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Optimum pH Value for Improving Postharvest Characteristics and Extending Vase Life of *Rosa hybrida* cv. Tereasa Cut Flowers

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

This experiment was conducted on cut *Rosa hybrida* L. (cv. Tereasa) during two summer seasons; 2015 and 2016 at the laboratory of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University. Stems of cut *Rosa* flowers were dipped in five different pH solutions; 3.0, 4.6, 5.6, 6.6 and 7.0 in the presence of 2% sucrose. The pH values were adjusted through using 0.2 M Na_2HPO_4 and 0.1 M citric acid. The results revealed that, the maximum values of vase-life and in turn the water uptake (4.54 ml/g) which was highly correlated with vase life duration, over the two seasons, was recorded with solution pH 3.0 followed by pH 4.6. Also, the lowest significant reduction in flower fresh weight was recorded with pH 3.0. The highest mean count of total spores forming bacteria in vase solution was recorded at pH 6.6, whereas the lowest was obtained with pH 3.0. Increasing pH level negatively influenced *Rose* flowers longevity through increasing free total amino acid as well as reducing total sugar and total chlorophyll and anthocyanin concentrations. Whereas, pH 3.0 was able to maintain a higher osmotic potential of cells through enhancing the total sugar and the total chlorophyll and anthocyanin concentrations which were in conformity with enhancing antioxidant enzymes, *i.e.* superoxide dismutase (SOD)

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and catalase (CAT) activities in leaves. In conclusion, decreasing the solution pH enabled the cut *Rose* flowers to maintain higher osmotic potential, delaying senescence and enhancing flowers longevity.

Keywords: Rosa hybrida cv. Tereasa; flower longevity; water uptake; antioxidant enzyme.

1. INTRODUCTION

Genus *Rosa* belongs to family *Rosaceae*, contains more than 150 species. *Rose* plants have highly valuable economic benefits as raw material for industrial especially in the cosmetics and perfumery. There was about 10-30% losses in rose cut flowers due to postharvest damage [1]. In the time that most customers like to enjoy the natural appearances of cut rose flowers for a longer period of time [2], about 20% of fresh rose cut flowers lose their quality, attractiveness and beauty appearance during harvest and packaging and about 10% through transportation till sale [3].

Recently, it is possible to extend the postharvest life of flowers by using different preservative solutions [4]. Wide ranges of floral preservatives solutions were applied to elongate the vase life longevity in order to fit with consumer satisfaction and for a good business. The main roles of the preservative materials, whatever their constructions, are supplying the flower with energy source, increase water uptake and reduce the amount of microorganisms which are the common source of vascular blockage [5].

Nowadays, the floriculture industry is getting popular in developing countries. The major problem of the horticulture sector in general and the floriculture industry in particular is the postharvest loss. Hence the crop was being alive for a certain period but was liable to deterioration and loss. It is clear that unless they are preserved, the ultimate fate of such product is senescent and die. However it is possible to extend the postharvest life of flowers by using different preservative solutions [6]. The use of sucrose with or without certain additive could be of practical significance for prolonging the life of many cultivars of cut roses [7].

The length of vase life is one of the most important factors for quality of cut flowers. The vase life of cut rose flowers is often short. Cut flower postharvest longevity is affected by many factors. Among them, the pH of vase solution which considered an important factor in controlling water uptake, reduce embolization and slow bacterial growth [8]. Solution pH has been found to be an important factor in vase life for many species of cut flowers, *i.e. Dendranthema* L. and *Helianthus* L. [9] as well as *Rosa* L. [10]. Vase solution can be acidified with citric acid, aluminum sulfate or other acids. Citric acid is a safe organic acid and is much cheaper and more effective than aluminum sulfate as well as induces stomatal closure, reducing transpiration and improving water balance [11].

Therefore, the aim of this work is to study the influence of different pH preservative solutions on the vase life and other postharvest characteristics of rose cut flowers.

2. MATERIALS AND METHODS

This experiment was conducted at the laboratory of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons, 2015-2016.

2.1 Plant Material

Cut stem of Rose flower (cv. Terease) at half opening stage were harvested from Flora Mix Farm at Mansouria, Giza and pre-cooled at 2°C and transferred immediately to the laboratory. In the laboratory, the cut stems each with 3 intact leaves, were labeled and re-cut diagonally using a sharp knife from the base of the stem to keep all the cut stems at one uniform lengths; 60 cm. The bottom of the cut flower stems were completely immersed in each treatment solution.

2.2 Treatments

The treatments were consisted of five pH preservative solutions, *i.e.* 3.0, 4.6, 5.6, 6.6 and 7.0. To adjust the solution pH; 0.2 M Na_2HPO_4 and 0.1 M citric acid were used as shown in Table 1. Also sucrose of 2% concentration was added. Two cut stem of rose flowers were placed in each pH solution (500 ml) and replicated 4 times. The cut stem of rose flowers were kept at room temperature (23-25°C), 60% relative humidity (RH) and 24 h photoperiod.

Table 1. Combinations of 0.2 M Na_2HPO_4 and 0.1 M citric acid to adjust different pH solution treatments

pH	0.2 M Na_2HPO_4 (ml)	0.1 M citric acid (ml)
3.0	20.55	79.45
4.6	49.75	53.25
5.6	58.00	42.00
6.6	72.75	27.25
7.0	82.35	17.65

2.3 Recorded Data

2.3.1 Flower vase life

Data were collected daily for vase life; the duration from placement of stems with flower in vases till the time when the termination symptoms were observed. The termination symptoms of rose flower were bent neck and petals loss pigments and browning or wilting. Each treatment was terminated when any of the above symptoms were appeared on about 50% of the flowers [5].

2.3.2 Solution uptake (S)

Solution uptake was determined according to formula suggested by Chamani et al. [12].

Solution Uptake = ((S t – 1) – St) /Initial Fresh Weight × 100

Where, St= Solution weight (g) daily; St-1 = initial solution weight (g).

Change in solution pH: Changes in solution pH for each treatment were measured till the termination of experiment time.

2.3.3 Relative fresh weight

Fresh weight of the flowers was determined just before immersion the flowers on the solution and repeated regularly every day until the vase life of the control flowers was ended (about 8 days in both seasons), according to Joyce and Jones [13].

2.3.4 Total sugar

Was determined using the phenol-sulfuric acid method described at Dubois et al. [14].

2.3.5 Total free amino acid

Was determined according to the method of Etsushiro et al. [15].

2.3.6 Total chlorophyll and anthocyanin

Total chlorophyll were determined in fresh leaf samples by using (SPAD) according to Netto et al. [16]. Anthocyanin was determined according to methods of Knee [17].

2.3.7 Bacteriological study

The microbiological examination of the holding solution was carried out on different periods according to Atlas [18].

2.3.8 SOD and Catalase activities

Enzymes extraction was carried out using fresh leaf tissues in a buffer solution (3:1 buffer: fresh weight v/w) in a pastel. It was mortared with 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3 mM dithiothreitol and 5% (w/v) insoluble polyvinylpyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were taken for assay Vitoria et al. [19]. Antioxidant enzymes were assayed as follows; catalase (CAT) by measuring the decrease in absorbance due to disappearance of H_2O_2 at 240 nm according to Chance and Maely [20] whereas superoxide dismutase (SOD) was assayed as described by Giannopolitis and Ries [21]. Enzymes activities were expressed as units/mg protein.

2.4 Statistical Analysis

A randomized complete design with one factor was used for analysis of all data with four replicates each including three flowers. Means were compared by least significant difference (L.S.D.) test as given by Snedecor and Cochran [22]. To study the relationship among different traits, simple correlation coefficient for each pair of the traits was calculated. The statistical significance of correlations was done according to Gomez and Gomez [23] by using of Assistat program.

3. RESULTS AND DISCUSSION

3.1 Flower Vase Life

It is evident from the data presented in Table 2, that the maximum recorded flower vase-life (13-14 days) over the two seasons were obtained with flowers kept in solution at pH 3.0. Moreover, it is clear that a negative relationship was found between the acidity of solution and the flower vase life, as the pH increased the flower vase life decreased. Generally, solution at pH 3.0 was likely more effective in enhancing flower vase life

compared with the other solutions pH. The flowers kept at solution pH 7.0 recorded the shortest periods of vase life (8.33 and 8.76 days) in both seasons, respectively. Also, it is worthy to notice, that the vase life of the flower kept in solution pH 3.0 was not significantly varied from those of pH 4.6.

In this regard, solution pH has been found to be an important factor in vase life for many species of cut flowers as mentioned by Regan and Dole [10] who found that an increase in vase life of Rose flowers with low pH 3-4. The solution acidity might improve stem water uptake [24], slowing of bacterial growth in the vase solution [25], and/or reduction of embolization [11]. Meanwhile, Carlson and Dole [9] found that vase life of cut *Zinnia* flowers was not affected by vase solution pH.

Citric acid is used to lowering solution pH, because it is the most common compound extensively used and control microbial populations in preservative solution and was effective for cut *Dianthus caryophyllus* L. [26], *Rosa* × *hybrida* L. [27], *Polianthes tuberosa* L. flowers [28]. It lowers the pH of cell sap and reduces vascular blockage, thereby improving water uptake and extending longevity. Similarly, citric acid also encouraged floral opening and maintained postharvest quality of cut tuberose spikes [28].

3.2 Solution Uptake

One can notice that a relatively small changes in pH concentration were recorded in solutions where the stems were kept continuously in the preservatives until termination. Data presented in (Table 2) revealed that the maximum solution uptake was recorded with flowers kept in preservative solution pH 3.0 or 4.6 in both seasons. The less amount of solution uptake by the flower was observed when increasing the pH solution. The flowers kept in solution pH 7.0 recorded the lowest amount of solution uptake, the reason might be due to the fact that there is a negative relationship between the acidity of preservative solution and the solution uptake. General speaking, preservative solution at low pH 3.0 was more effective in extended the flower vase life as a result of absorbing more amount of solution as compared to other treatments. Also, it was found a highly positive correlation coefficient between vase life and water uptake reaching + 0.87 over the two seasons as shown in Table 5.

In this regards, Carlson [11] proposed that low pH was beneficial for cut stems by helping to prevent and repair xylem air occlusions. Carlson added that pH below 3.0 prevented long-term declines in hydraulic conductivity, apparently by limiting bacterial growth. Moreover, Dole and Schnelle [29] reported that the solution uptake was increased below pH 3 and inhibited above pH 6, the effects being more marked with time. In addition Chandran et al. [30] suggested that, as water relations were improved and maintained, treated cut *Dendrobium* (Heang Beauty) orchids flowers with low pH solutions (sucrose 4% + 0.5mM AOA or 0.5 mM AOA) considerably delayed both abscission and petal wilting .–On the contrary, Carlson and Dole [9] found that solution uptake to be slightly higher in acidic solutions for *Dendranthema* and *Dianthus*, but this difference was not statistically significant from basic and neutral solutions and would not fully account for the difference in vase life.

3.3 Change in Solution pH

Vase solution pH is another important postharvest factor and low pH may improve stem water uptake, reduce embolization, and slow bacterial growth. The data in Table 3 represented the change in pH levels at the initial and the termination periods of the experiment during the two seasons. In all studied treatments a relatively small changes in pH (≤0.4 units) were recorded between starting and termination periods.

Table 2. Effect of different pH solutions on vase life (days) and solution uptake (ml/g flower f.wt.) of rose flowers during the two seasons

Treatment pH	Vase life (days)		Solution uptake (ml/g)	
	1st season	2nd season	1st season	2nd season
3.0	13.00	14.00	4.54	4.85
4.6	12.33	13.00	4.33	4.80
5.6	10.00	11.00	4.30	4.10
6.6	9.00	8.67	3.73	3.78
7.0	8.33	8.67	3.16	3.69
LSD 5%	**1.39**	**1.40**	**0.41**	**0.59**

Table 3. Changes in solution pH values at the termination of the experiment during the two seasons

Initial pH	pH at the termination	
	1st season	2nd season
3.0	3.20	3.09
4.6	4.60	4.72
5.6	5.81	5.56
6.6	6.13	6.25
7.0	6.27	6.37
LSD 5%	**0.38**	**0.35**

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

3.4 Flower Fresh Weight

The data in Fig. 1 indicated that the solution pH at 3.0 or 4.6 recorded the least significant reduction in flower fresh weight; reaching collecting (6 and 11%) and (5 and 8%) in the 1st and 2nd seasons, respectively. On the other hand, at pH values higher than 4.6, the reduction of flower fresh weight were progressively increased. Also, it was found a highly negative correlation coefficient between vase life and reduction of fresh weight reaching - 0.83 as shown in Table 5. In this regards, Ahmad et al. [5] reported that fresh weight of cut rose increased when hydrated in a low pH (4.0) solution, but was reduced at higher pH of 6.0 or 8.0.

3.5 Total Sugar

The data in Fig. 2 showed that total sugar concentration in cut stem of the flowers kept in solution pH 3.0 was significantly increased as compared to those in the other pH solutions in both studied seasons. Also, it was clearly indicated that total sugar in the flowers kept in solution pH 7.0 was significantly reduced than those in the other solutions of both seasons. Also, it was found a highly positive correlation coefficient between vase life and total sugar, reaching + 0.93 over the two seasons as shown in Table 5.

In this concern, it was proposed that there is positive correlation between the levels of endogenous sugars and the time to petal wilting [31]. Ahmad and Dole [32] stated that the senescence of cut rose flower is associated with a decrease in all sugar content. In addition, the senescence process of cut flowers is regulated by phytohormones and correlated with the carbohydrate status of the petals [33]. Also, it has been stated that the senescence process and longevity of cut flowers is closely correlated with the petal carbohydrate contents and solution uptake [34].

3.6 Total Free Amino Acids

Date in Fig. 3 showed that the longest flower vase life and the greatest amount of solution uptake were accompanied by the lowest total free amino acid concentration. Total free amino acids concentrations were significantly decreased with decreasing solutions pH. In other words, with increasing solution pH over than 3.0 or 4.6, the total free amino acid was increased. Also, it was found a highly negative correlation coefficient between vase life and total free amino acids, reaching -0.88 as shown in Table 5.

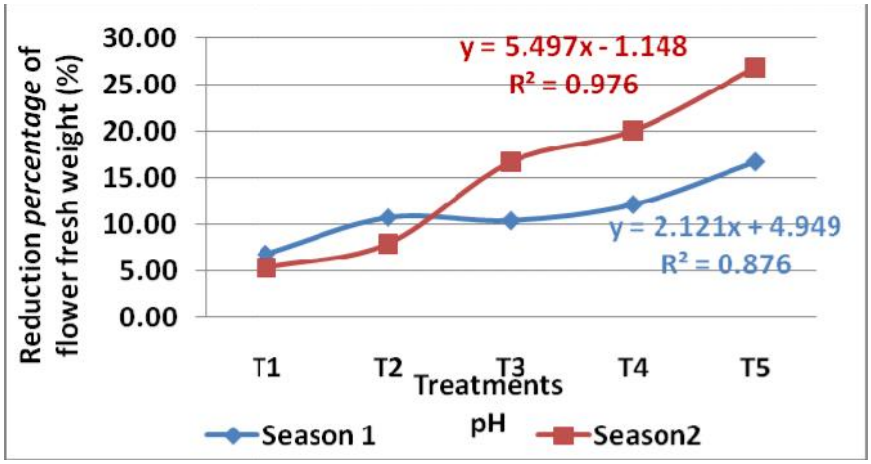

Fig. 1. Reduction percentage in the fresh weight of rose flower as affected by different solution pH

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

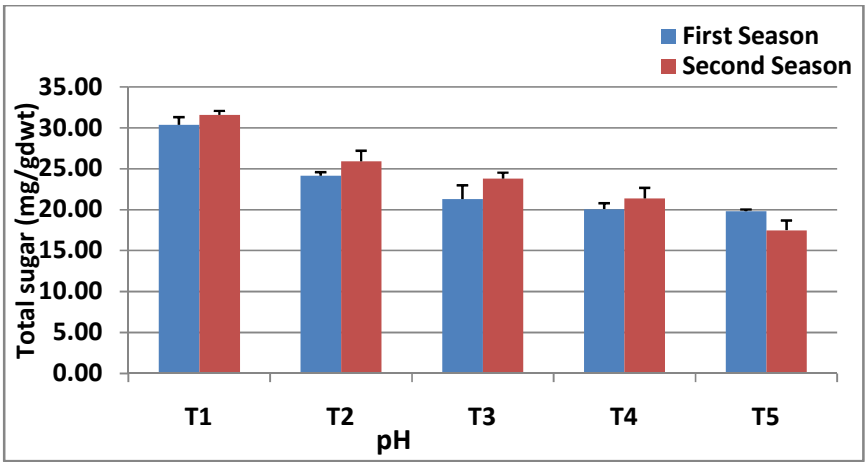

Fig. 2. Total sugar concentrations in rose flower as affected by different pH solutions during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

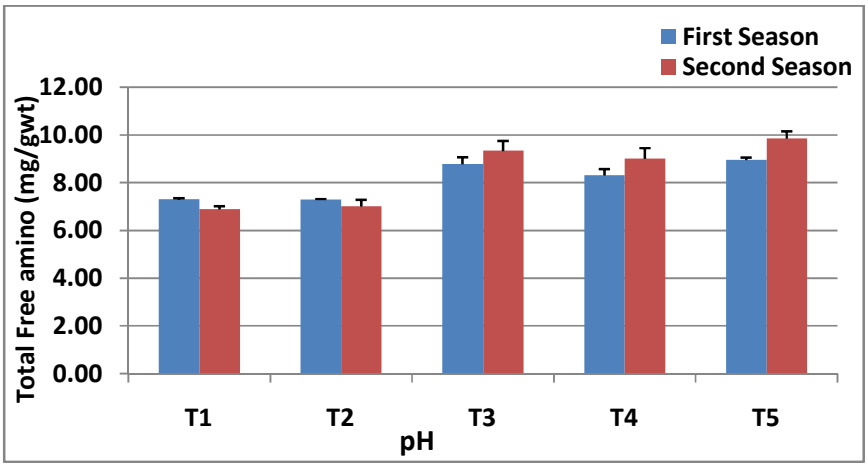

Fig. 3. Total free amino acids concentrations as affected by different pH solution during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

In this regard, Geo and Wu [35] reported that senescence of cut rose flower is associated with an increase in total free amino acids concentrations in petals. Also, increased longevity of lily flowers was associated with slowing down degradation processes, which was especially obvious in the first flower [36].

3.7 Total Chlorophyll and Anthocyanin

The results of total chlorophyll and anthocyanin concentrations during both seasons in the cut stem of the flowers kept at different pH solutions are presented in Figs. (4 and 5). At solution pH 3.0, the concentrations of total chlorophyll and anthocyanin were significantly increased as compared the other solutions. This increment was obvious in the 2nd season than in the 1st one. The concentration of anthocyanin in petals of the flower kept in solution pH 3.0 was increased by 137.1% over those flowers at pH 7.0, in the 2nd season. At the same time, the same trend for chlorophyll concentration was obtained (65.1%). Also, it was found a highly positive correlation co-efficient between vase life and either total chlorophyll or anthocyanin, reaching + 0.78 and + 0.91, respectively over the two seasons as shown in Table 5.

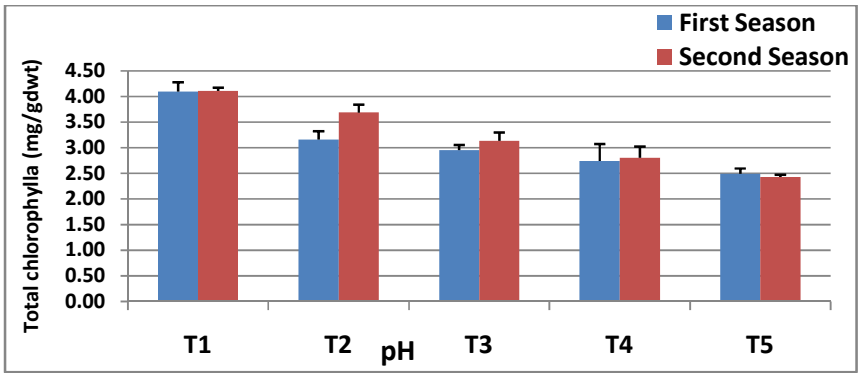

Fig. 4. Total chlorophyll concentrations in rose flower as affected by different pH solutions during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

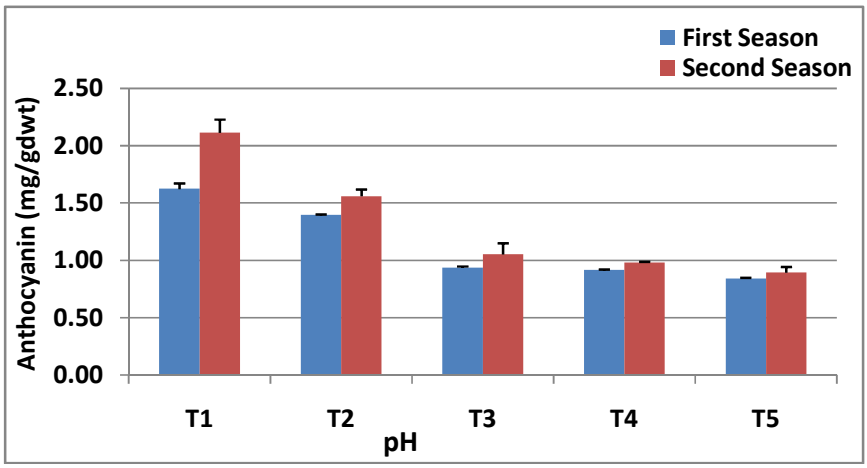

Fig. 5. Anthocyanin concentrations in rose flower as affected by different pH solutions during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

In this regard, chlorophyll content was significantly varied at different pH values [37]. The changes in petal color is significantly influenced by a change in the pH of the cell vacuole. Color fading and discoloration are important factors in determining visual quality of flowers and in many cases they are the main reasons for determination of post-production quality [38]. The improvement of petal color expression is at least partially due to the increase in anthocyanin contents. In some cases, stability of the pigments might cause the chemical degradation of the anthocyanin, which resulted in senescence process [36].

3.8 Bacteriological Study

In this research, it is worthy to mention that the amount of total bacteria (log CFU ml^{-1}) in vase solution had a positive relationship with the solution acidity either in the initial or termination time of the experiment, in both seasons (Table 4). In the 1st season, the amount of total bacteria in solution pH 7.0 was highly significant increased at the termination time (7.03) than the amount in solution pH 3.0 was (5.70). Also, it was found a highly negative correlation coefficient between vase life and total bacteria, reaching -0.92 as shown in Table 5.

In this concern, Dole and Wilkins [39] stated that, one of the main issues in cut flower postharvest is controlling microorganism growth. Bacterial growth in vase solutions can lead to stem vasculature blockage causing petal and leaf wilt, bent neck, or similar water stress related symptoms that reduce vase life [11]. Also, van Doorn [40] reported that microbial growth in vase

Table 4. Effect of different pH solutions on the amount of total bacteria (log CFU/ ml) at the initial and termination periods during the two seasons

Treatment pH	Total bacteria (Log CFU/ml)			
	1st season		2nd season	
	Initial	Termination	Initial	Termination
3.0	3.31	5.70	3.48	5.62
4.5	3.82	5.91	3.76	5.82
5.5	4.05	6.15	3.97	6.07
6.5	4.34	6.71	4.27	6.64
7.0	5.63	7.03	5.56	6.96
LSD 5%	**0.22**	**0.13**	**0.20**	**0.12**

solution caused obstruction of xylem vessels due to formation of tyloses, deposition of materials in the lumen of xylem vessels and the presence of air emboli in the vascular system. Carlson [11] also mentioned that lowering pH might improve stem water uptake, reduce embolization and slow bacterial growth. Acidic pH prevented and slow down bacterial growth, ensure proper water uptake and delayed senescence [6].

3.9 SOD and Catalase Activities

The data in Figs. (6 and 7) indicated that SOD and catalase enzymes activities were significantly increased at solution pH 3.0 or 4.6, in both seasons, as compared to the other pH values. The activity of SOD and catalase enzymes in the flowers kept in solution pH 3 or 4.6 recorded about 99.01 and 143% increase over those kept in pH 7.0 for both enzymes, respectively. Also, it was found a highly positive correlation coefficient between vase life and either SOD and catalase activities, reaching +0.94 and +0.93, respectively over the two seasons as shown in Table 5.

During senescence there is an over production of free radicals such as superoxide anion (O_2−), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2), which may cause damage and cell death. Superoxide dismutase (SOD) is the only enzyme capable of scavenging O_2^-, where as H_2O_2 it can be directly degraded by catalase (CAT) or peroxidase (POD) in the presence of a reductant [41].

The activities of CAT and SOD (as the components of antioxidative system) declined with the passage of time. Based on the available evidences, it is obvious that free oxygen radicals are involved in the senescence process via inducing oxidative stress. Salicylic acid treated peach fruit during postharvest life showed higher free radical scavenging activities, activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) as well as acceptability [42].

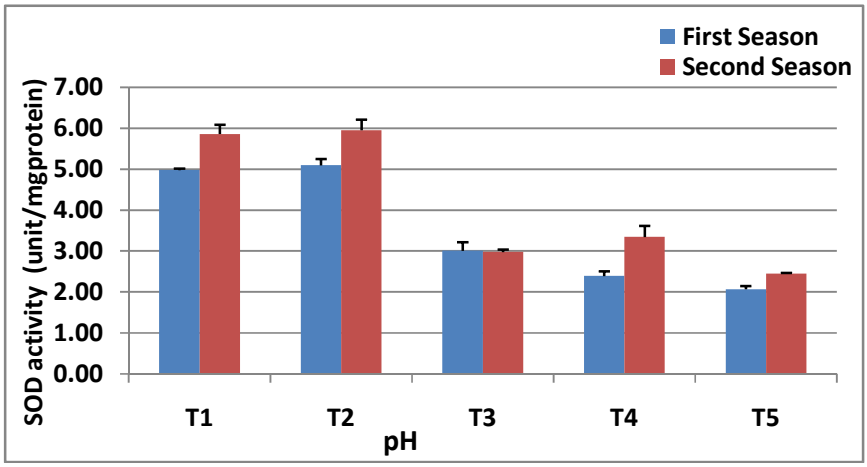

Fig. 6. SOD activity (unit/mg protein) in leaves of rose flowers kept in different pH solutions during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

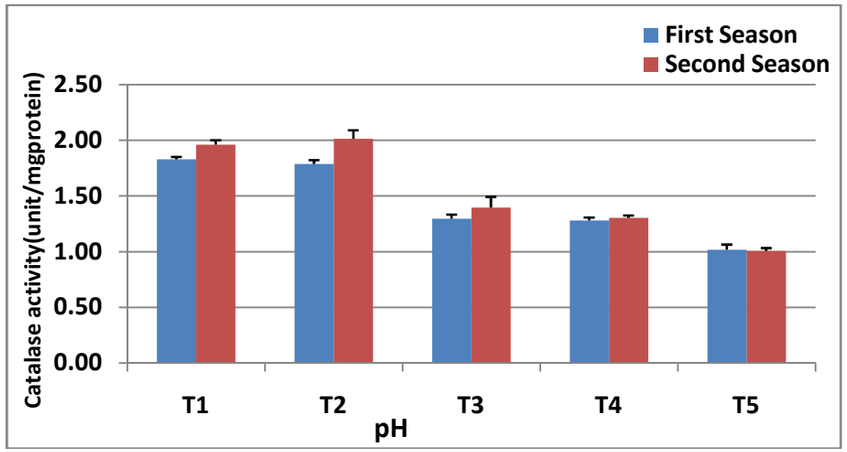

Fig. 7. Catalase activity (unit/mg protein) in leaves of rosa flowers kept in different pH solutions during the two seasons. Data are the mean of four replicaties ±SE

Key: T1: pH 3.0; T2: pH 4.6; T3: pH 5.6; T4: pH 6.6; T5: pH 7.0

Table 5. The correlation between all the studied growth and chemical characters under the influence of different pH solution of rose flowers over two seasons

	Reduction % of fresh weight	Vase life (days)	Solution up take (ml/g)	Total Free amino (mg/gwt)	Total sugar (mg/g dwt)	Anthocyanin (mg/g dwt)	Total Chlorophyll	Total bacteria log^{10} CFU ml^{-1}	SOD	CAT
Reduction % of fresh weight	1.00									
Vase life (days)	-0.83***	1.00								
Water up take (ml/g)	-0.89***	0.87***	1.00							
Total Free amino (mg/gwt)	0.84***	-0.88***	-0.84***	1.00						
Total sugar (mg/gdwt)	-0.85***	0.93***	0.84***	-0.85***	1.00					
Anthocyanin (mg/gdwt)	-0.87***	0.91***	0.85***	-0.92***	0.95***	1.00				
Total chlorophyll	0.85***	0.78***	0.73**	0.75***	-0.88***	-0.72**	1.00			
Total bacteria log^{10} CFU ml^{-1}	0.92***	-0.92***	-0.93***	0.83***	-0.90***	-0.86***	0.90***	1.00		
SOD	-0.85***	0.94***	0.86***	-0.96***	0.86***	0.93***	-0.76**	-0.87***	1.00	
CAT	-0.91***	0.93***	0.90***	-0.96***	0.87***	0.92***	-0.85***	-0.92***	0.99***	1.00

***, *** Significant at 0.01 and 0.001 levels of probability, respectively*

4. CONCLUSION

Finally, from this study it was concluded that maintaining cut *Rosa* L. (cv. Tereasa) flower stems in acidified solution (pH 3) containing Na_2HPO_4, citric acid and 2% sucrose significantly increased water uptake, total sugar concentration and the activities of both SOD and catalase enzymes as well as reduce the reduction in fresh weight, total free amino acids, total chlorophyll and anothyanin concentrations, which in turn enabled the cut rose flowers to delay senescence and enhance flowers longevity reaching about 13-14 days under this experiment conditions.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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