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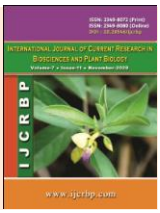

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Comparative assessment of sucrose, 8-hydroxyquinoline and 5-sulfosalicylic acid in the regulation of petal senescence in cut scapes of *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug.

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ABSTRACT

Comparative assessment was made between three chemicals viz. a metabolite (sucrose, 0.1M) a biocide (200 ppm of 8-hydroxyquinoline, 8-HQ) and a PGR (200 ppm of 5-sulfosalicylic acid, 5-SSA) regarding their performance to regulate petal senescence in cut scapes of three selected plants (*Aster novae belgii, Matricaria parthenium* and *Gaillardia pulchella).* These chemicals were used individually and also in combination of 8-HQ (200 ppm) + sucrose (0.1M) and 5-SSA (200 ppm) + sucrose (0.1M). Among these chemicals as holding solutions, individually sucrose was much more effective than 8-HQ and 5-SSA in minimizing the reduction in flower diameter and moisture loss. Sucrose was also more effective than 8-HQ and 5-SSA in reducing the protein loss by controlling protease activity. However, combined application of 8-HQ + sucrose yielded best result in controlling petal senescence followed by 5-SSA + sucrose in three selected plants. The holding solution having 8-HQ + sucrose could retain maximum protein and much lower protease activity in petals especially during 0 to 2-day stage than other stages in *A. novae belgii* and *M. parthenium*. Petals of *G. pulchella* recorded highest protease activity followed by *M. parthenium* and *A. novae belgii.*

Introduction

Senescence is a developmental process in all plants that starts after maturity. Various deteriorative changes viz. morphological, physiological and biochemical are witnessed at the onset of senescence. These changes shorten the functional life of plants and plant organs (Woolhouse, 1967; Beevers, 1976). In case of flowers or floral inflorescence, senescence begins after their complete development when they show the sign of wilting and abscission of whole flowers or flower parts (Stead and van Doorn, 1994). Cut flowers may be defined as flowers or an inflorescence containing more than one floral unit in the opened or unopened state which are harvested and marketed for ornamental purpose. Cut flowers and cut scapes (flowering twig from which leaves have been removed) experience very rapid loss of freshness and turgidity than attached flowers as the supply of water and nutrients becomes a limiting factor in the former. The breakdown of

proteins, carbohydrates and lipids and decrease in nucleic acids are most common features of flower senescence (van Doorn and Woltering, 2008; Khokhar et al. 2018). It is also characterized by rapid increase in lipid peroxidation, membrane leakage and breakdown of cell wall components (Buchanan - Wollaston, 1997).

Flower quality can be maintained and vase life can be extended by sugars like sucrose as they are carbon and energy source (Kuiper et al., 1995; Monteiro et al., 2002; van Doorn, 2004; Mukherjee and Mukherjee, 2017). When present in the vase solution, sucrose increases the osmotic concentration of petal cell sap and maintain turgidity in cut flowers (O'Donoghue et al., 2002). Sugars are also known to delay petal senescence by suppressing ethylene synthesis, which is a naturally occurring plant growth regulator (PGR) as opined by Woltering and van Doorn, 1988).

PGRs like abscisic acid (ABA), auxins, cytokinins, gibberellins, morphactins, salicylic acid, polyamines, etc. are also involved in the regulation of petal senescence (Kaur et al., 2017; Khokhar et al. 2018). Unlike cytokinins and some other PGRs, fewer studies have been carried out with 5-sufosalicylic acid (5-SSA) which is derived from salicylic acid (SA). SA is an important endogenous PGR which not only enhances flowering (Raskin, 1992) but also delays flower senescence (Kaur et al., 2017). The vase life of cut *Gladiolus* flowers was extended in presence of 5-SSA (Ezhilmathi et al., 2007). This treatment increased significantly solution uptake and number of opened florets; and lowered lipid peroxidation and lipoxygenase activity. It also increased membrane stability, soluble protein content and activities of superoxide dismutase (SOD) and catalase in comparison to controls. Experiments with cut scapes and cut flowers face the danger of microorganism invasion at the cut surface. Flow of holding solutions (water, nutrient solution, PGR, etc.) is slowed down due to bacterial multiplication and synthesis of extra cellular polysaccharides within the vessels. Release of pectinases and toxic compounds are also responsible for ethylene synthesis which in turn accelerates flower senescence (Jowkar et al., 2012).

Biocides like 8-hydroxyquinoline (8-HQ), 8-hydroxyquinoline citrate (8-HQC) and 8-hydroxyquinoline sulphate (8-HQS) are often used to prevent microbial attack. This property of biocides makes them very useful in floriculture industry. Working with cut roses, van Doorn and Perik (1990) have reported their effectiveness in lowering pH of the holding solution and preventing vascular blockage. Combined treatment of sucrose + 8-HQC+GA could extend the longevity of cut lily flowers (Rabiza - Swider et al., 2012).

Based upon above facts regarding the utility of sucrose, 5-SSA and 8-HQ representing a metabolite, a PGR and a biocide it was thought to undertake an investigation where one could assess their importance individually and in combination of two (5-SSA + sucrose and 8-HQ + sucrose) in relation to petal senescence of cut scapes. Furthermore, this study tried to assess effectiveness of these substances in three plants viz. *Aster novae belgii, Matricaria parthenium and Gaillardia pulchella* selecting some important paramenters like flower diameter, moisture content, absorption of holding solution, protein content and protease activity.

Materials and methods

Plant material

Three plants viz. *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug. were selected in the present investigation. Saplings were raised from the certified seeds of above plants in uniformly prepared experimental plots within wire net cage in the garden of Botany Department. Plants were allowed to grow and flower. At the time of flowering, cut flowers were harvested in the morning hours with a sharp scalpel. Flower twigs were cut in a bucket filled with water to prevent cavitation and were brought to the laboratory immediately. Leaves were removed from the cut flower twigs to have cut scapes which were put in the conical flasks containing different holding solutions already prepared.

Holding solutions and experimental set up

Holding solutions used here include 5-SSA (200 ppm), 8-HQ (200 ppm), sucrose (0.1M), 5-SSA (200 ppm) + sucrose (0.1M), 8-HQ (200 ppm) + sucrose (0.1M). Double distilled water (DDW) as a separate holding solution was also kept as a control set. For each holding solution, 10 conical flasks of

Borosil glass of 100 ml volume were used. Three scapes of 14 cm length were introduced in each flask having 30 ml of specific holding solution. Untreated control sets were similarly prepared. All experiments were set up at room temperature under 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* and *M. parthenium* was 25 ± 2°C while that of *G.pulchella* was 37 ± 2°C.

Samplings

Samples were collected at 0, 2, 4 and 6-day stages from petals of all cut scapes maintained in different holding solutions. Samples were made in such a manner that 3 replicates were available for each biochemical analysis. For collecting dry weight data triplicate samples were placed in the oven for 2 days at 80°C. Various observations such as visible effect, longevity, volume of holding solutions absorbed, moisture content and flower diameter were also recorded at specific time. These data are based upon mean of 10 replicates.

Extraction and estimation of protein

The method of extraction of protein has been described earlier (Singh et al., 2018). Protein content was estimated by the method of Bradford (1976) using Coomassie Brilliant blue G-250. The quantity of protein was calculated against a standard curve of bovine serum albumin (BSA, Sigma, USA).

Protease activity

The original method was described by Yemm and Cocking (1955) followed by its modification by Reimerdes and Klostermeyer (1976). One hundred milligram of petal sample was homogenized in 100 mM phosphate buffer (pH 7.2) and the final volume was raised to 10 ml. One percent casein (Sigma, USA) was prepared by dissolving 1 gm of casein in minimal volume (0.4 to 0.8 ml) of 0.1 N NaOH followed by raising the final volume to 100 ml with 100 mM phosphate buffer (pH 7.6). To 1 ml of casein 1 ml of enzyme extract was added and incubated for 3 hrs at 37°C. The pH of the reaction mixture was 7.5. One ml of boiled enzyme was taken in blank set to which 1 ml of casein and 1 ml of 10% trichloroacetic acid (TCA, 10 gm in 100 ml of distilled water) were added immediately to stop the reaction. The precipitate was then centrifuged. After incubation, 1 ml of TCA added to all the reaction sets; centrifuged them again and discarded the residue. Out of the resultant 3 ml filtrate 1 ml was taken to estimate the enzymatic activity. Ninhydrin solution was prepared by dissolving 1.25 gm of ninhydrin in 47 ml of methyl cellosolve (2-methoxy ethanol) and to this 15.5 ml of filtered solution of $SnCl_2$ (100 mg in 4N sodium acetate buffer, 25.01 ml; mentioned in next para) was added. The solution was collected in a dark bottle and stored in a refrigerator. To 1 ml of enzyme solution 1 ml of ninhydrin solution was added, mixed well and test tubes containing this mixture were placed in boiling water bath at 100°C for 15 min. Test tubes were cooled thereafter and 2 ml of 50% ethanol was added in each tube. The resultant violet colour was read at 550 nm in a UV-Vis spectrophotometer. Specific activity of protease was expressed in terms of μM Lysine equivalent per 100 mg dry weight of tissue hr^{-1}.

Preparation of 4N Sodium Acetate buffer

Stock solution number 1 (4N sodium acetate) was prepared by dissolving 5.44 gm of sodium acetate (hydrated, 3 H_2O) in double distilled water (DDW) and the volume of solution was raised to 1000 ml. A volume of 228.56 ml of glacial acetic acid was raised to 1000 ml with DDW to make stock solution number 2 (4N glacial acetic acid). Now 25 ml of stock solution 1 was mixed with 0.01 ml of stock solution 2. Thus we get 4N solution of sodium acetate buffer of pH 4.8.

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 and moisture content of petals of *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug. have been presented in Table 1-3 when scapes were

maintained in double distilled water, 5-sulfo-salicylic acid (5-SSA, 200 ppm), 8-hydroxy-quinoline (8-HQ, 200 ppm), sucrose (0.1M), 5-SSA (200 ppm) + sucrose (0.1M) and 8-HQ (200 ppm) + sucrose (01.M). A gradual decrease has been noticed in flower diameter of scapes belonging to three plants during 6-day. Maximum reduction was witnessed in untreated *A. novae belgii* petals followed by *G. pulchella* and *M. parthenium*. A comparison of 5-SSA and 8-HQ treated flower scapes with untreated ones revealed their effectiveness to check partially the reduction in flower diameter in all plants investigated. The use of sucrose (0.1 M) as holding solution was much better than either 5-SSA or 8-HQ application. It was indeed a better combination when sucrose was added to both 5-SSA and 8-HQ. Retention of flower diameter was the best with a combined application of 8-HQ + sucrose followed by 5-SSA + sucrose in these cut scapes.

Table 1. *Aster novae belgii* L. showing changes in flower diameter and moisture content of petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfo salicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 01.M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of flower diameter and moisture content were 6.9 cm and 80.10% respectively].

Treatments	Flower diameter (in cm)			% Difference between
	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
Moisture content (%)				
Control (DDW)	67.40	60.15	54.29	25.81
5-SSA	69.32	61.23	55.17	24.93
8-HQ	70.14	62.51	55.46	24.64
Sucrose	74.22	64.30	57.28	22.82
5-SSA+Sucrose	77.54	66.73	59.11	20.99
8-HQ+Sucrose	79.21	67.00	59.75	20.35

Each value indicates mean of 10 replicates.

Table 2. *Matricaria parthenium* L. showing changes in flower diameter and moisture content of petals when scapes were placed in various holding solutions (Double distilled water, DDW as control; 5-sulfo-salicylic acid, 5-SSA, 200 ppm; 8-hydroxyquinoline, 8-HQ, 200 ppm; sucrose, 0.1M; 5-SSA, 200 ppm + sucrose, 01.M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of flower diameter and moisture content were 7.2 cm and 89.12% respectively].

Treatments	Flower diameter (in cm)			% Difference between
	2-Day	4-Day	6-Day	0 to 6-Day
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
Moisture content (%)				
Control (DDW)	79.32	72.31	68.14	20.98
5-SSA	80.24	74.35	68.75	20.37
8-HQ	81.53	75.41	69.31	19.81
Sucrose	83.16	77.23	70.40	18.72
5-SSA+Sucrose	85.37	78.11	72.35	16.77
8-HQ+Sucrose	86.14	79.13	72.87	16.25

Each value indicates mean of 10 replicates.

Table 3. *Gaillardia pulchella* Foug. showing changes in flower diameter and moisture content of 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, 01.M; 8-HQ, 200 ppm + sucrose, 0.1M). [0-Day values of flower diameter and moisture content were 5.2 cm and 75.14% respectively].

Treatments	Flower diameter (in cm)			% Difference between
	2-Day	4-Day	6-Day	0 to 6-Day
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
Moisture content (%)				
Control (DDW)	67.22	59.33	50.00	25.14
5-SSA	67.75	59.88	50.25	24.89
8-HQ	68.16	60.14	50.16	24.98
Sucrose	70.35	62.37	52.22	22.92
5-SSA+Sucrose	73.14	64.44	53.14	22.00
8-HQ+Sucrose	73.95	65.12	53.76	21.38

Each value indicates mean of 10 replicates.

Percent moisture content was showing a gradual decline in petals of cut scapes during 6 days in all selected plants. The decline in percent moisture content in untreated petals of *A. novae belgii, M. parthenium* and *G. pulchella* was 25.81, 20.98 and 25.14 respectively during 6 days. Reduction in percent moisture content was also witnessed in the treated scapes of all these plants (Table 1-3). Petals of treated scapes exhibited slightly higher values of moisture content and the degree of effectiveness in retaining moisture was maximum in scapes having treatment of 8-HQ + sucrose > 5-SSA + sucrose > sucrose > 8-HQ > 5-SSA> control.

The volume of holding solution in each conical flask was 30 ml when experiment was set up and the volume of absorbed solution has been shown after 6-day (Table 4). An increasing trend in absorption was noticed in all treated scapes in comparison to control. Maximum absorption was found when holding solution was 8-HQ + sucrose > 5-SSA + sucrose > sucrose > 8-HQ > 5-SSA > control (DDW). Relatively larger absorption was witnessed by cut scapes of *A. novae belgii* and *G. pulchella* as compared to *M. parthenium* in both control and treated sets.

Table 4. Volume of holding solution absorbed (ml) scapes^{-3} of *Aster novae belgii* L., *Matricaria parthenium* L. and *Gaillardia pulchella* Foug during 6-Day 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). [Volume of holding solution on 0-Day = 30 ml].

Treatments	Volume of holding solution *A. novae belgii*	Volume of holding solution *M. parthenium*	Volume of holding solution *G. pulchella*
Control (DDW)	18.10	12.75	17.60
5-SSA	18.67	14.17	18.67
8-HQ	19.50	14.72	18.79
Sucrose	20.54	15.42	19.75
5-SSA+Sucrose	20.69	15.90	21.67
8-HQ+Sucrose	21.11	16.49	22.80

Each value indicates mean of 10 replicates.

Changes in the amount of protein and protease activity (in lysine equivalent) of petals from cut scapes of three selected plants have been incorporated in Table 5-7. The initial protein contents were 9.374 mg, 11.042 mg and 6.862 mg per 100 mg dry weight in *A. novae belgii, M. parthenium* and *G. pulchella* respectively. The protein quantity registered a steady decrease in untreated and treated petals of cut scapes. Treatments with 5-SSA, 8-HQ and sucrose, however, minimized the protein degradation in all selected plants. The holding solution containing 8-HQ + sucrose was unique to retain maximum protein specially during 0-2 day stage in *A. novae belgii* and *M. parthenium*. Among individual treatments, maximum effectiveness was observed with sucrose followed by 8-HQ and 5-SSA. Combined application was able to retain much higher protein than individual application in three plants studied. Unlike protein content, protease activity registered a steady increase from 0 to 6-day in controls and treated petals belonging to three plants. Highest protease activity was witnessed in *A. novae belgii* followed by *M. parthenium* and *G. pulchella* initially but 6-day stage of untreated petals showed maximum activity in *M. parthenium* followed by *A. novae belgii* and *G. pulchella*. Holding solutions of sucrose, 8-HQ and 5-SSA could bring down the enzymatic activity appreciably resulting comparatively higher amount of protein. The combined applications of 8-HQ + sucrose and 5-SSA + sucrose were again very effective in lowering the enzymatic activity in all these plants.

Table 5. *Aster novae belgii* L. showing changes in the amount of protein (mg / 100 mg dry weight±S.E.) and protease activity (µM Lysine equivalent / 100 mg 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 protein and protease activity were 9.374±0.434 and 8.716±0.053 respectively].

Treatments	2-Day	4-Day	6-Day
		Protein	
Control (DDW)	5.917±0.207^{aC}(-36.878)	4.463±0.393^{bB}(-52.390)	2.500±0.075^{cB}(-73.330)
5-SSA	6.013±0.125^{aC}(-35.854)	4.478±0.228^{bB}(-52.230)	2.650±0.171^{cB}(-71.730)
8-HQ	6.138±0.109^{aC}(-34.521)	5.058±0.671^{aAB}(-46.042)	2.839±0143^{bB}(-69.714)
Sucrose	6.684±0.371^{aBC}(-28.696)	5.257±0.100^{bAB}(-43.919)	3.882±0.095^{cA}(-58.588)
5-SSA+Sucrose	7.135±0.188^{aC}(-23.885)	5.371±0.062^{bAB}(-42.703)	4.011±0.195^{cA}(-57.211)
8-HQ+Sucrose	8.958±0.410^{aA}(-4.438)	5.776±0.125^{bA}(-38.383)	4.113±0.138^{cA}(-56.123)
		Protease activity	
Control (DDW)	14.721±0.048^{cA}(+68.896)	22.642±0.107^{bA}(+159.775)	28.463±0.062^{aA}(+226.560)
5-SSA	14.310±0.082^{cB}(+64.181)	20.414±0.066^{bB}(+134.213)	27.154±0.074^{aB}(+211.542)
8-HQ	14.111±0.034^{cB}(+61.898)	20.115±0.113^{bC}(+130.782)	25.332±0.090^{aC}(+190.638)
Sucrose	12.365±0.111^{cC}(+41.866)	18.110±0.071^{bD}(+107.779)	23.676±0.132^{aD}(+171.638)
5-SSA+Sucrose	10.674±0.109^{cD}(+22.464)	16.876±0.083^{bE}(+93.621)	21.881±0.136^{aE}(+151.044)
8-HQ+Sucrose	9.386±0.101^{cE}(+7.687)	15.421±0.114^{bF}(+76.927)	21.115±0.101^{aF}(+142.256)

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 the amount of protein (mg / 100 mg dry weight ± S.E.) and protease activity (µM Lysine equivalent / 100 mg 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 protein and protease activity were 11.042 ± 0.728 and 7.343 ± 0.054 respectively].

Treatments	2-Day	4-Day	6-Day
		Protein	
Control (DDW)	8.142±0.415^{aC}(-26.263)	6.901±0.199^{bB}(-37.502)	3.992±0.201^{cC}(-63.847)
5-SSA	8.332±0.161^{aC}(-24.543)	7.213±0.241^{bAB}(-34.677)	4.827±0.258^{cBC}(-56.285)
8-HQ	8.492±0.162^{aC}(-23.094)	7.440±0.172^{bAB}(-32.621)	5.328±0.244^{cAB}(-51.748)
Sucrose	9.809±0.434^{aB}(-11.166)	7.479±0.251^{bAB}(-32.268)	5.708±0.150^{cAB}(-48.306)
5-SSA+Sucrose	10.320±0.414^{aAB}(-6.539)	7.691±0.347^{bAB}(-30.348)	5.977±0.273^{cA}(-45.870)
8-HQ+Sucrose	10.915±0.183^{aA}(-1.150)	8.072±0.449^{bA}(-26.897)	6.345±0.647^{cA}(-42.538)
		Protease activity	
Control (DDW)	16.680±0.088^{cA}(+127.155)	23.644±0.076^{bA}(+221.994)	31.389±0.066^{aA}(+327.468)
5-SSA	16.093±0.036^{cB}(+119.161)	21.312±0.037^{bB}(+190.236)	30.214±0.056^{aB}(+311.467)
8-HQ	15.618±0.074^{cC}(+112.692)	20.531±0.047^{bC}(+179.600)	29.316±0.048^{aC}(+299.237)
Sucrose	14.131±0.057^{cD}(+92.442)	18.125±0.056^{bD}(+146.834)	26.651±0.062^{aD}(+262.944)
5-SSA+Sucrose	12.246±0.018^{cE}(+66.771)	16.845±0.079^{bE}(+129.402)	24.772±0.059^{aE}(+237.355)
8-HQ+Sucrose	10.196±0.027^{cF}(+38.853)	16.110±0.027^{bF}(+119.393)	22.262±0.038^{aF}(+203.173)

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 the amount of protein (mg / 100 mg dry weight ± S.E.) and protease activity (µM Lysine equivalent / 100 mg 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 protein and protease activity were 6.862 ± 0.062 and 4.550 ± 0.050 respectively].

Treatments	2-Day	4-Day	6-Day
		Protein	
Control (DDW)	4.032±0.055^{aE}(-41.242)	2.208±0.004^{bD}(-67.823)	0.436±0.004^{cD}(-93.646)
5-SSA	4.446±0.064^{aD}(-35.208)	2.898±0.082^{bC}(-57.767)	1.226±0.122^{cC}(-82.133)
8-HQ	4.511±0.044^{aD}(-34.261)	3.094±0.158^{bBC}(-54.911)	1.521±0.143^{cBC}(-77.834)
Sucrose	4.804±0.015^{aC}(-29.991)	3.362±0.079^{bB}(-51.006)	1.767±0.065^{cAB}(-74.249)
5-SSA+Sucrose	5.391±0.162^{aB}(-21.437)	3.746±0.085^{bA}(-45.410)	1.923±0.139^{cA}(-71.976)
8-HQ+Sucrose	5.997±0.072^{aA}(-12.606)	3.992±0.143^{bA}(-41.825)	2.040±0.056^{cA}(-70.271)
		Protease activity	
Control (DDW)	11.563±0.087^{cA}(+154.132)	18.771±0.042^{bA}(+312.549)	27.157±0.091^{aA}(+496.857)
5-SSA	10.632±0.051^{cB}(+133.670)	18.130±0.052^{bB}(+298.462)	25.312±0.019^{aB}(+456.308)
8-HQ	9.253±0.049^{cC}(+103.363)	17.234±0.030^{bC}(+278.769)	24.621±0.039^{aC}(+441.121)
Sucrose	7.680±0.044^{cD}(+68.791)	15.625±0.064^{bD}(+243.407)	22.110±0.078^{aD}(+385.934)
5-SSA+Sucrose	5.545±0.040^{cE}(+21.868)	14.821±0.057^{bE}(+225.736)	20.320±0.064^{aE}(+346.593)
8-HQ+Sucrose	4.990±0.039^{cF}(+9.670)	12.370±0.073^{bF}(+171.868)	19.123±0.033^{aF}(+320.286)

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

Results presented above indicate that individual application of 8-HQ (a biocide), 5-SSA (a plant growth regulator) and sucrose (a metabolite) and a combined treatment of 8-HQ + sucrose as well as 5-SSA + sucrose are very effective in reducing the shrinkage of flower diameter, and increasing the moisture content and longevity of all cut flowers investigated. The most promising holding solution is 8-HQ with sucrose in all three plants under investigation. When freshly cut scapes are placed in holding solutions, they gradually show shrinkage and decrease in flower diameter as observed in cut scapes of *Iris germanica, Hemerocallis fulva* and *Petunia hybrida* (Gulzar, 2003). Moisture content was maximum at the time of flower opening and it declined after transferring cut scapes to holding solutions irrespective of their specific nature.

Working with cut roses, Kumar and Pal (2005) have shown maximum vase life when 50 ppm of 8-hydroxyquinoline citrate (8-HQC) was used. *Chrysanthemum* cut flowers exhibited maximum vase life, solution uptake and flower size with 2% sucrose + 200 ppm 8 - HQC in comparison to water as control (Verma et al., 2007). Bacterial proliferation is responsible for the vascular blockage in cut flowers (Halevy and Mayak, 1979; van Doorn et al., 1989). It appears that sucrose, 8-HQ and 5-SSA have improved the water uptake of cut flowers by reducing the vascular blockage as one of these chemicals (8-HQ) is known to possess strong anti-microbial properties that eliminate vascular blockage resulting greater water uptake (Burdett, 1970).

Hydroxyquinolines (8-HQ, 8-hydroxyquinoline; 8-HQC, 8-hydroxyquinoline citrate and 8-HQS, -8-hydroxyquinoline sulphate) are responsible for rendering the holding solution acidified so that bacterial growth is reduced remarkably.

Another study with *Gladiolus grandiflora* (Ezhilmathi et al., 2007) using 5-SSA has shown significant increment in the uptake of vase solutioin, vase life and number of open florets as compared with control sets. They have also extended flower vase life using a combination of 100 ppm 5-SSA and 4% sucrose in 1:1 ratio. In cut carnation flowers, a combination of sugar and cytokinin were able to improve the longevity and performance in comparison to that situation where only one of these chemicals was present (van Staden et al., 1990). Combined applications of kinetin + sucrose and SA + sucrose were shown to be effective in arresting partly the reduction in flower diameter in *Aster novae belgii* (Mukherjee and Mukherjee, 2020). Sucrose is the source of energy and respiratory substrate which improves the ability of the tissue to absorb water and maintains turgidity (Bhaskar et al., 1999; Pun and Ichimura, 2003; Varu and Barad, 2008).

As far as alteration in the quantity of protein is concerned, individually 5-SSA and 8-HQ were slightly effective in minimizing the loss by lowering the protease activity in petals of cut scapes of *A. novae belgii, M. parthenium* and *G. pulchella*. Sucrose was a little more effective than other two compounds. Petal senescence has been associated with loss of protein as noticed in many plants including *Dianthus caryophyllus* (Kenis et al., 1985; Sugawara et al., 2002), cut daylily flowers (Lay-Yee et al., 1992), *Alstroemeria* (Wagstaff et al., 2002), Iris (Pak and van Doorn, 2005), *Petunia* (Jones et al., 2005) and gladiolus (Azeez et al., 2007). The rapid decline is due to little amount of de novo synthesis and considerable protein degradation (Lay-Yee et al., 1992). This leads to membrane disintegration (Woolhouse, 1984). Present study has noticed very high protease activity responsible for this breakdown. Combined applications of 8-HQ + sucrose and 5-SSA + sucrose were much more effective than their individual application as revealed in the present study. Shiva et al. (2002) found the effectiveness of 8-HQC (150 ppm) with sucrose (3%) in cut roses while Ezhilmathi et al (2007) found the importance of 5-SSA + sucrose in earlier studies. Studies carried out in our laboratory revealed efficacious nature of SA in *Calendula officinalis* (Renu et al., 2018) and 8-HQ in *C. officinalis* and *Gaillardia pulchella* (Mukherjee et al., 2019).

Conclusion

Among individual treatments, sucrose (0.1M) as a metabolite in the holding solution was the best followed by 8-HQ (200 ppm, a biocide) and 5-SSA (200 ppm, a PGR) in reducing petal shrinkage and minimizing moisture loss. Combined application was much better than individual treatment, the

best being 8-HQ + sucrose in cut scapes of all selected plants viz. *A. novae belgii*, *M. parthenium* and *G. pulchella*. Protein degradation was also effectively controlled by these treatments in comparison to control; the degree of competence was 8-HQ + sucrose > 5-SSA + sucrose > sucrose > 8-HQ > 5-SSA > control.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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