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The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of *Gerbera jamesonii* 'Sun Spot'

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Abstract: The present study investigates the roles of nitroprusside (SNP), salicylic acid (SA), methyl jasmonate (MJ), and their interaction with 8-hydroxyquinoline sulfate (8-HQS) in regulating the peroxidase activity (POX), water uptake, the relative water content (RWC), the contents of malondialdehyde (MDA), soluble sugar, proline, and protein content in the petals and the stem bending of *Gerbera jamesonii* 'Sun Spot' cut flowers. Cut flowers were treated with various concentrations (50, 100, and 200 µM) of hold-solutions containing 8-HQS, SA, MJ, and SNP. Hold solutions were used alone or in combination with 100 µM 8-HQS for 24 h. Distilled water was used as control and sucrose (4%, w/v) was added to all solutions. The findings showed that 50 µM SA+ 100 µM 8-HQS markedly improved the RWC, the contents of proline, anthocyanin, carotenoid, protein, and soluble sugar, and activities of POX in the petals and markedly reduced water loss and the contents of MDA in the petals, compared with other treatments, especially the control. Meanwhile, the combination of plant growth regulators (PGR) with 8-HQS markedly improved positive indexes than use alone PGR. This phenomenon seemed to be due to more absorption of PGR. Among different concentrations of PGR, 50 µM is the most effective treatment for the improvement of the vase life of *Gerbera jamesonii* cut flowers. The results also demonstrated that SA+8-HQS improves the vase life of gerbera cut flowers by enhancing the membrane stability and water retaining capacity as well as increasing proline, antioxidant activity, and pigment contents.

1. Introduction

Vase life and quality are key factors that contribute to the aesthetic and benefits of cut flowers (Mansouri, 2012). The short vase life of most cut flowers is mainly due to the water loss, which is an important physiological process that affects the main quality characteristics of cut flowers, such as appearance (Salehi Salmi *et al*., 2018).

Gerbera jamesonii is a commercially popular cut flower that ranks 10 in the globe auctions. This plant is a member of the family asteraceae that originates from Africa and Madagascar and extends to China (Parthasarathy and Nagaraju, 1999; Hind, 2007). Sensitivity to microbial contamination at the stem base is a major postharvest problem of this plant (Balestra *et al*., 2005). Microorganisms cause stem end blockage in cut flower (He *et al*., 2006; Liu *et al*., 2009) and also secretion of toxic compounds, and thereby accelerated wilting (Williamson *et al*., 2002). Vase life of gerbera has been studied extensively with different treatments (Nair *et al*., 2003; Solgi *et al*., 2009; Shabanian *et al*., 2018). Witte *et al.* (2014) reported that treating cultivars of gerbera stems by sucrose in combination with an antimicrobial compound (HQC, Chlorine) resulted in less bending than the same concentration of the antimicrobial compounds alone. Perik *et al.* (2014) noted that other factors might also be involved in bending and showed that a mixture of chemicals delayed the time to bending in six tested cultivars of gerbera.

Sodium nitroprusside (SNP), the inorganic nitrous compound (nitroferricyanide) with the formula $Na_2[Fe(CN)_5NO].2H_2O$, is an important signaling molecule. This molecule has diverse physiological functions for plants such as inducing tolerance to adverse environmental factors (Shi *et al*., 2016; Kumar Rai *et al*., 2018). Application of SNP in vase solution resulted in extending the vase life of gerbera cut flowers (Shabanian *et al*., 2018); however, there is very limited information regarding the positive effects of exogenously applied SNP in extending the vase life.

Ortho-hydroxybenzoic acid or salicylic acid is an endogenous plant growth regulator. Exogenous application of salicylic acid (SA) can affect the antioxidant capacity of plant cells and prolong vase life of cut flowers, such as rose (Alaey *et al*., 2011) and anthurium (Promyou *et al*., 2012). However, little work has been reported on the role of SA on cut flower vase-life improvement and physio-chemical attributes related to senescence.

Methyl Jasmonate (MJ) has been found as a naturally occurring substance in higher plants. In this context, the application of MJ induced antioxidant system activity can suppress fungal infection and enhance stress resistance (Kanani and Nazarideljou, 2017). To our knowledge, MJ effects on specific physiological and biochemical processes in gerbera cut flower have not been studied yet.

As a derivative of 8-hydroxyquinolines, 8-hydroxyquinoline sulfate is widely used as antibacterial since the beginning of the 1950s. This compound is active against gram-negative bacteria of the Enterobacteriaceae family, fungi of the Candida genus, and mycoplasma (Chupakhina *et al*., 2012). In previous studies, 8-hydroxyquinoline sulfate (8-HQS) remarkably increased vase life of rose cut flowers (Ichimura *et al*., 1999).

In the present study, the role of three plant growth regulators (i.e., SNP, MJ, and SA) in regulating the activities of the antioxidant enzyme, relative water content (RWC), water uptake, the contents of malondialdehyde (MDA), protein, pigments and soluble sugar in the petals, and time of stem bending of gerbera cut flowers were investigated. The objective of the present study is to provide a theoretical basis for the application and optimization dosage of plant growth regulators in combination with an antimicrobial compound, i.e., 8-HQS, in improving the vase life of gerbera cut flowers during the vase-holding period.

2. Materials and Methods

Plant material and treatment

'Sun Spot' cut-gerberas (*Gerbera jamesonii*), harvested at normal harvest maturity, were obtained from a commercial grower (Dezfol, Khuzestan, Iran). The length of the stem varied between 65 and 70 cm. The harvested flowers were packed into parchment paper and transported to the laboratory within 1-2 h. Then, stems were re-cut to a uniform length of 55, under distilled water to avoid air embolism. Each six-flower sample was placed randomly in 250 mL of various concentrations (50, 100, and 200 μM) of hold-solutions containing 8-HQS, SA, MJ, and SNP. Hold solutions were used alone or in combination with 100 μM 8-HQS for 24 h. Distilled water was used as control and sucrose (4%, w/v) was added to all solutions. To maintain the proper concentrations of hold-solutions, the mouths of the vases were covered with plastic wrap (around the stem) to minimize evaporation and to prevent contamination. Then, flowers were individually sited in glass bottles of 25 cm height, approximately 150 ml of distilled water in each bottle, under laboratory condition. The laboratory was maintained at 22°C, 60±5% relative humidity, and 16 mmol m^{-2} s^{-1} photons irradiance using cool fluorescent lamps for a 12 h photoperiod (07:00-19:00 h).

Measurements

- Time of stem bending was determined as described by Perik *et al*. (2012).
- Relative fresh weight (RFW) was calculated by the

following formula:

$$RFW\ (\%) = [(FW_{t=10} - FW_{t=0}) / FW_{t=0}] \times 100$$

where $FW_{t=10}$ is the fresh weight of flower (g) at 10th day and $FW_{t=0}$ is the fresh weight of the same flower (g) at first day (He *et al*., 2006).

- Water uptake (mL) was calculated by subtracting in the weight of the remaining water at the end of the experiment from the initial weight.
- Soluble carbohydrate content in petals was measured by the anthrone colorimetric method according to the method of Xue (1985).
- Total anthocyanin content of petal was measured by the pH differential method of Yang *et al*. (2009).
- Total carotenoid content of gerbera petal tissue was estimated using the method of Wellburn (1994).
- Flavonoid petal tissue was measured according to the method of Markham (1982).
- Protein content in petal was estimated by the method of Bradford (1976) using bovine serum albumin as the protein standard.
- Malondialdehyde (MDA) content in petals was measured by the thiobarbituric acid reaction following the procedure of Hodges *et al*. (1999).
- Peroxidase (POX) activity was evaluated by oxidation of guaiacol, as a substrate, according to Chance and Maehly (1955).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in SAS software. Means were compared by one-way ANOVA and Duncan's multiple range test at the 5% level of significance.

3. Results and Discussion

Vase life

As shown in figure 1, hold-solutions affected senescence of *Gerbera jamesonii* in a dose-dependent manner. Compared with control, different concentrations of SA, with or without 8-HQS, all prolonged the senescence of gerbera as shown by the tighter stem and more showy flowers. Compared with other concentrations of SA, 50 µM SA+ 100 µM 8-HQS markedly prolonged the longevity of the cut flowers. As shown in figure 1, different concentrations of SNP, with or without 8-HQS, all prolonged the length of vase life of gerbera cut flower, compared with control. Compared with control, 50 µM, 100 µM, and 200 µM SNP noticeably increased the length of vase life of *Gerbera jamesonii* cut flower by 65%, 78%, and 43%, respectively. However, there was no significant difference in the stem bending of flowers among different concentrations of SNP. Among various concentrations of 8-HQS, stem bending of cut flowers treated by 100 µM 8-HQS was significantly lower than those treated by other concentrations. Compared with control, 50, 100, and 200 µM 8-HQS increased the vase life by 69%, 100%, and 78%, respectively. Different concentrations of MJ all significantly increased vase life, compared with the control (Fig. 1). However, MJ treatments were less effective on vase life compared with 8-HQS, SA, and SNP treatments.

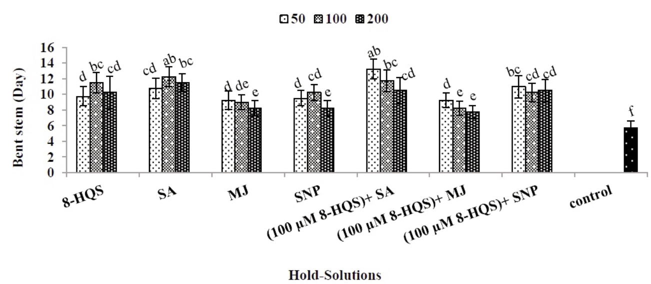

Fig. 1 - Effect of the different preservative solutions (µM) on time of stem bending in gerbera cut flowers. Columns followed by different letters are significantly different at P=0.05.

Shabanian *et al*. (2018) showed that SNP extended the vase life of gerbera cut flowers as compared with their respective control treated with water alone. In agreement with this finding, treatments with SNP extended the vase life of other cut flowers, e.g., carnation (Zeng *et al*., 2011), chrysanthemums (Mansouri, 2012), gladiolus (Dwivedi *et al*., 2016), and rose (Liao *et al*., 2013). The mechanism of SA action, as a hold solution, in vase life of cut flowers has not been clarified; however, published data suggest some association with ethylene production. Zhang *et al*. (2003) showed that application of SA resulted in suppression ACC synthase and ACC oxidase activities and biosynthesis of ethylene in kiwifruit. In Gladiolus, the maximum vase-life was obtained once flowers treated with a solution containing 100-ppm 5-sulfosalicylic acid + 4% sucrose (Ezhilmathi *et al*., 2007). 8-HQS is a subclass of quinolones with a wide variety of biological effects. The 8-hydroxyquinoline derivatives emerged as a hold-solutions being widely explored for several biological functions such as antifungal effects (Oliveri and Vecchio, 2016) and antimicrobial (Abouelhassan *et al*., 2017). According to van Doorn (1997), the bending of gerbera cut flowers was caused by low turgescence of the flower scape when facing water uptake problems. In addition, he notified that bacteria in the vase water were the most common cause of xylem blockage affecting water uptake. Accordingly, antimicrobial compounds such as 8-hydroxyquinoline citrate (Elhindi, 2012) and essential oils (Salehi Salmi *et al.*, 2018) were applied to improve vase-life of cut flowers. The effect of the application of MJ on vase life of cut flowers varies widely among species and cultivars. These reports indicated that the ethylene production rate might change with the kind of genes, which were stimulated by MJ (Salimi *et al.*, 2016). The results of the present study indicated that the decline in the vase life was significantly less in cut flowers in MJ-treated, compared with other treatments.

Water loss and water uptake

Comparing the results of the different hold-solutions revealed statistically significant differences such that the maximum and minimum amounts of water loss, in the 10th day, occurred on cut gerbera hold in 100 μM 8-HQS+ 200 μM MJ and 100 μM 8-HQS+ 100 μM SNP, respectively (Fig. 2). From figure 2 the maximum water loss occurred also on cut gerbera hold in MJ 200 μM alone (together with 100 μM 8-HQS+ 200 μM MJ). Also, data showed that cut flowers treated with 50 and 100 μM 8-HQS; 200 μM SA; 50 and 100 μM SNP; 50, 100, and 200 SA+ 100 μM 8-HQS; 50 μM MJ+ 100 μM 8-HQS; 50, 100, and 200 μM SNP + 100 μM 8-HQS hold solutions lost lower water the control (Fig. 2).

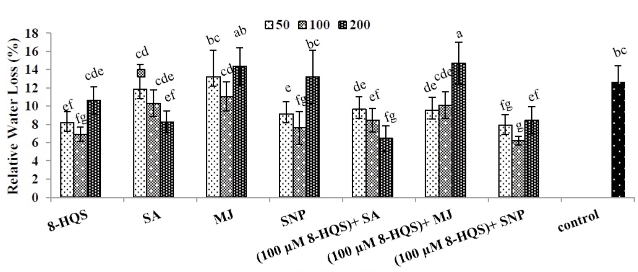

Fig. 2 - Relative water loss of cut flower of gerbera in various hold solutions with different concentrations (μM). The indicator was determined on 10st day. Columns followed by different letters are significantly different at P=0.05.

The lowest amount of water uptake appeared on 10th day in the cut flowers treated with 100 μM 8-HQS+ 200 μM MJ (Fig. 3). However, there was no significant difference between this treatment and 50 μM MJ, 200 μM MJ, and 200 μM SNP control treatments. In other treatments, water uptake was increased over 10 days of postharvest life in comparison with control. However, water uptake amounts showed significant differences among hold-solutions such that the maximum amount of it was observed in a cut flower treated with 100 μM 8-HQS+ 100 μM SNP (Fig. 3). Cut flower senescence is closely associated with water uptake stem and RWC of petals, whereas, these characteristics are closely related with the contents of osmoregulation substances such as soluble sugars and soluble proteins (Hou *et al.*, 2018).

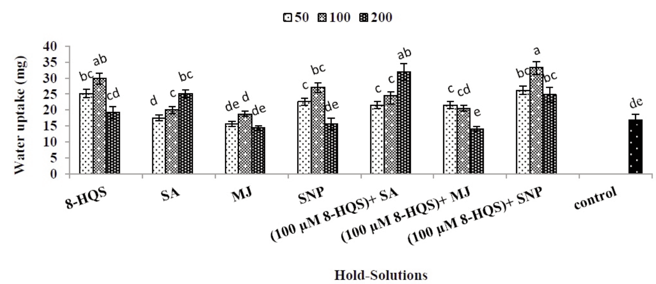

Fig. 3 - Effects of different concentrations (μM) of various hold solutions on the water uptake. The indicator was determined during 10 days. Columns followed by different letters are significantly different at P=0.05.

Soluble carbohydrates of petal

Changes of sugars content of gerbera petals are shown in figure 4. Maintenance of elevated total soluble carbohydrates content exhibited by the flowers under hold-solutions treatments can be correlated with the delay in senescence and the increase in vase life of Gerbera flowers. The results indicate that treatment with hold-solutions, except the high concentration of MJ, with or without 8-HQS, caused a significant decrease in reducing sugars compared with the control. Reducing carbohydrate starvation or its symptoms led to unwanted color changes and eventually increased susceptibility to microorganisms. Postharvest treatments can reduce carbohydrate starvation during the vase life phase. Postharvest treatments like sugar feeding often have

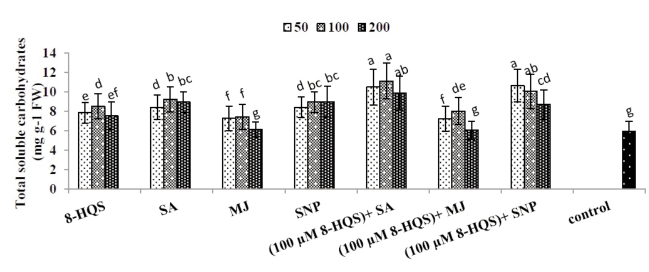

Fig. 4 - Total soluble carbohydrates in petals of hold-solutions-treated and untreated gerbera cut flowers stored at 22°C for 10 days.

a positive effect on vase life in general. It seems that 8-HQS, by preventing vascular blockage, caused sugars directly to reach flowers in the transpiration stream via xylem. Increased sugar caused by exogenous sucrose is well known from earlier studies (Ichimura *et al*., 1999; Promyou *et al*., 2012). Han *et al*. (2018) have illustrated that SNP treatment inhibited significantly the degradation of sucrose of peach fruit at the end of storage. They suggested sugars were significantly affected by SNP treatment probably due to the activities of sucrose metabolism enzymes. Yu *et al*. (2016) found that MJ treatment could increase the encoding level and enzyme activity of sucrose phosphate synthase, which resulted in the enhancement of sucrose content.

Petal pigments

The highest concentration of anthocyanin in gerbera florets was in the SA+ 8-HQS treatments, followed by the 8-HQS and SA treatments. The MJ, with or without 8-HQS, treatments did not result in a significant increase in anthocyanin concentration compared with that of the control (Fig. 5). Carotenoid content of petals were increased under treatment with all concentrations of SA with or without 8-HQS, all concentrations of SNP with or without 8-HQS, high concentrations of 8-HQS, and all concentrations of MJ with 8-HQS (Fig. 6) while the control had the lowest amount of carotenoid content among all treatments at the end of the experiment.

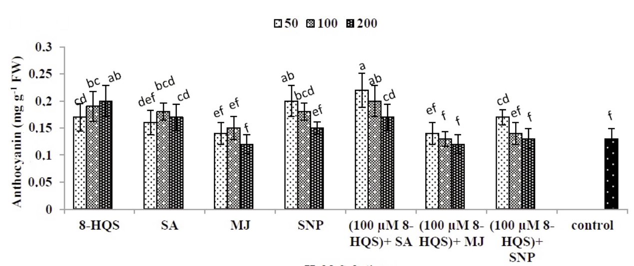

Fig. 5 - Effects of different concentrations (µM) of various hold solutions on anthocyanin content. The indicator was determined during 10 days. Columns followed by different letters are significantly different at P=0.05.

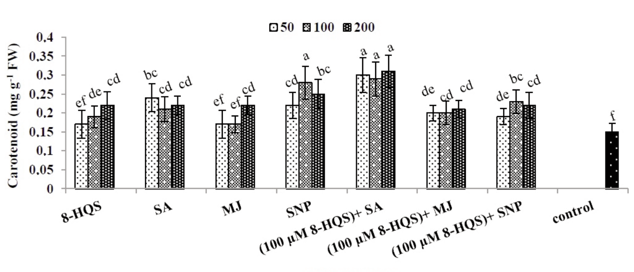

Fig. 6 - Effects of different concentrations (µM) of various hold solutions on amount of carotenoid. The indicator was determined during 10 days. Columns followed by different letters are significantly different at P=0.05.

Figure 7 depicts the contents (expressed as mg/g fresh weight) of flavonoid obtained from petals of gerbera cut flowers untreated and treated with hold solution at different concentrations. By comparing untreated-control samples, hold solutions differences could be clearly established (Fig. 7). In particular, the concentrations of petal flavonoid were significantly ($p<0.05$) higher in treated cut flowers with SNP, SA or MJ; except 50 µM SA, 200 µM MJ, 50 µM SNP treatments; than Control. As can be seen, cut flowers treated with 100 and 200 µM SNP+ 100 µM 8-HQS exhibit higher concentrations of flavonoid than the other treatments.

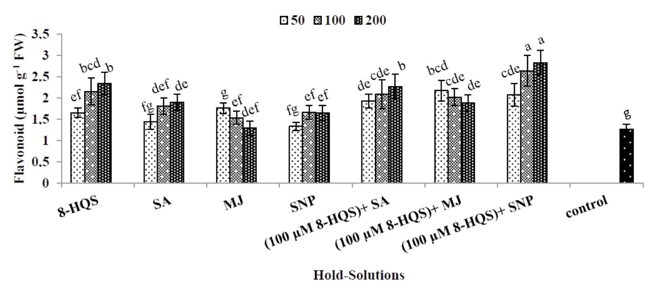

Fig. 7 - Effects of different concentrations (µM) of various hold solutions on flavonoid. The indicator was determined during 10 days. Columns followed by different letters are significantly different at P=0.05.

Despite the results of this study, most investigations on vase life of cut flowers do not present data on the changes in pigmentation and those that use subjective color grades for evaluation. Browning and discoloration are important factors in determining display quality of cut flowers and in many cases are the major reason for the termination of vase life (Elhindi, 2012; Khalaj *et al*., 2017; Salehi Salmi *et al*., 2018). Petal coloration is caused by the accumulation of pigment, including carotenoids, flavonoids, and betacyanins, within epidermal cells. Anthocyanins are synthesized via the phenylpropanoid and flavonoid pathways (Tanaka *et al*., 2008). Carbon metabolite levels, directly and indirectly, affect almost every metabolic process in a plant life. Anthocyanin and Carotenoid biosynthesis occur concomitantly with sugar accumulation in plant tissue (Hara *et al*., 2003; Zhang *et al*., 2015). Similarly, in our study, some vase solutions promoted soluble carbohydrates contents in the petals of gerbera cut flowers (Fig. 4), accompanying higher anthocyanin contents and presenting better ornamental quality of petal color (Figs. 5, 6, and 7). It is reported that application of MJ enhanced accumulation of flavonoids in *Daucus carota* (Sircar *et al*., 2012).

POX activity

The POX activity in the petal of gerbera flowers that were treated with different concentrations of all hold-solutions, except 100 µM MJ+ 100 µM 8-HQS, slightly increased during vase life (Fig. 8). The highest activity of POX was observed in cut flowers hold in 50 µM 8-HQS, 100 µM SA, and 200 µM 8-SNP solutions. The enzymatic antioxidant system can work against the accumulation of reactive oxygen species (ROS). Regulation of the antioxidant status and ROS production by SNP in plant cells subjected to either biotic or abiotic stressors is well established (Vidal *et al*., 2018). Previously, it has been shown that SNP provides protection in broccoli florets against rapid yellowing after harvest (Shi *et al*., 2016). This study was carried out to provide evidence for the ability of SNP to regulate flower senescence through regulation of the antioxidant status of gerbera petal cells. Salicylic acid can also act as a protector against several stressful impacts, scavenge free oxygen radicals, and counteract oxidative damage by regulating cellular redox balance and accelerating the transformation of superoxide anion and enhancing the activities of antioxidant enzymes (Zhang *et al*., 2003). SA treatment reduced chilling injury in anthurium via improving the activities of SOD, CAT, and POX (Promyou *et al*., 2012). Kumar Rai *et al*. (2018) reported that SA and SNP enhanced tolerance to heat stress in *Lablab purpureus*, by elevating antioxidant enzyme activity of POX, SOD, and CAT and thus alleviating heat-induced oxidative damage. The present study, for the first time, indicated that SA and SNP treatment delayed the senescence of gerbera flowers via improving the activity of antioxidant enzymes of POX. MJ was reported to stimulate POX activity in banana plants and reduce the level of O_2 and H_2O_2 (Sun *et al*., 2013). In this regard, Fan *et al*. (2016) reported inducing resistance responses in eggplant fruit by increasing the expression of POX genes.

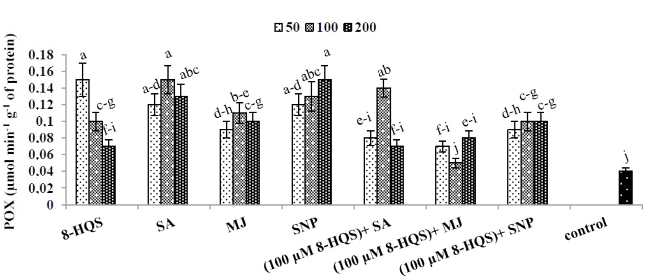

Fig. 8 - Effects of different concentrations (µM) of various hold solutions on activity of POX. The indicator was determined during 10 days. Columns followed by different letters are significantly different at P=0.05.

Protein

There was a significant difference among the type of hold-solution treatments on protein in the 10th day, although the protein content showed no significant differences among concentrations of a hold-solution. Protein content for SA treatments, with or without 8-HQS, increased compared with the control; however, it was not significantly different from other treatments to control (Fig. 9). The increase observed in the protein content, through treatment with SA, was likely the result of less protein degradation (Alaey *et al*., 2011) or an increase of protein synthesis (Ezhilmathi *et al*., 2007). Under flower senescence, the stimulation of protein synthesis leads to protein accumulation that may involve in the enhanced activity of enzymes as a defense mechanism (Promyou *et al*., 2012). To support the accumulation of proteins due to SA treatment, it was reported that SA results in a pronounced increase in total protein content and the formation of new proteins in roses (Alaey *et al*., 2011). It seems that MJ by increasing antioxidant defense enzymes leads to maintaining carbohydrate at high levels, as antioxidant inhibits the oxidation of cell biomolecules like proteins and carbohydrates (Kanani and Nazarideljou, 2017). In the present study, different concentrations of SNP and SA, with or without 8-HQS, could markedly increase the contents of soluble sugars and soluble proteins, which increased the RWC of petals and water uptake. These increases were helpful in increasing the water retaining capacity and also played an important role in increasing vase life of gerbera cut flowers. In addition, Schouten *et al*. (2018) suggested that SNP enhances flow through xylem vessels by increasing the ionic strength of the vase water.

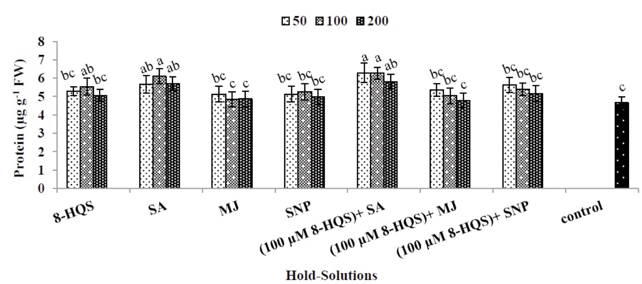

Fig. 9 - Petal proteins of gerbera cut flower in various hold solutions with different concentrations (µM). The indicator was determined on day 10. Columns followed by different letters are significantly different at P=0.05.

MDA

In the present experiment, a noticeable decrease in the MDA in control treated cut flowers compared with the other vase-solutions (Fig. 10). Accumulation of elevated amounts MDA in control treated cut flower was recorded. This accumulation indicates the presence of oxidative stress in gerbera petals. Zhang *et al*. (2015) reported over-reduction of the electron transport chain in mitochondria as the main source of O_2 production under specific stress conditions. Production of H_2O_2 can occur during lipid catabolism as a side-product of fatty acid oxidation. ROSs are also involved in the detoxifying reactions catalyzed by cytochromes in both the cytoplasm and the endoplasmic reticulum (Kumar Rai *et al*., 2018). Obviously, the observed high activity of POX in SA- and SNP-treated cut flowers are as a protective mechanism against senescence. It has been revealed that MJ mitigates the ROS effects in maize seedlings subjected to oxidative stress (Ahmadi *et al*., 2018).

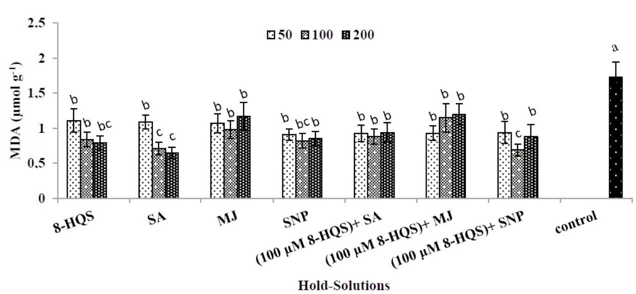

Fig. 10 - Effects of different concentrations (µM) hold solutions treatment on MDA of petal. The indicator was determined on day 10. Columns followed by different letters are significantly different at P=0.05.

4. Conclusions

Treatment with SA+8-HQS extends the vase life of gerbera cut flowers at relatively low SA concentrations and leads to generating the maximum cost-effectiveness. In conclusion, our results demonstrated that 8-HQS improves the vase life through increasing water uptake and consequently increases total soluble carbohydrates. Also, this effect may be exerted by improving the membrane stability and increasing proline, antioxidant activity, and pigment contents in the presence of SA.

Acknowledgements

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