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Assessment of Different Preservative Solutions on Vase Life of Cut Roses

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Abstract

The postharvest vase life of cut roses was studied to determine the effects of various physiological factors that influence the vase life of cut flower. Cut roses were obtained from commercial area of Siliguri. To assess the effect of preservatives on the postharvest life of rose, cut flowers were treated with following preservative solutions: fixed amount of citric acid (CA) (300 µg/ml) and 8-hydroxyquinoline (8-HQ) (200 µg/ml) with various concentrations (1% to 8%) of sucrose solution. Each treatment consisted of 3 replicates. Cut roses were treated with different solutions and were kept at normal room temperature (20° C) under normal day light and natural ventilation. The biochemical parameters such as phenol, flavonol content, total soluble sugar, reducing sugar, non-reducing sugar, and for antioxidant property 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined in flowers treated for 0, 2, 4, 6 and 8 days, respectively. The physiological parameters such as vase life, water uptake, transpiration rate and water balance were also observed. The results demonstrated that the flowers which were treated with 5% of sucrose extended the vase life of cut flower from 4 to 8 days by improving the carbohydrate supply and reducing oxidative stress mediated damages during rose flower senescence.

Keywords: Citric acid, DPPH, 8-Hydroxyquinoline, Postharvest, Rose, Sucrose, Vase life.

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INTRODUCTION

Rose (*Rosa hybrida*) belongs to the family Rosaceae under which comes more than 150 species and 1400 cultivars (Synge, 1971, Elgimabi, 2011). This family is recognized for their high economical values as they provide the best raw material for agro-based industry viz. cosmetic, perfumery and also has important role in medicine and nutrition (Butt, 2003). Rose is always regarded as the queen of flower but the vase life of cut roses are usually short (Gerailoo and Ghasemnezhad, 2011). Study done by Van Doorn and De (1997) suggested that the main reason for senescence of cut flower is wilting due to which the floral axis bent just below the flower head which stops the water supply to the flowers. The most important worsening factor in cut flower is the blockage of xylem vessels by air and microorganism (Elgimabi and Ahmed, 2009). This leads to decline in protein content, lipid fluidity of membranes and increase in protease activity (Arora *et al.*, 2007). Further it was observed that the cause of senescence is involvement of the reactive oxygen species (ROS) in plant tissues (Dhindsa *et al.,* 1981). Earlier authors also revealed that activated oxygen species (AOS) such as O2- or H_2O_2 and their interaction product, hydroxyl radicals (OH) degrade proteins, lipids and nucleic acids lead to senescence (Thompson *et al.,* 1987; Arora *et al.*, 2007).

There are three parameters which will affect the flower senescence: the water balance, the supply of carbohydrates and the susceptibility towards ethylene, as ethylene shortens the vase life and leads to senescence (Michael and Wu, 1992; Kazemi, 2012). Cut roses are harvested at bud stage but the flower opening needs huge amounts of carbohydrate, therefore treatment of cut roses with sucrose can extended the vase life of cut flower (Ichimura *et al.*, 2003). An experiment done by Hardenburg (1968) revealed that the cut flower should be in a healthy condition and should be free of any deterioration to avoid the entry point contamination with decaying microorganisms. But to keep the flower in fresh condition for longer period several methods have been developed and it was found that the use of preservative solution is helpful for delaying senescence and extending the postharvest life of cut flower which also controls ethylene synthesis and pathogen development (Halevy and Mayak, 1980, Gerailoo and Gasemnezhad, 2011).

According to Nowak and Rudinicki (1990), 8-hydroxyquinoline sulphate (8-HQS) is an important germicide used in flower industry. Different concentration of sucrose is used as preservatives for extending vase life of cut rose (Butt, 2005). Citric acid enhance vase life by reducing the risk of vascular blockage in cut flower through its anti embolism trait (Bhattacharjee *et al.*, 1993). But sucrose alone cannot be used without germicides, as sucrose promotes bacterial proliferation leading to shortening of vase life (Ichimura *et al*., 2002). In this study, the experiment was designed to assess the effects of different preservative solutions on vase life of cut yellow rose. Also the biochemical investigations related with sugar availability and free-radical scavenging capacity of petals after passing different post-harvest durations were quantified for evaluating the physiological changes associated with senescence and their influence on vase life.

MATERIALS AND METHODS

Plant material and preservative solutions

Cut flower of yellow rose [*Rosa hybrida* L. 'Sunpari' (Franco Rose Co.)] was collected from commercial area of Siliguri. Flower stems were trimmed to 10 cm underwater to avoid water embolisms. All leaves on the lower section of the stems were removed. Then the cut flowers were placed in the conical flasks containing the preservatives of various compositions (Table 1).

Chemicals used

8-hydroxyquinoline sulphate, citric acid, sucrose, 1,1-dyphenyl-2-picrylhydrazyl (DPPH), gallic acid, folin-ciocalteu reagent, sodium nitrate, aluminium chloride, sodium hydroxide, quercetin, anthrone, sulphuric acid, rochelle salt, di-nitrosalicylic acid.

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Determination of physiological status of flower

1. Experimental setup : To study the effects of sucrose in enhancing the vase life of cut flowers first of all the different concentration of sucrose was prepared viz. 1%, 2%, 3%, 5% and 8% with 250 ml of distilled water to that 50 mg of 8-HQ and 75 mg of CA was added and dissolved. Then the solution was transferred to 100 ml flask in triplicates and to avoid the surface evaporation solution surface was covered by adding neutral oil, after that the weight was taken. After putting flower trimmed to 10 cm, again weight was taken.

2. Fresh weight: The flask was weighted with flask + solution + flower and weight of flask and solution was subtracted the difference in the weight signifies fresh weight of flower. This process was repeated everyday till the flower was fresh. The flower weight was expressed in grams.

3. Water uptake: For determining water uptake, flasks were weight with solution without flower and the consecutive difference in weight signifies the water uptake by flowers, expressed in grams per flower.

4. Transpiration loss: Flowers were weighted daily along with solution and flowers and the consecutive difference in weights represents the transpiration loss, expressed in grams per flower.

5.Water Balance: Water balance was calculated by deducting the total transpiration loss from water uptake.

6. Vase Life: Vase life of cut flowers was determined on the percentage of wilting. When the percentage of wilting crosses 50%, there is discolouration and loss of petals then the vase life of flower is said to be terminated.

Biochemical and phytochemical analysis

1. Preparation of plant extracts: Out of six set of experiments sucrose at concentration of 5% and 8% gave the best results in experiment (1). Therefore further investigation on phytochemical changes and antioxidant activity of cut roses preserved in control (water), 8 HQ+CA, 8 HQ+CA+5% sucrose and 8 HQ+CA+8% sucrose for 0, 2, 4, 6 and 8 days respectively were studied.

The petal of cut rose flower (2 g) was crushed in motor and pestle using 10 ml of 80% ethanol to that another 15 ml methanol was added and refluxed for 30 min, the extract was filtered and volume was made 10 ml by heating. The extract was stored in dark glass bottles for further processing and kept in refrigerator for further analysis.

2. Evaluation of DPPH scavenging activity: The ability of the plant extract to scavenge 1,1-dyphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by the method Sidduraju et al. (2002). DPPH (4 mg) was dissolved in 100 ml of methanol. The stock solution of the flower extract was prepared in methanol to achieve the desired concentration and to 0.2 ml of sample 2 ml of DPPH was added and incubated for 20 min. and absorbance was measured against reagent blank at 517 nm.

The blank samples contained all the reagents except the extract. The percentage inhibition was calculated using equation given below, whilst IC50 values were estimated from the percent inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.

Percent inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

3. Determination of total phenol content: The total phenolic compounds in the different extracts of cut yellow rose were determined by Slinkard and Singleton (1977) method using Folin-Ciocalteu reagent. Gallic acid was taken as standard. To 1 ml of methanolic extract 1 ml of 95% ethanol and 5ml distilled water was added and mixed thoroughly with 0.5ml of Folin-Ciocalteu reagent. After 5 min, 1 ml of 5% sodium carbonate was added and the absorbance was measured at 765 nm after 1 h incubation. Using various concentration of gallic acid, a standard curve was obtained.

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4. Determination of flavonoid content: The total flavonoid content was measured by Kim *et al.* (2003) method. According to this method 0.5 ml of methanolic extract was first diluted with 4 ml distilled water. To the above mixture 0.3 ml of 5% $NaNO_3$ and 0.3 ml of 10% AlCl3 was added. After 6 min, 2 ml of 1(N) NaOH was added and the absorbance was measured against reagent blank at 510 nm. A standard curve was obtained using various concentrations of quercetin standard.

5. Estimation of total soluble sugars: The total soluble sugar was measured by Anthrone method (Hedge and Hofreiter, 1962). Anthrone was prepared dissolving 100 mg anthrone in 50 ml H2SO4. To 1ml of sample 4 ml of anthrone reagent was added, mixed thoroughly and then heated for 8 min in boiling water and then cooled rapidly. Absorbance was measured against reagent blank at 630 nm.

6. Estimation of reducing and non-reducing sugars: The reducing sugar was measured by DNS method (Sadasivam and Hanickam, 1992). To 1 ml of sample 1 ml of DNS was added then the sample was heated for 5 min in boiling water bath; to the warm sample 1 ml 40% Rochelle salt solution was added and then cooled under tap water. Absorbance was recorded against reagent blank at 510 nm. The non reducing sugar content was obtained by subtracting the content of reducing sugar from total soluble sugar.

RESULTS AND DISCUSSION

The following data were recorded: water uptake (g), transpirational loss (gm/flower), water balance and the changing of flower fresh weight (%) during the vase life period, flower longevity was determined when the petals showed symptoms of wilting and change of colour edge of petals.

Water uptake

When flowers are cut from the mother plant, water loss from these continues through transpiration. When cut flower absorbs water from the solution it maintains a better water balance and flower freshness is maintained for long duration increasing vase life (Reddy and Singh, 1996). The results from Fig. 2 indicated that there was decrease in water uptake within 2nd to 5th day in case of control as well as other preservatives, whereas 8HQ+CA+5% sucrose maintained steady water uptake till 8th day.

Transpirational rate

Wilting is the most common reason for the termination of vase life, not their natural senescence (Halevy, 1976). The important factor which causes wilting is water stress which occurs when rate of transpiration exceeds the rate of water uptake. The results from Fig. 3 showed decrease in transpiration rate within 4th to 5th day in case of control as well as other preservatives, whereas 8HQ+CA+5% sucrose maintained stable transpiration rate up to 8th day (Fig. 3).

Water balance

Water balance determined by the difference between water uptake and water loss (Halevy and Mayak, 1981). In case of many flowers, wilting is considered as the termination of vase life (He *et al.,* 2006). For determining the quality and longevity of cut flower, water balance is considered as the determining factor which is influenced by water uptake and transpiration rate (Da Silva, 2003). It is observed by Halevy and Mayak (1981) that wilting in cut flower occurs when transpiration rate was greater than the volume of water uptake. Lower water uptake is commonly caused by microbes when they block the xylem vessels resulting blockage of water to the upper part (He *et al.*, 2006, Van Doorn, 1997). The results from Fig. 4 indicated that water balance in

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preservative solution with 8HQ+CA+5% sucrose showed least negative value which confirmed the homeostatic balance between the rate of water uptake and transpiration.

Increase or decrease of flower weight percentage

Flower weight was assessed daily and from all observed data the percentage decrease in flower weight was minimum in the preservative set with 8HQ+CA+5% sucrose and also was stable up to 5th day when compared with other preservatives as well as control (Fig. 5). It is stated by Halevy and Mayak (1981) that this may be due to sucrose influences the vase life of cut flowers by improving their water balance.

Vase Life

Vase life was determined by the number of days in which flowers get wilted. It is observed from the result that there is a significant difference in the physiological characteristics of flowers, and the preservative set treated with 8HQ+CA+5%sucrose showed minimum percentage of wilting (Table 2).

Total soluble sugar

The total sugar content in the cut flowers varied throughout the observation period, rapid decrease in sugar content within day 4 and day 5 in case of most of other treatments except 8HQ+CA+5% sucrose was observed, possibly because of an increase in the rate of respiration, and therefore, the stored sugars were consumed which led to early senescence (Fig. 6). 8HQ+CA+5% sucrose which showed slow decrease, may be due to the optimum sucrose supplied in the preservative solution, at day 8, sugar content began to decrease and as the senescence progressed the rate of respiration increased resulting in exhausting of the available substrates. This slow decrease in the sugar content may be the reason for their prolonged vase life. The increase in soluble sugar content of cut roses treated with 8% could be because of its lower ability to translocate the sugars towards the flower, which leads to early senescence. Similar results were also reported in other rose cultivars, where significant differences were found in the total soluble sugar among flowers treated with different preservative solutions (Figueroa *et al.*, 2005).

Reducing and non-reducing soluble sugars

The results from Fig. 7 indicated that the total reducing sugar content was higher in the cut flowers treated with preservatives containing high amount of sucrose compared to control and 8HQ+CA. The preservatives containing 8% and 5% sucrose also had higher non-reducing sugar content may be due to the hydrolysis of exogenous sugar in the solutions (Fig. 8). This breakdown of exogenous sugar may be catalyzed by the hydrolytic enzymes produced during the process of senescence (Mayak and Halevy, 1980). Availability of exogenous sugar resulted in high content of metabolically utilizable reducing and non-reducing sugars for longer period may led to prolonged vase life.

Total phenolics content and DPPH scavenging activity

The results from Fig. 9 and 10 showed that the phenolics content and DPPH free radical scavenging activity of cut flower preserved with 8-HQ+CA+ 5% sucrose was low at initial stages but gradually increased during terminal phase except of control the result was inversed i.e. high at initial stages but gradually decreasing during terminal phase. As suggested by Schmitzer et al., (2010), at late senescence stage there will be low phenol content which makes the plant more susceptible to oxidative stress that leads to accelerated necrosis. Our observation also supports this opinion as it was found that the total phenolics content of the cut flowers used in our experiment was gradually reduced towards ageing of flowers after initial rise on 3rd day (Fig. 10). In contrast,

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total flavonol content during flower senescence enhanced markedly from 4th day onward through which oxidative stress management might be mitigated efficiently (Fig. 11).

The results of present study revealed that 8-HQ, sucrose and CA treatments were effective in improving the vase life of cut roses when compared to control. Among the applied treatments, 8-HQ+CA+5% sucrose maintained vase life of cut flowers for longer period. Vase life was extended from 4th day and for control from 8th day from treated with preservatives containing exogenous sucrose. The supplementation of sucrose in the preservative solution resulted in enhanced substrate mobilization as well as utilization which led to prolonged vase life of the treated cut flowers. On the other hand, preservative solution without sucrose were not found to influence the substrate levels over the experimental period. In addition, free radicals generated during accelerated ageing should be effectively mitigated through scavenging mechanism when prolonged vase life is expected. Further research is required for resolving exact physiological problems associated with senescence of cut flowers and optimizing the preservative doses for commercial purposes.

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Tables

Table 1. Composition of different preservative solutions for treatment

SET	TREATMENT
A	Water (Control)
B	8-HQ 200µg/ml+CA 300 µg/ml
C	8-HQ 200µg/ml+CA 300 µg/ml +1%Sucrose
D	8-HQ 200µg/ml+CA 300 µg/ml +2%Sucrose
E	8-HQ 200µg/ml+CA 300 µg/ml +3%Sucrose
F	8-HQ 200µg/ml+CA 300 µg/ml +5%Sucrose
G	8-HQ 200µg/ml+CA 300 µg/ml +8%Sucrose

Table 2. Effect of different preservative solutions on wilting percentage of yellow rose during vase life (days).

TREATMENTS	Wilting Percentage								
DAYS	1	2	3	4	5	6	7	8	9
CONTROL	0	0	18	48	100	100	100	100	100
8HQ+CA	0	0	0	0	6	24	68	100	100
8HQ+CA+1%Sucrose	0	0	12	16	64	100	100	100	100
8HQ+CA+2%Sucrose	0	0	13	36	78	100	100	100	100
8HQ+CA+3%Sucrose	0	0	33	88	100	100	100	100	100
8HQ+CA+5%Sucrose	0	0	0	0	0	5	10	33	55
8HQ+CA+8%Sucrose	0	0	0	13	51	62	100	100	100

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Figures

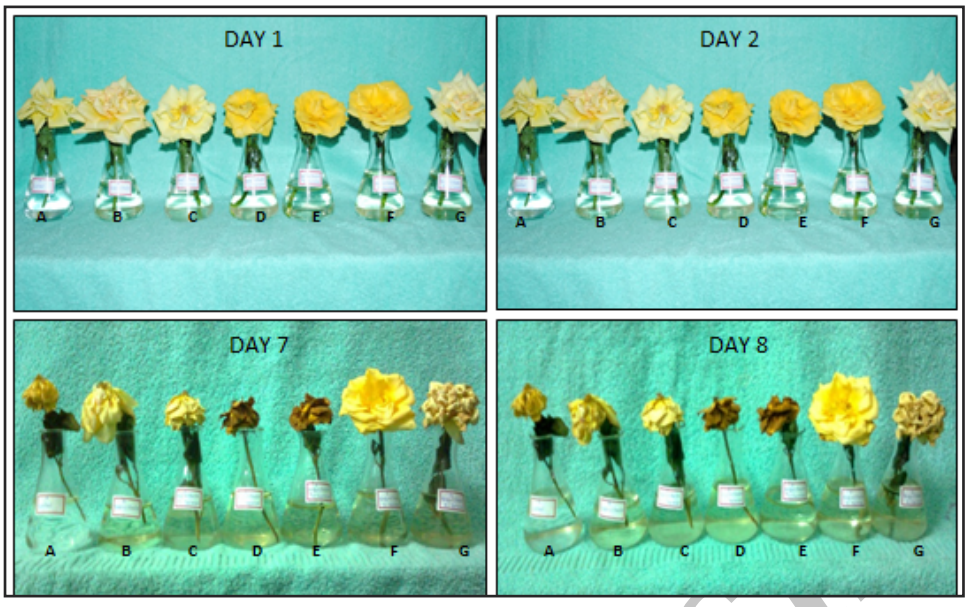

Fig.1. Photography of cut flower with various treatments of preservatives in different days.

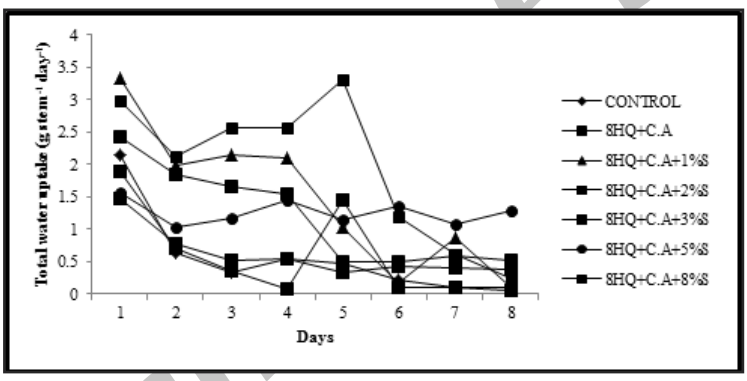

Fig.2. Effect of different preservative solutions on water uptake during vase life of yellow rose.

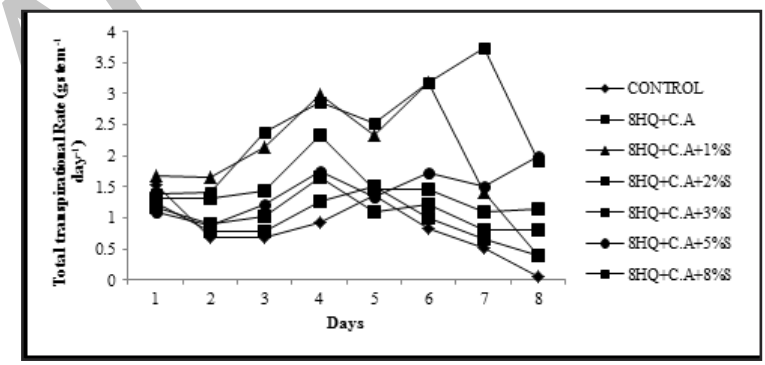

Fig.3. Effect of different preservative solutions on transpirational rate during vase life of yellow rose.

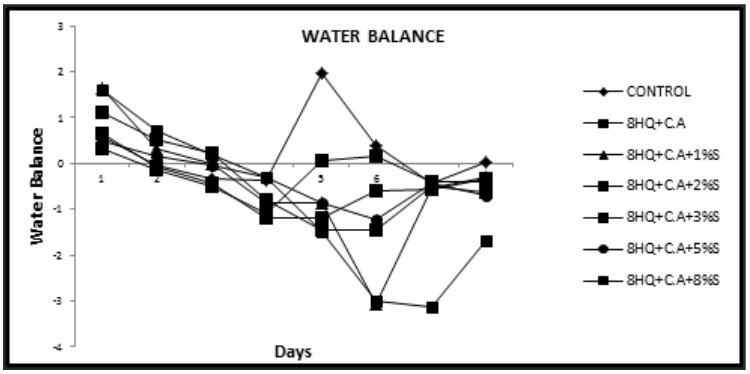

Fig.4.Effect of different preservative solutions on water balance during vase life of yellow rose.

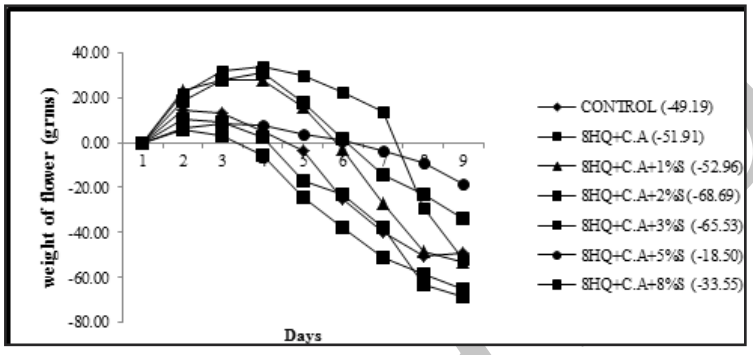

Fig.5. Effect of different preservative solutions on percentage of fresh weight during vase life of yellow rose.

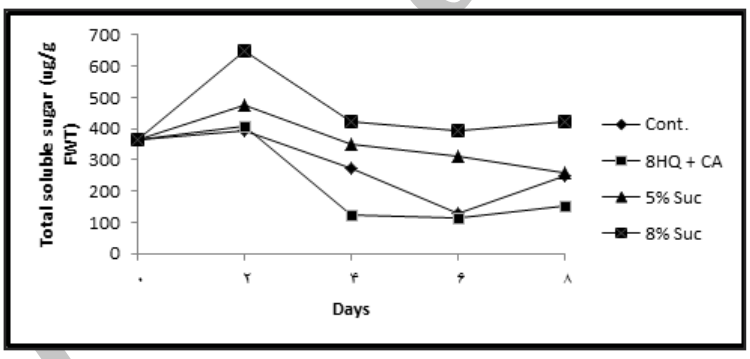

Fig.6. Effect of different preservative solutions on total soluble sugars content.

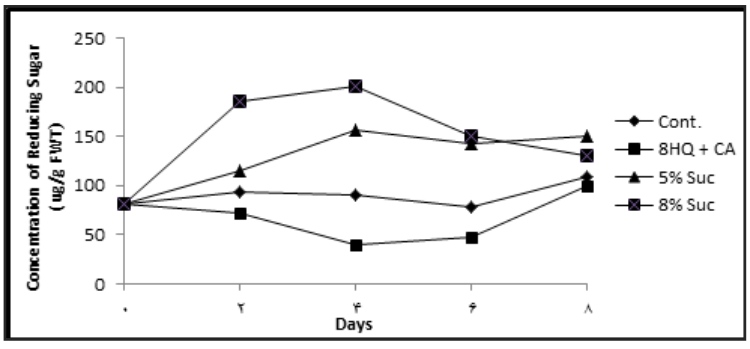

Fig.7. Effect of different preservative solutions on reducing sugar content.

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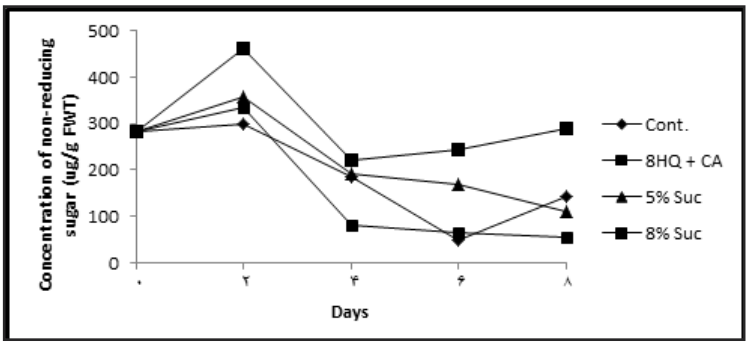

Fig.8. Effect of different preservative solutions on non reducing sugars content.

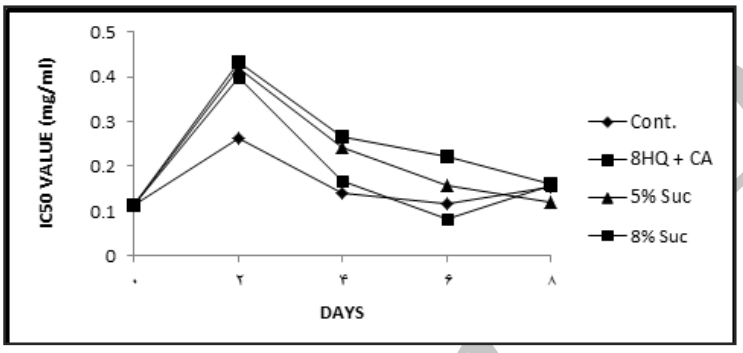

Fig.9. Effect of different preservative solutions on DPPH free radical scavenging activity.

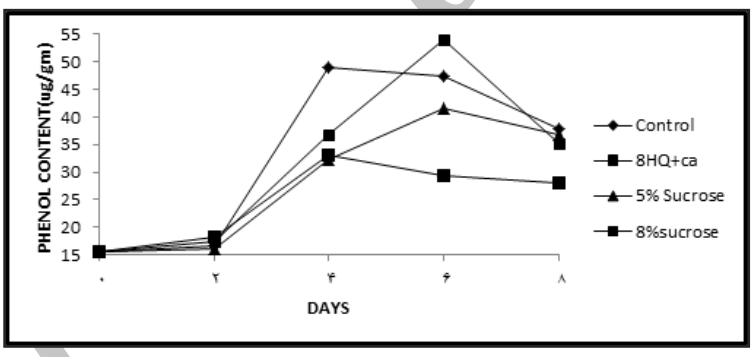

Fig.10. Effect of different preservative solutions on phenol content of fresh weight during vase life of yellow rose.

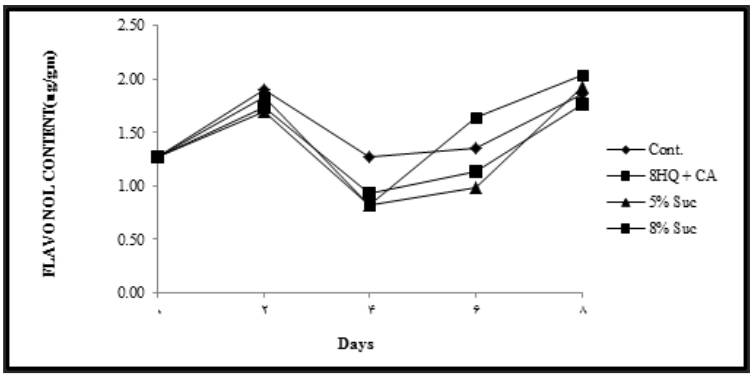

Fig.11. Effect of different preservative solutions on flavonol content of fresh weight during vase life of yellow rose.