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Research article

EFFECT OF GIBBERELLIC ACID AND CALCIUM CHLORIDE ON KEEPING QUALITY AND VASE LIFE OF NARCISSUS (*NARCISSUS TAZETTA*) CUT FLOWERS

Ali Salehi Sardoei

Young Researchers Club, Jiroft Islamic Azad University, Branch Jiroft, Iran.
alisalehisardoei1987@gmail.com

ABSTRACT: The effects of Gibberellic Acid and Calcium Chloride, on cut Narcissus was studied. gibberellic acid (0, 20 and 40 mg L^{-1}) and calcium chloride (0, 10, 20, 40 mM), their combinations were tested as preservative mixture. This study was conducted in a factorial experiment with complete randomized design on 108 Narcissus cut flowers in horticulture laboratory of agriculture faculty of Islamic Azad University, jiroft branch. The recorded traits included vase life, microbial count, relative fresh weight and solution uptake. The results shown using gibberellic acid and calcium chloride as a preservative significantly increased the vase life, Fresh weight changes, Microbial Count and Solution uptake. The results showed that calcium chloride increased cut flower vase life, while decreased the Microbial Count with total delay of senescence. Maximum flower vase life was recorded in 20 Mm calcium chloride treatments. A direct relationship between vase life and, increasing of relative fresh weight and water uptake was observed as well.
Key words: Microbial Count, Relative Fresh Weight, Narcissus, Vase Life.
Abbreviations: GA_3, Gibberellic Acid; $CaCl_2$, Calcium Chloride.

INTRODUCTION

Cut flowers are precious products of horticulture. Maintaining good quality of cut flowers and extending the vase life, is considered important and practical for having acceptable products for the markets. In general, many studies have been under taken for this purpose. [26, 31, 38, 45, 46]. Vase life of cut flowers is mainly affected by two main factors, namely ethylene which accelerates the senescence of many flowers and by microorganisms which cause vascular blockage and thus reduces the vase life of cut flowers [42, 45, 46]. Narcissus is a genus of hardy, spring-blooming, bulbous plants in the family Amaryllidaceae. Earlier reports suggested that the genus Narcissus contained around 26 wild species [40]. The number has been reported to be between 50 and 100 including species variants and wild hybrids [3]. The species *Narcissus tazetta* derives its name from the word ‘‘Tazetta’’ which in Italian means ‘‘little cups’’ with reference to the centrally placed little yellow corona cups. It is the most widespread species of the genus Narcissus found in region with Mediterranean type of climate extending from Spain, Iran, Kashmir to China and Japan [4]. Found out that using giberrelic acid with amounts of 2.5, 5, 7.5 $mg.L^{-1}$ can delay the withering of the flower and the falling of its petals [6]. Cycokenins in gerbera preservation solution can increase anthocyanin of petals and decrease the speed of respiration [18]. Role of calcium in maintenance and modulation of various cell functions is based on its presence in the in membrane and in on cell wall Ca^{2+} in an integral part of the cell wall where it provides stability, resulting in cell-wall rigidity. Several research studies have tested the effects of different sources of calcium (Ca) on various cut flower species. The effects of calcium chloride ($CaCl_2$) have been studied on *Gladiolus* cv. ‘Happy End’ [30], *Gerbera jamesonii* ‘Campitano’, ‘Dino’, ‘Sangria’ and ‘Testarossa’ [8], and *Rosa hybrid* cvs ‘Mercedes’ and ‘Baroness’ [41]. Other research studies have looked at the effects of calcium nitrate on *Rosa hybrida*‘ Raktagandha’, ‘Sonia’, ‘Celica’, ‘Samantha’, ‘Mercedes’ and ‘Ilseta’ [28]; Chrysanthemum indicumand *Tagetes erecta* [29], and *Dianthus caryophyllus* [27]. Calcium sulfate has also been the subject of research on *Rosa hybrida* cv. ‘Kiss’ [5].

The GA_3 is considered to be a senescence-delaying plant growth regulator [1]. Pulsing alstromeria for 24 h with a 0.01 m mol GA_3 solution increased longevity of cut flowers [22]. Sabehat and Zieslin [34] also noted that GA_3 treatment increased the vase life of roses. Found that treatment with GA_3 repressed accumulation of the seven senescence associated transcripts in daffodil [17]. For example [37] demonstrated that the vase solution treatment combinations of GA_3 and benzyladenine with sucrose significantly increased the vase life of cut spikes of gladiolus as compared to the sucrose alone treatment or the control. However, the results obtained have been variable.
Treatment with gibberellic acid has also been shown to enhanced post harvest life and quality of gerbera cut flowers [6]. Conversely, the effect of gibberellic acid and benzyl adenine to reduce oxidative enzyme activity in cut leaves of Hosta has been demonstrated [32]. Supplementary treatments, such as the use of plant growth regulators (PGR), have been employed to improve flower longevity. Gibberellic acid (GA_3) is a PGR known to promote growth processes in plants [10, 24] including cut flower opening and stem elongation [42]. GA_3 application has also been shown to extend rose flower vase life which was attributed to the inhibition of senescence-related increases in cell membrane permeability and protein decomposition [35]. Similarly, GA_3 treated rose flowers stored better under low temperature than untreated flowers [11]. Furthermore, GA_3 was found to reduce Botrytis blight in cut roses as a result of its senescence-inhibitory effect [36] and the possible production of antimicrobial compounds such as various phenolics [43]. The aim of this work was to study the responses Narcissus to the interactive effects of Gibberellic Acid and Calcium Chloride.

MATERIALS AND METHODS

Cut Narcissus flowers were obtained from a local the village Nargesi, jiroft, and transported with proper covers immediately to Laboratory. Solutions were freshly prepared at the start of experiments. Stems were recut to 35 cm length. The study was arranged in a factorial test with completely randomized design with four replications. Each replication consisted of three cut flowers. Three levels of Gibberellic Acid (0, 20 and 40 mg L^{-1}), three levels of and Three levels of Calcium Chloride (0, 10, 20, 40 mM) were applied (total of 12 treatments). After recording the fresh weight, each flower was placed in a bottle containing 400 ml preservative solutions. The flowers were held at ambient temperature (22±2°C). Except vase life all measurements including flower diameter and stem curvature were made at the 10^{th} day of the experiment.

Vase life: The average vase life of the spikes was counted from the day of transfer of spikes to the holding solution and was assessed to be terminated when 50% flowers had senesced, which was characterized by loss of turgor followed by petal wilting. Petal senescence was marked by the loss of turgor in the petal tissue followed by complete wilting.

Microbial Count: Microbial count was determined by taking 1ml vase solution samples at 2 days intervals with 4 replications during the first 11 days of the experiment. 1ml from each sample was diluted in 10 fold serial dilution. 0.1 ml from each concentration of diluted samples was plated on nutrient agar and all were incubated at 35ºC for 48 hours. Microorganisms were counted by standard plate counting method (by counting the number of colonies formed after incubation) to generate the number of colony forming units.ml–1 (CFU ml^{-1}) [21].

relative fresh weight: In order to record fresh weight changes of cut flowers, flower stems were taken out of vase making sure that stem end is not dry and weighted as quickly as possible by a balance on a daily basis. Data were obtained to calculate fresh weight changes (g and %) and relative fresh weight (RFW) changes of the stems. Relative fresh weight was calculated as: RFW (%) = (Wt/$Wt0$)×100; where, Wt is weight of stem (g) at t = day 0, 1, 2, etc., and$Wt0$ is weight of the same stem (g) at t=day 0 [15, 25].

Solution uptake*:* Solution uptake of flowers was measured using a balance by weighting each vase containing its solution without its flowers and correcting the evaporation from the 4 evapo-control vases (vases which did not contain any flowers and were located between the vases that contained flowers at different places) by subtracting the average of 4 evaporation data from solution uptake on a daily basis. Daily vase solution uptake was calculated as: vase solution uptake rate (g $stem^{-1}$ day^{-1})=(St-1-St); where, St is weight of vase solution (g) at t = day 1, 2, 3, etc., and St-1 is weight of vase solution (g) on the previous day [15, 21, 25].

Experimental Design and Statistical Analysis: Experiment was arranged in a factorial test with completely 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 dancan analysis in the same software (p= 0.05).

RESULTS AND DISCUSSION

Vase Life: Result showed that the effects of $CaCl_2$ and interaction of $GA_3 \times CaCl_2$ were significant at ($P < 0.01$) and ($P < 0.05$) levels (Table 1). Moreover, interaction of $GA_3 \times CaCl_2$ revealed that there is a significantly increase in flower stability by increasing in $CaCl_2$ concentration compared to other treatments. These results had accordance with results of [2] and [19]. In fact, Ca leads to decrease the respiration rate, osmotic adjustment and stability of cell membrane [2]. Obstruction of the xylems by bacteria, therefore, inability of water absorption by flower steams is one of the current problems that lead to decrease in flowers post harvest longevity and also early welter of them. Infection of flower containers can be the reason of this problem [33]. Therefore, different retentive solutions have been used to increase flowers post harvest longevity. Different compounds via retarding of physiological processes related to senescence cause an increase cut flowers longevity and also quality retention [7, 12]. In this study, GA_3 had not significant effect on flower senescence delay that it was in agreement with results [7]. In general, at this experiment the highest longevity of cutting flowers was obtained at 20 mM $CaCl_2$ also this treatment had positive effect on relative fresh weight of stem in 8 and 10 days. Application of Ca is necessary for improve longevity. Ca decreases respiration rate [2] and also increases cell wall resistance [9] therefore, it had been increased longevity of narcissus cutting flowers.

Table 1: Effects of Gibberellic Acid and Calcium Chloride in preservative mixture on Vase life and Solution uptake in Narcissus cut flowers

GA_3 (mg L^{-1})	$CaCl_2$ (mM)	Vase life (day)	Solution uptake (g $stem^{-1}$ day^{-1})			
			8	10	12	14
0	0	13/66 c	82/07 bcd	86/70 ab	95/99 ab	95/67 a
	10	16/33ab	84/12 bc	85/77 ab	93/78 abc	96/02 a
	20	18 a	89/72 a	89/90 a	95/41 ab	90/67 a
	40	16/66ab	80/87 cd	86/23 ab	92/99 bc	95/62 a
20	0	15/66 b	81/71 bcd	87/74 a	94/60 ab	95/96 a
	10	16/66 ab	85/96 ab	88/21 a	95/60 ab	97/38 a
	20	16 ab	81/75 bcd	86/02 ab	94/77 ab	95/44 a
	40	16/33 ab	82/60 bcd	87/13 ab	94/24 ab	96/35 a
40	0	16/33 ab	83/52 bc	87/96 a	96/93 a	96/35 a
	10	16 ab	77/99 d	82/70 bc	90/73 c	89/13 a
	20	16/66 ab	80/55 cd	80/80 c	90/62 c	90/07 a
	40	16/66 ab	83/02 bc	88/50 a	95/54 ab	93/25 a
GA_3		n.s	0.018	0.050	0.039	0.027
$CaCl_2$		0.000	0.041	0.031	n.s	n.s
$GA_3 \times CaCl_2$		0.044	n.s	0.018	0.039	0.000

Microbial Count: The effect of $CaCl_2$ on microbial count was significant at ($P < 0.05$) level. Mean comparison of GA_3 and $CaCl_2$ was significant at ($P < 0.05$) level. The highest microbial count growth was observed in 40 mg L^{-1} GA_3 and the lowest was observed in mixture of 40 mg L^{-1} GA_3 and 10 mM $CaCl_2$ (Table 2). Obstruction of the vessels by microbes inside cutting flowers retentive solutions are the main problem that lead to decrease in flower post harvest longevity [5, 6]. Intensity of vessels obstruction after harvest is one of the limiting factors and effectiveness on cutting flowers longevity and quality that usually is because of bacteria activity [23] and physiological processes induced in flower stem base [44]. It seems that application of anti microbial compound at pots solution is necessary in order to prevent of microbial growth. As same as our findings, sterilized distilled water did not have any pleasing effect in controlling or reducing microbial population of narcissus vase solution [21]. As the main role of integrated biocide in floral preservatives is to sustain clarity in vase solution and to avoid blockage of xylem elements by microorganisms [23].

Relative Fresh Weight: Result showed that GA_3 effect on relative fresh weight was significant on days 8, 10, 12 and 14 at ($P < 0.05$) level (Table 1). But the effect of $CaCl_2$ on relative fresh weight was significant on days 8 and 10 at ($P < 0.05$) level although it was or non significant on days 12 and 14. Interaction of $GA_3 \times CaCl_2$ was significant at ($P < 0.01$) and ($P < 0.05$) level and on 10 and 12 days at ($P < 0.05$) and 14 day was significant at ($P < 0.01$) level.

Change of relative fresh weight at estimation of different days revealed that relative fresh weight decreased from beginning of experiment to day 10 but it increased significantly on 12 day (Table 1). In evaluation of change in relative fresh weight between treatments, 40 mg L^{-1} GA_3 on 12 day of experiment had the highest relative fresh weight (Table 1). But on 8 and 10 days of experiment the highest relative fresh weight was obtained at 20 m molar $CaCl_2$ while it cause to delay in senescence and increase in cutting flower longevity [16].

Table-2: Effects of Gibberellic Acid and Calcium Chloride in preservative mixture on Microbial Count and Relative Fresh Weight in Narcissus cut flowers

GA_3 (mg L^{-1})	$CaCl_2$ (mM)	Microbial Count (log10 (CFU ml^{-1})	Relative Fresh Weight (% of the initial)			
			8	10	12	14
0	0	2/64 bc	0/78 a	2/24 a	1/10 bc	1/45 a
	10	2/40 bc	0/35 a	1/72 c	0/85 c	1/22 abc
	20	1/82 bc	1/64 a	1/88 bc	0/90 c	1/19 abc
	40	2/56 bc	1/15 a	1/54 c	0/86 c	1/16 abc
20	0	2/54 bc	0/85 a	1/95 bc	1/44 ab	1/46 a
	10	3/81 bc	1/09 a	1/77 bc	1/10 bc	1/01 bc
	20	2/52 bc	0/33 a	1/84 bc	0/95 c	1/10 bc
	40	3/02 bc	2/45	1/88 bc	0/85 c	1/20 abc
40	0	5/97 a	2/06 a	2/79 a	1/08 bc	1/25 abc
	10	1/61 c	1/75 a	1/99 bc	1/12 bc	1/32 ab
	20	3/72 b	1/74 a	1/68 c	1/03 bc	0/99 b
	40	1/53 c	1/61a	2/20 a	1/73 a	1/07 bc
GA_3		n.s	0.045	n.s	0.02	n.s
$CaCl_2$		0.056	n.s	0.012	n.s	0.005
$GA_3 \times CaCl_2$		0.002	n.s	0.042	0.011	0.058

Solution Uptake: The effect of GA_3 on solution uptake was significant at ($P < 0.05$) level on 8 day but the effect of $CaCl_2$ on relative fresh weight on 10 day of experiment was significant at ($P < 0.05$) level. Also, on 10 day solution uptake was significant at ($P < 0.01$) level (table 2). The interaction of $GA_3 \times CaCl_2$ was significant at ($P < 0.05$) level on 10, 12 and 14 days. The amount of solution uptake was increased by GA_3 treatment during the evaluation. Change in relative fresh weight in different days of assessment showed that relative fresh weight decreased from beginning of experiment to 10 day but it was increased significantly on 12 day of experiment [20]. With increasing in $CaCl_2$ concentration during 10 and 14 days of experiment the amount of Solution uptake decreased. High concentration of Ca leads to decrease in cell permeability and reduction in solution uptake and ion absorption [14].

CONCLUSIONS

In present study, Gibberellic Acid and Calcium Chloride increased vase life of cut flowers and had the least bacterial clonies population in vase solution.

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