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Postharvest Quality Studies in Tuberose (*Polianthestuberosa* cv. Peril) Cut Flower as Affected by Vase Preservative Solutions

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Abstract – **Suitable preservatives play an important role on tuberose vase life therefore a factorial experiment was conducted based on completely randomized design with three replications. Treatments were sucrose, 5-sulfosalcylic acid, salicylic acid, Malic acid, Boric acid, mio-inositol and distilled water. Results showed that 5-sulfo Salicylic acid components maintained more chlorophyll, longest vase life and also when flowers treated with 1.5 mM 5-SSA created more open florets and fresh weight than the other treatments during vase lifewhich was very effective in display life of flowers. The lowest pH in preservative solutions and highest membrane stability were belonged to 5-SSA with 3 mM concentration and their stability was approximately three fold stable than the control. The accumulations of bacteria population were increased in all treatments during experiment performance; however, acidic solutions had the lowest bacteria accumulation. It is concluded that the 5-SSA had a key rolein perseverating of tuberose cut flowers.**

Keywords – **Longevity, Membrane Stability, Preservative, Solution Contamination, Tuberose (*Polianthestuberosa*).**

Abbreviations – **Boric 1 (Boric acid 1mM); Boric 2 (Boric acid 2mM); Control (Tap water); MA 100 (Malic acid 100 mgL^{-1}); MA 150 (Malic acid 150 mgL^{-1}); Mio 100 (Mio-inositol 100 mg^{L-1}); Mio 150 (Mio-inositol 150 mgL^{-1}); SA 1.5 (Salicylic acid 1.5 mM); SA 3 (Salicylic acid 3 mM); SA 5 (Salicylic acid 5 mM); Su 3(Sucrose 3%); 5-SSA 1(5-sulfosalicylic acid 1.5 mM) and 5-SSA 3(5-sulfosalicylic acid 3 mM); Chl (Chlorophyll(spad reading)); MSI (Membrane stability index); RWC (Relative water content)**

I. Introduction

Tuberose (*Polianthestuberosa* L.), as an ornamental bulbous plant native to Mexico, is a perennial plant which successfully is grown for cut flowers in Iran [17]. People like long spike of flowers because of their sweet fragrance [4] and perfume industry also uses the plant extensively [9]. Although tuberose has a high potential for a long vase life after harvesting, but it declines rapidly at home conditions and more than 50% of the buds normally open after harvest and florets and buds usually abscised in a few days [37]. Approximately 45% of world floriculture trade goes to cut flower which their life spans are very critical for the industry of floriculture [12]. Senescence is a programmed event responding to external and internal signals [15]. The senescence of flower petals is associated with a series of highly regulated physiological and biochemical processes [22]. The studies of reference [24] show two major senescing agents attributed inpostharvest life of tuberose; ethylene sensitivity and vascular blockage. However besides those factors, vase life of cut flower is influenced by many factors such as climate, crop variety, harvesting time, post-harvest handling [20], constant water supply [29], microbial growth [35], ethylene formation ([41], [42] [8]) and carbohydrate depletion [39]. Furthermore the ideal floral preservatives should contain sucrose as an energy source and acidifier as an inhibitor of microorganisms ([20], [34]) and also an ethylene action or synthesis inhibitor like STS and SA [34]. Many organic and inorganic materials have been examined for improving quality and vase life of cut flowers such as Malic acid [18], ethanol and aluminioumsulphate([14], [31]), Mio inositol [16], Boric acid [30] and sucrose [7]. RecentlySA and its derivativesas an antagonist for ethylene action, use in cut flower preservation such as Gladiolus [10], rose [1], Lilium[13]. The present research aimed to find the effectiveness of different solutions of sucrose, 5-sulfosalcylic acid, salicylic acid, Malic acid, Boric acid and mio-inositol to improve vase life of tuberose cut spikes withoutre-cutting or changing solution at home conditionsto improve decorative life of cut tuberose flowers.

II. Materials and Methods

A. Plant material- Cut tuberose (*Polianthestuberosa* cv. peril) spikes were obtained from commercial growers in Tehran-Iran. Inflorescences were harvested with two or three open florets immediately placed in water and were trimmed to 60 cm ([37], [36]) and in order to prevent of decay, all leaves under water surface were cut and every four cut flowers were placed in a 1000 *ml* flask with 500 *ml* of solution. Cut flowers placed in different concentration of Salicylic acid (1.5, 3 and 5 mM), Malic acid (100 and 150 mg L^{-1}), Boric acid (1 and 2 mM), mio-inositol (100 and 150 mg L^{-1}), sucrose (3%), 5-SSA (1.5 and 3 mM) and distilled water was used as a control. All treatments were placed in chambers at room temperature 20±2 ˚C. The relative humidity was about 70% while 12h photoperiod was maintained using fluorescence lamps whit a light intensity of 15-20 $\mu mol\ m^2 s^{-1}$at the top of corolla. Another flask with 500 ml Distilled water and without flower set up for calculating the rate of evaporation. Then morpho-physiological traits were measured through every 3 days sampling times.

B. Morpho-physiological parameters

Solution pH- For measuring acidic changes in preservative solutions the amount of solutions pH was recorded every 3 days by pH meter (Model SP-701, Taiwan) during vase life of flowers.

Relative fresh weight (RFW)- The weight of flower stalk on the first day of each experiment was assumed to be 100 per cent. Subsequent weights were referred to as percentage of the initial value.

Membrane Stability Index (MSI)- Membrane stability index of petals was determined by recording the electrical conductivity of leachates in double distilled water at 40 and 100˚C [28].

Chlorophyll content- Total chlorophyll content was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan). Average of 3 measurements from different spots of a single leaves was considered.

Water uptake- For measuring amount of water uptake the vases evaporation rate and reduction of water amount in evaporation flasks were recorded as well. Then, by subtracting the water evaporation from solution reduction, water absorption was calculated.

Vase life and floret opening- The vase life of cut inflorescences was considered terminated when the number of senesced florets exceeded the number of open ones. On the other hand, loss or wilting of 50% of florets mark the end of vase life (Wilkins et al., 2005) and Number of opened and withered florets was counted daily.

Microbe population- Microbe population was isolated from vase solutions of tuberose in 3 sampling times including 0: start day, 5: after 5 days and 10: after 10 days. The aliquots of the vase solutions were diluted 100-times and 25 µL aliquots of the diluted solution were spread on sterile nutrient agar, in sterile petri plates. The plates were allowed to incubate for 24 h at 37˚C in the incubator and individual colonies of microorganisms, representing the most common colony morphology types, then were picked off the agar media with a sterile loop and streaked on EMB medium for purification. Purified microbe population were maintained axenically on EMB medium and transferred daily to fresh medium.

C. Statistical analysis- A factorial experiment was conducted in a completely randomized design with tree replications. Four stems of tuberose cut flowers were used for each replication. Results were analyzed by SAS software. Mean comparisons were performed using Duncan's multiple range tests at the 5% level.

III. Results and Discussion

A. pH variation in the solutions- The pH value significantly changed ($p \leq 0.01$) during vase life in solutions, as shown in the Table 1, different treatments had different values. The lowest and the highest pH belong to 5-SSA (3 mM) and sucrose, respectively. Differences among Boric acid, Mio-inositol and Sucrose were not statistically noticeable. Preservatives containing SA and 5-SSA remarkably declined the values compared to the control. The high concentration of acidic treatments lowered the values sharply. Rely on our findings it is very interesting that all other treatments including sucrose and mio-inositol even boric acid have high pH than the control. Results also revealed a significant difference in solutions pH ($p \leq 0.01$) during longevity period. The pH value increased to 5.5 in 6th day and after that point the value decreased to 4.8. Reference [27] reported that solution pH about 3 and 3.5 is a good pH (travels faster in the water-conducting system (xylem)) to prevent of plugging of the cut surface of the stem which is agree with our findings.Reference [40] demonstrated that there was reduction of pH in vase solutions with the presence of microorganisms.It seems that flowers which are in acidic preservatives should have more water uptake because of having no bacteria growth and vascular embolism in the stems. However it is demonstrated in flowers within 5-SSA solution at 1.5mM concentration but not in 5-SSA solution at 5mM concentration.

B. Microbial contamination (CFU)- Study of treatments on CFU showed a significant difference at $p \leq 0.01$ level. The lowest and the highest bacterial growth were recorded by the 5-SSA (3 mM) and the sucrose, respectively. Acidic preservatives had a range of prevention on microbial growth (Table 1). In comparison with the control, the SA derivatives suppressed bacterial growth and the best results recorded from 5-SSA treatments. During flower storing, the pH of Boric acid increased faster than malic acid and the microorganism's growth showed similar trends, so using of malic acid in preservative solutions is more advisable than the boric acid. With the pass of the time, the number of microorganisms had an increasing trend and the number of bacteria at 10^{th} day (~900) was almost 5 times more than 5^{th} day (~180) of experiment.Also the results of interaction between all preservative solutions during vase life illustrated that solutions prepared with sucrose at 10^{th} day had maximum bacterial growth (~2150 CUF) whereas at the same time, the rate for 5-SSA treatments was lower than 5 CUF. It is clear that the presence of microorganisms in vase solutions can cause physical plugging of cut stems, release toxic metabolites and result in programmed cell death [3].According to the results there is a significant relation between microbial growth and vase life, but it is very noticeable that the mentioned relation depended to the kind of microbe[40]. It is suggested that microorganisms may physically block the cut flower stem (Put and Klop, 1990), produce enzymes which break down walls of conducting xylem vessels (Burdett, 1970), produce ethylene or induce its production in the flower and thus accelerate deterioration, or produce toxic metabolites[40].

C. Vase life- The analysis of variance revealed that the vase life of *Polianthestuberosa* cut flower was significantly affected by treatments with a 99% level of confidence. Means comparison indicated that only the 5-SSA treatments improved longevity by 11 days and the others did not affect flower life span compared to control which had 8 days longevity at room temperature (Table 1). With increasing in concentration of the treatment, further improvements did not observed. It is clear that SA is

effective in decreasing in ethylene production and or sensitivity to ethylene which is one of the important factors in last long cut flowers either climacteric or non-climacteric. On the other hand 5-SSA is one of the SA derivate, but according to little longevity of flowers which kept in SA solutions It seems that, a factor beyond to ethylene plays a key role in positive effect of 5-SSA in vase solutions. According to our results, it seems that there is no significant relation between pH of solution and vase life. Our findings are agreed with [40], Lee *et al*. (2005).It is suggested that cavitation induced by decreasing stem water potential and not by bacterial cells is a factor affecting on water uptake more [5].

D. Water uptake- The water balance of a fresh cut flower determines its hydration status, turgidity and ultimately its flower vase life and freshness. Our results showed that there is a significant difference between all treatments in water uptake ($p \leq 0.01$), as flowers in 5-SSA preservative solution with 1.5 Mm concentration has more absorption compare to control. On the other hand there were no difference between all other treatments expect for control (Table 1). Flowers placed in 5-SSA solution had more amount of water uptake than control about 2 folded (163.34 ml). There is a notable difference between cut flowers and other perishable commodities from their water relations point of view, since they are so susceptible to desiccation, due both to transpiration from leaves and to their high surface area to volume ratio [27] and also This could be due to air embolism of cut stem, proliferation of microbes, and plant reaction to woundingas described by [33].

Water uptake of control reduced gradually with the progress of vase life until day 8 whereas the flowers placed in solution with 1.5 and 3 mM 5-SSA have high water uptake with 11 days longevity which is the best factor in extending their vase life. Similar results are obtained from Knee (2000) reports. Also, the display life of cut flowers especially at room conditions was better than the control. Our results are agreed with [10]. Higher water uptake in solutions including 5-SSA allowed more increase in fresh weight and mainly due to better corolla development and also it is likely due to acidifying and stress alleviating properties of SA.

E. Relative Fresh weight percentage (%RFW)-

According to the results the interaction between all preservative solutions during storing period on quality and specially relative fresh weight and display life of tuberose was significantly difference ($p<0.01$). In general % relative fresh weight at the beginning of experiment increase during start day and day 3 and then all cut flowers have a decreasing trend until the end of vase life. Our results are agreed with [11].The increment in RFW at initial vase life days could be due to the higher solution uptake during theearly storage time as supported by [31] and [34]. According to the Table 1, the lowest percentage of relative fresh weight belongs to sucrose and control, respectively and the highest one is for 5-SSA with 1.5 mM concentration. The declined RFW during prolonged storage time might be due to high water loss and the declining solution uptake as confirmed by [3].It seems that storing flowers in sucrose and distilled water with less water content either for having problems in water uptake or losing of their internal water faster than other treatments, leads to wilting and short vase life of them. It is suggested that more flower water content save flower display life for more time which is clear in flowers kept in 5-SSA solutions with high amount of water uptake and vase life as demonstrated in Table 1.

F. Rate of Bud opening- Our results showed that all treatments had different effects on the number of opened florets through the spikes ($p \leq 0.01$). tuberose stored in 5-SSA (1.5 mM) solution had more opened buds rather than control and also flowers were kept in Boric acid (2mM) solution had a least opened buds compare to the other treatments (Table 1). Our results are agree with [8] who reported that 5-SSA significantly increased cumulative uptake of vase solution, vase life and number of opened florets of Gladiolus cut flowers compared to the control. It is suggested that 5-SSA (1.5mM) not only has a key role in extending vase life of flowers along with increasing in water uptake, but also it is very effective in opening of other buds on the inflorescence which is very important in display life of cut flowers.

G. Leaf chlorophyll contents- The results showed that there was a significant difference ($p<0.05$) among treatments in leaf chlorophyll contents. Means comparison illustrated that except for maleic acid treatments and SA at 1.5 mM concentration, the other treatments slightly increased the chlorophyllcontents compared to control. However no significant difference was observed in leaf chlorophyll along the vase life. According to Table 1 cut flowers in preservatives including 5-SSA had high level of leaf chlorophyll. These findings are agree with [25] and [10] reports.

H. Petal Membrane Stability Index (MSI)- Analysis of variance revealed that membrane stability was significantly influenced by treatments ($p \leq 0.01$). According to table 1, the 5-SSA treatment at 3 mM concentration has remarkably increased membrane stability index in comparison with other treatments and control. The lowest value was measured by sucrose that had no differences with untreated treatment.as demonstrated from table 1, 5-SSAwith 3mM concentration was the best preservative in maintaining MSI compare to sucrose which was about 3 times more stable than flowers stored in solution containing 3% sucrose.Our results rely on the positive effect of salicylic acid and its derivate on the integrity of cell membrane is in agreement with Shirasu*et al*. (1997) and [10].

IV. Conclusion

Among all treatments preservative solutions including 5-SSA with both concentrations (1.5 and 3 mM) were the best floral solutions as compared to control with regard to all qualitative attributes and specially vase life. The vase solution having 5-SSA significantly increased cumulative uptake of vase solution, vase life, number of opened florets and decreased the number of unopened florets compared to the controls. Totally 5-SSA and SA had better

performance in preventing of wilting and senescing of cut tuberose flowers.

It is clear that adding a biocide and sucrose lead to preventing of rapid multiplication of bacteria growth and increasing in mechanical rigidity of the stem by inducing cell wall thickening and lignification of vascular tissues, respectively. Salicylic acid and its derivate as natural, cheap, safe and biodegradable compounds are suitable alternatives for conventional chemical treatments in order to prolong vase life of cut flowers of tuberose. Commercialization of these compounds for optimum formulations needs further experiments. However, more investigations are needed to find the best solution as a floral preservative to preserve postharvest quality parameters of tuberose (*Polianthestuberosa* cv. peril) cut flower.

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Table 1: Means comparison of preservative solutions on Tuberose cut flower

	Traits							
Treatments	Bacteria (CFU)	Chlo (spad)	pH	MSI	RWC	No. of fully opened florets	Vase Life (day)	Water up take
Control	597 c	25.1 bcd	5.89 bc	21.2 bc	92.6 de	15.3 cdef	8 b	170 d
Su3	1196 a	25.9 abcd	6.70 a	12.7 c	90.8 e	16.0 bcdef	8 b	241 bc
Boric1	584 bc	26.1 abcd	6.37 a	18.6 bc	95.9 cde	14.3 def	8 b	259 bc
Boric2	386 cd	27.0 abcd	6.62 a	19.0 bc	99.0 abc	13.0 f	8 b	240 bc
MA100	123 d	24.8 cd	6.28ab	16.5 bc	97.2 bcd	13.6 ef	8 b	234 bc
MA150	254 cd	24.6 cd	5.59 cd	19.2 bc	98.3 abcd	17.6 abcd	8 b	262 bc
Mio100	675 b	26.4 abcd	6.58 a	17.0 bc	95.7 cde	19.3 ab	8 b	221 c
Mio150	713 b	28.0 abc	6.45 a	15.7 bc	93.8 cde	17.3 bcde		230 c
SA1.5	13 d	23.8 d	5.38 d	15.5 bc	96.0 cde	15.0 def	8 b	224 c
SA3	19 d	27.1 abcd	3.61 e	16.7 bc	102.4 ab	16.6 bcdef	8 b	259 bc
SA5	16 d	29.8 a	3.13 f	19.4 bc	93.8 cde	14.0 def	8 b	218 c
SulfSA1.5	1 d	28.9 ab	3.14 f	23.5 b	103.8 a	21.3 a	11 a	333 a
SulfSA3	3 d	27.7 abcd	2.62 g	32.5 a	99.5 abc	19.0 abc	11 a	278 b

Different letters in each column indicating significant different at $p \leq 0.05$.
Boric 1 (Boric acid 1mM); Boric 2 (Boric acid 2mM); Control (Tap water); MA 100 (Malic acid 100 mgL^{-1}); MA 150 (Malic acid 150 mgL^{-1}); Mio 100 (Mio-inositol 100 mg^{L-1}); Mio 150 (Mio-inositol 150 mgL^{-1}); SA 1.5 (Salicylic acid 1.5 mM); SA 3 (Salicylic acid 3 mM); SA 5 (Salicylic acid 5 mM); Su 3(Sucrose 3%); 5-SSA 1(5-sulfosalicylic acid 1.5 mM) and 5-SSA 3(5-sulfosalicylic acid 3 mM); Chl (Chlorophyll(spad reading)); MSI (Membrane stability index); RWC (Relative water content);