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Interaction Effect of Salicylic Acid and Putrescine on Vase life of Cut Narcissus Flowers

Ali Salehi Sardoei[1*], Gholam Abbas Mohammadi[2], Parviz Rahbarian[3]

1. Young Researchers and Elite Club, Islamic Azad University, Jiroft Branch, Jiroft, Iran.
2. Assistant of Plant Science, Islamic Azad University, Jiroft Branch, Jiroft, Iran.
3. Department of Horticulture, Islamic Azad University, Jiroft Branch, Iran.

ABSTRACT

The effects of salicylic acid (SA) and Putrescine (Put), on cut Narcissus was studied. SA (0, 100 and 200 mg l^{-1}) and Put (0, 150 and 300 mg l^{-1}), 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, Fresh weight changes and Solution uptake. the results shown using SA and PUT as a preservative significantly increased the vase life, Fresh weight changes, Microbial Count and Solution uptake ($P \leq 5\%$). The results showed that salicylic acid and Putrescine treatments increased cut flower vase life, while descreased the Microbial Count with total delay of senescence. Maximum flower vase life was recorded in SA 100 mgl^{-1} + PU 100 mgl^{-1} treatments. A direct relationship between vase life and, increasing of Fresh weight changes and water uptake was observed as well.

Key words: Cut flowers, Fresh weight changes, Narcissus, Vase life.

Abbreviations: SA, Salicylic acid; Put, Putrescine.

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. (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 by microorganisms which cause vascular blockage and thus reduces the vase life of cut flowers (Van Doorn, 1994; Zencirkiran, 2005; Zencirkiran, 2010). Narcissus is a genus of hardy, spring-blooming, bulbous

Corresponding Author E-mail: alisalehisardoei1987@yahoo.com

plants in the family Amaryllidaceae. Earlier reports suggested that the genus Narcissus contained around 26 wild species (Third, 1976). The number has been reported to be between50 and 100 including species variants and wild hybrids (Brent and Becky, 2001). 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 Meditteranean type of climate extending from Spain, Iran, Kashmir to China and Japan (Coats, 1971).Salicylic acid (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 also suppressed 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. Also, the upward gravitropic bending of snapdragon was inhibited using SA (Friedman et al, 2003). PAs are low molecular weight polycations, organic, biogenic amines that are found in all eukaryotic and most prokaryotic cells (Kumar et al., 1997; Mahgoub et al., 2006) and have profound effects on growth, development and senescence in eukaryotic cells (Casiro and Marton, 2007). In plants, di-amine putrescine (Put), triamine spermidine (Spd) and tetra-amine spermine (Spm) are frequently present in amounts varying from micromolars to more than millimolars (Kakkar and Sawhney, 2002). Polyamines (Put, Spm and Spd) are recognized as a new class of plant growth bio-regulators (Dantuluri et al., 2008). They influence many biochemical and physiological processes such as cell division, cell elongation, flowering, fruit set and development, fruit ripening, senescence, storage life (Cohen, 1998; Nambeesan et al., 2010). The various plant growth and developmental processes affected by PAs include stimulation of cell division, response to environmental stress and regulation of rhizogenesis, embryogenesis, fruit and flower development (Bouchereau et al., 1999). PAs mainly Spm, retarded the senescence of leaf discs of two diverse species of roses (*R. damascena and R. bourboniana*) whereas, PAs synthesis inhibitors such as difluoromethylarginine (DFMA) and methylglyoxal-bis-guanylhydrazone (MGBG) promoted senescence (Iman Talaat et al., 2005). PAs significantly improved fresh weight, uptake of vase solution, flower opening and vase life of gladiolus. Spermidine at a concentration of 100 ppm+4% sucrose, 500 ppm Spm+4% sucrose, 100 ppm Put+4% sucrose, 100 ppm Spd+4% sucrose and 500 ppm Spd+4% sucrose significantly improved vase life over both control and4%sucrose. PAs delayed senescence and improved vase life of cut spikes by improving membrane stability (Mahgoub et al., 2011). The aim of this work was to study the responses Narcissus to the interactive effects of salicylic acid and Putrescine.

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 complete randomized design

with four replications. Each replication consisted of three cut flowers. Three levels of SA (0, 100 and 200 mg L^{-1}), Three levels of and Three levels of Putrescine (0, 150 and 300 mg L^{-1}) were applied (total of 9 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}) (Jowkar, 2006).

Fresh weight changes: 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 [He et al, 2006; Liu et al, 2009].

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 (He et al, 2006; Jowkar, 2006; Liu et al, 2009).

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 dancan analysis in the same software (p= 0.05).

RESULTS AND DISCUSSION

Vase life: According to the results shown in Table 1, using SA and PUT as a preservative significantly increased the vase life (P≤5%). The highest values observed in are 14.24 respectively and on the other hand, the lowest values are 12.66 for the same cut flowers (Table 1). This was while there was a significant difference between SA treatments. A large number of factors such as pre-harvest conditions, packaging and post harvest handling as well as storage interfere in the vase life. Salicylic acid has been found to play a key role in regulating the plant growth and in the responses to environmental stresses (Yalpani et al., 1994; Senaratna et al., 2000). SA treatments extended vase life in association with

inhibition of ethylene production (Srivastava, 2000). Pathogens also affect vase life due to vascular blockage (Van Dome, 1994). In Free State, SA has a pH of 2.4 and acidic solution inhibits bacteria growth and proliferation (Raskin, 1992). 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). Salicylic acid (SA) is known as an inhibitor of ethylene biosynthesis thereby delaying the senescence process. In addition to involvement of SA in local and systemic resistance to pathogens (Yalpani et al., 1994; Kang et al., 2003), it has been stated that SA suppress the conversion of ACC into ethylene by inhibiting the ACC oxidase activity (Fan al., 1996). Bacteria and their decay products caused stem blockage and water deficit (Loubaud and Van Doorn, 2004; Van Doorn, 1977) as well as secreted pectic enzymes and toxic compounds by bacteria, and/or produced ethylene resulting in the accelerated senescence (Williamson et al., 2002) are main limiting factors during the postharvest life of cut flowers. As the restricted solution uptake by the stem blockage caused by the microbial contamination and/or air emboli is one of the most limiting factors in postharvest life of cut flowers, acidic solutions (unsuitable condition for bacterial growth) are usually considered to have desirable effects for the most cut flowers. The microbial contamination, air emboli and physiological wound induced have been mentioned as typical reason for stem blockage (Loubaud and Van Doorn, 2004; He et al., 2006; Van Meeteren et al., 2006).

Table 1- Effects of Salicylic acid and Putrescine in preservative mixture on Vase life and Solution uptake in Narcissus cut flowers

Salicylic acid ($Mg.L^{-1}$)	Putrescine ($Mg.L^{-1}$)	Vase life (day)	Solution uptake (g $stem^{-1}$ day^{-1})		
			7	9	11
0	0	12.66b	1.77bc	3.89a	4.15ab
	100	12.66b	2.37ab	2.73b	2.42bc
	200	12.66b	2.19abc	3.88a	4.48a
100	0	13.58b	2.52a	0.73c	0.61c
	100	14.24a	1.91abc	3.16ab	5.14a
	200	13.49b	1.71c	1.04c	0.68c
200	0	13.24ab	1.93abc	0.61c	0.61c
	100	13.41ab	2.18abc	1.19c	1.05c
	200	13.41ab	2.1abc	0.7c	0.65c
CV (%)	5.77	15.71	20.44	42.04	

Microbial Count: According to the results shown in Table 2, using SA and PUT as a preservative significantly increased the vase life (P≤5%). The highest values observed in are concentrations of SA 100 mgl^{-1}+PU 200 mgl^{-1} and SA 200 mgl-$^{-1}$+PU 200 mgl^{-1} respectively and on the other hand, the lowest values are 12.66 for the same cut flowers (Table 2). This was while there was a significant difference between SA treatments. As same as our findings, sterilized distilled water did not have any pleasing effect in controlling or reducing microbial population of *Narcissus* vase solution (Jowkar, 2006). 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 (Knee, 2000), our results suggest the application of SA and PUT and vase solution. The presented research indicated that SA treatments, especially at highest used concentration, were effective to affect postharvest life of cut flowers probably via the declined bacterial growth, reduced vascular blockage, higher soluble solid contents, reduced transpiration, prevented ethylene formation and induced antioxidant system in treated cut flowers thereby delaying the senescence process. The application of a germicide and lowering the pH of the vase solution may extend the longevity of Acacia cut flowers (Horlock et al., 2000).

Fresh weight changes: According to the results shown in Table 2, using SA and PUT as a preservative significantly increased the vase life (P≤5%). As seen in Table 2, there is a general sharp Reduction in relative fresh weight during the Ninth day of the experiment. During the 9 days, relative fresh weight of SA treated flowers showed a slight Reduction until day-11, while control flowers showed a slight increase until day-11. On the other hand van Meeteren et al., (van Doorn et al., 1991) observed a decrease in fresh weight of deionized treated cut flowers during the first days of vase life. According to our observations, SA 100 mgl^{-1}+PU 100 mgl^{-1} treatment best retained relative fresh weight.

Table 2- Effects of Salicylic acid and Putrescine in preservative mixture on Microbial Count and Fresh weight changes in Narcissus cut flowers

Salicylic acid ($Mg.L^{-1}$)	Putrescine ($Mg.L^{-1}$)	Microbial Count (log10 (CFU ml^{-1}))	Fresh weight changes (% of the initial)		
			7	9	11
0	0	6.14ab	96.47abc	94.36a	73.56abc
	100	7.39a	97.48abc	86.51ab	79.09ab
	200	7.28a	94.86abc	81.6ab	73.99abc
100	0	6.84a	93.54bc	82.14ab	78.91abc
	100	5.59abc	101.08a	91.08ab	77.84abc
	200	3.11d	99.61ab	86.13ab	80.39a
200	0	4.28bcd	92.17c	81.14b	72.04bc
	100	4.19bcd	91.1c	79.3b	71.18c
	200	3.95dc	94.59abc	81.9ab	77.45abc
CV (%)	18.21	3.67	7.82	5.3	

Solution uptake: According to the results shown in Table 2, using SA and PUT as a preservative significantly increased the vase life (P≤5%). the most water uptake (2.52 g $stem^{-1}$ day^{-1}) was observed in 100 mg l^{-1} SA sevennth and Ninth day of the experiment. The effectiveness of this compound can be due to water relations enhancement, prevent vascular occlusion due to antimicrobial effect, anti-ethylene effect which reduces respiration rate of cut flowers and increased dry matter percent (Edrisi, 2009; Gast Karen, 1997). Zamani et al., (2011) reported that 1.5 mM salicylic acid (SA) increased water uptake about 13 ml compared to the control in cut chrysanthemum (*Chrysanthemum morifolium* L.).

CONCLUSIONS: In present study, SA and PUT increased vase life of cut flowers and had the least bacterial clonies population in vase solution.

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