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Efficacy of Salicylic Acid Treatment in Delaying Petal Senescence and Improving the Quality of Gladiolus Cut Spikes

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A B S T R A C T

Gladiolus is very important cut flower crop in floriculture industry and maintaining the quality of cut spikes is very imperative topic. Therefore, the current study was conducted to investigate the effects of salicylic acid (SA) on keeping the quality and extending the vase life of gladiolus cut spikes. Gladiolus spikes were put in holding solutions of SA at 0.2, 0.4, 0.6 and 0.8 mM while control spikes were placed in distilled water. The vase life of gladiolus spikes was considerably extended due to SA application relative to untreated spikes. Treating gladiolus spikes with SA at 0.6 mM resulted in 9 days longer than the untreated spikes. The number of opened florets, relative water content (RWC) and chlorophyll content were significantly enhanced in treated spikes compared to the control. The proline content was increased unlike the malondialdehyde (MDA) content that decreased in SA treated spikes and hence resulted in maintaining the membrane integrity compared with the control. The total phenolics in florets were increase as a result of SA treatment compared to untreated spikes. The positive effects of SA treatment in maintaining the quality of gladiolus cut spikes were more observed when 0.6 mM concentration was used while further higher level (0.8 mM) causes no improvement in spike longevity. Conclusively, SA had a sustainable effect on the physiological and biochemical investigated parameters that mitigated the oxidative stress in gladiolus cut spikes. Application of SA in floral preservative industry of cut flowers is recommended.

Introduction

The main challenge for most florists worldwide is how to keep the quality of cut flowers after harvest. Therefore, mitigating the senescence onset to extend the vase life of various cut flowers is very important research area and still the focus of several scientists (Hassan and Ali, 2014). Gladiolus, queen of bulbous crops, is a very valuable cut flower crop (Bhattacharjee and De, 2005) and the vase life of its spike depends on floret opening on the spike and the floret life (Ezhilmathi *et al.,* 2007).

The senescence of gladiolus flowers is induced by several physiological and bio-chemical processes that lead to short vase life. Otherwise, oxidative stress caused after harvest induces the flower senescence as well

and hence the spikes loose the ornamental value (Hassan and Ali, 2014; Saeed *et al.*, 2014).

Senescence is an oxidative process involving general cellular structure degradation and the products of degradation transport to other plant parts (Wang *et al.,* 2006). The flower senescence has been found to be correlated with over production of reactive oxygen species (ROS) and higher permeability of petal cells (Reezi *et al.,* 2009). Therefore, oxidative damage can enhance the senescence process in cut flowers while; mitigation of such stress is very important factor in keeping the quality of cut flower crops. It is well known that hormones are involved in the flower senescence regulation and the levels of hormones act as regulating signals for the discontinuation of specific reactions (Mansouri, 2012).

Salicylic acid (SA) is a phenolic compound that involved in the regulation of various plant growth and development processes (Esan *et al.,* 2017) and inhibits ACC-oxidase activity, a precursor of ethylene biosynthesis, (Zhang *et al.,* 2003). SA also plays an important role in stomatal conductance, photosynthetic rate and transpiration (Khan *et al.,* 2003; Arfan *et al.,* 2007) and enhancing the antioxidative protection (Xu *et al.,* 2008). It has been reported that SA reduced lipid peroxidation via motivation of antioxidant enzymes and therefore retains the membrane stability (Kazemi *et al.,* 2011; Hatamzadeh *et al.,* 2012). SA extended the vase life of gladiolus, attributable to reduced ROS, maintained membrane stability of floret cells, overcome fresh weight loss and increased antioxidant enzyme activities (Ezhilmathi *et al.,* 2007; Marandi *et al.,* 2011; Hatamzadeh *et al.,* 2012, Hassan and Ali, 2014). In a recent study on rose, Kazemi *et al.,* (2018) observed an increase in vase life due to SA treatment through decreasing lipid peroxidation as well as suppressing the increase in CAT and POD activities and hence improving membrane stability.

In gladiolus, several applications have been used to extend the spike longevity by blocking microbial agents, regulating water balance and motivating antioxidant defense system (Ezhilmathi *et al.,* 2007; Hassan and Ali, 2014; Saeed *et al.,* 2014). SA treatment significantly reduced the respiration rate, alleviated the moisture stress and extended the vase life of cut roses (Senaratna *et al.,* 2000). Moreover, the treatment of SA enhanced the postharvest life in different cut flowers (Bleeksma and van Doorn, 2003; Hayat *et al.,* 2010).

Although the impact of SA in plant growth has been well investigated, information concerning its role on extending the vase life via improvement of physiological and biochemical parameters is scarce. More information about the physiological response of gladiolus cut spikes to SA application will provide a better understanding of the optimum requirements for introducing satisfactory flowers to the market. Little work has been published on the role of SA on lipid peroxidation and total phenolics and their relation to the senescence of cut glagiolus spikes. The present study was, therefore, undertaken to invsstigate the effects of SA on the vase life of gladiolus cut spikes. Several physiological and biochemical attributes that involved in flower senescence were also evaluated in relation to SA treatment.

Materials and Methods

Flower materials

Cut spikes of *Gladiolus grandiflorus* cv. "White Prosperity" were used in current investigation. After obtaining from a commercial grower, the spikes were directly

transported to the laboratory of Horticulture Department, Faculty of Agriculture, Menoufia University during January to March 2017 season. Homogenous spikes, having 14-16 buds each, were selected at tight bud stage but the first floret was shown its color. The spikes were trimmed to 70 cm length after removing the lower leaves.

SA treatments

Aqueous solutions of salicylic acid (SA; 2-hydroxybenzoic acid) at 0.2, 0.4, 0.6 and 0.8 mM SA were prepared using distilled water after dissolving the proper weight of SA in 50 mL dimethyl sulfoxide. SA concentrations were applied as holding solutions and the spikes were placed in 500 mL beakers. Control spikes were not treated with SA and were put in 500 mL beakers with distilled water. Each treatment had three replicates and five spikes were placed in each replicate.

Vase life assessment

The vase life of cut spikes was evaluated at 21 °C, 75 ± 5 % RH under lab conditions. The vase life of cut gladiolus spikes was terminated when the ornamental value of 50 % of the florets in each spike were lost (lost turgor and wilted) as reported by (Hassan and Ali, 2014).

Number of opened florets

The number of opened florets on each spike was evaluated from the beginning of the study until the end of control vase life.

Relative water content (RWC)

The RWC of gladiolus leaves were assessed as described by Weatherley (1950) as follows: $(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry}) \times 100$, where W_{fresh} is fresh weight of sample, W_{turgid} is turgid weight of sample after saturating with distilled water at 4 °C for 24 h, and W_{dry} is oven-dry (at 70 °C for 48 h) weight of sample. RWC was measured in the third leaf from the inflorescence base at days 2, 4, 6, 8 and 10 from the beginning of the investigation.

Chlorophyll determination

The total chlorophyll content was investigated in the third leaf from the spike base on days 2, 4, 6, 8 and 10 by the method of Metzner *et al.*, (1965). Leaf discs (0.2 g) were homogenized in 50 mL acetone (80 %). For slurry straining, a cheese cloth was used and then the extract was centrifuged for 10 min at 15000 *g*. The acetone extract was spectrophotometrically observed at 663 nm for chlorophyll (a) and 645 nm for chlorophyll (b) by the following equations:

Chlorophyll (a) = $10.3_{E663} - 0.918_{E644}$
Chlorophyll (b) = $19.3_{E644} - 3.87_{E663}$
The total chlorophyll was calculated and presented as mg g^{-1} FW.

Proline determination

All subsequent physiological and biochemical analysis were assessed in floret samples from the third floret at the spike base on days 1, 2, 3, 4 and 5. The free proline content was determined as described by Bates *et al.*, (1973). Briefly, frozen floret sample (0.2 g) was homogenized in 10 mL of 3 % sulfosalicylic acid at 4 °C. After the extract is being filtered, 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed and incubated for 1 h at 100 °C in a test tube. After terminating the reaction on ice, the reaction mixture was extracted with 4 mL of toluene. The optical density was spectrophotometrically determined at 520 nm with toluene as a blank. Proline content was calculated based on a standard curve and was expressed as µmol g^{-1} FW.

Lipid peroxidation assay

Malondialdehyde (MDA) content was determined as an indicator to lipid peroxidation. MDA was assessed by the method of Hodges *et al.,* (1999). Floret samples of (0.2 g) were homogenized with 2 mL of 0.1 % trichloroacetic acid (TCA) then centrifuged for 15 min at 14000 *g*. A mixture of 2 mL of supernatant and 3 mL of 0.5 % TBA in 5 % TCA was incubated for 30 min in hot water (95 °C). To stop the reaction, the mixture was immediately cooled on ice and centrifuged for 15 min at 5000 *g*. The supernatant was spectrophotometrically observed at 450, 532 and 600 nm. The MDA content of was estimated using the formula: MDA content = 6.45 × (A_{532} - A_{600}) - 0.56 × A_{450}, where A_{450}, A_{532} and A_{600} are the absorbance at 450, 532 and 600 nm, respectively and was expressed as µmol mL^{-1}.

Membrane stability index (MSI)

Determining the ions leakage was assessed using the method of Sairam *et al.,* (1997). Two samples (0.2 g) were placed in 20 mL of double distilled water in two 50 mL flasks. The first one was kept at 40 °C for 30 min while the second was kept in boiling water bath for 15 min at 100 °C. A conductivity meter was used to measure the electric conductivity of the first (C_1) and second (C_2) samples. The ions leakage was expressed as the MSI according to the following formula, MSI = [1- (C_1/C_2)] X 100

Total phenol content assay

Samples of 0.5 g floret material were stirred at room temperature in 50 mL of methanol (80 %) for two days. Then, the solvent was removed and the extract was kept below 4˚C for total phenolics evaluation (McDonald *et al.,* 2001). To assay the phenol content, diluted extract (0.5 mL of 0.1 kg L^{-1}) or standard phenolic compound (Gallic acid) was mixed with the Folin-Ciocalteu reagent (5 mL, 1:10 using distilled water) and 4 mL of 1 M aqueous sodium carbonate. Finally, the total phenolic was spectrophotometrically observed at 765 nm and expressed as g kg^{-1} GAE.

Statistical analysis

The SA treatments were arranged in a complete randomized design. The experiment was repeated three times and had qualitative and quantitative results. The results of three experiments were pooled. The analysis of variance (ANOVA) was performed using MSTAT program, USA. Means were separated using LSD at *P*=0.05. The values are means ± SE of the three experiments (*n* = 9).

Results and Discussion

Vase life

All concentrations of SA significantly increased the longevity of cut gladiolus spikes compared with untreated spikes, more so with higher two levels without significant difference between them (Fig. 1A). Spikes treated with SA at 0.6 mM resulted in the longest vase life (16.72 days) while the control recorded the lowest vase life (7.45 days).

Number of opened and unopened florets

Data in Fig. 1B clearly show that the number of opened florets on gladiolus spike was increased as a result of SA treatment and the impact was more observed with 0.6 mM concentration. The lower number of opened florets was obtained by untreated control. Relative to the control, the increment in

percentage of opened florets was 65.06, 120.71, 177.61 and 166.94 % for SA at 0.2, 0.4, 0.6 and 0.8 mM, respectively.

Relative water content (RWC)

In treated and non-treated spikes, the RWC was decreased with the progressive development in vase life days (Fig. 2A). However, SA treatment considerably decreased this decline in treated leaves relative to the control that recorded a sharp decrease in RWC over vase life period. This effect was clearer from day 4 and the best results were observed with 0.6 followed by 0.8 mM SA.

Chlorophyll content

The chlorophyll content in gladiolus leaves was gradually decreased in treated and non-treated spikes during the vase life evaluation period and the chlorophyll reduction in the control was sharp compared to the other treatments (Fig. 2B). The chlorophyll reduction was significantly retarded by SA application, more so with higher levels (0.6 or 0.8 mM). By day 10, control leaves kept with 52.54 % of the initial chlorophyll content, while, the treated spikes maintained the chlorophyll by 70.09, 78.99, 93.16 and 85.59 % for SA at 0.2, 0.4, 0.6 and 0.8 mM, respectively.

Proline content

Free proline content in SA treated spikes relative to the control was presented in Fig. (3A). The proline content was significantly increased due to SA treatment compared to untreated spikes. A gradual increase was observed till day 4 over floret life period then decreased thereafter. The highest proline accumulation was recorded by 0.6 mM concentration while control florets gave the lowest proline values.

Malondialdehyde (MDA) content

In untreated florets, a significant increase in MDA accumulation was observed reaching the peak at day 4 and then decreased. However, SA treatment decreased MDA accumulation compared to the control throughout the floret life days. The treatment of SA at 0.6 mM recorded the lowest accumulation of (Fig. 3B).

Membrane stability index (MSI)

It is very clear from data in Fig. (4A) that MSI was sharply lost in control florets upon the floret senescence progression over floret life days. However, SA application retained the MSI relative to the control florets. By day 5, the MSI was 53 % in control florets compared to 71.45, 74.67, 80.12 and 77.67 % SA at 0.2, 0.4, 0.6 and 0.8 mM, respectively.

Total phenol content

During the gladiolus floret life, the total phenolics in untreated spikes was slightly increased till day 3 and decreased thereafter however this change was not significant. Otherwise, SA treatments appreciably increased the floret phenol content relative to the control, more so with 0.6 mM concentration (Fig. 4B). Relative to the control, the increase in total phenolics at day 4 was 39.91, 73.39, 138.30 and 115.43 % for SA at 0.2, 0.4, 0.6 and 0.8 mM, respectively.

In current study, the effects of SA on the longevity and postharvest quality of gladiolus cut spikes were investigated. All concentrations of SA significantly prolonged the vase life compared to the control. Increasing the vase life could be explained through the higher number of opened florets observed in treated spikes due to SA treatments. These results are in accordance with the results of Ezhilmathi *et al.*, (2007),

Hatamzadeh *et al.*, (2012) and Hassan and Ali (2014) on gladiolus. RWC refers to the ability of plant organs to keep the water and therefore, SA treated spikes was in favorable conditions to uptake and maintain water consequently, the RWC was higher in treated spikes. Otherwise, control flowers were under oxidative stress conditions and could not maintain water properly therefore, recorded lower RWC. It has been reported that maintaining water relations has shown to be very critical to prolong the vase life while the flower senescence was observed when water balance was disturbed (Ezhilmathi *et al.*, 2007; Hassan *et al.*, 2014). In this respect, Mori *et al.*, (2001) explained the the improved water balance due to SA treatment through the germicidal effect of SA which acting as an antimicrobial that inhibit the vascular blockage. SA also regulates stomatal closure and transpiration rate that increases the capacity of water-retaining. In accordance with current data, previous reports showed that SA improved the water relations and therefore increased the RWC (Hassan and Ali, 2014).

Fig.1 Vase life (A) and number of opened florets (B) of gladiolus cut spikes treated with salicylic acid (SA). The values (mean ± SE) are the average of three independent experiments (n = 9). Columns had different letters are significantly differ from each other according to LSD test ($P \leq 0.05$)

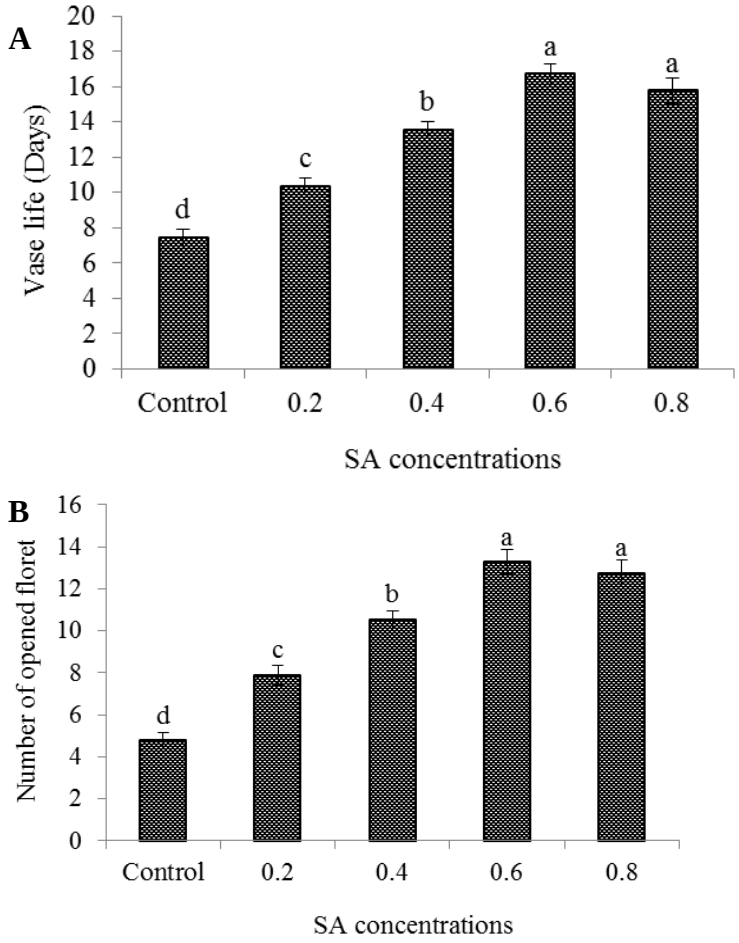


Fig.2 Relative water content (A) and Chlorophyll content (B) of gladiolus cut spikes treated with salicylic acid (SA). The values (mean ± SE) are the average of three independent experiments (n = 9)

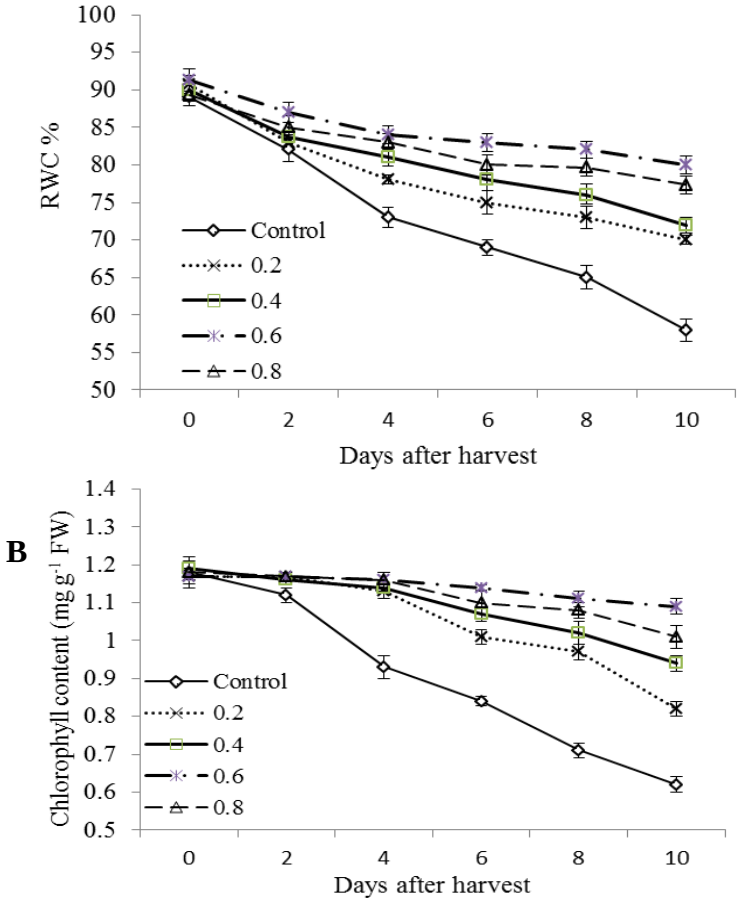
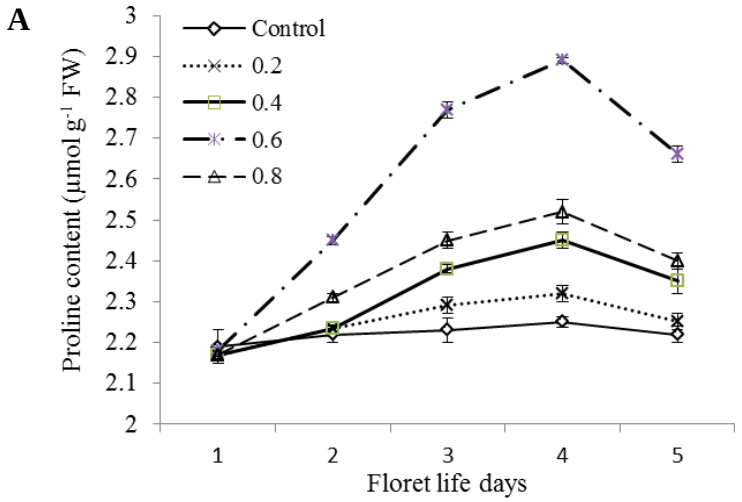


Fig.3 Proline content (A) and Malondialdehyde (MDA) content (B) in gladiolus florets treated with salicylic acid (SA). Samples were taken from the third floret at the spike base on days 1, 2, 3, 4 and 5. The values (mean ± SE) are the average of three independent experiments (n = 9)



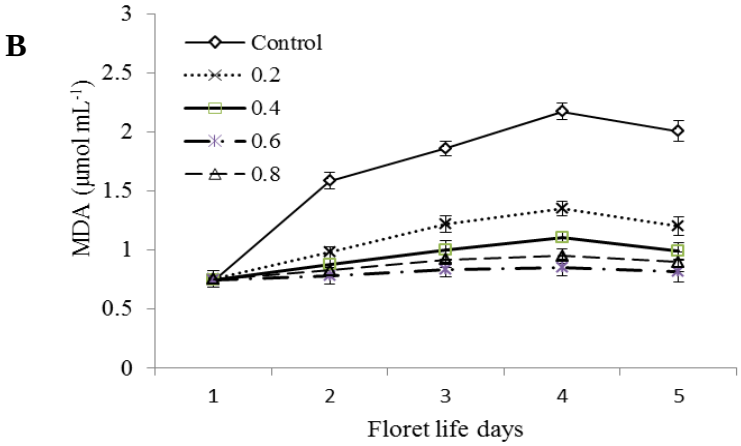
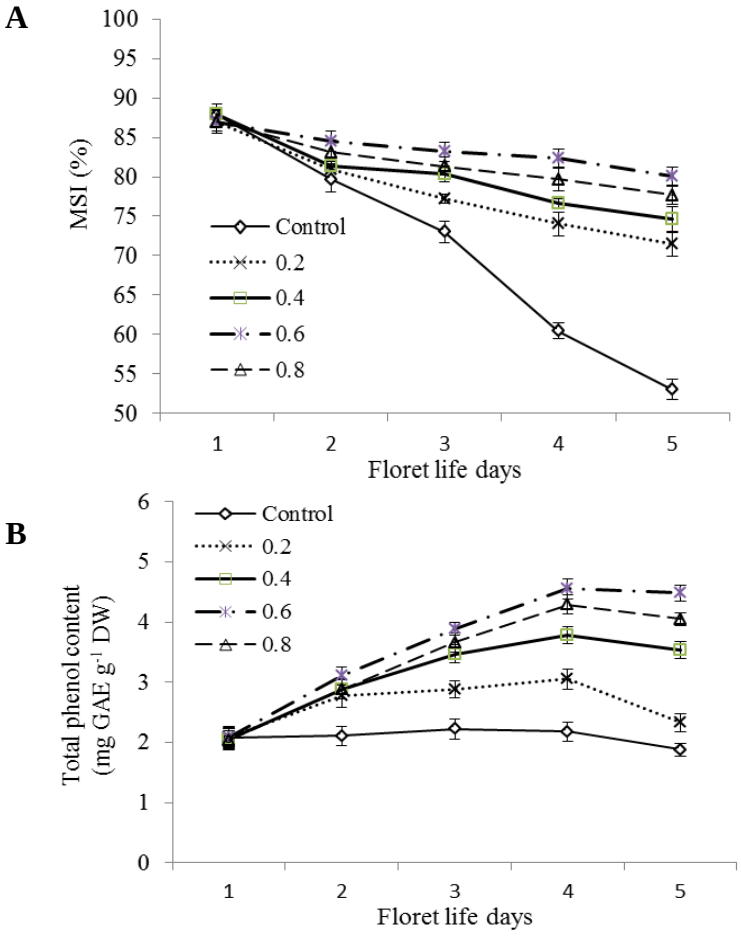


Fig.4 Membrane stability index (A) and total phenol content (B) in gladiolus florets treated with salicylic acid (SA). Samples were taken from the third floret at the spike base on days 1, 2, 3, 4 and 5. The values (mean ± SE) are the average of three independent experiments (n = 9)



The results of this study indicate that SA treatment considerably mitigated the chlorophyll reduction that observed in control spikes and therefore higher chlorophyll

content was recorded in treated spikes over the vase life period relative to the control. Poor water relation in control spikes could be ascribed to oxidative stresses after harvest (Hassan and Ali, 2014) which led to chlorophyll reduction due to the disorganization of thylakoid membrane and motivation of chlorophyllase enzyme that associated with chlorophyll degradation (Rong-Hua *et al.,* 2006). These results support the previous reports of Fariduddin *et al.,* (2003), Kazemi *et al.,* (2011) and Zamani *et al.,* (2011) who found an improvement in chlorophyll content due to SA treatment.

In this investigation, SA regulates gladiolus floret senescence through other mechanisms including proline accumulation, reducing lipid peroxidation and maintaining membrane stability. Over the floret life period, SA treatment increased the proline accumulation but decreased the MDA content relative to untreated spikes. The free proline accumulation is considered a possible mechanism for cell protection against oxidative damage (Olga *et al.,* 2003). Under oxidative stress, proline plays an adaptive role in osmotic adjustment mediation and preserving the subcellular structures (Ashraf and Harris, 2004).

The increase in MDA has been reported as a biomarker of lipid peroxidation (Bailly *et al.,* 1996) and hence the reduction in MDA level means lipid peroxidation reduction. In this study, reduced lipid peroxidation and hence increased MSI were observed with SA treatment. During the senescence of gladiolus florets, the reduction in lipid peroxidation and maintained the membrane integrity have been reported to be reversely proportional (Hatamzadeh *et al.,* 2012). Reduced MDA probably mitigates gladiolus flower senescence in response to SA treatment, which is in accordance with the reports of Hassan and Ali (2014) who indicate SA role in lipid peroxidation reduction. In this regard, Kazemi *et al.,* (2018) reported that increased the membrane leakage as well as MDA by lipoxygenase activity were associated with the senescence process. Interestingly, SA treatment increased the total phenolic content in gladiolus florets relative to the control. This observation is consistent with the decreased MDA content in treated florets as phenols are known to have non-enzymatic antioxidive function (Gan *et al.,* 2017).

In conclusion, this study was an attempt to evaluate the impact of SA in extending the vase life of gladiolus cut spikes. SA treatment prolonged the vase life, increased the number of opened florets by enhancing the water relation, maintaining the chlorophyll content, improving the proline accumulation, reducing the MDA and hence maintaing the membrane integrity as well as increasing the total phenol content.

The current results suggest that SA could be considered as an effective commercial substance for the cut gladiolus industry.

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