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ORIGINAL ARTICLE

Additive Effect of 8-Hydroxyquinoline and 5-Sulfosalicylic Acid with Sucrose in regulating Petal Senescence of Cut Scapes of *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug.

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ABSTRACT
To evaluate individual role of sucrose (a metabolite), 5-sulfosalicylic acid (5-SSA, a plant growth regulator) and 8-hydroxyquinoline (8-HQ, a biocide) and also combined role of 5-SSA + sucrose and 8-HQ + sucrose, in the regulation of petal senescence of cut scapes, experiments were carried out with Aster novae belgii L., Matricaria parthenium L. and Gaillardia pulchella Foug. The major aim was to find out how do these chemicals affect the breakdown of starch, modify lipid peroxidation and lipoxygenase activity; and regulate SOD activity which are some of the important processes associated with petal senescence. Selected concentrations of sucrose, 5-SSA and 8-HQ were 0.1M, 200 ppm and 200 ppm respectively. Petals of untreated and treated scapes showed a gradual decrease in flower diameter with noticeable shrinkage and loss in turgidity. But applied substances as vase solutions were able to reduce this loss partially. This was evident not only in flower diameter but also in starch values and superoxide dismutase (SOD) activity. Treatments were also responsible for exerting a check over lipid peroxidation and lipoxygenase activity. Among individual applications, sucrose was the best followed by 8-HQ and 5-SSA; in combined form 8-HQ + sucrose was more effective than 5-SSA + sucrose in arresting starch degradation and membrane disintegration. 8-HQ + sucrose was also the best combination in minimizing loss of SOD activity in petals. Although three selected plants exhibited usefulness of the applied chemicals in controlling senescence but some variations noticed in per cent effectiveness between specific stages as discussed in the results.
Key words : Cut scapes, Lipoxygenase activity, MDA content, Petal senescence, Starch breakdown, SOD activity.

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INTRODUCTION

Plant senescence has been described as a developmental process witnessed in all plants and plant parts. Various morphological, physiological and biochemical alterations have been noticed during this process which lead to deterioration at tissue, cell and organelle level. Functional life of plants becomes shortened as a result of such changes [1]. In case of flowers and flower inflorescence, senescence starts after their complete development when sign of wilting and abscission are visible. Cut flowers and cut scapes (flowering twig or branch from which leaves have been removed) experience very quick loss of turgidity and freshness as compared to attached and uncut flowers as the supply of water and nutrients becomes a limiting factor in the former. Senescence as a whole including petal senescence is characterized by rapid increase in lipid peroxidation, membrane leakage and degradation of cell wall components [2-5].

Vase life of cut flowers and scapes can be extended in presence of sucrose, an important sugar and metabolite [6-7]. It is the source of carbon and energy. It can maintain the turgidity in cut flowers by increasing the osmotic concentration of petal cell sap [8]. External supply of sugars suppresses ethylene synthesis so that petal senescence is delayed [9].

Plant growth regulators (PGRs) like auxins, gibberellins, cytokinins, abscisic acid (ABA), ethylene, morphactins (MOR), salicylic acid (SA), polyamines (PAs), etc. also play major role in the regulation of petal senescence [1,10]. Senescence studies with SA are fewer in comparison to other PGRs like cytokinins, ABA and ethylene. SA is not only important for enhancing flowering [11] but also known to delay flower senescence [10]. 5-Sulfosalicylic acid (5-SSA) is derived from SA and the former plays important role in prolonging vase life of cut flowers like *Gladiolus* [12]. This treatment was responsible for significant increase in the number of opened florets and solution uptake. It also increased membrane stability, soluble protein quantity and activities of superoxide dismutase (SOD) and catalase in treated plants as compared to control. Moreover, lipid peroxidation and lipoxygenase activity were lowered by 5-SSA.

Biocides like 8-hydroxyquinoline (8-HQ), 8-hydroxyquinoline citrate (8-HQC) and 8-hydroxyquinoline sulphate (8-HQS) are often used as holding solution to prevent invasion and growth of microorganisms. Such a situation is very common when experiments are conducted with cut flowers. Microbes prevent the flow of holding solutions (e.g. water, nutrient solution, PGR, etc.) at the cut end of flower twig by blocking vessels as extra cellular polysaccharides are synthesized. The release of pectinases and toxic compounds are also responsible for ethylene synthesis; which in turn accelerate flower senescence [13]. Use of biocide will not allow this to happen as noticed by van Doorn and Perik [14] while working with cut roses. Biocides will maintain low pH of the vase solution that will prevent microbial growth. Rabiza-Swider *et al* [15] have noticed that the longevity of cut lily flowers could be extended by combined application of sucrose + 5 - SSA + 8 - HQ.

In view of the important role played by a metabolite (e.g. sucrose), PGR like 5-SSA and biocide such as 8-HQ, an investigation was planned to assess not only the individual role of these biochemical substances but also when 5-SSA and 8-HQ were separately combined with sucrose in the regulation of petal senescence of cut scapes. There is a direct association between reactive oxygen species (ROS) and senescence. ROS include both free radicals and hydrogen peroxide (H_2O_2). They are produced during normal metabolism in many organelles. ROS are probably formed when lipoxygenase peroxidizes double bonds in membrane c^{18} - c^{22} fatty acids. Polyunsaturated fatty acid oxidation also results in the formation of malondialdehyde (MDA) and other thiobarbituric acid (TBA) - reactive compounds [4]. A number of free radical - scavenging enzymes like superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX) and glutathione reductase (GR) are available to counteract lipid peroxidation and lipoxygenase activity. The aim of this investigation was, therefore, to assess the role of selected metabolite, PGR and biocide to know how do they modify starch depletion, lipid peroxidation, lipoxygenase activity and SOD activity in petals of cut scapes of three selected plants viz. *Aster novae belgii, Matricaria parthenium* and *Gaillardia pulchella.*

MATERIAL AND METHODS

Plant material and holding solutions

Aster novae belgii L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug. were the selected plants, seeds of which sown in uniformly prepared experimental plots to raise their saplings. Each one of these plots measuring 3 x 1 m^2 were maintained within wirenet cage of the garden of Botany Department, Kurukshetra University, Kurukshetra. During flowering, cut flowers were harvested in early morning. Flower twigs were cut in a bucket filled with water to prevent cavitation and brought to the laboratory. Leaves were removed from twigs to obtain cut scapes. Length of these scapes was 14 cm. Meanwhile 100 ml conical flasks of Borosil glass were arranged to have different vase solutions. Holding solutions prepared were : 5-sulfosalicylic acid (5-SSA, 200 ppm), 8-hydroxyquinoline (8-HQ, 200 ppm), sucrose (0.1M), 5-SSA (200 ppm) + sucrose (0.1M), 8-HQ (200 ppm) + sucrose (0.1M) and double distilled water (DDW). Ten conical flasks were maintained for each holding solution. The volume of vase solution was 30 ml and three scapes were introduced in each conical flask. All flasks having vase solutions and scapes were placed below the fluorescent tube with day and night light intensity of 2.24 μmol m^{-2} s^{-1} and 1.13 μmol m^{-2} s^{-1} respectively. The room temperature for cut scapes experiment with *A. novae belgii* L. and *M.* parthenium L. was 25 ± 2 ^{0}C while that of *G. pulchella* Foug. was 37 ± 2 ^{0}C.

Collection of petal samples

Petal samples were collected at 0, 2, 4 and 6-day stages from cut scapes placed in various holding solutions. For collecting dry weight data, triplicate samples were placed in the oven at 80 ^{0}C for 2 days. Various observations such as visible effect, longevity, volume of holding solutions absorbed, flower diameter, etc. were based upon mean of 10 replicates and also recorded at specific time.

Estimation of starch and sugars

Starch, reducing sugars and non-reducing sugars were estimated with the help of anthrone - sulphuric acid reagent as described by Hart and Fisher [16]. It has been dealt in detail elsewhere [7]. The absorbance was recorded at 630 nm in a UV - Vis spectrophotometer. For reducing and non-reducing sugars, absorbance recorded at 600 and 625 nm respectively. The amount of non-reducing sugars was obtained by subtracting the value of reducing sugars from that of total sugars. Starch and sugars were quantified in terms of glucose using standard curves.

α-Amylase activity

The specific activity of α-amylase was measured by the method of Bernfeld [17]. Petal extract was prepared by taking 100 mg of the sample and 10 ml of double distilled water (DDW). The extract was centrifuged at 5000 rpm (2124 RCF) and supernatant was used to determine enzyme activity. The reaction mixture included 1 ml of 1% starch and 1 ml of enzyme. It was incubated at 20 ^{0}C for 3 min. The activity was interrupted by the addition of 2 ml of 3, 5 - dinitrosalicylic acid reagent. Test tubes were then placed in water bath at 100 ^{0}C for 5 min followed by cooling in running tap water or ice cold water. Ten millilitre of DDW was added to each test tube. Absorbance was recorded at 540 nm. Blank set was prepared in the same manner except that 1 ml of DDW was taken in place of l ml of petal extract, i.e. enzyme. Specific activity was expressed as α-amylase activity per mg protein. From remaining enzyme extract, 0.2 ml was taken and raised to 1 ml with DDW, to which 5 ml of Coomassie brilliant blue dye G-250 was added and mixed properly. The absorbance was noted at 595 nm using a spectrophotometer and protein content was estimated in terms of BSA by the method of Bradford [18].

Superoxide dismutase activity

The methodology of Giannopolitis and Ries [19] was followed to find out superoxide dismutase (SOD) activity of fresh petals of selected plants. Fifty milligram of fresh petals homogenized in 2 ml of 0.1 M EDTA - phosphate buffer of pH 7.8. It was centrifuged at 15000 g and resultant supernatant was used as crude extract. The reaction mixture was prepared by adding 0.1 ml of crude extract followed by 0.9 ml of DDW, 0.5 ml of 300 mM Na_2CO_3 (pH 10.2), 0.5 ml of 378 μM p-nitroblue tetrazolium chloride (NBT), 0.5 ml of 78 mM L-methionine and 0.5 ml of 7.8 μM riboflavin. The final reaction mixture was 3 ml. The reaction was carried out in similar test tubes at 25 ^{0}C for 15 min in 100 μmol photon per m^2 per s PFD from fluorescent lamp. The initial rate of reaction as measured by the difference in increase in absorbance at 560 nm in the presence and absence of extract was proportional to the amount of enzyme. The unit of SOD activity was obtained as that amount of enzyme which under the experimental conditions caused a 50% inhibition of the reaction observed in the absence of enzyme. The SOD activity was measured by the following formula :

$$\text{Units / mg protein} = \frac{d \times 1000}{H \times P\ (\mu g/ml)}$$

where,

d = Blank OD (Sample in light without extract) - OD (Sample having crude extract)

H = OD of blank / 2 (Kept in light without extract)

P = Amount of protein

Lipid peroxidation

Lipid peroxidation was measured in petals in terms of malondialdehyde (MDH) content as described by Heath and Packer [20]. Two hundred milligram petal sample was homogenized in 3 ml of 50 mM phosphate buffer of pH 7.0. The homogenate was centrifuged at 8497 RCF (10000 rpm) for 20 min in a Remi centrifuge (Remi Compufuge, CPR-24, India). To 0.5 ml aliquot of the supernatant, 2 ml of 5g L^{-1} thiobarbituric acid (TBA) in 200 g L^{-1} trichloroacetic acid (TCA) was added. The mixture was heated at 90 ^{0}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 non-specific 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 method described by Doderer *et al*. [21] was followed for determining the lipoxygenase activity of flower petals at various stages of vase life.

Preparation of substrate solution : Five millilitre DDW containing 50 μl Tween 20 was added to 35 μl linoleic acid (substrate) and pH of this solution was around 8.5 - 8.6 and the final pH was adjusted to 9 by adding 0.2 M NaOH drop by drop so that all linoleic acid was dissolved. Again the pH was adjusted to 6.5

by adding 0.2 M 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 : Both 0.1 M potassium dihydrogen orthophosphate and dipotassium orthophosphate solutions were mixed in the ratio of 16:84 respectively and the pH was adjusted to 7.5 with a pH meter. In 100 ml of buffer solution of pH 7.5, 0.186 gm of EDTA (0.5 μM) was added and this solution was used for enzyme extraction.

Preparation of enzyme extract : Petal sample (0.2 gm) was homogenized in ice cold 0.1 M 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. 0.05 ml 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 [18].

Statistical analysis

The experiments were laid out in a completely randomised design and repeated twice with mostly three replicates. Flower diameter was based upon ten replicates. Analysis of variance (ANOVA) was performed and means were compared by the least significant difference (P=0.05).

RESULTS

Flower diameter data of three plants under investigation revealed maximum values in *M. parthenium* followed by *A. novae belgii* and *G. pulchella* (Table 1). Cut flower showed shrinkage and reduction in diameter between 0 to 6-day in all of them but these were maximum in *A. novae belgii* and least in *M. parthenium* irrespective of treatments and control. These three plants exhibited the same trend with regard to effectiveness of holding solutions. It was greatest with 8-HQ + sucrose followed by 5-SSA + sucrose, sucrose, 8-HQ, 5-SSA and DDW.

Amount of starch and specific activity of α-amylase were presented in Table 2-4. Starch concentration was fairly high when scapes were introduced in vase solutions (0-day), the highest in *Aster* and lowest in *Gaillardia*. Breakdown of starch continued in petals during 6-day in all plants under control and treated conditions. Control petals registered as high as about 84 per cent decline in *Aster* while it was about 81 and 78 per cent in *Gaillardia* and *Matricaria* respectively. Applications of 5-SSA, 8-HQ and sucrose individually and in combination were able to reduce this degradation partly and the order of effectiveness was 8-HQ + sucrose > 5-SSA + sucrose > sucrose > 8-HQ > 5-SSA > DDW. Vase solution containing 8-HQ + sucrose was able to reduce starch degradation by about 23 percent in *Matricaria* while it was around 15 and 12 percent in case of *Gaillardia* and *Aster* respectively.

To begin with, at 0-day α-amylase activity was very low in petals; the highest in *Gaillardia* and lowest in *Aster.* From 0 to 2-day, among three selected plants, highest increment in α-amylase activity was noticed in *Gaillardia* and lowest in Aster; while between 2 to 4-day, highest activity recorded in *Matricaria* and lowest in *Aster.* However, between 4 to 6-day, highest was found again in *Matricaria* and lowest partly in *Gaillardia* and partly in *Aster.* In fact, very small increment in α-amylase activity was found in *Aster* after 2-day with 8-HQ + sucrose and 5-SSA + sucrose. Effectiveness of 8-HQ + sucrose could also be noted in *Matricaria* and *Gaillardia* upto 2-day as this treatment could bring down α-amylase activity remarkably (Table 3-4). In untreated and treated scapes regular increments of higher values could be seen in most of the cases between 0 to 2-day, 2 to 4-day and 4 to 6-day stages.

Reducing, non-reducing and total sugars of petals collected from these plants as depicted in Table 5-7 indicated much higher values of reducing than non-reducing sugars at 0-day. This trend was maintained even at 2, 4 and 6-day stages in petals of scapes placed in different holding solutions. Maximum accumulation of both kinds of sugars was noticed in untreated scapes and minimum in those held in 8-HQ + sucrose solution. The order of effectiveness in reducing sugar accumulation was 8-HQ + sucrose > 5-SSA + sucrose > sucrose > 8-HQ > 5-SSA > DDW (control). However, sucrose was better than 8-HQ and 5-SSA in arresting sugar concentration when comparison is made between individual treatments. One of the important observations was efficacy of combined application of 8-HQ + sucrose followed by 5-SSA + sucrose that remarkably arrested sugar accumulation upto 2-day in cut scape petals. The distribution and alteration pattern of sugars on different days exhibited a similarity in the selected plants.

Superoxide dismutase (SOD) activity was considerably high in petals of the selected plants at 0-day, maximum and minimum values were recorded in *M. parthenium* and *A. novae belgii* respectively (Table 8). As expected, values declined gradually between 0 to 6-day period in petals of all plants irrespective of

treatments. However, per cent decline was least between 2 to 4-day stage. Further, selected treatments could partly check the decline of enzymatic activity. Here again, the combination of biocide (8-HQ) with sucrose was the best in retaining the activity partly. The performance of 8-HQ + sucrose was the best in *G. pulchella* petals than other plants when 6-day values were compared with respective control.

Tables 9-11 show changes in lipid peroxidation, as measured by levels of malondialdehyde (MDA content) and lipoxygenase activity of *A. novae belgii, M. parthenium* and *G. pulchella*. The degree of lipid peroxidation in cell membranes can be judged with the amount of breakdown products produced. The amount of MDA increased from 0 to 6-day in all cases of petals. The rise in MDA - content was highest in control sets of scapes and individual treatments with 5-SSA and 8-HQ were able to reduce it partially in *A. novae belgii* and *M. parthenium;* while more effectively in *G. pulchella.* Sucrose as a holding solution showed better results individually and also in combination with 5-SSA and 8-HQ in these plants.

Lipoxygenase activity also showed regular increments like MDA-content but the percent increment at every stages was much greater than changes in MDA-content in three plants under investigation. On a comparison among three plants regarding percent increment in lipoxygenase activity, it was very high and maximum in *A. novae belgii* and minimum in *G. pulchella*. Data presented in Tables 9-11 clearly revealed spectacular decrease in lipoxygenase activity in petals of scapes received a combined treatment of 8-HQ + sucrose and 5-SSA + sucrose in all selected plants. Further, maximum control over lipid peroxidation and lipoxygenase activity was achieved by combined application.

Table 1. *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug. showing values of flower diameter (in cm) on different days when scapes were maintained in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1 M; 5-SSA, 200 ppm + sucrose, 0.1 M; 8-HQ, 200 ppm + sucrose, 01. M) [0-Day values of flower diameter - *Aster novae belgii* L. 6.9 cm, *M. parthenium* L. 7.2 cm and *G. pulchella* Foug. 5.2 cm.].

Treatments		**Flower diameter**		**% Difference between**
A.novae belgii L.	2-Day	4-Day	6-Day	0 to 6-Day
Control (DDW)	5.1	4.2	2.1	69.57
5-SSA	5.4	4.6	2.3	66.67
8-HQ	5.7	4.8	2.5	63.77
Sucrose	6.1	5.3	2.8	59.42
5-SSA+Sucrose	6.5	5.7	3.0	56.52
8-HQ+Sucrose	6.8	5.9	3.5	49.28
M.parthenium L.				
Control (DDW)	5.8	4.9	3.7	48.61
5-SSA	6.3	5.2	3.9	45,83
8-HQ	6.7	5.4	4.1	43.06
Sucrose	6.9	5.5	4.3	40.28
5-SSA + Sucrose	7.1	5.7	4.5	37.50
8 - HQ + Sucrose	7.2	5.8	4.8	33.33
G.pulchella Foug.				
Control (DDW)	4.3	3.1	1.9	63.46
5-SSA	4.5	3.4	2.4	53.85
8-HQ	4.6	3.6	2.6	50.00
Sucrose	4.7	4.0	3.0	42.31
5-SSA + Sucrose	4.9	4.2	3.1	40.38
8-HQ + Sucrose	5.1	4.3	3.2	38.46

Each value indicates mean of 10 replicates

Table 2. *Aster novae belgii* L. showing changes in the amount of starch (mg/100mg dry weight ± S.E.) and α-amylase activity (units mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of starch and α-amylase activity were 41.305± 0.753 and 0.498 ± 0.064 respectively].

Treatments	2-Day	4-Day	6-Day
Starch			
Control (DDW)	18.321±0.359^{aD}(-55.645)	11.617±0.243^{bB}(-71.875)	6.446±0.365^{cD}(-84.394)
5-SSA	20.656±0.760^{aC}(- 49.992)	11.735±0.804^{bB}(-71.589)	8.151±0.083^{cC}(-80.266)
8-HQ	21.239±0.467^{aC}(-48.580)	13.218±0.268^{bB}(-67.999)	9.223±0.293^{cBC}(-77.671)
Sucrose	24.585±0.500^{aB}(-40.479)	15.284±0.787^{bA}(-62.997)	9.999±0.407^{cAB}(-75.792)
5-SSA+Sucrose	25.408±0.458^{aB}(-38.487)	15.777±0.255^{bA}(-61.804)	10.406±0.331^{cAB}(-74.807)
8-HQ+Sucrose	27.721±0.669^{aA}(-32.887)	16.187±0.744^{bA}(-60.811)	11.232±0.670^{cA}(-72.807)
α-Amylase activity			
Control (DDW)	1.152±0.012^{cA}(+131.325)	3.075±0.059^{bA}(+517.470)	5.222±0.210^{aA}(+948.594)
5-SSA	1.124±0.077^{cA}(+125.703)	2.912±0.020^{bA}(+484.739)	4.288±0.169^{aB}(+761.044)
8-HQ	0.864±0.012^{cB}(+73.494)	2.333±0.039^{bB}(+368.474)	3.911±0.030^{aBC}(+685.341)
Sucrose	0.732±0.018^{cC}(+46.988)	2.116±0.096^{bBC}(+324.900)	3.534±0.259^{aCD}(+609.639)
5-SSA+Sucrose	0.528±0.040^{cD}(+6.024)	1.889±0.075^{bC}(+279.317)	3.211±0.299^{aD}(+544.779)
8-HQ+Sucrose	0.516±0.016^{cD}(+3.614)	1.748±0.248^{bC}(+251.004)	2.987±0.225^{aD}(+499.799)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).

Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).

Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 3. *Matricaria parthenium* L. showing changes in the amount of starch (mg/100mg dry weight ± S.E.) and α-amylase activity (units mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of starch and α-amylase activity were 37.214± 0.161 and 0.539 ± 0.016 respectively].

Treatments	2-Day	4-Day	6-Day
Starch			
Control (DDW)	23.959±0.553^{aD}(-35.618)	17.510±0.459^{bE}(-52.948)	8.216±0.170^{cE}(-77.922)
5-SSA	24.564±0.786^{aD}(-33.993)	18.786±0.856^{bDE}(-49.519)	11.814±0.538^{cD}(-68.254)
8-HQ	24.907±0.781^{aD}(-33.071)	19.926±0.495^{bCD}(-46.456)	13.762±0.624^{cC}(-63.019)
Sucrose	27.214±0.816^{aC}(-26.872)	21.602±0.653^{bBC}(-41.952)	14.652±0.424^{cBC}(-60.628)
5-SSA+Sucrose	31.324±0.507^{aB}(-15.827)	23.215±0.416^{bAB}(-37.618)	15.815±0.335^{cAB}(-57.503)
8-HQ+Sucrose	35.675±0.797^{aA}(-4.136)	23.769±0.497^{bA}(-36.129)	16.925±0.429^{cA}(-54.520)
α-Amylase activity			
Control (DDW)	2.018±0.222^{cA}(+274.397)	5.584±0.195^{bA}(+935.993)	9.432±0.230^{aA}(+1649.907)
5-SSA	1.664±0.130^{cB}(+208.720)	5.384±0.334^{bA}(+898.887)	9.211±0.227^{aA}(+1608.905)
8-HQ	0.960±0.059^{cC}(+78.108)	4.280±0.148^{bB}(+694.063)	8.920±0.079^{aA}(+1554.917)
Sucrose	0.840±0.044^{cC}(+55.844)	3.736±0.158^{bBC}(+593.135)	7.283±0.086^{aB}(+1251.206)
5-SSA+Sucrose	0.726±0.052^{cC}(+34.694)	3.138±0.304^{bC}(+482.189)	7.156±0.200^{aB}(+1227.644)
8-HQ+Sucrose	0.592±0.059^{cC}(+9.833)	2.147±0.130^{bD}(+298.330)	6.242±0.126^{aC}(+1058.071)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).

Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).

Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 4. *Gaillardia pulchella* Foug. showing changes in the amount of starch (mg/100mg dry weight ± S.E.) and α-amylase activity (units mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of starch and α-amylase activity were 34.446± 0.568 and 2.032 ± 0.532 respectively].

Treatments	2-Day	4-Day	6-Day
Starch			
Control (DDW)	20.816±0.534^{aE}(-39.569)	12.772±0.196^{bC}(-62.922)	6.616±0.411^{cC}(-80.793)
5-SSA	21.432±0.453^{aCD}(-37.781)	13.606±0.491^{bC}(-60.500)	6.984±0.499^{cC}(-79.725)
8-HQ	21.995±0.463^{aCD}(-36.146)	14.635±0.426^{bC}(-57.513)	7.318±0.261^{cC}(-78.755)
Sucrose	22.844±0.788^{aBC}(-33.682)	16.498±0.722^{bB}(-52.105)	9.810±0.175^{cB}(-71.521)
5-SSA+Sucrose	23.913±0.530^{aB}(-30.578)	18.876±0.791^{bA}(-45.201)	11.195±0.798^{cAB}(-67.500)
8-HQ+Sucrose	25.816±0.508^{aA}(-25.054)	19.885±0.679^{bA}(-42.272)	11.809±0.562^{cA}(-65.717)
α-Amylase activity			
Control (DDW)	8.024±0.287^{cA}(+294.882)	13.424±0.337^{bA}(+560.630)	16.136±0.517^{aA}(+694.094)
5-SSA	7.625±0.125^{cAB}(+275.246)	12.774±0.210^{bB}(+528.642)	15.693±0.198^{aAB}(+672.293)
8-HQ	7.208±0.173^{cB}(+254.724)	11.854±0.116^{bC}(+483.366)	15.111±0.168^{aABC}(+643.652)
Sucrose	5.421±0.098^{cC}(+166.782)	9.642±0.154^{bD}(+374.508)	14.760±0.425^{aBC}(+626.378)
5-SSA+Sucrose	2.926±0.314^{cD}(+43.996)	9.102±0.123^{bD}(+347.933)	14.296±0.289^{aCD}(+603.543)
8-HQ+Sucrose	2.268±0.079^{cE}(+11.614)	8.224±0.238^{bE}(+304.724)	13.540±0.217^{aD}(+566.339)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 5. *Aster novae belgii* L. showing changes in reducing, non-reducing and total sugars (mg/100mg dry weight ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of reducing, non-reducing and total sugars were 2.899 ± 0.315, 1.650 ± 0.181 and 4.549 ± 0.135 respectively].

Treatments	2-Day	4-Day	6-Day
Reducing sugars			
Control (DDW)	5.996±0.190^{cA}(+106.830)	9.211±0.118^{bA}(+217.730)	16.722±0.200^{aA}(+476.820)
5-SSA	5.883±0.513^{cAB}(+102.932)	8.944±0.155^{bAB}(+208.520)	16.128±0.116^{aB}(+456.330)
8-HQ	5.398±0.246^{cAB}(+86.202)	8.580±0.166^{bB}(+195.964)	15.512±0.119^{aC}(+435.081)
Sucrose	5.014±0.151^{cBC}(+72.956)	7.670±0.150^{bC}(+164.574)	14.423±0.132^{aD}(+397.516)
5-SSA+Sucrose	4.258±0.308^{cCD}(+46.878)	7.282±0.089^{bC}(+151.190)	12.828±0.198^{aE}(+342.497)
8-HQ+Sucrose	3.502±0.220^{cD}(+20.800)	6.106±0.124^{bD}(+110.624)	11.306±0.166^{aF}(+289.997)
Non-reducing sugars			
Control (DDW)	3.446±0.190^{cA}(+108.848)	6.711±0.148^{bA}(+306.727)	13.608±0.147^{aA}(+724.727)
5-SSA	3.108±0.182^{cA}(+88.364)	5.426±0.140^{bB}(+228.848)	12.848±0.162^{aB}(+678.667)
8-HQ	2.876±0.228^{cAB}(+74.303)	5.111±0.161^{bB}(+209.758)	11.775±0.119^{aC}(+613.636)
Sucrose	2.433±0.180^{cB}(+47.455)	4.377±0.105^{bC}(+165.273)	10.634±0.174^{aD}(+544.485)
5-SSA+Sucrose	2.365±0.131^{cB}(+43.333)	4.109±0.157^{bC}(+149.030)	8.337±0.167^{aE}(+405.273)
8-HQ+Sucrose	1.744±0.162^{cC}(+5.697)	3.224±0.155^{bD}(+95.394)	7.842±0.152^{aF}(+375.273)
Total sugars			
Control (DDW)	9.442±0.022^{cA}(+107.562)	15.922±0.068^{bA}(+250.011)	30.330±0.053^{aA}(+566.740)
5-SSA	8.991±0.371^{cA}(+97.648)	14.370±0.098^{bB}(+215.894)	28.976±0.081^{aB}(+536.975)
8-HQ	8.274±0.087^{cB}(+81.886)	13.691±0.092^{bC}(+200.967)	27.287±0.025^{aC}(+499.848)
Sucrose	7.447±0.095^{cC}(+63.706)	12.047±0.049^{bD}(+164.827)	25.057±0.042^{aD}(+450.824)
5-SSA+Sucrose	6.623±0.187^{cD}(+45.592)	11.391±0.072^{bE}(+150.407)	21.165±0.049^{aE}(+365.207)
8-HQ+Sucrose	5.246±0.081^{cE}(+15.322)	9.330±0.081^{bF}(+105.100)	19.148±0.021^{aF}(+320.928)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 6. *Matricaria parthenium* L. showing changes in reducing, non-reducing and total sugars (mg/100mg dry weight ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of reducing, non-reducing and total sugars were 3.788 ± 0.123, 1.218 ± 0.271 and 5.006 ± 0.203 respectively].

Treatments	2-Day	4-Day	6-Day
Reducing sugars			
Control (DDW)	8.088±0.080^{cA}(+113.516)	14.609±0.148^{bA}(+285.665)	19.762±0.159^{aA}(+421.700)
5-SSA	7.816±0.153^{cA}(+106.336)	13.123±0.148^{bB}(+246.436)	17.315±0.164^{aB}(+357.101)
8-HQ	7.244±0.173^{cB}(+91.235)	12.326±0.165^{bC}(+225.396)	16.884±0.087^{aB}(+345.723)
Sucrose	6.292±0.099^{cC}(+66.103)	11.275±0.197^{bD}(+197.650)	15.598±0.197^{aC}(+311.774)
5-SSA+Sucrose	5.574±0.129^{cD}(+47.149)	9.912±0.142^{bE}(+161.668)	14.808±0.190^{aD}(+290.919)
8-HQ+Sucrose	5.231±0.143^{cD}(+38.094)	9.205±0.145^{bF}(+143.004)	14.211±0.186^{aE}(+275.158)
Non-reducing sugars			
Control (DDW)	5.345±0.120^{cA}(+338.834)	7.346±0.159^{bA}(+503.120)	10.378±0.181^{aA}(+752.053)
5-SSA	4.622±0.170^{cB}(+279.475)	6.240±0.164^{bB}(+412.315)	8.273±0.180^{aB}(+579.228)
8-HQ	2.875±0.194^{bC}(+136.043)	5.631±0.210^{aC}(+362.315)	6.330±0.254^{aC}(+419.704)
Sucrose	2.411±0.150^{cCD}(+97.947)	3.429±0.184^{bD}(+181.527)	6.117±0.321^{aC}(+402.217)
5-SSA+Sucrose	1.945±0.300^{cDE}(+59.688)	2.875±0.063^{bE}(+136.043)	4.642±0.150^{aD}(+281.117)
8-HQ+Sucrose	1.649±0.149^{bE}(+35.386)	2.106±0.154^{bF}(+72.906)	4.393±0.176^{aD}(+260.673)
Total sugars			
Control (DDW)	13.433±0.041^{cA}(+168.338)	21.955±0.013^{bA}(+338.574)	30.140±0.022^{aA}(+502.078)
5-SSA	12.438±0.063^{cB}(+148.462)	19.363±0.034^{bB}(+286.796)	25.588±0.016^{aB}(+411.147)
8-HQ	10.119±0.075^{cC}(+102.137)	17.957±0.070^{bC}(+258.710)	23.214±0.169^{aC}(+363.724)
Sucrose	8.703±0.052^{cD}(+73.851)	14.704±0.097^{bD}(+193.728)	21.715±0.127^{aD}(+333.779)
5-SSA+Sucrose	7.519±0.183^{cE}(+50.200)	12.787±0.098^{bE}(+155.433)	19.450±0.054^{aE}(+288.534)
8-HQ+Sucrose	6.880±0.008^{cF}(+37.435)	11.311±0.038^{bF}(+125.949)	18.604±0.056^{aF}(+271.634)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 7. *Gaillardia pulchella* Foug. showing changes in reducing, non-reducing and total sugars (mg/100mg dry weight ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of reducing, non-reducing and total sugars were 2.074 ± 0.133, 0.869 ± 0.239 and 2.943 ± 0.113 respectively].

Treatments	2-Day	4-Day	6-Day
Reducing sugars			
Control (DDW)	9.833±0.203^{cA}(+374.108)	15.500±0.147^{bA}(+647.348)	20.343±0.179^{aA}(+880.858)
5-SSA	8.672±0.178^{cB}(+318.129)	14.967±0.137^{bB}(+621.649)	20.074±0.162^{aA}(+867.888)
8-HQ	6.797±0.188^{cC}(+227.724)	14.240±0.176^{bC}(+586.596)	19.842±0.128^{aA}(+856.702)
Sucrose	4.790±0.230^{cD}(+130.955)	13.039±0.157^{bD}(+528.689)	19.118±0.141^{aB}(+821.794)
5-SSA+Sucrose	3.342±0.192^{cE}(+61.138)	12.313±0.183^{bE}(+493.684)	17.331±0.127^{aC}(+735.632)
8-HQ+Sucrose	2.947±0.153^{cE}(+42.093)	10.105±0.136^{bF}(+387.223)	16.208±0.192^{aD}(+681.485)
Non-reducing sugars			
Control (DDW)	4.642±0.179^{cA}(+434.177)	6.542±0.171^{bA}(+652.819)	8.315±0.292^{aA}(+856.847)
5-SSA	3.876±0.339^{cB}(+346.030)	5.889±0.342^{bA}(+577.675)	7.446±0.200^{aB}(+756.847)
8-HQ	3.148±0.251^{cC}(+262.255)	5.137±0.177^{bB}(+491.139)	7.132±0.223^{aBC}(+720.713)
Sucrose	2.208±0.181^{cD}(+154.085)	4.320±0.175^{bC}(+397.123)	6.554±0.203^{aC}(+654.200)
5-SSA+Sucrose	1.345±0.217^{cE}(+54.776)	3.168±0.208^{bD}(+264.557)	4.321±0.169^{aD}(+397.238)
8-HQ+Sucrose	1.167±0.132^{bE}(+34.292)	2.887±0.225^{aD}(+232.221)	3.244±0.224^{aE}(+273.303)
Total sugars			
Control (DDW)	14.475±0.060^{cA}(+391.845)	22.042±0.028^{bA}(+648.964)	28.658±0.118^{cA}(+873.768)
5-SSA	12.548±0.161^{cB}(+326.368)	20.856±0.270^{bB}(+608.665)	27.520±0.038^{aB}(+835.100)
8-HQ	9.945±0.096^{cC}(+237.920)	19.382±0.179^{bC}(+558.580)	26.974±0.094^{aC}(+816.548)
Sucrose	7.107±0.221^{cD}(+141.488)	17.359±0.030^{bD}(+489.840)	25.672±0.066^{aD}(+772.307)
5-SSA+Sucrose	4.687±0.091^{cE}(+59.259)	15.481±0.025^{bE}(+426.028)	21.652±0.059^{aE}(+635.712)
8-HQ+Sucrose	4.114±0.029^{cF}(+39.789)	12.992±0.108^{bF}(+341.454)	19.451±0.037^{aF}(+560.890)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 8. Changes in superoxide dismutase (SOD) activity (units mg^{-1} protein min^{-1} ± S.E.) in petals of *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug. when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of SOD activity were 10.581 ± 0.483, 24.620 ± 0.177 and 13.162 ± 0.143 respectively in petals of above three genera].

Treatments	2-Day	4-Day	6-Day
***A. novae belgii* L.**			
Control	4.060±0.279^{aC}(-61.629)	3.189±0.147^{bB}(-69.861)	1.410±0.157^{cC}(-86.674)
5-SSA	4.187±0.103^{aC}(-60.429)	3.479±0.202^{bAB}(-67.120)	1.524±0.084^{cC}(-85.597)
8-HQ	4.618±0.216^{aC}(-56.356)	3.543±0.163^{bAB}(-66.515)	1.743±0.102^{cBC}(-83.527)
Sucrose	4.706±0.213^{aC}(-55.524)	3.727±0.149^{bAB}(-64.776)	1.995±0.121^{cB}(-81.145)
5-SSA+Sucrose	6.126±0.255^{aB}(-42.104)	3.883±0.214^{bA}(-63.302)	2.402±0.102^{cA}(-77.299)
8-HQ+Sucrose	7.159±0.131^{aA}(-32.341)	3.926±0.163^{bA}(-62.896)	2.621±0.100^{cA}(-75.229)
***M. parthenium* L.**			
Control	16.058±0.103^{aE}(-34.777)	13.071±0.079^{bE}(-46.909)	9.847±0.101^{cF}(-60.004)
5-SSA	16.322±0.120^{aE}(-33.704)	13.365±0.144^{bD}(-45.715)	10.126±0.069^{cE}(-58.871)
8-HQ	17.174±0.627^{aD}(-30.244)	13.977±0.076^{bC}(-43.229)	10.414±0.113^{cD}(-57.701)
Sucrose	18.087±0.078^{aC}(-26.535)	14.021±0.033^{bC}(-43.050)	11.216±0.069^{cC}(-54.444)
5-SSA+Sucrose	19.464±0.127^{aB}(-20.942)	14.774±0.113^{bB}(-39.992)	11.578±0.117^{cB}(-52.973)
8-HQ+Sucrose	20.549±0.124^{aA}(-16.535)	15.249±0.080^{bA}(-38.063)	12.680±0.051^{cA}(-48.497)
***G. pulchella* Foug.**			
Control	9.159±0.050^{aC}(-30.413)	7.748±0.111^{bD}(-41.134)	3.209±0.110^{cD}(-75.619)
5-SSA	9.213±0.059^{aC}(-30.003)	7.944±0.111^{bCD}(-39.644)	4.111±0.095^{cC}(-68.766)
8-HQ	9.316±0.115^{aC}(-29.220)	8.223±0.070^{bC}(-37.525)	4.426±0.103^{cC}(-66.373)
Sucrose	9.744±0.113^{aB}(-25.969)	8.618±0.112^{bB}(-34.524)	5.110±0.077^{cB}(-61.176)
5-SSA+Sucrose	9.889±0.041^{aB}(-24.867)	8.875±0.120^{bAB}(-32.571)	5.321±0.118^{cB}(-59.573)
8-HQ+Sucrose	10.367±0.093^{aA}(-21.235)	9.021±0.078^{bA}(-31.462)	6.569±0.110^{cA}(-50.091)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 9. *Aster novae belgii* L. showing changes in MDA content (nM g^{-1} fresh weight ± S.E.) and lipoxygenase activity (units min^{-1} mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of MDA content and lipoxygenase activity were 0.00396± 0.00033 and 0.619 ± 0.055 respectively].

Treatments	2-Day	4-Day	6-Day
MDA content			
Control (DDW)	0.00651±0.00018^{cA}(+64.394)	0.00751±0.00022^{bA}(+89.646)	0.00998±0.00030^{aA}(+152.020)
5-SSA	0.00648±0.00023^{bA}(+63.636)	0.00712±0.00056^{abA}(+79.798)	0.00879±0.00060^{aAB}(+121.970)
8-HQ	0.00634±0.00018^{bAB}(+60.101)	0.00700±0.00026^{abA}(+76.768)	0.00838±0.00069^{aAB}(+111.616)
Sucrose	0.00597±0.00045^{aAB}(+50.758)	0.00674±0.00070^{aA}(+70.202)	0.00788±0.00090^{aAB}(+98.990)
5-SSA+Sucrose	0.00551±0.00015^{cAB}(+39.141)	0.00670±0.00028^{bA}(+69.192)	0.00777±0.00015^{aB}(+96.212)
8-HQ+Sucrose	0.00529±0.00060^{bB}(+33.586)	0.00663±0.00012^{abA}(+67.424)	0.00765±0.00081^{aB}(+93.182)
LOX activity			
Control (DDW)	2.215±0.094^{cA}(+257.835)	3.458±0.107^{bA}(+458.643)	7.115±0.061^{aA}(+1049.435)
5-SSA	2.157±0.079^{cA}(+248.465)	3.188±0.072^{bB}(+415.024)	6.485±0.099^{aB}(+947.658)
8-HQ	2.087±0.061^{cA}(+237.157)	2.887±0.059^{bC}(+366.397)	6.308±0.051^{aB}(+919.063)
Sucrose	1.777±0.124^{cB}(+187.076)	2.343±0.036^{bD}(+278.514)	5.878±0.059^{aC}(+849.596)
5-SSA+Sucrose	1.121±0.052^{cC}(+81.099)	1.527±0.063^{bE}(+146.688)	5.522±0.057^{aD}(+792.084)
8-HQ+Sucrose	0.859±0.048^{bD}(+38.772)	1.042±0.075^{bF}(+68.336)	5.210±0.054^{aE}(+741.680)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 10. *Matricaria parthenium* L. showing changes in MDA content (nM g^{-1} fresh weight ± S.E.) and lipoxygenase activity (units min^{-1} mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of MDA content and lipoxygenase activity were 0.00318± 0.00023 and 2.089 ± 0.050 respectively].

Treatments	2-Day	4-Day	6-Day
MDA content			
Control (DDW)	0.00446±0.00009^{cA}(+40.252)	0.00532±0.00016^{bA}(+67.296)	0.00871±0.00035^{aA}(+173.899)
5-SSA	0.00433±0.00014^{cA}(+36.164)	0.00514±0.00007^{bAB}(+61.635)	0.00805±0.00009^{aB}(+153.145)
8-HQ	0.00429±0.00011^{cA}(+34.906)	0.00500±0.00015^{bABC}(+57.233)	0.00735±0.00018^{aC}(+131.132)
Sucrose	0.00379±0.00013^{cB}(+19.182)	0.00494±0.00016^{bABC}(+55.346)	0.00685±0.00007^{aCD}(+115.409)
5-SSA+Sucrose	0.00375±0.00026^{cB}(+17.925)	0.00472±0.00015^{bBC}(+48.428)	0.00672±0.00020^{aD}(+111.321)
8-HQ+Sucrose	0.00340±0.00006^{cB}(+6.918)	0.00455±0.00022^{bC}(+43.082)	0.00541±0.00013^{aE}(+70.126)
LOX activity			
Control (DDW)	5.252±0.063^{cA}(+151.412)	8.485±0.086^{bA}(+306.175)	12.142±0.048^{aA}(+481.235)
5-SSA	4.485±0.096^{cB}(+114.696)	7.567±0.058^{bB}(+262.231)	11.881±0.081^{aAB}(+468.741)
8-HQ	4.245±0.047^{cB}(+103.207)	7.232±0.041^{bC}(+246.194)	11.710±0.054^{aB}(+460.555)
Sucrose	3.872±0.214^{cC}(+85.352)	6.598±0.048^{bD}(+215.845)	9.919±0.136^{aC}(+374.820)
5-SSA+Sucrose	2.712±0.114^{cD}(+29.823)	6.317±0.058^{bE}(+202.393)	9.664±0.138^{aC}(+362.614)
8-HQ+Sucrose	2.409±0.054^{cD}(+15.318)	5.773±0.058^{bF}(+176.352)	8.372±0.113^{aD}(+300.766)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

Table 11. *Gaillardia pulchella* Foug. showing changes in MDA content (nM g^{-1} fresh weight ± S.E.) and lipoxygenase activity (units min^{-1} mg^{-1} protein ± S.E.) in petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfosalicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 0.1M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of MDA content and lipoxygenase activity were 0.0435± 0.00265 and 3.774 ± 0.086 respectively].

Treatments	2-Day	4-Day	6-Day
MDA content			
Control (DDW)	0.0716±0.00082^{cA}(+64.598)	0.0880±0.00045^{bA}(+102.299)	0.0998±0.00153^{aA}(+129.425)
5-SSA	0.0681±0.00215^{cAB}(+56.552)	0.0869±0.00047^{bAB}(+99.770)	0.0973±0.00074^{aAB}(+123.678)
8-HQ	0.0651±0.00128^{cB}(+49.655)	0.0853±0.00047^{bB}(+96.092)	0.0942±0.00045^{aBC}(+116.552)
Sucrose	0.0587±0.00364^{cC}(+34.943)	0.0819±0.00072^{bC}(+88.276)	0.0911±0.00085^{aCD}(+109.425)
5-SSA+Sucrose	0.0542±0.00131^{cCD}(+24.598)	0.0797±0.00123^{bC}(+83.218)	0.0899±0.00162^{aD}(+106.667)
8-HQ+Sucrose	0.0521±0.00067^{cD}(+19.770)	0.0745±0.00051^{bD}(+71.264)	0.0881±0.00057^{aD}(+102.529)
LOX activity			
Control (DDW)	6.868±0.113^{cA}(+81.982)	9.310±0.050^{bA}(+146.688)	12.205±0.098^{aA}(+223.397)
5-SSA	6.111±0.113^{cB}(+61.924)	8.672±0.110^{bB}(+129.783)	11.350±0.082^{aB}(+200.742)
8-HQ	5.835±0.097^{cB}(+54.610)	8.356±0.083^{bC}(+121.410)	10.665±0.125^{aC}(+182.591)
Sucrose	5.126±0.052^{cC}(+35.824)	6.444±0.118^{bD}(+70.747)	8.337±0.071^{aD}(+120.906)
5-SSA+Sucrose	3.994±0.132^{cD}(+5.829)	5.321±0.060^{bE}(+40.991)	7.642±0.114^{aE}(+102.491)
8-HQ+Sucrose	3.821±0.050^{cD}(+1.245)	5.110±0.068^{bE}(+35.400)	7.144±0.052^{aF}(+89.295)

Means with different lower case letters in the same row are statistically (P ≤0.05) different (i.e. according to days, using DMRT).
Means with different upper case letters in the column of same box are statistically (P ≤0.05) different (i.e. according to treatments, using DMRT).
Data in parenthesis indicate percent change from the initial (0-Day) value.

DISCUSSION

From the overall findings, it was apparent that sucrose at a concentration of 0.1 M was the best among individual treatments to reduce the shrinkage of petals so that reduction in flower diameter could be controlled to some extent. Effectiveness of sucrose was also evident when it was present along with either 8-HQ or 5-SSA. Working on cut rose flowers, Kumar and Pal [22] reported maximum vase life with 50 ppm of 8-hydroxy quinoline citrate (8-HQC). Regarding combined application of metabolite with biocide, Verma et al. [23] have noticed maximum vase life, uptake of holding solution and flower size of *Chrysanthemum* cut flowers with 2% sucrose + 200 ppm 8-HQC in comparison to water as control. In fact, carbohydrate status of cut flowers decides how long they will remain fresh and turgid.

The rapid depletion of starch in untreated scapes of *A. novae belgii*, *M. parthenium* and *G. pulchella* was due to much higher activity of α-amylase in comparison to treated ones. This investigation has clearly revealed that sucrose alone as vase solution was very useful in arresting starch degradation and the combination of 8-HQ + sucrose and 5-SSA + sucrose were much more effective than the former. Earlier study with two cultivars of *Chrysanthemum* correlated the enhancement of α-amylase with first sign of petal wilting [24]. Continuous supply of substrates is most essential for petal growth, flower opening, sustaining turgidity and freshness of flowers; and also to fulfil membrane constituents and energy requirement. In addition, osmotic potential of cells has to be maintained. Cut scapes have undergone stress and the breakdown products of starch and sucrose can serve as substrates in the cells of stressed tissue.

Distribution of sugars in petals at different stages of cut scapes showed larger amount of reducing sugars than non-reducing sugars in all selected plants. However, per cent increment in non-reducing sugars was much greater than reducing sugars between 0 to 6-day after most of the treatments in *A. novae belgii* and *M. parthenium.* In *G. pulchella,* per cent increment values of non-reducing sugars are lower than reducing sugars during 6-day period. Present study clearly indicated that use of any one substance out of sucrose, 5-SSA and 8-HQ did not favour rise in the amount of reducing and non-reducing sugars. It was observed earlier that presence of sucrose in vase solution could retard starch hydrolysis and delay biosynthesis of ethylene - the phytohormone that triggers senescence [25].

A comparison between *Aster, Matricaria* and *Gaillardia* regarding SOD activity revealed very high degree of decline between 0 to 6-day but per cent decline was higher between 0 to 2-day stage in *Aster* in comparison to other two cut flowers. Flower petals of these three plants were unique in showing very little decline in SOD activity between 2 to 4-day stage. Showing very high SOD value even at 6-day of control in *Matricaria* cut flowers than other two flowers may indicate endogenous mechanism that minimizes activities of oxidants. It was interesting to note that individually sucrose and 8-HQ were effective slightly in minimizing the decline in SOD activity but when present together, the activity increased sharply. Significant decline in total SOD activity was also observed during senescence of carnation petals [26]. Reactive oxygen species (ROS) play very important role in the regulation of petal senescence and generally it increased with the onset of senescence [27]. ROS are associated with the decline in SOD activity [28]. It has been noticed that SOD activity increased during early development of flowers but declined after opening of flowers in *Gladiolus* [12]. From the present study, it appears that sucrose, 8-HQ and 5-SSA have important role either in the induction of antioxidant enzymes (like SOD) or they partly help in scavenging ROS. As a result of these changes petal senescence is delayed.

One of the key markers for petal and leaf senescence is a sharp rise in ion leakage, associated with a loss in membrane integrity [27]. Lipid peroxidation induced by ROS is considered as an important phenomenon of membrane deterioration and may also be induced by the action of lipoxygenase which causes oxidation of fatly acids liberated from membranes [29]. Available reports indicate that antioxidants reduce lipid peroxidation, delay senescence and increase vase life of flowers [30,12,27]. With the available results from the study of *Aster, Matricaria* and *Gaillardia* it can be pointed out that sucrose, 8-HQ and 5-SSA when present alone can minimize both the phenomena of lipid peroxidation and lipoxygenase activity. Further, combined application of 8-HQ + sucrose was the best to reduce these processes to postpone petal senescence. 8-HQ and 5-SSA in association with sucrose exhibited additive effects in delaying petal senescence.

CONCLUSION

Control and untreated petals showed a steady decline in flower diameter, starch content and SOD activity while increment was registered in both kinds of sugars, MDA - content and lipoxygenase activity. Cut scape petals of *Matricaria* exhibited very high SOD value at 6-day in comparison to that of other plants. Sucrose, 8-HQ and 5-SSA at chosen concentrations when present individually in vase solutions were able to check partially the shrinkage and reduction in flower diameter in all selected plants. However, better results were obtained when sucrose was combined either with 8-HQ or 5-SSA. A gradual increase in

starch degradation, lipid peroxidation and lipoxygenase activity; and a decline in SOD activity were noticed in petals of *Aster*, *Matricaria* and *Gaillardia*. Vase solution containing 8-HQ + sucrose was the best among all treatments and control in minimizing α-amylase activity, membrane degradation and lipoxygenase activity by accelerating SOD activity.

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CONFLICT OF INTEREST
Authors declare that they have no conflict of interest.

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