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Evaluation of the Foliar Spraying Effects of Chitosan Nanoparticles and Salicylic Acid on the Petal Senescence and Postharvest Quality of Cut Roses cv. "Samuraie"

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Abstract

Extending the flower vase life is very important in the floriculture industry. In present study, the effectiveness of chitosan and salicylic acid nanoparticles in delaying of the senescence of cut roses was evaluated. Experiments were performed in a completely randomized design with 9 treatments in six repetitions, and the treatments were applied by petal spraying every three days. Chitosan nanoparticles with two concentrations (0.1 and 0.5 mg L^{-1}) and SA with two concentrations (0.1 and 0.3 mM) on the postharvest quality of cut roses were tested. Biochemical and enzymatic traits were measured during a period of 15 days (1, 4, 7, 11, 15 days after harvest), whereas, the vase life, RWU and electrolyte leakage were recorded till the end of flowers vase life. The maximum vase life (17.8 days) observed in flowers subjected to foliar spraying of 0.5 mg L^{-1} of chitosan nanoparticles with 0.3 mM salicylic acid, while shortest flower longevity (13.5 days) obtained in untreated flowers. Also, the amount of relative solution uptake of flowers and flavonoid content with significant increase were observed in 0.5 mg L^{-1} chitosan nanoparticles with 0.3 mM salicylic acid treatment. Treatments containing chitosan nanoparticles, especially the combined treatment of 0.5 mg L^{-1} of chitosan nanoparticles with 0.3 mM SA, reduced the amount of electrolyte leakage of the petals and increasing the vase life. Moreover, the lowest activity of antioxidant enzymes including GPX, PPO, POD and hydrogen peroxide were observed in flowers sprayed with 0.5 mg L^{-1} chitosan nanoparticles + 0.3 mM SA. Among chitosan nanoparticle treatments, the longest vase life was related to 0.5 mg L^{-1} (15 days), which increased flowers vase life by 11% compared to the control. Between SA treatments, the concentration of 0.3 mM was the most effective with a 6% increase in flowers vase life comparing untreated flowers. Finally, the combined treatments of chitosan nanoparticles and SA beside improving postharvest quality of cut roses, caused a 31% increase in flower longevity comparing untreated flowers.

Keywords: Chitosan, Longevity, Rose, SA, Spray treatment.

INTRODUCTION

Rose from Rosaceae family is considered as the most important cut flower in the world. The export value of this cut flower in the floriculture industry of the world is about 11 billion dollars (Chalabi and Nasreen Khalil, 2013). Cut roses are the most important cut flowers from the economic point of view. Rose cut flowers are sensitive to water stress under postharvest adverse conditions and often lose rapidly their marketability as well as their beauty value under such conditions (In *et al.,* 2016). Postharvest technology is able to increase the lifespan of cut flowers (Scariot *et al*., 2014). The vase life of cut roses is determined by many factors, including water relations (Doi *et al*., 2000; Hassan *et al*., 2020; In *et al.,* 2017). Therefore, increasing the vase life of cut flowers through proper management and care after harvesting is important from a commercial and economic point of view. In this regard, the lack of nutrients, bacterial and fungal contamination, wilting caused by water stress and blockage of vessels are considered to be the main factors that shorten the life of cut flowers. By using techniques to delay the senescence of cut flowers, the marketability of cut flowers can be significantly increased because the postharvest life is a vital and important factor for cut flowers. In this regard, to increase the longevity of cut flowers, a suitable preservative solution should be used (Parween and Gupta, 2022). Various postharvest treatments using growth regulators, sugars, signaling molecules and substances such as salicylic acid can inhibit post-harvest senescence and increase the postharvest life of the flower (Zulfiqar *et al*., 2020).

The pre-harvest life of cut flowers depends on various factors such as environment, genetics, handling systems and harvesting time, however, the postharvest life is determined by the water relations, microorganism's infections, storage conditions and packaging methods. Microbes have the ability to grow rapidly and settle at the ends of the cut stems after harvesting the flowers, which results in the blockage of the xylem vessels. Due to microbial blockage, the water absorption via stems is disturbed and ultimately causes water imbalance and unwanted wilting of cut flowers (He *et al*., 2018). Therefore, to prevent or slowing senescence process in cut flowers, preventing microbial blockage is considered a useful approach to increase postharvest quality of cut flowers. The use of different chemicals, including silver nitrate, silver thiosulfate, silver nanoparticles, calcium and hydrogen gas, was used in the past to increase the longevity of cut flowers (Ahmad *et al*., 2016; Alimoradi *et al.,* 2013; Bai *et al.,* 2009). However, the high cost of these chemicals and their hazards on the environment and human health have caused the attention of researchers to change to the use of environmentally friendly agents.

The use of nanoparticles plays an important role in postharvest management of horticultural products and plant protection due to their biocidal properties. For instance, in gerbera cut flowers, the floral preservative solution containing silver nanoparticles with antimicrobial activity increased their postharvest life (Solgi *et al*., 2009). Previous studies have shown that the use of different antimicrobial compounds can increase the life of cut flowers. The process of petal senescence is very complex and includes physiological and biochemical changes such as changes in the permeability of cell membranes, which lead to loss of color, wilting and finally senescence of petals (Arora and Singh, 2004). During the last decade, nanotechnology has made significant progress and various types of nanoparticles have been synthesized and introduced in different laboratories, and with the increase in the use of nanoparticles in various industrial and research sectors, studies on the applications of nanoparticles in the field of agriculture. Also, the interaction of plants and nanoparticles, the impact of nanoparticles on the environment, food chain and human health have been the focus of researchers all over the world (Ioannou *et al*., 2020).

Chitosan is a valuable biopolymer with many remarkable properties. This composition is

compatible with plants and has antioxidant, antiperspirant and antimicrobial properties. Also, it is a biodegradable and economic compound that is obtained from the skin of animals such as crabs and shrimps. In ornamental plants, chitosan has been used as an antitrannspirant compound to increase the longevity of cut flowers (Bañuelos-Hernández *et al*., 2017). Based on research, compounds containing chitosan called chito-oligosaccharide (COS) by improving the water absorption capacity of roses increased the vase life up to 6.4 days comparing control.

These compounds, while increasing the activity of antioxidant enzymes such as glutathione reductase, have improved the amount of glutathione in cut rose petals, so they are recommended as a commercial protective solution to increase the lifespan of cut roses (Hong-Juan and Huan- Qing, 2015). The use of chitosan or its derivatives increase the quality and postharvest life of various horticultural crops due to its germicidal properties and also by stimulating the defense mechanism of plant tissues (Terry and Joyce, 2004).

Salicylic acid (SA) as a plant growth regulator causes many physiological and biochemical effects in plants. Meanwhile, salicylic acid delays senescence process in cut flowers by increasing the activity of antioxidant enzymes and strengthening the cellular antioxidant system (Armitage and Laushman, 2003). They concluded that the salicylic acid treatment significantly prevents the formation of lignin. The reduction of lignin formation is directly related to the inhibitory effect of SA on the activity of enzymes related to lignin production and indirectly to the reduction of oxidative damage, which is caused by the reduction of O_2 and H_2O_2 accumulation (Wang *et al*., 2016). It has been reported that the pre- and postharvest treatments of SA influenced the physico-chemical properties of cut roses , improved the longevity of roses via increase of enzymatic antioxidant capacity, the improvement of water relations and the increase of cumulative water absorption (Alaey *et al*., 2011).

In some ethylene sensitive cut flowers, ethylene is the main responsible for petal senescence. As internally produced ethylene causes senescence and regulates gene expression coordination in flower petals (Mohammadi Kabari and Jadid Soleimandarabi, 2019; Hassan *et al*., 2020), but rose is not more sensitive to ethylene, and petal senescence may occur due to internal factors other than ethylene, such as the increase of reactive oxygen species (ROS) (Jones and McConchie, 1995). Antioxidant enzymes such as peroxidase and guaiacol peroxidase (GPX) play an important role in the defense against oxygen free radicals (Okigbo and Ogbonnaya, 2006; Bayat and Aminifard, 2017).

The aim of this study was to investigate the role of chitosan nanoparticles and salicylic acid as a preservative treatment to slowing the senescence process of rose cut flowers. Therefore, in this research, the optimal concentrations of preservative treatments (chitosan nanoparticles and salicylic acid in the form of foliar spraying) in order to increase the vase life, maintain moisture and improve water absorption (preventing the xylem blockage) and especially preventing the ROS accumulation has been tested.

MATERIALS AND METHODS

Plant materials and treatments

Cut roses were obtained from a commercial greenhouse located in Ajabshir, Iran. The flowers were harvested early morning and homogenized based on their visual appearance and openness. The flowers were cut to a height of 35 cm and then placed in containers containing distilled water. Cut roses were sprayed with different solutions prepared using various concentrations of salicylic acid and/or chitosan nanoparticles. The treatments included salicylic acid in two concentrations (0.1 and 0.3 mM), chitosan nanoparticles in two levels (0.1 and 0.5 mg L^{-1}) and four levels of combined aforementioned substances (CSNPs 0.1 mg L^{-1} + SA 0.1 mM), (CSNPs 0.5 mg L^{-1} + SA 0.1 mM), (CSNPs 0.1 mg L^{-1}+

SA 0.3 mM), (CSNPs 0.5 mg L^{-1} + SA 0.3 mM) in addition with distilled water as control (Figs. 1 and 2). The treated cut flowers were kept at a temperature of 10 ± 2 °C, relative humidity of 60-70% and a photoperiod of 12 hours at an intensity of 15 µmol m^2 S^{-1}.

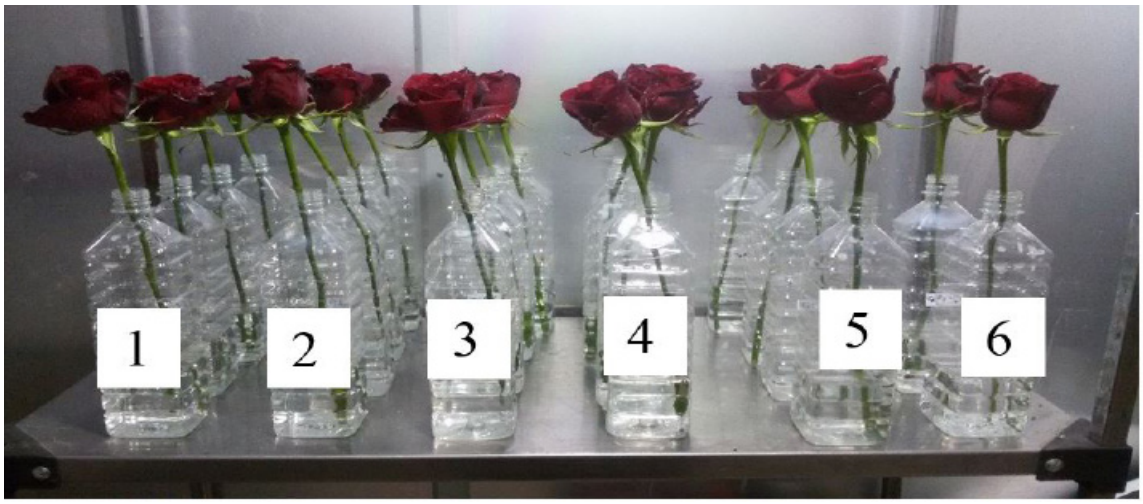

Fig. 1. Effect of chitosan nanoparticles (CSNPs) and salicylic acid on cut rose "Sammuraie" 13th day after harvest. 1: SA 0.1 mM; 2: SA 0.1 mM+CSNPs 0.5 mg/L; 3: Control; 4: CSNPs 0.5 mg/L; 5: SA 0.3 mM+CSNPs 0.1 mg/L; 6: SA0.3 mM+CSNPs 0.1 mg/L.

Fig. 2. Effect of chitosan nanoparticles (CSNPs) and salicylic acid on cut rose "Sammuraie" on the 11th day of vase life.

The flowers were evaluated for morphological traits (such as relative water uptake, and vase life) and membrane electrolyte leakage, while the petals of each treated groups were collected, frozen in liquid nitrogen and stored at - 40 ˚C for further biochemical and enzymatic assays.

Preparation of chitosan nanoparticles solution

Pure chitosan with molecular characteristics of Mw = 100 kD, DD = 85%, and purity = 97% obtained from Sabz Gostaresh Aazin Turkan Company (Maragheh, Iran), tripolyphosphate (TPP) from Merck (Germany) and salicylic acid with a purity 99 % were obtained from Sigma Aldrich (USA). To prepare 5 L of chitosan solution with a concentration of 1% by weight, first, 50 g of chitosan powder was mixed with 4950 ml of distilled water for one hour on a heater until a uniform solution was obtained. Then 50 ml of acetic acid was added to the chitosan

dispersion and completely dissolved using stirrer. Using this solution, we prepared the desired concentrations of chitosan nanoparticles (Ahmadi *et al*., 2018). Chitosan nanoparticle solution in two concentrations of 0.1 and 0.5% by weight was obtained using chitosan stock solution and TPP ionic binder. Solutions of salicylic acid with concentrations of 0.1 and 0.3 mM were prepared by dissolving 0.373 and 1.12 g of SA in two liters of distilled water, respectively. To prepare chitosan nanoparticles with concentrations of 0.1 and 0.5 mg L^{-1}, 200 and 1000 mL of chitosan solution were diluted with 1800 and 1000 mL of distilled water, respectively. SA was dissolved in distilled water and added to the chitosan solution with a certain concentration and the solution was reached to a volume of two liters. The desired amount of TPP as an ionic interface based on the amount of chitosan was dissolved in 25 ml of distilled water and the TPP solution was slowly added into the chitosan solution, and the chitosan nanoparticle solution was produced.

Traits measurment

Vase life

During the postharvest life, the visual quality of cut roses were inspected daily. In present study, vase life was defined as the period (days) from the time of spray treatments until 50% of flower petals were wilted or abscised or flower necks were bent as symptoms of flower senescence process (Jiang *et al*., 2015).

Relative water uptake (RWU)

Relative water uptake of the flowers was traced by the formula:

Formula (1): Relative water uptake (RWU) = V_t-V_{t-1}/ stem weight at the day zero

Where V_t refers to the vase water volume during the measurements, and V_{t-1} is the water volume at the day before.

This trait was measured at one- day intervals till the end of vase life (van Meeteren and van Gelder, 1999; Pompodakis *et al*., 2004).

Membrane electrolyte leakage (EL)

To measure membrane turgidity using the method (Sairam *et al*., 2002), 0.1 g of petal samples from each treatment were transferred separately (in duplicate) to test tubes containing 20 ml of deionized water. Then, a series of samples were placed at a temperature of 40°C for 30 minutes and another series at a temperature of 100 °C for 15 minutes in a Ben-Marie. The conductance of the samples was measured by EC meter (AL 10Con, AquaLytic, Germany). Then the percentage of ion leakage was calculated in the following way:

Formula (2) $$EL = \frac{EC(2)-EC(1)}{EC(2)} * 100$$

EC (1): Conductance amount in temperature 1, EC (2): Conductance amount in temperature 2.

Measurement of guaiacol peroxidase (GPX)

The activity of GPX enzyme was measured according to method described by Mencarelli *et al*. (1995). 0.5 g petal tissue was homogenized by potassium phosphate buffer (pH=7, 100 mM). The homogenate was centrifuged at 4°C for 15 min. at 15000 g and supernatant ware used for recording enzyme activity. The GPX activity was calculated at 470 nm based on the extinction coefficient of tetraguaiacol (26.16 mmol cm^{-1}) and expressed as micromoles of oxidized guaiacol per minute per gram of fresh weight.

Measurement of peroxidase (POD) and polyphenol oxidase (PPO) activities

Peroxidase enzyme activity was traced based on the conversion of guaiacol to tetraguaiacol by the method (Maehly and Chance, 1995). For this purpose, 200 mg of frozen petal tissue was grinded in sodium phosphate buffer containing 2% PVP and 1.3 mM EDTA. To measure each sample, 450 µL of hydrogen peroxide buffer and 450 µL of guaiacol buffer are mixed together at a low temperature (container containing ice) and 10 µL of enzyme extract is added to it, and finally oxidation of guaiacol at a temperature of 25 °C with a spectrophotometer two visible-ultraviolet rays, model UV-1800 of Shimadzu, Japan, were measured at a wavelength of 470 nm.

Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) activity was assayed by measuring the oxidation of catechol as substrate according to Nguyen *et al*. (2003) and was expressed as IU mg^{-1} protein min^{-1}.

Measurement of H_2O_2 amount

The H_2O_2 content was measured according to Alexieva *et al*. (2001) and expressed in µmol g^{-1} FW.

Measurement of total flavonoids

The amount of total flavonoids was measured as described by Chang *et al*. (2002) which is based on aluminum chloride colorimetric. The absorbance of the mixture was read at 415 nm with a UV-1800 model UV-1800 spectrophotometer from Shimadzu, Japan. Total flavonoids content was expressed as mg Quercetin g^{-1} FW.

Statistical analysis of data

Two-way ANOVA was used for all the traits except vase life which is measured only once at the end of the experiment. The experiment was designed as factorial based on completely randomized design (CRD) with six replications. First factor was 8 levels of treatment solutions in addition with distilled water as control and the second factor was time of sampling for measurements which were first, fourth, seventh, eleventh and fifteenth day of the experiment. For vase life which was measured once at the end of the experiment, one-way ANOVA was used. Mean comparisons were done by Tukey's honestly significant difference (HSD) test ($P<0.05$). All statistical analyses was performed using R statistical software (R foundation for statistical computing, version 4.3.2).

RESULTS AND DISCUSSION

Vase life

ANOVA (Table 1) showed the significant ($P < 0.01$) difference in the vase life of cut roses under tested treatments. The mean comparison indicates that foliar spraying of the combined treatment of SA 0.3 Mm + CSNP 0.5 mg L^{-1} has shown the longest vase life (17.83 days) compared to the control (13.5 days), showed 4.33 days more longevity comparing untreated flowers (Fig. 3).

Table 1. Summary of two-way ANOVA for physiological traits of cut rose cv. "Sammuraie".

S.o.V	df	Vase life	RWU	EL
Treatment	8	108.00**	2.454**	2390.5**
Time	4	58.83	15.872**	196.8**
Treatment * Time	32	166.83	0.09**	15.187**
Error	225	12.18	0.013	5.593
Total	269		0.331	80.504
CV (%)			12.18	6.94

**: Significant at P < 0.01 based on the HSD test. RWU: Relative water uptake, EL: Electrolyte leakage.

The flowers treated with 0.1 Mm SA, alone and without the application of chitosan had the lowest flower longevity (13.6 days) after the control treatment (13.5 days), which did not show a significant difference. Our results are consistent with Jing and Li's study which demonstrated chitosan positive impacts on the longevity of cut roses, which recorded about 6.4 days more than the control (Jing and Li, 2015).

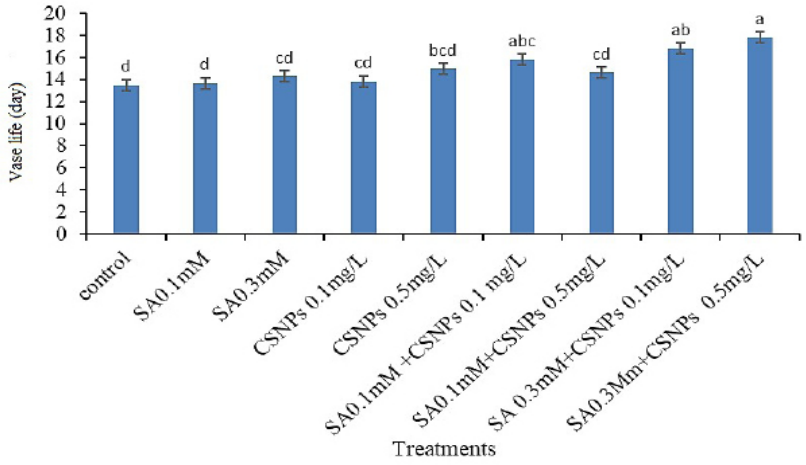

Fig. 3. The effect of different levels of chitosan and salicylic acid nanoparticle treatments on the vase life of cut roses CV. "Samuraie". *In each column, means with similar letter(s) are not significantly different (P < 0.05) using the HSD test.

Relative water uptake (RWU)

The results revealed that the amount of water uptake by rose cut flowers influenced significantly is significant (P < 0.01) by interactive effects of chemical treatments and time. The mean comparisons showed that in all the treatments until the fifth day, the uptake of the water was high. With the increase of time after harvest, the amount of uptake of the water showed a significant decrease. After the fifth day, the amount of water uptake in the treatment containing SA 0.1 mM and CSNP 0.1 mg L^{-1} decreased, while in treatments containing SA 0.3 mM + CSNP 0.5 mg L^{-1} and SA 0.3 mM + CSNP 0.1 mg L^{-1} absorption rate decreased just after 9th day. So, in the last days of flower vase life, the flowers treated with SA 0.3 mM + CSNP 0.5 mg L^{-1} had the highest amount of water uptake (1.29 ml g^{-1} FW), which was significantly different from the treated flowers with 0.1 mM of SA. 0.1 mM SA treatment alone had the lowest average water uptake (0.64 ml g^{-1} FW) (Fig. 4). The minimum amount of water absorption (0.47 ml g^{-1} FW) was recorded in control flowers comparing other treatments.

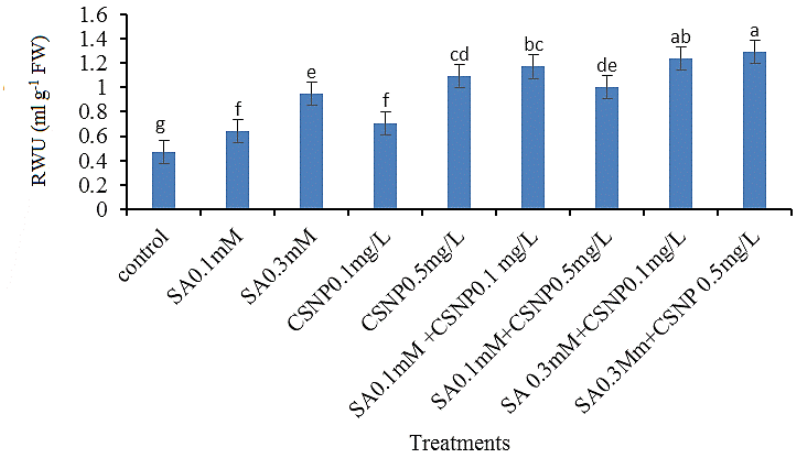

Fig. 4. The effect of different concentrations of chitosan and salicylic acid nanoparticles on the relative solution uptake in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Since chitosan nanoparticles and salicylic acid act as antibacterial agents, they reduce bacterial infection and prevent vascular occlusion, thus improve solution uptake (van Meeteren, 1978; Mehdikhah *et al*., 2016). The present study in agreement with previous findings showed similar results. In this research, foliar spraying of chitosan nanoparticles along with increasing the solution uptake, significantly improved postharvest quality of cut rose flowers.

The reduction of water solution uptake due to xylem occlusion has the greatest effect on the early wilting of the petals and bending of the stem (Xue *et al*., 2009). The vessels in cut flowers are vital tissues for water absorption, so that the amount of solution absorption decreases with the obstruction of the vessels by bacteria. In this regard, the role and importance of chitosan nanoparticles against bacteria has been investigated, and the current research is in accordance with the research conducted regarding the improvement of water absorption in cut roses due to the antibacterial property of chitosan nanoparticles (Jing and Li, 2015).

Electrolyte leakage (EL)

The analysis of variance of the data showed that the interaction effect of these treatments with the time after harvesting on the amount of electrolyte leakage of petals was significant at the level of 1% ($P < 0.01$) (Table 1). The mean comparisons showed a notable decrease in ion leakage index in the higher concentrations of the preservative treatment, especially in the combined treatment of 0.3 Mm SA + 0.5 mg L^{-1} CSNP by 21.84%, comparing untreated flowers those showed the highest amount of petal electrolyte leakage (49.26%) (Fig. 5).

The mean comparisons among treatments showed that in all the treatments until the third day, the amount of electrolyte leakage was low. With time passage after harvest, the amount of electrolyte leakage increased. In the treatment containing 0.1 mM SA and 0.1 mg L^{-1} CSNP, an increase in the amount of electrolyte leakage after the fifth day, and in the treatments containing 0.3 mM SA + 0.5 mg L^{-1} CSNP and 0.3 mM SA + 0.1 mg L^{-1} CSNP, the amount of petal electrolyte leakage was increased from 10th day after harvest. So, in the last days of the vase life flowers, the petals that were treated with 0.3 mM SA + 0.5 mg L^{-1} CSNP had the lowest amount of electrolyte leakage. Therefore, the membrane integrity of petals sprayed with 0.3 mM SA + 0.5 mg L^{-1} CSNP treatment was higher than other treatments, and as a result of this treatment, the life span of cut flowers was increased compared to other treatments.

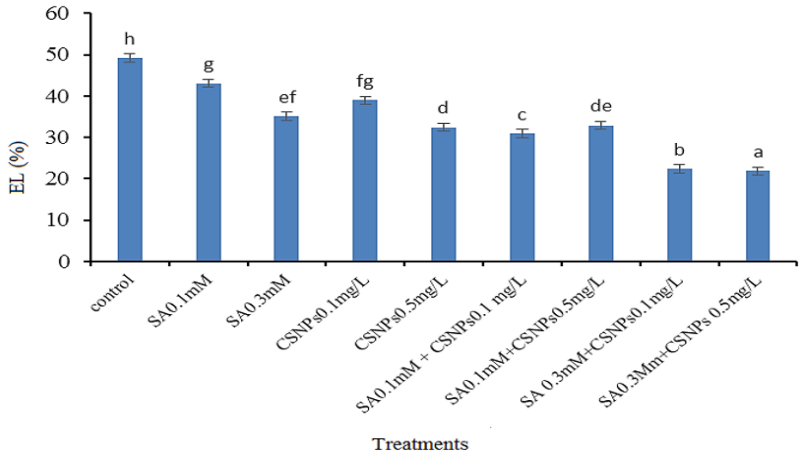

Fig. 5. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the amount of electrolyte leakage on cut roses cv. "Samuraie". *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Shortly, the combined treatments of chitosan nanoparticles and salicylic acid in this research maintained the strength of the cell wall and increased the vase life of rose cut flowers. Obtained results by Mohammadi and Mortazavi (2014) about the effect of salicylic acid on alstromeria cut flowers are consistent with our research. The findings of Parween and Gupta (2022) regarding the effect of chitosan treatment on membrane stability in gerbera cut flowers are completely consistent with our results.

Flavonoid content

The effect of different concentrations of chitosan nanoparticles with salicylic acid indicate that these treatments increased the flavonoids content compared to the control during post-harvest period. Comparison of the means showed that the highest amount of flavonoid (580.38 µg g^{-1} FW) was in the combined treatment chitosan nanoparticles with a concentration of 0.5 mg L^{-1} along with salicylic acid with a concentration of 0.3 mM. The lowest amount of flavonoids (480 µg g^{-1} FW) was obtained in the control treatment. In foliar spraying with of chitosan nanoparticles with a concentration of 0.5 mg L^{-1} (alone) and salicylic acid with a concentration of 0.3 mM (alone) compared to the combined treatments of SA 0.1 mM + 0.1 mg L^{-1} CSNP and SA 0.1 mM + 0.5 mg L^{-1} CSNP, no significant difference was observed in terms of petal flavonoid content (Fig. 6).

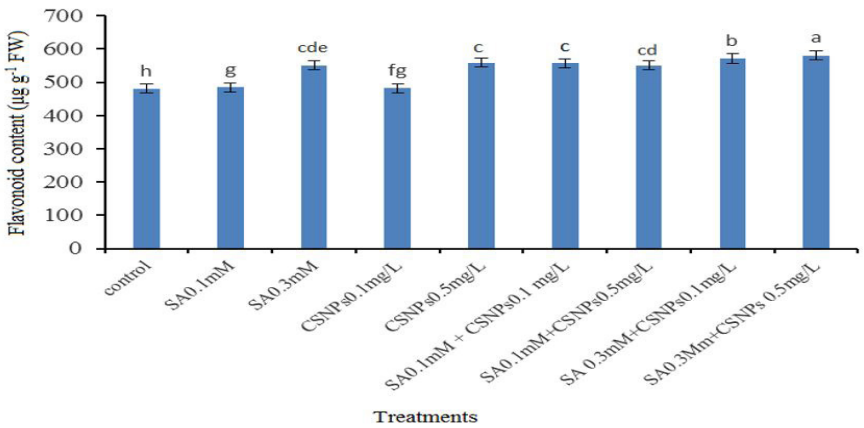

Fig. 6. The effect of different concentrations of chitosan and salicylic acid nanoparticle on the flavonoid content of cut rose flowers cv. "Samuraie". *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

The results showed that the interaction of the nanoparticle treatments with post-harvest times was significant at the 1% probability level. Mean comparison of data showed that with increasing concentration of chitosan nanoparticle complex with salicylic acid, the flavonoid content increased, so that in foliar spraying with 0.3 mM SA + 0.5 mg L^{-1} CSNP, the highest amount of flavonoid in petals (Fig. 4).

Guaiacol peroxidase (GPX) activity

According to the results of analysis of variance (Table 2), the interaction of different concentrations of salicylic acid and chitosan nanoparticles and time caused significantly ($P < 0.05$) the guaiacol peroxidase enzyme activity, where, guaiacol peroxidase activity in flowers treated with different concentrations of salicylic acid and chitosan nanoparticles was lower compared to untreated flowers regardless of their concentration.

Table 2. Summary of two-way ANOVA for biochemical traits of cut rose cv. "Sammuraie".

S.o.V	df	Flavonoid	GPX	PPO	POD	H_2O_2
Treatment	8	49628.12**	13.166**	10.610**	0.0077**	4.909**
Time	4	8532.5**	0.0035**	5.214**	0.00087**	4.83**
Treatment × Time	32	611.19**	0.0003**	0.7080**	0.000054*	0.240**
Error	225	127.4	0.0000	0.170	0.000028	0.0101
Total	269	1782.07	0.3916	0.620	2.741	0.255
CV (%)		2.11	0.05	17.6	7.54	4.01

**: Significant at $P < 0.01$ based on the HSD test.

As shown in Fig. 7, the change of peroxidase enzyme was decreasing with the progress of time, and the highest level of enzyme activity was in the control treatment (3.81 IU mg^{-1} protein min^{-1}) and the lowest level was observed in 0.3 mM salicylic acid chitosan nanoparticles 0.5 mg L^{-1} (1.81 IU mg^{-1} protein min^{-1}).

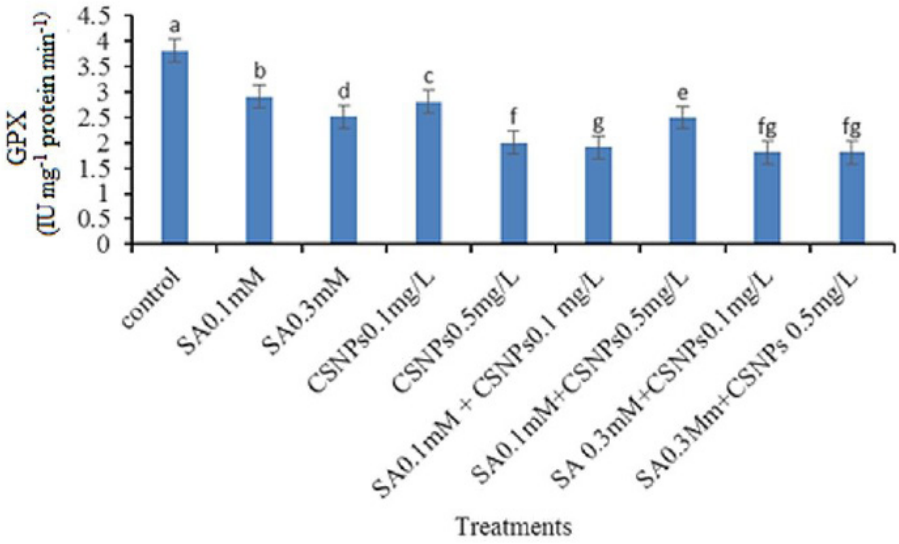

Fig. 7. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the guaiacol peroxidase activity in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

In this research, the activity of guaicol peroxidase enzyme shows an increasing trend in rose cut flowers at postharvest and the senescence stage of petal cells. These findings confirm similar results recently reported for carnation flowers (Moulaei *et al*., 2021). So the results of our research indicate that chitosan nanoparticles of and salicylic acid treatments have a protective effect and increasing the vase life of rose flower petals, which prevent the creation of various stresses (such as osmotic potential) in the petals and as a result increase the activity of antioxidant enzymes, including peroxidase.

Peroxidase (POD) activity

According to the results of analysis of variance (Table 2), the interaction of different concentrations of salicylic acid and chitosan nanoparticles and time caused significantly ($P < 0.05$) the peroxidase enzyme activity, where, POD activity in flowers treated with different concentrations of salicylic acid and chitosan nanoparticles was lower compared to untreated flowers regardless of their concentration. As shown in fig. 8, the change of peroxidase enzyme was decreasing with the progress of time, and the highest level of enzyme activity was in the control treatment (0.091 µmol mg^{-1} FW min^{-1}) and the lowest level was observed in the treatment of 0.3 mM salicylic acid with chitosan nanoparticles 0.5 mg L^{-1} (0.047 µmol mg^{-1} FW min^{-1}).

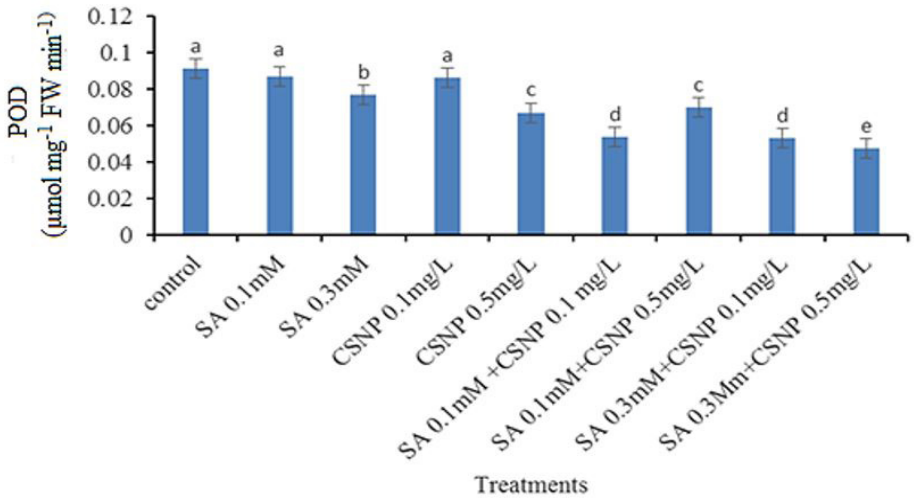

Fig. 8. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the peroxidase activity in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

Peroxidases play an important role in oxidative stress, removal of oxygen free radicals, involvement in auxin metabolism, wound healing in plants, and reaction to environmental pollution. This enzyme breakdown hydrogen peroxide through compounds such as ascorbate or oxidation of substrates such as phenolic compounds (Reddy *et al*., 2008). Peroxidase enzyme is able to remove malondialdehyde and hydrogen peroxide (Hojati *et al*., 2011). In our research, salicylic acid and chitosan nanoparticles caused a significant decrease in peroxidase enzyme activity. One of the causes of senescence in plant tissues are active oxygen species such as O_2^- and H_2O_2, which cause flower senescence by destroying proteins, lipids and nucleic acids (Choudhary *et al*., 2017). Antioxidant enzymes are very effective systems that protect cells against ROS. According to the results of Ezhilmathi *et al*. (2007) 5-sulfo-salicylic acid treatment and the results of Hatamzadeh *et al*. (2012), salicylic acid treatment increases the activities of catalase

and peroxidase antioxidant enzymes, which scavenging ROS resulted to reduce senescence process in cut flowers.

Polyphenol oxidase (PPO) activity

The results of ANOVA revealed that the interaction effects of different concentrations of chitosan-salicylic acid nanoparticle complex and postharvest period was significant ($P<0.01$) on the activity of polyphenol oxidase (PPO) enzyme (Table 2). Mean comparison of data showed that the highest and lowest PPO activity was observed with an average of 3.42 IU mg^{-1} protein min^{-1} in the control treatment and 1.62 IU mg^{-1} protein min^{-1} in 0.3 mM salicylic acid with 0.5 mg chitosan (Fig. 9).

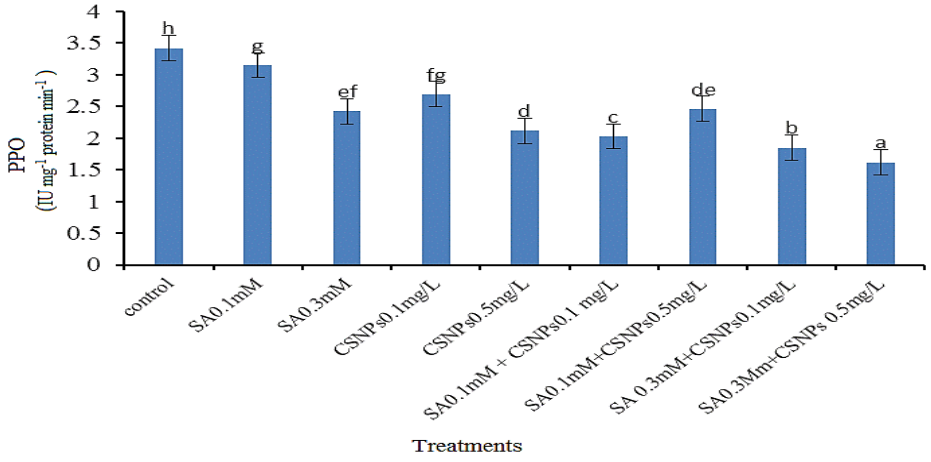

Fig. 9. The effect of different concentrations of chitosan nanoparticles and salicylic acid treatments on the PPO activity in "Samuraie" rose cut flowers. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

During the days after harvest, the activity of PPO increased. So, in the control treatment, the increasing trend of this enzyme started on the fifth day after harvest, and in the treatments containing chitosan nanoparticles and salicylic acid containing treatments, respectively, from the seventh and sixth days, and in the combined treatments of chitosan nanoparticles with salicylic acid, the increasing trend of the activity of this enzyme was started from 9th day. Therefore, considering that foliar spraying with combined treatments of chitosan nanoparticles with salicylic acid slows down PPO activity rate. So, combined treatments of chitosan nanoparticles with salicylic acid, especially the treatment of 0.3 mM SA + 0.5 mg L^{-1} CSNP in increasing the flower longevity showed the greatest effect comparing other treatments. According to previous studies, the reactions related to the polyphenol oxidase enzyme are very important in the postharvest stage (Zeeshan *et al*., 2020), because this enzyme is able to convert the hydrogen peroxide produced in the organs and cytosol to water and oxygen and reduce its harmful effects. Also, the PPO plays an effective role in cleaning these compounds (Michalak, 2006). In addition, increasing the activity of these enzymes may increase the concentration of $NADP^+$ to release electrons from the photosynthetic electron transport chain and thus reduce the production of ROS (Gozukirmizi *et al*., 2015).

Peroxide hydrogen

The results of variance analysis of the data showed that the interaction effects of chitosan and SA nanoparticle treatments with the duration of treatment on hydrogen peroxide (H_2O_2)

were significant ($P < 0.01$) (Table 2). During the days after harvest, the hydrogen peroxide content increased. H_2O_2 increasing was started in untreated flowers from the 4th day after harvest, but in the treatments containing chitosan nanoparticles, from the seventh day, and in the combined treatments of chitosan nanoparticles with salicylic acid, the increasing trend of the hydrogen peroxide content started from the 10th day. Therefore, the combined treatments of chitosan nanoparticles with SA showed notable capacity in slowing down of the H_2O_2 content which resulted to the vase life of treated flowers by compared to other treatments. Mean comparison of the data showed that the highest concentration of hydrogen peroxide with an average (3.31 µmol g^{-1} FW) was recorded in control flowers and the lowest amount of it with an average (1.96 µmol g^{-1} FW) was observed in combined treatment of 0.3 mM salicylic acid with 0.5 mg L^{-1} of chitosan (Fig. 10). Considering that two types of ROS (reactive oxygen species), including hydrogen peroxide and superoxide anion, play an important role in the plant senescence process, especially in cut flower senescence process, where the content of O_2 and H_2O_2 are increasing. But the trend of this increase with the application of chitosan nanoparticles and salicylic acid treatments is less than that of the control, and there is a significant difference between the amount of hydrogen peroxide in the petals of roses treated with chitosan nanoparticles and salicylic acid compared to the control (Fig. 10). According to the obtained results, treatments containing chitosan nanoparticles are able to reduce the activity of reactive oxygen species (ROS) in rose cut flowers.

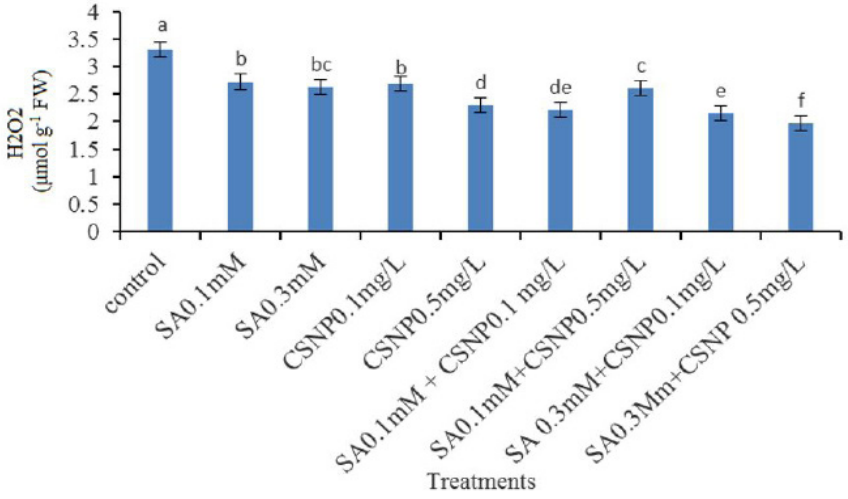

Fig. 10. The effect of different concentrations of chitosan and salicylic acid nanoparticle treatments on hydrogen peroxide content in the cut flowers of the "Samuraie" rose. *In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the HSD test.

In recent decades, H_2O_2 has received much attention as a reactive oxygen species (ROS). This molecule accumulates more in plants in most environmental stresses, both biotic and non-biotic, and causes more damage to the plant. Thus, hydrogen peroxide plays a role in most physiological processes such as senescence, stomatal opening control, photorespiration and photosynthesis and plant development. On the other hand, the increase and accumulation of H_2O_2 causes oxidative stress, which starts the process of cell death. Therefore, the survival of all aerobic organisms depends on hydrogen peroxide homeostasis. This homeostasis includes the production of H_2O_2 from different pathways and its removal. H_2O_2 removal pathways include enzymatic pathways and non-enzymatic pathways (Shahroodi *et al*., 2020).

Earlier study (Wan *et al*., 2023) has shown that chitosan and its oligosaccharins improve the absorption of the solution in rose cut flowers cv. Gaoyuanhong. Moreover, chitosan activates nutrients and defense mechanisms of cut flowers. In this regard, Ahmed *et al*. (2020), showed the role of chitosan in the growth regulating and improving resistance mechanism to senescence, resulting increase the vase life of cut rose flowers.

CONCLUSION

Rose, as the most important cut flower in the world, has a great economic value and importance in terms of popularity among consumers worldwide. The most basic problem of this cut flower is its relatively short vase life due to the failure in water uptake coming from xylem occlusion beside diverse effects of ROS. Therefore, choosing the proper preservative solution for the post-harvest stage of cut roses is very important considering the various side effects of chemicals. In this research, we use nanoparticles of chitosan and salicylic acid in the form of foliar spray for its bioavailability and environmental friendly nature. In addition, as appropriate biochemical compounds they have the ability to control or inhibit ROS activity and also due to having antibacterial properties, we have used them to increase the vase life of cut flowers. The results showed that the postharvest spraying of chitosan nanoparticles and salicylic acid treatments significantly increased the vase life of rose cut flowers. In short, in cut roses sprayed with solutions containing chitosan nanoparticle +SA, the relative solution uptake and the flavonoid content of the petals increased. In addition, the amount of electrolyte leakage and the activity of POD, PPO and GPX enzymes and the amount of hydrogen peroxide in petal tissues of treated roses sprayed were decreased. Finally, results were demonstrated that the among of different concentrations of solution treatments, the combined treatment of 0.5 mg/L of chitosan nanoparticles with 0.3 mM salicylic acid has the greatest impact on increasing the flower longevity and improving postharvest physiological and biochemical attributes of cut roses.

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