East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online)
Published By East African Scholars Publisher, Kenya

Volume-5 | Issue-6 | June-2022| DOI:10.36349/easjals.2022.v05i06.001

OPEN ACCESS

Original Research Article

Salicylic Acid Enhances the Vase Life and Improves the Postharvest Attributes — A Case Study of *Antirrhinum majus* L.

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Article History
Received: 06.03.2022
Accepted: 15.04.2022
Published: 04.06.2022

Journal homepage:
http://www.easpublisher.com

Quick Response Code

Abstract: *Antirrhinum majus* L. is one of the most significant ornamental plants in the international flower industry, but has a relatively limited vase life, as evidenced by petal abscission and flower spike bending. The current study focuses on examining the efficacy of salicylic acid on the vase life and postharvest attributes of *A. majus* flower spikes. The spikes with the oldest floral bud at one day before anthesis stage were harvested and divided into 4 sets, with one set of spikes held in distilled water (DW) designated as control. The remaining three sets were supplemented with various concentrations of SA viz., 1, 2 and 3 mM. Among the concentrations studied, 2 mM was found to be the most promising SA concentration for increasing the postharvest performance of flowers of *A. majus* cut spikes. The enhanced vase life of *A.majus* spikes was characterised by increased floral diameter, a higher membrane stability index and elevated levels of total phenols and sugars as compared to the control. SA has been proven to suppress protein degradation and lipoxygenase activity during the vase life of flower spikes. Furthermore, SA-treated flower spikes with enhanced postharvest properties were shown to have higher levels of antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase activity.

Keywords: Antioxidant enzymes, Membrane stability index (MSI), Salicylic acid, Sugars, Vase life.

INTRODUCTION

Flowers have long been an important facet of human existence and are particularly vital in the Indian traditional way of living. Being extremely perishable, preserving their quality is one of the most difficult tasks for florists in the floriculture industry (Poonam *et al.*, 2021). Premature postharvest senescence is a major bottleneck in the marketing of cut flowers and considerable effort has been devoted in creating postharvest treatments to extend the marketing period (Vehniwal and Abbey, 2019). Membrane degradation is an early and distinctive feature of cut flower petal senescence (Davoud *et al.*, 2015). Increased lipid peroxidation, regulated and sustained by phospholipid-degrading enzymes, results in a loss of membrane integrity and increased reactive oxygen species (ROS) production (Reezi *et al.*, 2009). The accumulation of hazardous ROS such as superoxide, hydrogen peroxide and hydroxyl radicals causes oxidation of the cell's essential components and eventually damage to DNA and RNA, resulting in oxidative stress (Aghdam and Bodbodak, 2014). For the survival of plants, the antioxidant system must work properly in order to maintain a balance between ROS generation and scavenging. Several enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are involved in ROS scavenging in plant systems (Davoud *et al*, 2015). There are several chemicals that are widely employed in postharvest conditions to delay floral senescence. Many compounds that affect ethylene production or activity and are currently used to enhance the postharvest longevity of flowers may not be suitable in the near future owing to environmental and public health concerns (Shisarena *et al.*, 2017). Over the last decades, environmentally and eco-friendly production methods and conscientious use of resources have become more important in order to achieve the objective of more sustainable plant production. Salicylic acid (SA) has been identified as a new viable option for this purpose due to its natural, safe, low cost and biodegradability and has been discovered to impact different physiological and biochemical activities in plants (Alaey *et al.*, 2011). SA, A signalling molecule of phenolic nature is an old hormone playing new tricks while orchestrating various physiological processes in plants such as growth, photosynthesis, flowering, stomatal conductance and

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defence against biotic and abiotic challenges (Saeed *et al.,* 2016). A positive role in the reduction of ethylene synthesis, induction of disease resistance, deterrence to oxidative stress, diminution in respiratory kinetics and delay in senescence have been manifested in plants by the application of SA (Asghari and Aghdam, 2010). SA improves the postharvest life of horticultural crops by preserving cell membrane integrity, enhancing solution uptake, regulating stomatal transpiration and magnifying the activities of several antioxidant enzymes (Radwan *et al.,* 2019; Abdelaal *et al.,* 2020). SA is a well-known phenol that may inhibit ACC oxidase, which is a direct precursor of ethylene, as well as reduce ROS by increasing enzyme antioxidant activity (Heidarnezhadian *et al.,* 2017).

Antirrhinum majus L. (Snapdragon) plants belong to the family *Plantaginaceae*. The species is frequently planted as landscaping in gardens because of its gorgeous blossoms (Christopher *et al.,* 2020). Snapdragons are popular ornamental plants with a variety of petal colors and a pleasant aroma, making them an excellent choice for cut flower arrangements. The vase life of cut snapdragon flowers is relatively short, terminating due to petal abscission, incomplete flower opening, pigmentation suppression and flower spike bending (Miki *et al.,* 2022). This short vase life on the other hand is a serious postharvest concern of cut snapdragon flowers, posing a hazard to flower producers and consumers (Flower *et al.,* 2002). As a result, extending the vase life of *A. majus* is critical in preserving its essence and marketing of this popular cut flower for a longer period. In the light of importance of this cut flower and its short vase life, the current study was undertaken to investigate how SA improves vase life and postharvest attributes in *A. majus* flower spikes to boost its ornamental value.

MATERIALS AND METHODS

For this investigation, healthy and uniform flower spikes of *Antirrhinum majus* were taken from the Kashmir University Botanic Garden (KUBG) with the oldest bud at one day before anthesis stage and transported to the laboratory in small sterilized buckets with the cut ends immersed in distilled water. To prevent embolism, the flower spikes were defoliated and re-cut to a uniform length of 30 cm under water. The spikes were then placed in 100 mL flasks with various concentrations of SA, including 1, 2, 3, 4, 5 and 6 mM. A separate set of spikes in distilled water was used as a control. Each treatment, including the control, comprised ten replicates (flasks), with two spikes in each flask. After standardization, SA concentrations of 1, 2 and 3 mM were found to be optimum for enhancing various postharvest characteristics. The values above the optimum concentrations were found to be hazardous, causing premature senescence. The day on which flower spikes were treated with different treatments was designated as day zero (D0). On day 2 and day 6 after transfer into test solutions, the oldest flowers present on the spikes corresponding to the same stage of development were employed for the analysis of several physiological and biochemical characteristics. The experiment was carried out in a controlled environment with a relative humidity (RH) of 60 ± 10%, a light duration of 12 h per day and an average temperature of 23 ± 2 ^{o}C.

Assessment of Vase life and floral Diameter

The vase life of the cut flower spikes was calculated as the number of days from day 1 (D1) of the experiment up to the day when 60% or more flowers had wilted or senesced and only 40% or less were fresh and fully open, the spike at this stage was considered to have lost its ornamental value. During the experiment, floral diameter was recorded on day 2 and day 6 as the mean of two perpendicular measurements of the top surface of the flower.

Bacterial density and solution uptake

The bacterial density was estimated by measuring the optical density (absorbance) of 1 mL of holding solution taken from each treatment, including the control, at 600 nm with a PC-based UV-VIS spectrophotometer (Systronics) using Escherichia coli as the reference (1 OD = 8x108) (Naing *et al.,* 2017). Solution uptake (mL) was evaluated as the difference between the volume of vase solution at the end of the experiment and the total volume of vase solution.

Membrane stability index (MSI)

The MSI evaluated in the form of solute leakage of the petal tissues was calculated by incubating 100 mg of petal tissue in 5 ml deionized water at 25 ^{0}C for 30 min and 100 ^{0}C for 15 min (Sairam, 1994). MSI was computed as under

$$\text{MSI} = \left[1 - \frac{\text{C1}}{\text{C2}}\right] \text{X } 100$$

C1 represents the conductivity of the samples incubated at 25 ^{0}C and C2 represents the conductivity at 100 ^{0}C, after recording the values on Elico CM180 Conductivity meter.

Protein estimation

For the estimation of proteins, 1 g of petal tissue was macerated in 100 mM phosphate buffer of pH 7.2 containing 10 % polyvinyl pyrrolidone (PVP), 1 mM EDTA, 150 mM NaCl, 10 % glycerol, 1 % Triton X-100, and 1 mM Dithiothreitol (DTT). The mixture was centrifuged utilizing a refrigerated centrifuge at 12,000*x*g at 5°C for 15 min. Proteins were estimated from a suitable volume of aliquot taken from the collected supernatant following the method of Lowry *et al.,* (1951).

Estimation of sugar fractions and phenols

One g of chopped petal tissue from each treatment was fixed in hot 70% ethanol, macerated and centrifuged thrice. From the suitable aliquot taken from

the supernatant total phenols, reducing, non-reducing and total sugars were estimated. Total phenolics were quantified by Swain and Hillis method (1959) using gallic acid as standard. Nelson's method (1944) was employed for estimating reducing sugars with glucose acting as standard. For the estimation of total sugars, non-reducing sugars were converted to reducing sugars by invertase. Amount of non-reducing sugars were calculated from difference between total and reducing sugars. Total phenols and sugar fractions (reducing, non-reducing and total sugars) were determined at day 2 and 6 of the transfer of the flowers to the respective test solutions.

Enzyme extraction and assays

Superoxide dismutase activity (SOD)

One g of petal tissue was homogenized and thoroughly mixed with 0.1 mM potassium phosphate buffer (pH = 7.8) containing 0.5 % (v/v) Triton X-100, 0.1 mM EDTA and 1 % PVP .The mixture was centrifuged at 15,000*x*g for 10 min. The collected supernatant was first filtered through Mira cloth and then used for the enzyme assay. The activity of SOD was calculated following the method of Dhindsa *et al.*, (1981), by observing the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 50 mM sodium carbonate, 0.1 mM EDTA, 75 µM nitroblue tetrazolium (NBT), 0.1 ml of the enzyme extract, and 13 mM methionine in 50 mM phosphate buffer (pH = 7.8) in a total volume of 3 ml. To the reaction mixture adding of 2 µM riboflavin and putting the test tubes in water bath at 25°C and illuminating with a 30 W fluorescent lamp initiated the reaction. The test tubes were kept in dark after the reaction was stopped by switching off the light. Other identical, unilluminated test tubes acted as blanks. Absorbance was measured at 560 nm. The amount of enzyme that prevents photoreduction of NBT to blue formazan by 50 percent compared to the reaction mixture kept in darkness without the enzyme extract has been defined as one unit of SOD activity. The SOD activity was expressed as units min^{-1} mg^{-1} protein.

Catalase activity (CAT)

Catalase activity was calculated following the method of Aebi, (1984). 1 g of petal tissue was macerated and homogenized in 100 mM potassium phosphate buffer of pH 7.0 containing 1 mM EDTA. The reaction mixture contained 50 mM potassium phosphate buffer (pH = 7.0), 50 µl enzyme extract, 12.5 mM H_2O_2, was added with distilled water to make the final volume to 3 ml. Addition of H_2O_2 initiated the reaction and the catalase activity was assayed by determining H_2O_2 consumption for 3 minutes at 240 nm and was expressed as µmol H_2O_2 red.$min^{-1}mg^{-1}$ protein.

Ascorbate peroxidase activity (APX)

Petal tissue of 1 g was macerated and homogenized in 100 mM sodium phosphate buffer containing 10 % glycerol, 1 mM EDTA and 5 mM ascorbate. The APX activity was determined in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH = 7.0), 0.3 mM H_2O_2 and 0.1 mM ascorbate. The reduction in the absorbance was noted for 3 min at 290 nm (Chen and Asada, 1989).

Lipoxygenase activity (LOX)

LOX activity was determined by employing the method of Axerold *et al.*, (1981). 1 g of petal tissue was thoroughly mixed in 1 ml extraction buffer containing 50 mM potassium phosphate buffer (pH = 6.5), 10 % polyvinyl pyrrolidone (PVP), 0.25 % Triton X-100 and 1 mM phenyl methyl sulfonyl fluoride (PMSF). The 1 ml reaction mixture contained 50 mM Tris–HCl buffer (pH = 6.5) and 0.4 mM linoleic acid. Addition of 10 µl crude petal extract to the reaction mixture initiated the reaction and absorbance was recorded at 234 nm for 5 min. The activity was expressed as µmol min^{-1} mg^{-1} protein.

Statistical Analysis

During the experiment, a completely randomized experimental design was adopted. Treatment means were compared via analysis of variance using SPSS (SPSS version 16; Chicago, USA). Standard error between the replicates was also calculated. The Duncan's multiple range test (DMRT) has been applied to the data to separate the means.

RESULTS

Vase life and floral diameter

A. majus cut spikes in holding solutions containing SA dramatically improved vase life as compared to flowers in distilled water (Figure 1). Spikes treated with varying concentrations of SA, viz., 1, 2 and 3 mM, were found to last 10.5, 14 and 12 days respectively. Spikes kept in distilled water lasted for 8 days, indicating that higher SA concentration was most effective in delaying flower senescence in the cut spikes of *A. majus* against the control (Figure 2a). In addition to vase life, the flower diameter also exhibited a maximum increase at 2 mM SA. However, a small decrease in flower diameter was seen as time progressed from day 2 to day 6 (Figure 2b).

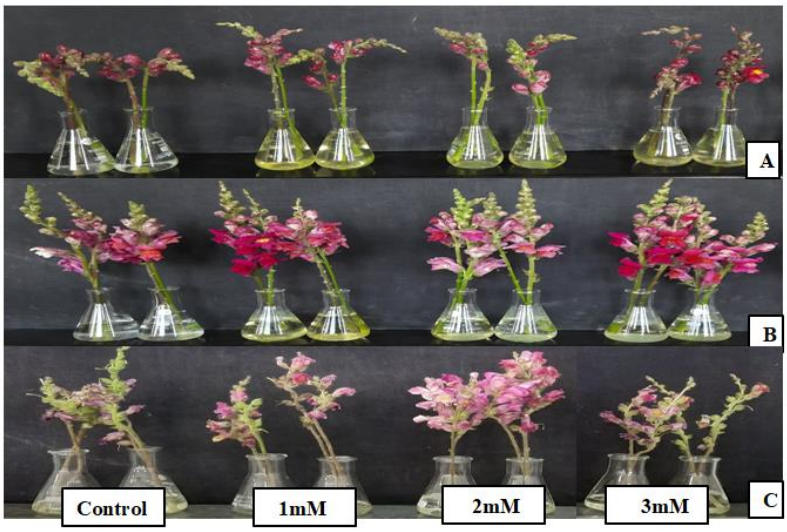

Fig-1: The effect of different SA concentrations on the postharvest performance and vase life of *A. majus* cut spikes on day 0 (A), day 3 (B), and day 13 (C)

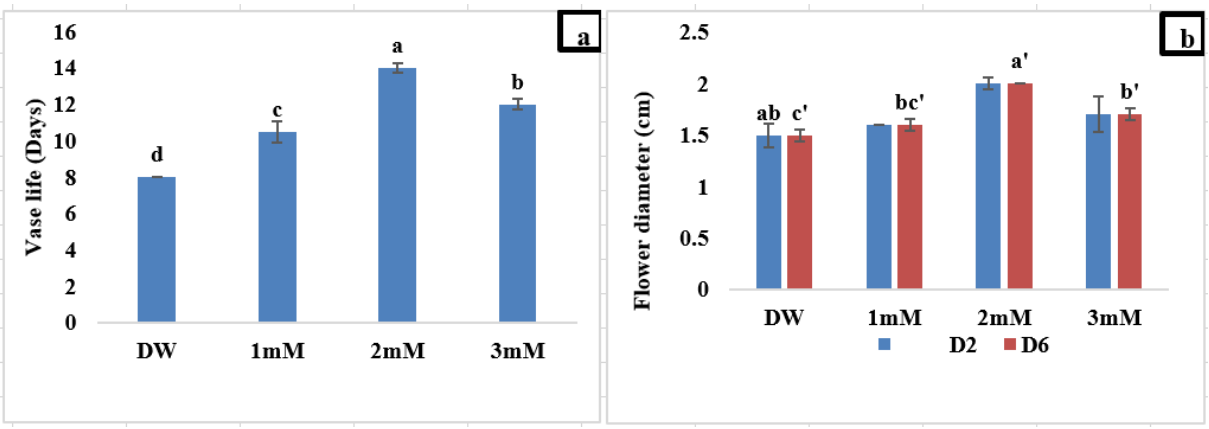

Fig-2: Variation in Vase life (a) Flower diameter (b) of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at p <0.05 by DMRT

Bacterial density and solution uptake

SA significantly improved solution absorption by reducing bacterial density in holding solutions containing *A. majus* cut spikes (Figures 3a and 3b). The least amount of bacterial growth was observed in vase solutions containing 2 mM SA, resulting in the greatest amount of solution uptake. The highest bacterial density with the lowest solution uptake was recorded in the control.

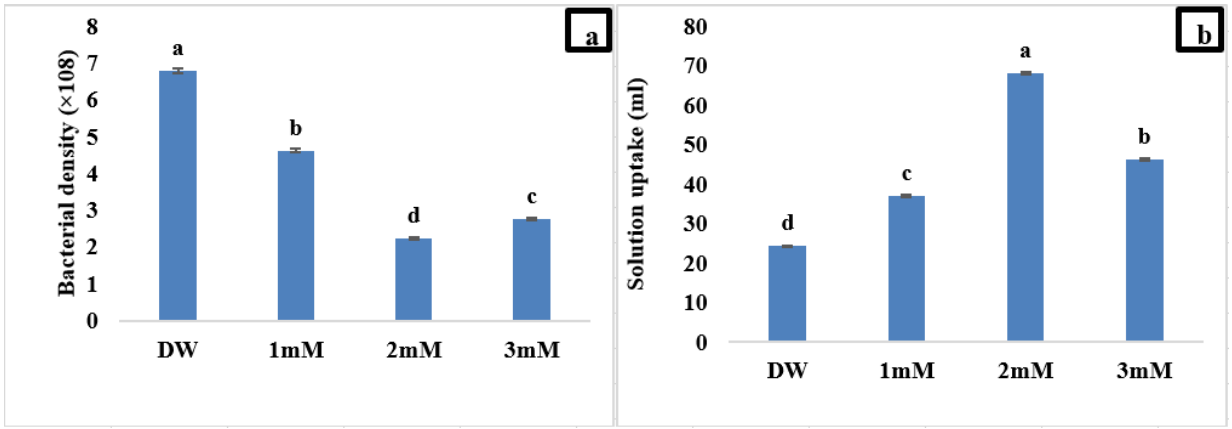

Fig-3: Variation in Bacterial density (a) Solution uptake (b) of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at p <0.05 by DMRT

Membrane stability index (MSI) and lipoxygenase activity

In the current study, SA at 2 mM was found to be very effective in maintaining membrane stability in petal tissues compared to control by reducing membrane lipid peroxidation via inhibition of LOX activity (Figures 4a and 4b). However, MSI values decreased while LOX activity increased as the flower spikes approached senescence.

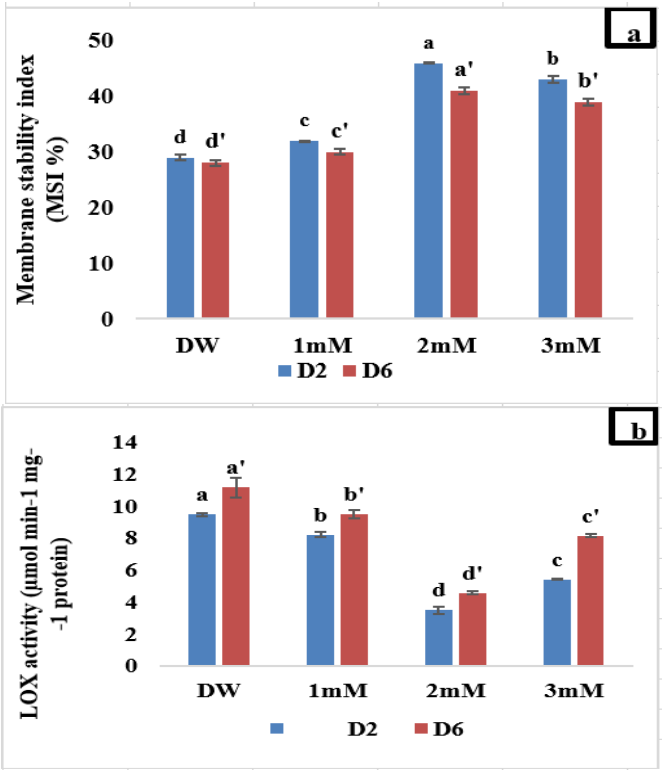

Fig-4: Variation in Membrane stability index (a) LOX activity (b) of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at p <0.05 by DMRT

Soluble proteins

Exogenous inclusion of SA resulted in a considerable increase in the total protein content of petal tissues in comparison to control. Maximum protein enrichment was recorded in the petal tissues treated with 2 mM SA (Figure 5).

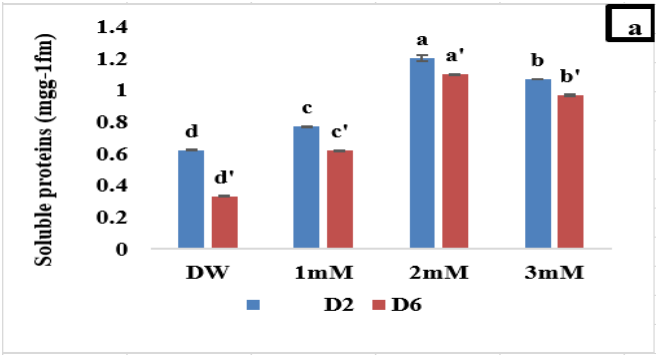

Fig-5: Variation in Soluble protein content of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at *p* < 0.05 by DMRT

Sugar fractions and total phenols

Flowers treated with SA at 1, 2 and 3 mM had significantly higher levels of sugar fractions and total phenols. 2 mM SA was found to be the most appropriate treatment in maintaining high levels of these parameters, followed by 3 mM SA. However, with the progression of time from day 2 to day 6, both

the sugar and phenolic concentrations decreased (Figures 6a, 6b, 6c and 6d).

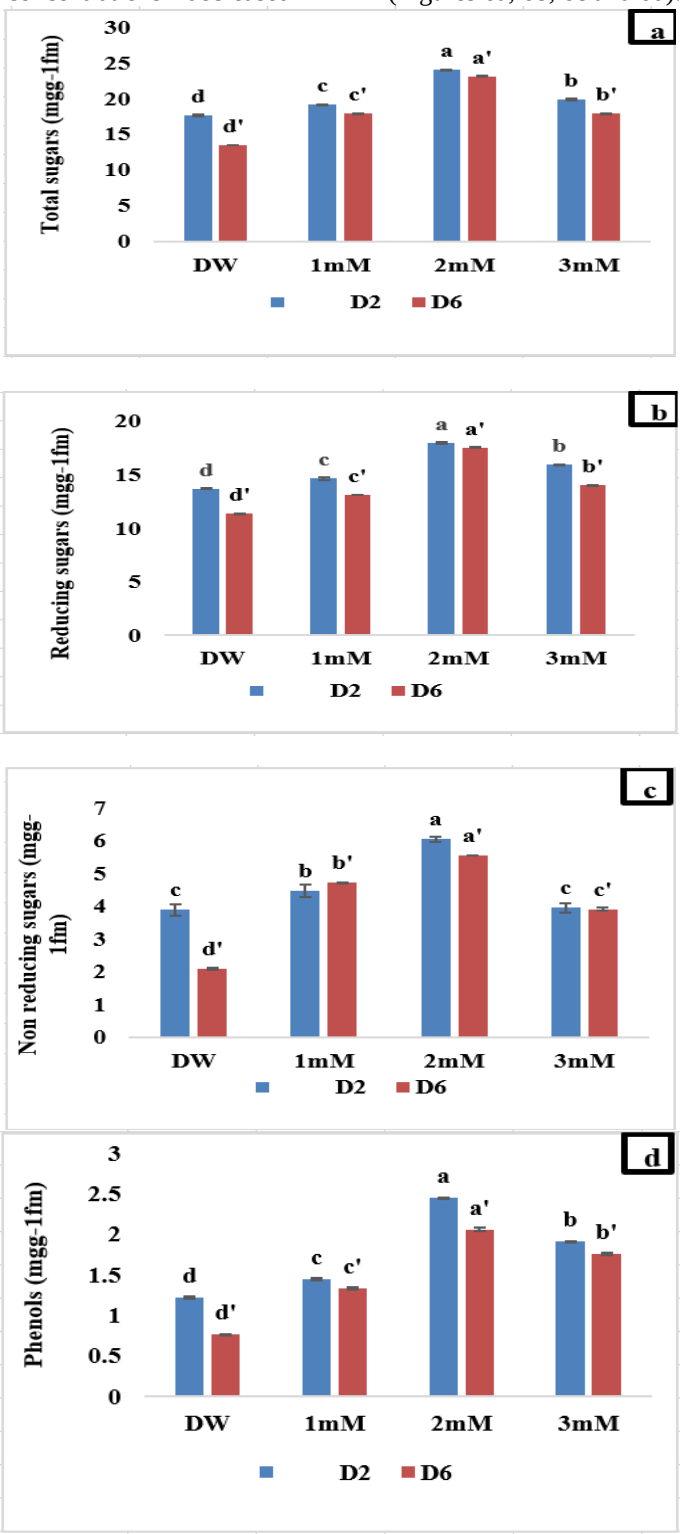

Fig-6: Variation in Total sugars (a) Reducing sugars (b) Non reducing sugars (c) Total phenols (d) of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at $p < 0.05$ by DMRT

Activities of Antioxidant Enzymes

The activities of antioxidant enzymes (SOD, CAT and APX) were substantially augmented in SA-treated petal tissues as compared to untreated tissues. The peak in antioxidant enzyme activity was registered in flower petals treated with 2 mM SA. However, as the flower spikes approached senescence, the enzyme activity decreased, as shown in Figures 7a, 7b and 7c.

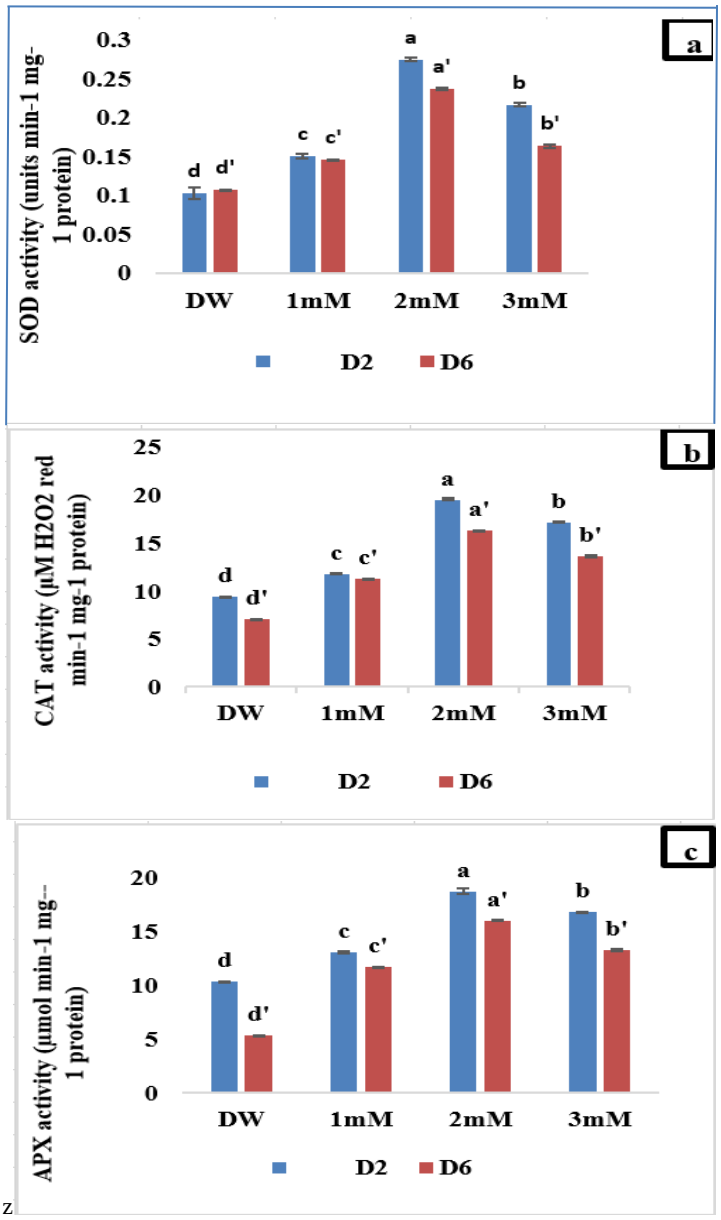

Fig-7: Variation in SOD (a) CAT (b) APX (c) activity of cut spikes of *A. majus* held in distilled water (control) and treated with different concentrations of SA. Each value is the mean of 3 replicates and error bars represent ± SE (standard error). Bars with different letters differ significantly at $p < 0.05$ by DMRT

DISCUSSION

The most important challenge for postharvest researchers is to delay the mechanisms controlling flower senescence to enable cut flowers with the longest vase life and best quality to reach distant markets (Ebrahimzadeh *et al.*, 2008; Ahmad and Tahir 2018). As a result, measures for maintaining quality, as well as methods for extending vase life, are of immense importance. Several chemical treatments like silver thiosulphate, cytokinins, sodium nitroprusside and boric acid have been explored in diverse floral systems such as *Dianthus*, *Calendula*, *Consolida* and *Digitalis* and have proven beneficial in increasing postharvest performance (Dar *et al.*, 2014; Lone *et al.*, 2021; Ul *et al.*, 2021, Farooq *et al.*, 2021). The present research was carried out to investigate the efficacy of SA on the vase life of *A. majus*, which has a lot of potential in the cut flower business. In our study, application of varying concentrations of SA to *A. majus* spikes revealed that 2 mM SA was found to be the most efficacious concentration in accentuating the vase life by 6 days as compared to control. Increased postharvest life due to SA application has also been reported in *Cut Roses*, *Gladiolus*, *Lisianthus* and *Gerbera* which might be related to SA's activity as ACC oxidase inhibitor, which is a direct precursor of ethylene (Alaey *et al.*, 2011; Hatamzadeh *et al.*, 2012; Davoud *et al.*, 2015; Bayat & Aminifard, 2017; Heidarnezhadian *et al.*, 2017). In comparison to the control, spikes from SA containing solutions had the maximum solution absorption and the lowest bacterial count. Higher solution uptake in SA-treated flowers may be due to its ability to reduce microbial proliferation, hence preventing vascular occlusion (Sevana *et al.*, 2020). The addition of SA to

the vase solution enhanced floral diameter compared to the control, which might be due to increased solution absorption in the xylem, which maintained the turgidity of the petal tissues, hence increasing floral diameter. Treatment of *Gladiolus* flowers with SA also demonstrated delayed dehydration, increased fresh weight and floral diameter (Saeed *et al.,* 2016). The postharvest application of SA prolonged the vase life of *A. majus* spikes by boosting the membrane stability index and lowering lipid peroxidation properties. The decreased values for lipid peroxidation in SA treated spikes are mainly due to lower lipoxygenase activity, which in turn helps to maintain sufficient phospholipids, proteins and thiols by limiting the protease leakage from vacuoles into the cytoplasm (Fukuchi-Mizutani *et al.*, 2000). Increased lipoxygenase activity has been identified as the fundamental change that takes place during the onset of senescence in various flowers like *Gladiolus, Dianthus, Gerbera* and *Cut Roses* (Saeed *et al.,* 2014; Kazimi *et al.,* 2017). SA treated spikes exhibited profound phenolic enrichment in the petal tissues as compared to untreated ones. The biosynthesis of phenolic compounds starts with the activity of phenylalanine ammonia-lyase (PAL). SA promotes PAL (phenyl ammonia lyase) activity, thereby resulting in the accumulation of phenolic compounds. This phenolic enrichment reinforces antioxidant defence mechanisms and protects flowers against oxidative stress via free radical scavenging (Rahmani *et al.,* 2015; Ahmad and Tahir, 2017; Shabanian *et al.,* 2019). An overall increase in soluble sugar content was found in SA treated cut spikes. SA inhibits the activity of ACC synthase and the formation of ethylene, it may be argued that this compound delays the decomposition of carbohydrates by lowering the respiration rate, extending the vase life by preserving a larger sugar content (Hemati *et al.,* 2021). The present investigation showed that the content of soluble proteins reduced in spikes held in DW, while the content increased with the supplementation of SA preservative into the vase solution. Protein degradation is a key component of membrane disintegration during petal senescence (Azeez *et al.,* 2007). This progressive degeneration of the membrane bilayer, accompanied by senescence might be caused by a decrease of membrane protein activity (Nisar *et al,* 2021). The findings of the current study are similar with the previous studies on *Gladiolus* and *Statice* cut flowers (Hatamzadeh *et al.,* 2012; Khandan-Mirkohi *et al.,* 2021). The addition of SA to cut *A. majus* flower spikes increased the activity of antioxidant enzymes (SOD, CAT and APX). Plants use antioxidative enzyme defence mechanisms to defend themselves from the negative effects of reactive oxygen species (ROS), which play a critical role in petal senescence (Chakrabarty *et al.,* 2007; Gill & Tuteja 2010). SA, whether administered exogenously or synthesised endogenously, has been shown to boost antioxidative enzyme activity, enhancing plant tolerance to stress conditions (Alaey *et al.,* 2011). SA treatments before or after harvest can increase the activity of the antioxidant enzymes catalase and peroxidase, extending the vaselife of cut flowers such as *Gladiolus* and *roses* (Alaey *et al.,* 2011; Kazemi *et al.,* 2018; Saeed *et al.,* 2016;). Furthermore, SA increases the antioxidant enzyme activity by accumulating abscisic acid for a short time, delaying cell component hydrolysis and lowering ROS generation (Hayat *et al.,* 2010). In general, it can be concluded that the use of SA in cut flowers has a beneficial influence on the activity of antioxidant enzymes SOD, CAT and APX, resulting in a delay in their ageing.

CONCLUSION

In conclusion, the study was an attempt to evaluate the potential effect of SA in extending the vase life of cut *A. majus* flower spikes. SA at optimal concentration was able to prolong vase life and delay flower senescence by maintaining membrane integrity, which was achieved by decreasing LOX activity and increasing the antioxidant enzyme activities of SOD, CAT and APX resulting in decreased H_2O_2 accumulation. Furthermore, the examination of several biochemical parameters was also helpful in establishing a complex interplay of various biomolecules like proteins, phenols and carbohydrates in the regulation of flower senescence.

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Cite This Article: Sumira Farooq, Aehsan ul Haq, Mohammad Lateef Lone, Shazia Parveen, Foziya Altaf, Inayatullah Tahir (2022). Salicylic Acid Enhances the Vase Life and Improves the Postharvest Attributes — A Case Study of Antirrhinum majus L. *East African Scholars J Agri Life Sci*, *5*(6), 87-96.