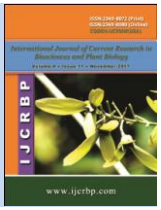
International Journal of Current Research in
Biosciences and Plant Biology
Volume 4 • Number 11 (November-2017) • ISSN: 2349-8080 (Online)
Journal homepage: www.ijcrbp.com



Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2017.411.010

Additive Effects of Sucrose with Kinetin and Salicylic Acid in Delaying Petal Senescence of Cut Flowers of *Matricaria parthenium* L.

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Abstract

Selecting 0.1 M sucrose solution and two concentrations (4µM and 40µM) of kinetin (Kn) and salicylic acid (SA), roles of the sugar and two plant growth regulators (PGRs) were assessed and compared individually as well as in combination in the regulation of petal senescence in *Matricaria parthenium* L. Among these chemical compounds (one metabolite and two concentrations each of two PGRs), 0.1M sucrose was the most effective individually as holding solution to minimize reduction in flower diameter and starch content. Maximum decline in α-amylase activity, lipoxygenase activity and MDA content was brought by sucrose. Higher concentration of both PGRs was also very effective. Further, in combined applications both Kn and SA could induce additive effects in presence of sucrose to make the flower more turgid, fresh with larger diameter even after 6-day. Kinetin at a concentration of 40µM along with sucrose (0.1M) was the best in keeping least reduction in the amount of starch while maintaining relatively lower values of lipoxygenase activity and MDA content in comparison to other holding solutions. This combined application was able to delay petal senescence in cut flower quite effectively.

Article Info

Accepted: 25 October 2017
Available Online: 06 November 2017

Keywords

Cut flowers
Kinetin
Matricaria parthenium
Petal senescence
Salicylic acid
Sucrose

Introduction

Flower senescence represents the last stage of floral development exhibiting shrinkage, wilting and / or abscission of whole flowers or flower parts and fading of blossoms (Stead and van Doorn, 1994; Jones and Mc Conchie, 1995; Tripathi and Tuteja, 2007). It is generally rapid and continuous similar to whole plant or leaf senescence (Voleti et al., 2000). A cut flower may be defined as flower or an inflorescence containing more than one floral unit in the opened or unopened state which is harvested and marketed for ornamental purpose. Cut flowers are highly perishable commodities and highly vulnerable to huge post-harvest losses. Once severed from the plant, they are deprived of their natural sources of water and nutrients and they wilt rapidly. The biochemical changes associated with petal senescence (in cut flowers too) include the expression of different hydrolases associated with degradation of macromolecules such as carbohydrates, proteins, lipids and nucleic acids. Controlled degradation, remobilization and reutilization of cell components can be witnessed during this process (Rubinstein, 2000; O'Donoghue et al., 2002). Short post-harvest life is one of the most important problems of cut flowers. Studies have been carried out extensively to find out how vase

life of cut flowers can be prolonged (Rubinstein, 2000). Various kinds of holding solutions like sugar, biocides, plant growth regulators (PGRs) and mineral ions, besides water have been used to study their effects on the longevity of cut flowers (Halevy and Mayak, 1981). Flower quality is maintained by sugars during post-harvest stages as they are the source for carbon and energy (Ho and Nichols, 1977; Kuiper et al., 1995; Monteiro et al., 2002; van Doorn, 2004). Translocated sugars in the petals increase osmotic concentration of cell sap to enable it to absorb more water and maintain turgidity (O'Donoghue et al., 2002). Sugars are also used as substrate for respiration and for suppressing ethylene synthesis. PGRs are also known to delay senescence of both intact and cut flowers. Some of these studies have also been carried out specially with cytokinins (Zubko et al., 2002; Asil and Karimi, 2010; Moneruzzaman et al., 2010), morphactins and salicylic acid (Khokhar and Mukherjee, 2010) and polyamines (Mahgoub et al., 2011; Sardoei et al., 2013). However, fewer studies have been carried out to find out how sugars (like sucrose) will behave when present along with a specific PGR at different concentrations to improve the vase life of cut flowers.

It was, therefore, thought to assess and compare the role of sucrose, kinetin (Kn) and salicylic acid (SA) individually as well as when sucrose was present in combination with either Kn or SA in the regulation of petal senescence. Plants of *Matricaria parthenium* which are dwarf (around 30 cm in length), compact and bushy with very fine cut leaves and very attractive yellow and ivory white flowers were selected to carry out this investigation. Here, flower heads are borne on the terminal ends of thin stems. Leaves and flowers, both have medicinal value. To use those flowers which are ornamental and medicinal also but not commonly used by most research workers prompted us to select it for petal senescence investigation.

Materials and methods

Flowers of *Matricaria parthenium* L. of family Asteraceae were harvested from plants growing in the experimental plots of University Botanical Garden, Kurukshetra. Uniform flowers of the same age and morphological appearance were collected for nine treatments. Flowers were cut under water to prevent cavitation and were immediately brought to the laboratory. Leaves were removed from flower twigs and were recut under distilled water to obtain an uniform length of 14 cm. These scapes were transferred to 100 ml Borosil conical flasks containing 30 ml holding solutions in each. Five conical flasks were maintained for each holding solution and three scapes were placed in each flask. Holding solutions were sucrose (0.1M), kinetin (Kn, Kn_1 = 4μM) and Kn_2 = 40μM), sucrose + Kn_1, sucrose + Kn_2, salicylic acid (SA, SA_1 = 4μM and SA_2 = 40μM), sucrose + SA_1 and sucrose + SA_2. Control sets having double distilled water (DDW) were also maintained, each flask containing 30 ml DDW. Change in flower diameter and its external appearance noted regularly at 2, 4, and 6-day.

Petal samples were collected in triplicates for various biochemical estimations like reducing and non-reducing sugars, starch, specific activities of α-amylase and lipoxygenase, and malondialdehyde (MDA) content.

Estimation of starch and sugars

Anthrone method of Hart and Fisher (1971) was followed for the determination of starch and sugars. One hundred milligram sample was extracted in 10 ml double distilled water (DDW) and centrifuged at 5000 rpm (2124 RCF) for 10 min in a Remi centrifuge. The residue left after the separation of aqueous extract was later used for the determination of starch. It was mixed with 10 ml of DDW and stirred. This mixture was cooled in ice water bath for 3-4 min and 13 ml of 52% perchloric acid was added followed by constant stirring for 5 min. The final volume was raised up to 100 ml with DDW. Three ml of diluted extract was taken in triplicate for determination of starch in terms of glucose by anthrone method. The absorbance was read at 630 nm in a UV-Vis Spectrophotometer. The amount of starch was calculated by multiplying the starch value in terms of glucose with 0.90.

For the estimation of sugars, after separating the residue, the aqueous extract was collected and final volume was raised to 50ml with DDW. The pH was also noted. Three millilitre of extract was taken in triplicate and kept in water bath at 100^0C for 5 min. Six millilitre of anthrone reagent was added slowly when test tubes were still placed in water bath at 100°C. Test tube contents were shaken carefully to get bluish-green colour without any turbidity. Test tubes were then cooled in ice water. Blank set was also prepared similarly taking 3 ml DDW and 6 ml anthrone reagent. Finally the absorbance was noted at 600 nm in Spectrophotometer. Quantified absorbance values will indicate reducing sugars.

The remaining extract was hydrolysed with 10 ml of 50% HCl at room temperature and kept as such for 24 hrs. Next day, the original p^H was reset (as it was previous day before hydrolysis) with 6 N NaOH. This was followed by raising the volume to 100 ml with DDW. Here again, reaction and blank sets were prepared as mentioned above in case of reducing sugars. However, the absorbance was recorded at 625 nm. This will reveal the amount of total sugars (already existing reducing sugars + non-reducing sugars hydrolyzed to reducing sugars). Deducting the amount of reducing sugars from the values of total sugars will show the values of non-reducing sugars. Sugars were quantified in terms of glucose.

α-Amylase activity

The specific activity of α-amylase was measured by the method of Bernfeld (1951). The extract was prepared from 100 mg of petals with 10 ml of DDW; centrifuged at 5000 rpm (2124 RCF) and the supernatant was used for determination of the enzyme activity. The reaction mixture contained 1% starch as substrate besides enzyme extract (1ml each) and was incubated for 3 min. at 20 ^{0}C. The enzyme reaction was interrupted by the addition of 2ml of 3, 5-dinitrosalicylic acid reagent. Then test tubes were placed in boiling water bath at 100 ^{0}C for 5 min. Thereafter, they were cooled in running tap water. To each test tube, 10 ml DDW was added. Absorbance was then noted at 540 nm. Blank was prepared in the same manner without petal extract; 1 ml DDW was taken instead of it. Specific activity was expressed as α-amylase activity per mg protein. From remaining enzyme extract, 0.2 ml was taken and raised to 1ml with DDW, to which 5 ml of Coomassie brilliant blue dye G-250 was added and mixed by inversion. The absorbance was recorded at 595 nm using Spectrophotometer and protein content was estimated in terms of BSA by the method of Bradford (1976).

Lipid peroxidation

It was measured in petals in terms of malondialdehyde (MDA) content following the method of Heath and Packer (1968). Two hundred milligram petal sample was homogenized in 3ml of 50 mM phosphate buffer (p^H 7.0). The homogenate was centrifuged at 8497 RCF (10000 rpm) for 20 min. in a Remi centrifuge (Remi Compufuge, CPR-24). To 0.5 ml aliquot of the supernatant, 2 ml of 5gL^{-1} TBA in 200g L^{-1} TCA was added. The mixture was heated at 90°C for 30 min. in a water bath and then quickly cooled in an ice water bath. After centrifugation at 10000 rpm for 10 min. the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption of each sample at 600 nm was also recorded and subtracted from the absorption recorded at 532 nm. The concentration of MDA, an end product of lipid peroxidation was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1}.

Lipoxygenase activity

The activity has been estimated according to the method described by Doderer et al. (1991).

(a) Preparation of substrate solution: Five millilitre DDW containing 50 µl Tween 20 was added to 35 µl linoleic acid (substrate) and p^H of this solution was around 8.5-8.6 and the final pH was adjusted to 9 by adding 0.2M NaOH drop by drop so that all the linoleic acid was dissolved. Again the pH was adjusted to 6.5 by adding 0.2M HCl. To this solution, 0.1M phosphate buffer of pH 6.5 was added and the final volume of the substrate solution was raised to 100 ml with the same buffer.

Preparation of buffer solution for enzyme extraction: 0.1M potassium dihydrogen orthophosphate and 01.M dispotassium orthophosphate solutions were mixed in the ratio of 16:84 respectively and the p^H was adjusted to 7.5 with p^H meter. In 100 ml of the buffer solution (pH 7.5), 0.186gm of EDTA (0.5µM) was added and this solution was used for enzyme extraction.

Preparation of enzyme extract: Petal sample (0.2gm) was homogenized in ice cold 0.1M phosphate buffer (pH 7.5) containing 0.5 µM EDTA using pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes, centrifuged at 4^{0}C in a Remi Centrifuge (Compufuge, CPR-24) for 15 min at 10000 rpm (8497 RCF). The supernatant was transferred to test tubes and referred as enzyme extract.

Enzyme assay: 2.95 ml of substrate solution was taken in a cuvette and considered as blank set. In reaction set, 0.05ml of the enzyme extract was added to the cuvette containing 2.95 ml of the substrate solution at zero time. Absorbance was noted at 234 nm for every minute up to 5 min. The activity was expressed as change in absorbance per minute per mg protein. The amount of protein in the enzyme extract was estimated by the method of Bradford (1976).

Flower diameter is based upon mean value of 15 flowers. Values of biochemical analysis have been expressed as means of three replicates and each replicate value is the mean of three aliquots ±standard error (S.E.). The experiment has been repeated to confirm the trend of results.

Results and discussion

Results have been summarized in Tables 1-5. The change in flower diameter of *M. parthenium* after putting cut flower scapes in different holding solutions can be seen in Table 1. All scapes are characterized by shrinkage and decrease in flower diameter at different interval during 6-day. Petals of untreated flowers had higher degree of shrinkage and loss of turgidity than those having sucrose or PGRs. A comparison of flower diameter (noted values as well as percent difference between different days) when scapes were subjected to individual treatment [sucrose, two concentrations each of kinetin (Kn) and salicylic acid (SA)] revealed that 0.1M sucrose solution was the best as holding solution as it caused least decline during 6-day period. It was very effective in comparison to distilled water (DDW) as control.

Kn and SA as PGRs are also effective to reduce flower shrinkage when compared to control but the degree of effectiveness was maximum in sucrose followed by Kn and SA. In both PGRs, the higher concentration (40µM) was more useful than the lower one (4µM). It was interesting to note that the combined applications of sucrose + Kn and sucrose + SA were able to minimize the flower shrinkage and dehydration more efficiently than when they were used individually. Combined application resulted more freshness in the flower appearance. However, sucrose + Kn treatment was better than sucrose + SA in maintaining flower diameter.

Table 1. *Matricaria parthenium* showing changes in flower diameter (in cm) of scapes maintained in holding solutions of sucrose (0.1M), kinetin (Kn, Kn_1 = 4.0 µM; Kn_2 = 40µM), salicylic acid (SA, SA_1 = 4.0 µM; SA_2 = 40 µM); salicylic acid (SA, SA1 = 4.0 µM; SA_2=40 µM); also a combination of sucrose+Kn_1/sucrose + Kn_2 and sucrose + SA_1 / sucrose + SA_2 (Initial diameter 7.20 cm).

Holding Solutions	Flower Diameter*			Percent Difference Between			
	2-day	4-day	6-day	0-2 day	2-4 day	4-6 day	0-6 day
Double distilled water (DDW)	5.8	5.0	3.9	-19.44	-13.79	-22.00	-45.83
Sucrose	6.8	6.1	5.4	-5.56	-10.29	-11.48	-25.00
Kn_1	6.3	5.8	5.1	-12.50	-7.94	-12.07	-29.17
Kn_2	6.6	6.0	5.2	-8.33	-9.09	-13.33	-27.78
Sucrose + Kn_1	7.0	6.3	5.7	-2.78	-10.00	-9.52	-20.83
Sucrose + Kn_2	7.1	6.6	6.0	-1.38	-7.04	-9.09	-16.67
SA_1	6.1	5.6	4.9	-15.28	-8.20	-12.50	-31.94
SA_2	6.6	5.8	5.0	-8.33	-12.12	13.79	-30.56
Sucrose + SA_1	6.6	6.0	5.4	-8.33	-9.09	10.00	25.00
Sucrose + SA_2	6.8	6.0	5.6	-5.56	-11.76	-6.67	-22.22

* Average value of 15 flowers.

While working on effects of sucrose and alcohols on post-harvest senescence of *Matricaria* flowers, gradual decline in the diameter in control flowers was noticed which could be minimized by 4% sucrose, 2% ethanol and a combination of 2% ethanol + 4% sucrose (Kaur and Mukherjee, 2012). Steady decline in flower diameter has also been recorded in *Chrysanthemum* cut flowers (Khokhar et al., 2013; Kaur and Mukherjee, 2016).

Cut flowers suffer from adequate supply of water and nutrients (both inorganic and organic) that is reflected in loss of turgidity, dehydration, appearance of wrinkles and shrinkage. The freshness and lustre are lost from the petals; which can be restored partially by sucrose and the PGRs as observed in this investigation.

Control flowers of *M.parthenium* exhibited considerably lower amount of reducing and non-reducing sugars; the former was 3 times higher than the latter when severed from plants (Table 2). Cut flowers were unique in having regular significant increment in both kinds of sugars at 2,4 and 6-day. Among the individual treatments, percent increase in sugars was maximum in control flowers and minimum in those maintained in 01. M sucrose solution. Kinetin and salicylic acid were also responsible in bringing down the amount of sugars in petals but their effectiveness was slightly lower then that of sucrose. When sucrose was combined either with Kn or SA the amount of petal sugar was further decreased.

It appears that Kn and SA in combinations with sucrose bring an additive effect; which is greater in case of sucrose + Kn. Sucrose, Kn and SA have effective control over the increment in petal sugars.

Table 2. *M. parthenium* showing changes in reducing and non-reducing sugars of flower petals (mg / 100 mg dry weight ± S.E) of scapes maintained in holding solutions of double distilled water (DDW, control), sucrose (01.M), kinetin (Kn, Kn_1 = 4 μM; Kn_2 = 40μM) and salicylic acid (SA, SA_1 = 4μM; SA_2 = 40μM) during 6-day.

Days	Holding Solutions	Reducing sugars	Non-reducing sugar
0-day	Initial value	3.23 ± 0.30	1.05 ± 0.25
2-day	Control (DDW)	13.61±0.12 (+321.36)	3.85 ± 0.08 (+(266.67)
	Sucrose	7.50 ± 0.13 (+132.20)	1.93 + 0.03 (+83.81)
	Kn_1	8.24 ± 0.35 (+155.11)	2.05 ± 0.21 (+95.24)
	Kn_2	7.75 ± 0.17 (+139.93)	1.90 ± 0.15 (+80.95)
	Sucrose + Kn_1	6.90 ± 0.41 (+113.62)	1.70 ± 0.31 (+61.90)
	Sucrose + Kn_2	6.14 ± 0.22 (+90.09)	1.50 ± 0.12 (+42.86)
	SA_1	10.90 ± 0.12 (+237.46)	2.10 ± 0.13 (+100.00)
	SA_2	10.10 ± 0.24 (+212.69)	2.00 ± 014 (+90.48)
	Sucrose + SA_1	10.00 ± 0.11 (+209.59)	1.95 ± 0.22 (+85.71)
	Sucrose + SA_2	8.15 ± 0.17 (+152.32)	1.92 ± 0.32 (+82.86)
4-day	DDW	18.05 ± 0.35 (+458.82)	4.92 ± 0.14 (+368.57)
	Sucrose	10.40 ± 0.11 (+221.98)	2.25 ± 0.09 (+114.29)
	Kn_1	11.81 ± 0.05 (+265.63)	3.00 ± 0.17 (+185.71)
	Kn_2	11.00 ± 0.07 (+240.56)	2.40 ± 0.20 (+128.57)
	Sucrose + Kn_1	9.40 ± 0.22 (+191.02)	1.95 ± 0.05 (+85.71)
	Sucrose + Kn_2	8.55 ± 0.18 (+164.70)	1.75 ± 0.10 (+66.67)
	SA_1	13.45 ± 0.15 (+316.41)	3.50 ± 0.20 (+233.33)
	SA_2	12.22 ± 0.04 (+278.33)	3.35 ± 0.05 (+219.05)
	Sucrose + SA_1	11.35 ± 0.17 (+251.39)	2.52 ± 0.09) (+140.00)
	Sucrose + SA_2	10.20 ± 0.10 (+215.79)	2.15 ± 0.07 (+104.76)
6-day	DDW	26.14± 0.25 (+709.29)	13.50 ± 0.16 (+1185.71)
	Sucrose	12.16 ± 0.12 (+276.47)	3.00 ± 0.05 (+185.71)
	Kn_1	13.60 ± 0.10 (+321.05)	3.42 ± 0.11 (+225.71)
	Kn_2	12.76 ± 0.09 (+295.05)	3.10 ± 0.07 (+195.24)
	Sucrose + Kn_1	11.40 ± 0.12 (+252.94)	2.47 ± 0.12 (+135.24)
	Sucrose + Kn_2	10.35 ± 0.05 (+220.43)	1.91 ± 0.10 (+81.90)
	SA_1	14.94 ± 0.12 (+362.54)	4.40 ± 0.09 (+319.05)
	SA_2	14.26 ± 0.10 (+341.49)	4.25 ± 0.10 (+304.76)
	Sucrose + SA_1	13.05 ± 0.17 (+304.02)	3.23 ± 0.04 (+207.62)
	Sucrose + SA_2	11.50 ± 0.11 (+256.04)	2.81 ± 0.05 (+167.62)

Data in parenthesis indicate percent change from initial values.

The change in the quantity of starch and specific activity of α-amylase clearly indicate rapid depletion of starch with steady increase in α-amylase activity in control *M. parthenium* flowers (Table 3). Treatments with sucrose, Kn and SA, however, put a partial break over starch degradation. Lesser starch degradation was due to comparatively lower α-amylase activity as revealed in this study. Combined applications of sucrose + Kn and sucrose + SA were again showing comparatively higher level of starch and lower α-amylase activity as compared with control and other treatments. Plant cells require more sugars under stressed conditions to fulfil energy and carbon needs (Koizuka et al., 1995; Yakimova, 1997). Cut flowers experience stressed situation leading to senescence. Invertase and α-amylase are the important enzymes that participate in this degradation process. α-Amylase is capable of releasing glucans from starch granules (Steup et al., 1983; Sun et al., 1995; Scheidig et al., 2002). Some of the earlier workers suggested important role of α-amylase in the petal opening mechanism (Hammond, 1982; Tirosh and Mayak, 1988). While working on the sugar status of carnation flowers, reducing sugars rather than sucrose were found to be the main constituents of the sugar pool

at maturity (Nichols, 1973; Ho and Nichols, 1975). In cut flowers of carnation, they have noticed a decline in both types of sugars at senescent phase. However, Trivellini et al. (2011) reported a decline in reducing sugars and increase in sucrose in petals of *Hibiscus rosa sinensis.* Earlier studies carried out in our laboratory on *M.parthenium* with various alcohols also showed rapid degradation of starch with an increment in α-amylase activity and the level of sugars during senescence of petals in untreated cut flowers (Kaur and Mukherjee, 2012). Ethanol application at a concentration of 2% could appreciably reduce starch breakdown and lower the activity of α-amylase. Senescing petals have shown an accumulation of sugars in *Calendula officinalis* (Khokhar and Mukherjee, 2010; Kaur and Mukherjee, 2013), *Arctotis grandis* (Khokhar and Mukherjee, 2010) and *Chrysanthemum dendranthema grandiflorum* var. Chandrima (Kaur and Mukherjee, 2016).

Table 3. *M. parthenium* showing amount of starch (mg/100 mg dry weight ± S.E.) and specific activity of α-amylase (unit mg^{-1} protein ± S.E.) in cut flowers with holding solutions of double distilled water (DDW, control), sucrose (0.1M), kinetin (Kn, Kn_1 = 4μM; Kn_2 = 40μM) and salicylic acid (SA, SA_1 = 4μM; SA_2 = 40μM) during 6-day.

Days	Holding Solutions	Starch	Specific Activity of α-Amylase
0-day	Initial value	17.25 ± 1.04	2.51 ± 0.15
2-day	Control (DDW)	6.60±0.45(-61.74)	8.60 ± 0.21 (+241.27)
	Sucrose	10.51 ± 0.30 (-39.07)	4.05 + 0.09 (+60.71)
	Kn_1	9.50 ± 0.25 (-44.93)	4.18 ± 0.32 (+65.87)
	Kn_2	10.16 ± 0.51 (-41.10)	4.10 ± 0.07 (+62.70)
	Sucrose + Kn_1	11.41 ± 0.27 (-33.86)	3.10 ± 0.11 (+23.02)
	Sucrose + Kn_2	13.65 ± 1.05 (-20.87)	2.68 ± 0.06 (+6.35)
	SA_1	8.32 ± 0.40 (-51.77)	5.10 ± 0.09 (+102.38)
	SA_2	8.70 ± 0.22 (-49.57)	4.50 ± 0.40 (+78.57)
	Sucrose + SA_1	10.72 ± 0.42 (-37.86)	3.70 ± 0.21 (+46.83)
	Sucrose + SA_2	11.05 ± 0.57 (-35.94)	3.31 ± 0.18 (+31.35)
4-day	Control (DDW)	4.75 ± 0.12 (-72.46)	14.45 ± 0.81 (+473.41)
	Sucrose	9.10 ± 0.15 (-47.25)	6.72 ± 0.55 (+166.67)
	Kn_1	7.90 ± 0.21 (-54.20)	7.10 ± 0.32 (+181.75)
	Kn_2	8.70 ± 0.32 (-49.57)	6.90 ± 0.24 (+173.81)
	Sucrose + Kn_1	10.42 ± 0.41 (-39.59)	5.10 ± 0.52 (+102.38)
	Sucrose + Kn_2	10.75 ± 0.17 (-37.68)	4.70 ± 0.16 (+86.51)
	SA_1	6.50 ± 0.28 (-62.32)	9.15 ± 0.26 (+263.10)
	SA_2	7.57 ± 0.50 (-56.12)	7.10 ± 0.35 (+181.75)
	Sucrose + SA_1	8.35 ± 0.75 (-51.59)	6.90 ± 0.22) (+173.81)
	Sucrose + SA_2	9.60 ± 0.92 (-44.35)	5.82 ± 0.17 (+130.95)
6-day	Control (DDW)	2.70 ± 1.05 (-84.35)	21.45 ± 0.35 (+751.19)
	Sucrose	5.90 ± 0.36 (-65.80)	9.85 ± 0.42 (+290.87)
	Kn_1	5.05 ± 0.24 (-70.72)	12.35 ± 0.16 (+390.08)
	Kn_2	5.70 ± 0.40 (-66.96)	10.04 ± 0.31 (+298.41)
	Sucrose + Kn_1	6.52 ± 0.35 (-62.20)	8.48 ± 0.17 (+236.51)
	Sucrose + Kn_2	8.65 ± 1.10 (-49.86)	6.36 ± 0.26 (+151.98)
	SA_1	3.94 ± 0.28 (-77.16)	13.32 ± 0.34 (+428.57)
	SA_2	4.70 ± 0.16 (-72.75)	13.00 ± 0.19 (+415.87)
	Sucrose + SA_1	4.95 ± 0.25 (-71.30)	10.20 ± 0.42 (+304.76)
	Sucrose + SA_2	6.05 ± 0.18 (-64.93)	9.05 ± 0.17 (+259.13)

Data in parenthesis indicate percent change from initial values.

MDA content in untreated cut flowers of *Matricaria* increased more than 500 percent during 6-day while sucrose, Kn and SA treatments reduced this increment considerably when applied individually (Table 4). They were greater effective when sucrose was combined with either Kn or SA. The best effective treatment was sucrose + 40μM Kn concentration in minimizing the membrane leakage of petals. Lipid peroxidation (expressed as MDA level) is considered to be an important mechanism of leaf and petal senescence

(Khokhar et al., 2013). Loss of membrance integrity is the final and irreversible phase of senescence and is closely linked with membrane lipid peroxidation (Paulin et al., 1986). Various studies have indicated an increment in MDA content during flower senescence as in carnation (Sylvestre et al., 1989), *Chrysanthemum morifolium* (Bartoli et al., 1995), tulips (Jones and Mc Conchie, 1995), roses (Fukuchi-Mizutani et al., 2000) and gladiolus (Ezhilmathi et al., 2007). Studies on cut flowers in our laboratory also revealed a gradual increase in MDA content with the advancement in petal senescence (Kaur and Mukherjee, 2012; Khokhar et al., 2013; Kaur and Mukherjee, 2016). Lipid peroxidation damages membranes, membranous proteins and produces a variety of aldehydes and ketones (Valentine et al., 1998; Wilhelmova et al., 2006). MDA is a common end product and a sensitive diagnostic index of oxidative injury (Janero, 1990).

Table 4. *M. parthenium* showing MDA content (mM g^{-1} fresh weight ± S.E.) in petals of cut flowers with sucrose (0.1M), kinetin (Kn_1, 4.0 µM; Kn_2, 40µM) and salicylic acid (SA_1, 4.0 µM; SA_2, 40 µM) as holding solutions during 6-day [Initial value 0.00230 ± 0.00005].

Holding Solutions	2-day	4-day	6-day
Double distilled water (DDW)	0.00635 ± 0.00009 (+176.09)	0.01050 ± 0.00015 (+356.52)	0.01400 ± 0.00011 (+508.70)
Sucrose	0.00455 ± 0.00005 (+97.83)	0.00586 ± 0.00009 (+154.78)	0.00880 ± 0.00008 (+282.61)
Kn_1	0.00490 ± 0.0006 (+113.04)	0.00660 ± 0.00007 (+186.96)	0.01100 ± 0.00007 (+378.26)
Kn_2	0.00460 ± 0.00007 (+100.00)	0.00630 ± 0.00008 (+173.91)	0.00920 ± 0.00006 (+300.00)
Sucrose + Kn_1	0.00395 ± 0.00005 (+71.74)	0.00465 ± 0.00007 (+102.17)	0.00611 ± 0.00004 (+165.65)
Sucrose + Kn_2	0.00325 ± 0.00010 (+41.30)	0.00410 ± 0.00005 (+78.26)	0.00526 ± 0.00005 (+128.70)
SA_1	0.00570 ± 0.00006 (+147.83)	0.00755 ± 0.00008 (+228.26)	0.01200 ± 0.00011 (+421.74)
SA_2	0.00540 ± 0.00006 (+134.78)	0.00710 ± 0.00007 (+208.70)	0.01070 ± 0.00007 (+365.22)
Sucrose + SA_1	0.00431 ± 0.00007 (+87.39)	0.00530 ± 0.00005 (+130.43)	0.00760 ± 0.00005 (+230.43)
Sucrose + SA_2	0.00425 ± 0.00006 (+84.72)	0.00504 ± 0.00007 (+119.13)	0.00691 ± 0.00006 (+200.43)

Data in parenthesis indicate percent change from the initial value.

Table 5. *M. parthenium* showing lipoxygenase activity (units min^{-1} mg^{-1} protein ± S.E.) in petals of cut flowers with sucrose (0.1M), kinetin (Kn, Kn_1 = 4.0 µM; Kn_2 = 40 µM) salicylic acid (SA, SA_1 = 4.0 µM; SA_2 = 40µM), also a combination of sucrose + Kn_1 / sucrose + Kn_2 and sucrose + SA_1 / Sucrose + SA_2 [Initial value 2.12 ± 0.041].

Holding Solutions	2-day	4-day	6-day
Double distilled water (DDW)	5.31 ± 0.052 (+150.47)	8.50 ± 0.056 (+300.94)	13.23 ± 0.061 (+524.06)
Sucrose	3.92 ± 0.025 (+84.91)	6.62 ± 0.044 (+212.26)	9.95 ± 0.056 (+369.34)
Kn_1	4.24 ± 0.035 (+100.00)	6.95 ± 0.048 (+227.83)	10.24 ± 0.021 (+383.02)
Kn_2	4.05 ± 0.047 (+91.04)	6.81 ± 0.051 (+221.23)	10.05 ± 0.027 (+374.06)
Sucrose + Kn_1	3.65 ± 0.049 (+72.17)	6.33 ± 0.028 (+198.58)	9.61 ± 0.035 (+353.30)
Sucrose + Kn_2	3.51 ± 0.037 (+65.57)	6.21 ± 0.031 (+192.92)	9.45 ± 0.045 (+345.75)
SA_1	4.62 ± 0.031 (+117.92)	7.35 ± 0.032 (+246.70)	10.54 ± 0.044 (+397.17)
SA_2	4.51 ± 0.042 (+112.74)	7.20 ± 0.027 (+239.62)	10.43 ± 0.051 (+391.98)
Sucrose + SA_1	3.85 ± 0.037 (+81.60)	6.92 ± 0.027 (+226.42)	10.36± 0.029 (+388.68)
Sucrose + SA_2	3.75 ± 0.035 (+76.89)	6.75 ± 0.021 (+218.40)	10.30± 0.018 (+385.85)

Data in parenthesis indicate percent change from the initial value.

Lipoxygenase (LOX) activity of cut flowers has been shown in Table 5. A regular increment in its activity was noticed at 2, 4 and 6-day. However, the activity was decreased when sucrose, Kn and SA were used as holding solutions. Again, sucrose was maximum effective among all applications including Kn and SA. Combined application of sucrose + Kn_2 (higher concentration of kinetin) was the best to control the activity of this enzyme. Higher LOX activity is said to be a common feature of senescent plant tissue as noticed in petals of various flowers including carnation (Rouet-Mayer et al., 1992), tulips (Jones and Mc Conchie, 1995), fully open *Gladiolus* flowers (Ezhilmathi et al., 2007), *Chrysanthemum* cut flowers (Khokhar et al., 2013). Lipoxygenase causes peroxidative damage in membrane lipids affecting membrane fluidity (Khokhar

et al., 2013). Various active oxygen species (AOS) including superoxide radicals may be generated by LOX (Roy et al., 1994). The decline in phospholipid level enhances the permeability of the plasma membrane and makes the cell leaky (Simon, 1974).

Conclusion

Petal senescence in cut flowers of *M.parthenium* was characterized by dehydration, loss of turgidity, decline in starch values and rapid increase in total sugars (reducing and non-reducing sugars), MDA content and lipoxygenase activity. Individually, sucrose (0.1M) and in combined applications, sucrose (0.1M) + Kn (40μM) were the best holding solutions that delayed petal senescence.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The financial assistance to D. Mukherjee from Indian Science Congress Association, Kolkata for providing Asutosh Mookerjee Fellowship is gratefully acknowledged.

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How to cite this article:
Mukherjee, S., Mukherjee, D., 2017. Additive effects of sucrose with kinetin and salicylic acid in delaying petal senescence of cut flowers of *Matricaria parthenium* L. Int. J. Curr. Res. Biosci. Plant Biol. 4(11), 86-95. **doi:** https://doi.org/10.20546/ijcrbp.2017.411.010