.1 DEFG	14.3 G	19.3 B
Mean	**30.8 A**	**23.6 B**	**15.5 C**	

* C. A.: citric acid.

** Means with the same letter are not significantly different at $P < 0.05$ level according to Duncan's Multiple Range Test.

3- Vase life

Data presented in **Table (3)** indicate that the longest vase life was determined with treatments C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol & C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm with values of 8.7 & 8.7 days and treatment C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol with value of 7.9 days, also the shortest vase life was noticed was with treatment distilled water (control) with values of 6.3 & 5.2 days and the differences were significant. In addition, the longest vase life was found with cold storage period for 1 week was valued at 10.5 and 9.3 days, meanwhile the shortest vase life occurred with cold storage period for 3 weeks 3.3 & 3.2 days and the differences in between them were significant. The longest vase life was obtained was with treatments C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm & C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol and one week of cold storage period as valued by 11.7 & 10.3 days, while, the shortest vase life occurred with treatment distilled water (control) and 3 weeks of cold storage period which gave 2.2 & 1.8 days and the differences were significant. These data are in agreement with **Shimizu-Yumoto and Ichimura (2013)** who found that spraying or pulsing Dahlia cut flowers with (BA) 6 - benzylaminopurine treatments significantly extended the vase life of florets more than those treated with distilled water. Also, **Macnish et al (2010)** found that Iris cut flowers when treated with 20 % sucrose plus GA_3 had an additive effect on increasing the vase life by a further 0.8 d up to 1.5 day and pulsing stems with combined treatment prior to storing flowers dry for 14 day at 0°C provided maximum display life after storage.

4- Percentage of total chlorophyll decrease (%)

Data presented in **Table (4)** show that the lowest decrease of chlorophyll content occurred from treatment C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol with 1 week storage period by 20 and 18%, while the highest decrease of chlorophyll content was obtained from treatment distilled water (control) with 3 weeks storage period by 50 & 45 %. The data were in agreement with **(Pandya and Saxena 2003)** who found an increase in the total

Table 3. Effect of pulsing with preservative solutions, growth regulators and cold storage periods on vase life (days) of chrysanthemum cv. Royal Accent during the two seasons of 2012 and 2013

Treatments / Cold storage	First season			
	1 Week	2 Weeks	3 Weeks	Mean
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	11.5 A	10.6 AB	3.8 E	8.7 A
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	11.7 A	10.6 AB	3.9 E	8.7 A
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	10.6 AB	10.5 AB	3.5 E	8.2 A
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	10 BC	9.2 C	3.2 EF	7.5 B
5- Distilled water (control)	8.8 CD	7.8 D	2.2 F	6.3 C
Mean	**10.5 A**	**9.8 B**	**3.3 C**	
Treatments / Cold storage	**Second season**			
	1 Week	**2 Weeks**	**3 Weeks**	**Mean**
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	10.3 A	8.9 AB	4.5 CD	7.9 A
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	10.2 A	8.5 B	3.8 DE	7.5 AB
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	9.2 AB	8.2 B	3.2 DEF	6.9 BC
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	8.9 AB	7.8 B	2.8 EF	6.5 C
5- Distilled water (control)	7.8 B	5.9 C	1.8 F	5.2 D
Mean	**9.3 A**	**7.9 B**	**3.2 C**	

* C. A.: citric acid.

** Means with the same letter are not significantly different at P < 0.05 level according to Duncan's Multiple Range Test.

Table 4. Effect of pulsing with preservative solutions, growth regulators and cold storage periods on mean of decreasing rate of total chlorophyll (%) in the leaves of chrysanthemum cv. Royal Accent during the two seasons of 2012 and 2013

Treatments / Cold storage	First season		
	1 Week	2 Weeks	3 Weeks
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	20	23	25
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	22	28	35
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	22	26	29
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	25	29	30
5- Distilled water (control)	35	45	50
Treatments / Cold storage	**Second season**		
	1 Week	**2 Weeks**	**3 Weeks**
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	18	22	27
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	21	25	32
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	20	26	30
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	23	28	35
5- Distilled water (control)	32	38	45

* C. A.: citric acid.

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chlorophyll content of leaves in cut flowers of *Dendranthema grandiflora* when treated with 6 % sucrose solutions as improved the quality and vase life. Notably, **(Mutui et al 2001)** found that *Alstroemeria aurantiaca* cut flowering stems held in 25 mg / litre BA as a commercial preservative, significantly increased the chlorophyll content.

5- Total carbohydrates (%)

Data presented in **Table (5)** show that the highest total carbohydrates (%) was recorded from treatment C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm with one week cold storage period which gave 31.2 & 46.2 %, whereas the lowest total carbohydrates content was found with treatment distilled water (control) and three weeks cold storage period which gave 1.9 & 6.2 %. The data are in agreement with **(Pandya and Saxena 2003)** who found that there was a correlation between carbohydrates content and vase life of *Chrysanthemum morifolium* cut flowers. Also, **(Ichimura et al 1999)** on cut Roses (*Rosa hybrida* cv. Sonia) flowers found that soluble carbohydrates concentration in petals is an important factor in determining the vase life.

Table 5. Effect of pulsing with preservative solutions, growth regulators and cold storage periods on mean of total carbohydrates (%) in chrysanthemum cv. Royal Accent during the two seasons of 2012 and 2013

Treatments / Cold storage	First season		
	1 Week	2 Weeks	3 Weeks
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	20.2	17.2	13.2
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	20.2	13.2	6.2
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	25.2	20.2	12.5
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	31.2	24.2	13.2
5- Distilled water (control)	13.5	6.2	1.9
Treatments / Cold storage	**Second season**		
	1 Week	**2 Weeks**	**3 Weeks**
1- C. A. 75 ppm + sucrose 3 % + BA 0.5 ml mol	34.2	16.5	8.2
2- C. A. 75 ppm + sucrose 3 % + $AgNO_3$ 25 ppm	27.2	16.2	7.5
3- C. A. 75 ppm + sucrose 3 % + $CoSO_4$ 50 ppm	34.9	27.9	14.5
4- C. A. 75 ppm + sucrose 3 % + NaOCl 50 ppm	46.2	33.2	18.6
5- Distilled water (control)	18.5	8.9	6.2

* C. A.: citric acid.

** Means with the same letter are not significantly different at $P < 0.05$ level according to Duncan's Multiple Range Test.

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