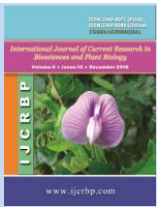
International Journal of Current Research in
Biosciences and Plant Biology
Volume 5 • Number 12 (December-2018) • ISSN: 2349-8080 (Online)
Journal homepage: www.ijcrbp.com



Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2018.512.004

Interaction of Sucrose, a Morphactin and 8-Hydroxyquinoline in the Regulation of Petal Senescence of *Calendula officinalis* L. Cut Flowers

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Article Info

Date of Acceptance:
22 November 2018
Date of Publication:
06 December 2018

Keywords

Calendula officinalis
Chemical substances
Guaiacol peroxidase
Lipid peroxidation
Protein content
Senescence regulation

ABSTRACT

Cut flowers of *Calendula officinalis* L. as scapes were maintained in holding solutions of 0.1M sucrose, 20 µM methyl chlorflurenol (a morphactin, MOR), 1.5 mM 8-hydroxyquinoline (8-HQ), sucrose + MOR, sucrose + 8-HQ and sucrose + MOR + 8-HQ in the laboratory under continuous light. Untreated sets were also present where scapes getting the supply of double distilled water (DDW). Combined applications of sucrose + 8-HQ and sucrose + MOR + 8-HQ were very effective in minimizing the loss of fresh weight of scapes and moisture content. These combinations and also individual sucrose treatment as well as sucrose + MOR were very useful to reduce the loss in flower diameter by maintaining hydration and turgidity. Sucrose + MOR + 8-HQ combination was also unique in exhibiting maximum rise in protein amount on fresh weight basis between 0 and 3 and 3 and 6-day and on dry weight basis between 0 and 3-day. Lesser GPOX activity in petals of flower scapes subjected to 8-HQ, MOR and sucrose treatments may suggest lesser production of H_2O_2. Further, used chemicals such as sucrose, MOR and 8-HQ could reduce the lipid peroxidation process in petals and the best treatment was sucrose + MOR + 8-HQ followed by sucrose + 8-HQ > sucrose + MOR > MOR> 8-HQ > sucrose > DDW.

Introduction

Flower senescence represents the last stage of floral development where various deteriorative changes are initiated. Such alterations lead to petal wilting, colour changes and abscission of whole flowers or flower part (Stead and van Doorn, 1994). Some other changes like inrolling or outrolling of petals, dehydration and shrinkage can also be seen. It is very rapid and continuous process especially in cut scapes (leafless flowers) where no scope is left to carry out photosynthesis. Petal senescence is considered to be a subset of developmental programmed cell death (PCD), and in case of petal senescence the term 'senescence' is considered to be a synonym of 'PCD' (van Doorn and Woltering, 2008).

Once detached from the plant, the life of cut flowers becomes shorter than attached flowers. Cut flowers not only suffer from dehydration but also show loss of assimilates, ethylene induced senescence, greater release of reactive oxygen species (ROS), etc. which will collectively affect the vase life. Interruption in the uptake of water and various holding solutions is due to rapid activities of microorganisms which block the basal end of the cut flowers. Besides bacterial multiplication, other factors such as synthesis of extra cellular polysaccharides and degradation product of dead cells are also responsible for vessel blockage. Release of pectinases and toxic compounds are also responsible for ethylene production which accelerate flower (also petals) senescence (Jowkar et al., 2012). Biocides are often used in cut flower industry to prevent or minimize the attack by microbes and to extend the vase life as the senescence process is delayed. 8-Hydroxy quinoline (8-HQ) is an important biocide that can be directly used as holding solution or two other derivatives viz., hydroxy quinoline sulphate (HQS) and hydroxy quinoline citrate (HQC) can also be used for the same purpose. They lower the pH of the holding solution preventing vascular blockage in stem of may flowers including cut roses (van Doorn and Perik, 1990). Besides biocides, sucrose and specific plant growth regulators (PGRs) can also be used to increase the vase life of cut flowers (Rabiza-Swider et al., 2012). Presence of sucrose in holding solution increases the endogenous carbohydrate pool in petals and enhances respiration, thus increasing flower longevity (Song et al., 1996). Presence of adequate sucrose plays a major role in prolonging vase life of cut flowers by delaying the release of ethylene (Pun and Ichimura, 2003). Reports are available that sometimes negative sugar effect can be seen along with 8-HQC (van Doorn, 2008). PGR is required to reduce this effect (Pemberton et al., 1997). Methyl chlorflurenol (MCF, a morphactin) which was developed earlier by Schneider (1970) and reintroducted by Repar Corp., Maryland, USA in recent years, is a synthetic PGR having large number of important functions. It arrests the apical meristem activity temporarily and favours lateral growth. It can also delay senescence in leaves (Jain and Mukherjee, 1980) and petals (Khokhar and Mukherjee, 2010).

In recent years, it has been noticed that flowers like *Arctotis, Aster, Calendula, Chrysanthemum, Gaillardia, Matricaria,* etc. are useful for bedding, and also for growing in pots. They are used for decoration. *Calendula* is an ornamental aromatic annual plant having valuable medicinal properties. Some studies have already been carried out with cut flowers of *C. officinalis* (Kaur et al., 2015, Kaur and Mukherjee, 2015, Khokhar et. al., 2018). In continuation with that, effectiveness of these chemicals individually and also in combination in relation to petal senescence was assessed.

Materials and methods

Plant material

Calendula officinalis L. plants were grown in the experimental beds maintained in the garden of Botany Department, Kurukshetra University, Kurukshetra. Uniformly developed flowers of the same physiological age and almost similar diameter were identified and flower twigs were cut under water in a bucket and brought to the laboratory. Twigs with flowers were recut in the laboratory to have an uniform length of 14 cm. Leaves were also removed from these twigs and placed in 100 ml Borosil-make conical flasks having 30 ml holding solution in each of them. Five conical flasks were maintained for each holding solution and two scapes were placed in each flask. Holding solutions were : double distilled water (DDW, control), sucrose (0.1M), methylchlorflurenol (a morphactin, MOR, 20 μM), 8-hydroxyquinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20 μM), sucrose (0.1M) + 8-HQ (1.5 mM) and sucrose (0.1M) + MOR (20 μM) + 8-HQ (1.5 mM). Experiment was 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. Flower diameter and external appearance were noted at 0, 3 and 6-day. Petal samples were collected in triplicates to find out moisture content, dry weight, total soluble protein,

guaiacol peroxidase (GPOX) activity and lipid peroxidation (MDA content). The experiment has been repeated once to confirm the trend of results.

Fresh weight of scapes, flower diameter and moisture content

Fresh weight of scapes was recorded individually on the initial (0-day) and final day (6-day) of experiment and percent difference was calculated as the difference between two. Flower diameter of individual scape was determined as the mean of two perpendicular measurements across a flower. Data related to fresh weight of scapes and flower diameter, were based upon 10 replicates. One hundred milligram fresh sample (in triplicates) was placed in an oven at 80^0C for 2-day to find out dry weight and moisture content.

Total soluble protein

One hundred mg petal sample was used for an extraction. The sample was dropped in 10 ml of 80% boiling ethanol in a test tube placed in a water bath for 1 min. It was cooled and homogenized in same ethanol using pestle and mortar. The extract was centrifuged in a Remi centrifuge at 2124 RCF (5000 rpm) for 15 min. Supernatant was discarded and the residue was re-extracted with 10 ml of 5% perchloric acid and centrifuged again at 2124 RCF for 15 min. Supernatant was discarded again and 5 ml of 1 N NaOH was added to the residue and collected it in a test tube. The residue in the alkali was tried to dissolve by shaking and putting in water bath at a temperature of 40-50 ^{0}C for 20 min. It was centrifuged again at 2124 RCF for 15 min. and supernatant was collected separately for protein estimation by the method of Bradford (1976). To 0.3 ml of protein extract, 0.7 ml of double distilled water (DDW) was added. Further, 5 ml of Coomassie brilliant blue G-250 reagent was added and shaken well at room temperature. Blank was prepared by mixing 1 ml DDW and 5 ml of this reagent. Absorbance was recorded at 595 nm in a UV-Visible Spectrophotometer (Systronics, Double Beam Spectrophotometer 2203), India). The protein contents of samples were calculated against a standard curve of bovine serum albumin (BSA, Sigma, USA).

Guaiacol peroxidase (GPOX) activity

The method of Maehly (1954) was followed to find out GPOX activity. Petal sample (100 mg for 1 sample) was homogenized with 10 ml of ice cold 0.1M KH_2PO_4 - Na_2HPO_4 buffer of pH 7.0 and centrifuged in a Remi centrifuge at 2124 RCF for 15 min. Supernatant was collected and raised to 10 ml with the above ice cold phosphate buffer. Reaction set was prepared by mixing 2 ml of enzyme extract, 2 ml of phosphate buffer (pH 7.0), 2 ml of guaiacol (20 mM) and 2ml of H_2O_2 (10 mM) in a sequence. Blank set contains 2 ml of enzyme extract, 2 ml of phosphate buffer (pH 7.0) and 4 ml of DDW. After 10 min. the absorbance was recorded at 420 nm in a Systronics Double Beam Spectrophotometer. Specific GPOX activity was expressed in terms of mg protein per 10 min. Protein was estimated from the enzyme extract using Coomassie brilliant blue reagent as mentioned earlier (Bradford, 1976).

Lipid peroxidation (MDA content)

The level of lipid peroxidation of petal sample was estimated in terms of MDA content (Heath and Packer, 1968). Two hundred mg petal sample was homogenized in 2ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 8497 RCF (10000 rpm) for 20 min in a Remi refrigerated centrifuge. To 0.5 ml aliquot of the supernatant, 2ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 90^0C for 30 min. in the water bath and then quickly cooled in an ice water bath. After centrifugation at 8497 RCF (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 in accordance to its extinction coefficient of 155 per mM per cm.

Results and discussion

Fig. 1 shows experimental plots of *C. officinalis* with open flowers. Various morphological changes in flowers and petals of *C. officinalis* after main taining these scapes in different holding solutions can be seen in Fig. 2. Fresh weight changes of scapes having various combinations of holding solutions placed at 27 ± 1 °C and 33 ± 1°C can be seen in Table 1-2. In both the experiments, percent decrease in fresh weight of scapes (leafless flowers) was slightly higher in presence of sucrose than control. However, individual treatments with morphactin (MOR, 20 μM) and 8-hydroxyquinoline (8-HQ, 1.5 mM) could reduce this decrease appreciably. Another notable feature was the effective nature of combined applications with holding solutions of sucrose + 8-HQ and sucrose + MOR + 8-HQ in minimizing the loss of fresh weight in scapes. The order of effectiveness was sucrose + MOR + 8-HQ > Sucrose + 8-HQ > Sucrose + MOR > MOR > 8-HQ > control > sucrose at 27 ± 1^0C (Table 1).

Alterations in moisture content of petals and flower diameter have been shown in Table 3-4. Between 0 and 3-day, moisture content registered a slight increment in petals but 3 to 6-day showed a sharp decline irrespective of individual treatment. Percent moisture content was slightly higher in petals of scapes placed in sucrose or MOR or 8-HQ. However, combined application of sucrose + 8-HQ and sucrose + MOR exhibited considerably higher retention of moisture in petals at 6-day in comparison to control (Table 3).

Earlier studies with sucrose (0.1M) and lower concentration of spermine ($1x10^{-4}$M) (Kaur and Mukherjee, 2015) as well as sucrose (4%) and 0.4 mM sulfosalicylic acid (SAA) (Kaur et al., 2015) in *C. officinalis* also exhibited remarkable retention of fresh weight, moisture content and cumulative uptake of vase solution. Morphactins are synthetic plant growth regulators (PGRs) which are known to maintain water balance in plant tissues (Schneider, 1972) and are able to retain adequate moisture content. In *C. officinalis* cut flowers, while working with kinetin (KN, 40 μM), salicylic acid (SA, 40 μM) and a morphactin (MOR; methyl chlorfluerenol - MCF, 40 μM), the retention of moisture content was witnessed by these PGRs and also sucrose individually (Khokhar et al., 2018) which increased further when sucrose was being combined with these PGRs. The most effective combination was sucrose + KN followed by sucrose + SA and sucrose + MOR.

Fig. 1: (a) General view of experimental plants in the garden and (b) *Calendula officinalis* plants with open flowers.

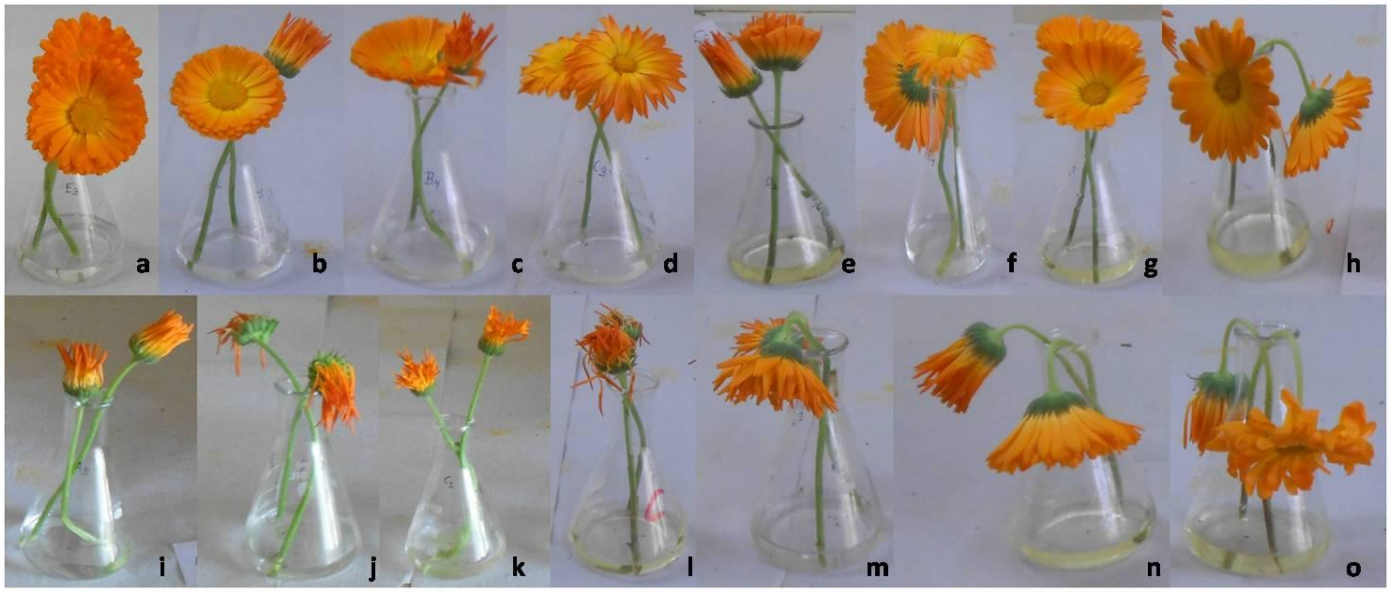

Fig. 2: *Calendula officinalis* showing scapes of various stages having different holding solutions like (a) 0-day control (distilled water, DDW), (b) to (h) 3-day stage, (i) to (o) 6-day stage. (b) and (i) untreated control (DDW); (c) and (j) sucrose (0.1M); (d) and (k) morphactin (MOR, 20 µM); (e) and (l) 8-hydroxyquinoline (8-HQ, 1.5mM); (f) and (m) sucrose (0.1M) + MOR (20 µM); (g) and (n) sucrose (0.1M) + 8-HQ (1.5mM); (h) and (O) sucrose (0.1M) + MOR (20 µM) + 8-HQ (1.5mM).

Data presented in Table 4 indicate 47.38 and 47 percent increase in flower diameter in scapes having morphactin and sucrose + MOR + 8+HQ holding solutions respectively after 3-day but these values reduced considerably between 3 and 6 day. All other holding solutions except 8-HQ brought about some increment during 0 to 3-day but 3 to 6-day stage of cut scapes showed substantial decrease in flower diameter. The combined treatment of sucrose + MOR + 8-HQ was the best in having least decline in flower diameter.

Table 1. *Calendula officinalis* showing fresh weight* change per scape in various holding solutions during 6 days at 27 ± 1^{0}C.

Holding Solutions	0-DAY	6-DAY	Percent decrease / increase in fresh weight
	Fresh Weight ± S.E. (in gm)		
Double distilled water	2.10±0.089	1.65±.0.065	-21.43
Sucrose (0.1M)	2.40±0.253	1.81±0.147	-24.58
Morphactin (20µM)	2.40±0.109	2.08±0.209	-13.33
8-Hydroxyquinoline (8-HQ, 1.5 mM)	2.34±0.168	1.90±0.160	-18.80
Sucrose (0.1M) + Morphactin (20 µM)	2.55±0.154	2.23±0.124	-12.55
Sucrose (0.1M)+8-HQ (1.5 mM)	2.19±0.109	2.17±0.137	-0.91
Sucrose (0.1M) + Morphactin (20 µM) + 8-HQ (1.5 mM)	2.36±0.136	2.44±0.107	+3.39

*Data are mean values of 10 scapes.

Table 2. *C. officinalis* showing fresh weight* change per scape in various holding solutions during 4 days at 33±1°C.

Holding Solutions	0-DAY	6-DAY	Percent decrease / increase in fresh weight
	Fresh Weight ± S.E. (in gm)		
Double distilled water	2.22 ± 0.080	1.68 ± 0.075	-24.32
Sucrose (0.1M)	2.03 ± 0.106	1.44 ± 0.081	-29.06
Morphactin (20µM)	1.83 ± 0.092	1.43 ± 0.105	-21.86
8-Hydroxyquinoline (8-HQ, 1.5 mM)	2.36 ± 0.132	2.09 ± 0.122	-11.44
Sucrose (0.1M) + Morphactin (20 µM)	2.51 ± 0.140	1.49 ± 0.104	-40.64
Sucrose (0.1M)+8-HQ (1.5 mM)	2.33 ± 0.110	2.28 ± 0.115	-2.15
Sucrose (0.1M) + Morphactin (20 µM) + 8-HQ (1.5 mM)	2.28 ± 0.125	2.37 ± 0.120	+3.95

*Data are mean values of 10 scapes.

Table 3. *C. officinalis* showing changes in flower diameter (in cm. ± S.E.) in scapes maintained in double distilled water (DDW, control), sucrose (0.1M), morphactin (MOR, 20µm), 8-hydroxyquinoline (8-HQ, 1.5mM), sucrose (0.1M) + MOR(20 µM), sucrose (0.1M) + 8-HQ (1.5 mM) and sucrose (0.1M)+MOR (20µM) +8-HQ (1.5mM).

Holding Solutions				Percent Difference Between		
	0-DAY	3-DAY	6-DAY	0 to 3-DAY	3 to 6-DAY	0 to 6-DAY
DDW (Control)	4.26±0.27	4.41±0.40	2.03±0.51	+3.64	-53.97	-52.29
Sucrose	3.91±0.17	4.49±0.55	3.03±0.29	+14.83	-32.63	-22.63
MOR	4.00±0.20	5.90±0.42	2.65±0.22	+47.38	-55.05	-33.75
8-HQ	4.71±0.27	3.49±0.44	2.33±0.26	-25.82	-33.23	-50.47
Sucrose+MOR	4.21±0.17	5.77±0.72	3.12±0.26	+36.94	-45.88	-25.89
Sucrose + 8-HQ	4.26±0.21	4.92±0.65	3.06±0.46	+15.63	-37.81	-28.09
Sucrose +MOR + 8-HQ	4.55±0.20	6.68±0.20	4.05±0.81	+47.00	-39.38	-10.89

Table 4. *C. officinalis* showing changes in dry weight (in mg) and percent moisture content in flower petals of scapes maintained in sucrose (0.1M), morphactin (MOR, 20 µM), 8- hydroxyquinoline (8-HQ,1.5 mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M)+8-HQ (1.5 mM) and sucrose (0.1M) + MOR (20 µM) + 8-HQ (1.5 mM).

	Holding solutions	Fresh weight	Dry weight	Moisture content
0-DAY	Initial	100	14	86
3-DAY	Control	100	10	90
	Sucrose	100	11	89
	MOR	100	09	91
	8-HQ	100	10	90
	Sucrose + MOR	100	09	91
	Sucrose + 8-HQ	100	08	92
	Sucrose +MOR+8-HQ	100	08	92
6-DAY	Control	100	42	58
	Sucrose	100	44	56
	MOR	100	36	64
	8-HQ	100	39	61
	Sucrose+ MOR	100	25	75
	Sucrose+8-HQ	100	11	89
	Sucrose+MOR+8-HQ	100	28	72

It seems that flowers picked up from experimental plots of *C. officinalis* and put in different holding solutions were still in the developing stage. This process continued for 3 days and selected chemicals had different degree of impact. While working with the same cut flowers (*C. officinalis*) but different PGRs like KN, SA and a MOR along with sucrose earlier, consecutive decline was noticed in flower diameter between 0 and 4 and 4 and 8-day stage (Khokhar et al., 2018). Since, these were already developed and fully matured flowers, no further increment could be seen in flower diameter. Reduction in flower diameter is due to constant dehydration and shrinkage. Variation in flower diameter can be noticed in these flowers depending upon the month (November to mid April at Kurukshetra) and the prevailing temperature.

Changes in total soluble proteins indicated an increase between 0 and 3-day on both fresh and dry weight basis in all flower petals; both control and treated ones (Table 5). The increment on fresh weight basis was almost similar in flowers having distilled water and sucrose (0.1M). However, much higher increments were noticed in petals of scapes getting MOR and 8-HQ as holding solutions during first 3 days on fresh and dry weight basis. Sucrose (0.1M) + MOR (20 μM) + 8-HQ (1.5 mM) as a combined holding solution was unique to register maximum increment in petal proteins on both fresh and dry weight basis after 3-day. On fresh weight basis, further rise in proteins was recorded between 3 and 6-day but on dry weight basis a sharp fall was noticed in all cases except flowers having sucrose (0.1M) + 8-HQ (1.5 mM) as holding solution.

Flower diameter and protein content of petals followed a similar trend as they registered an increment between 0 and 3-day. Between 3 and 6-day, however, flower diameter and the amount of protein on dry weight basis showed a decline. But, protein content on fresh weight basis during 3 to 6-day again showed a rise in *C. officinalis* petals.

Protein degradation in cut flowers was observed in earlier studies with *Chrysanthemum dendranthema grandiflorum, Chrysanthemum coronarium* and *Calendula officinalis* (Kaur and Mukherjee, 2014; Kaur and Mukherjee, 2016) and also in *Salvia splendens* (Kaur et al., 2015). While working with uncut flower petals of *Calendula officinalis* and *Aster novae belgii*, gradual rise in proteins was recorded with the flower development but a sharp decline was also witnessed with the onset of senescence (Kaur et al., 2014). The decrease in the amount of protein is due to decreased synthesis and increased degradation (Celikel and van Doorn, 1995). The decrease in protein has been shown to precede the visible symptoms of senescence (Lay-yee et al., 1992).

Guaiacol peroxidase (GPOX) activity and its increment between 0 and 3-day, 3 and 6-day and 0 and 6-day have been presented in Table 6. Petals of cut scapes maintained as control or having some treatments in holding solutions showed increment in GPOX activity during 6-day. Petals receiving double distilled water and morphactin exhibited greater rise in peroxidase activity between 3 and 6-day than 0 and 3-day whereas in rest of the cases larger increase was recorded between 0 and 3-day in comparison to 3 and 6-day. However, degree of increase was much higher in control than other holding solutions during 0 to 6-day. Percent increase between 0 and 6-day was comparatively lower in petals getting the supply of 8-HQ (Table 6). Earlier work on *Calendula officinalis* and *Salvia splendens* cut flowers with some metabolites (ethyl alcohol, sugar) and other PGRs like 5-sulfosalicylic acid (5-SSA) and 6-benzyl aminopurine (6-BAP) revealed gradual increment in GPOX activity in control flower petals and much lower values were obtained with ethyl alcohol, sugar, 5-SSA and 6-BAP (Kaur et al. 2015). Another study with L-serine and spermine in *C. officinalis* also showed rise in GPOX activity in untreated cut flowers (Kaur and Mukherjee, 2015). Gradual increase in GPOX activity was also observed in uncut flowers of *C. officinalis* and *Aster novae belgii* from 'half open' to 'advanced senescence' stage. However, the percent increment between any two stages was quite different (Kaur et al., 2014). Higher GPOX activity catalyzes the breakdown of H_2O_2 and higher MDA level indicates the rapid disintegration

of membranes due to peroxidation (Paulin et al., 1986). Much lower GPOX activity after individual application of 8-HQ, morphactin and sucrose in comparison to untreated control and combined application may suggest comparatively lesser requirement of GPOX as production of H_2O_2 might have been reduced in former in the present investigation.

Table 5. *C. officinalis* showing changes in total protein ± S.E. (in fresh and dry weight basis, mg/100 mg) in flower petals of scapes maintained in sucrose (0.1M), morphactin (MOR,20µM), 8-hydroxy quinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M)+8-HQ (1.5mM) and sucrose (0.1M)+MOR (20µM)+ 8-HQ (1.5mM). [Initial Value: 1.578 ± 0.148 mg/100mg (fr.wt.), 11.019 ± 1.059 mg/100mg (dry wt.)].

Holding solutions	3-DAY		6-DAY	
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Control (DDW)	1.805±0.136	18.053±1.363	2.571±0.197	6.121±0.470
Sucrose	1.845±0.126	16.770±1.153	3.043±0.187	6.916±0.427
Morphactin	2.089±0.071	23.211±0.796	2.331±0.069	6.475±0.194
8-HQ	2.114±0.061	21.134±0.610	3.317±0.460	8.506±1.180
Sucrose +MOR	1.939±0.066	21.535±0.734	2.231±0.286	8.923±1.147
Sucrose +8-HQ	2.035±0.514	25.441±6.425	3.410±0.272	30.987±2.485
Sucrose +MOR+8-HQ	2.525±0.123	31.554±1.538	4.235±0.266	15.125±0.950

Holding solutions	% Difference in Protein on fresh weight		% Difference in Protein on dry weight	
	0 to 3-DAY	3 to 6-DAY	0 to 3-DAY	3 to 6-DAY
Control (DDW)	+14.385	+42.438	+63.835	-66.094
Sucrose	+14.472	+64.932	+52.192	-58.760
Morphactin	+32.383	+11.584	+110.645	-72.104
8-HQ	+33.967	+56.906	+91.796	-59.752
Sucrose +MOR	+22.877	+15.059	+95.435	-58.565
Sucrose +8-HQ	+28.961	+67.568	+130.883	+ 21.800
Sucrose + MOR + 8-HQ	+60.013	+67.723	+186.361	-52.066

Table 6. *C. officinalis* showing changes in guaiacol peroxidase (GPOX) activity (per mg protein min^{10}) ±S.E. in flower petals of scapes maintained in sucrose (0.1M), morphactin (MOR, 20µM), 8-hydroxyquinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20µm), sucrose (0.1M) + 8-HQ (1.5mM) and sucrose (0.1M) + MOR (20µM) +8-HQ (1.5mM). [Initial value: 0.040 ± 0.010].

Holding solutions			Percent Increment Between		
	3-DAY	6-DAY	0 to 3-DAY	3 to 6-DAY	0 to 6-DAY
Control (DDW)	0.078±0.015	0.276±0.016	95.00	253.85	590.0
Sucrose	0.121±0.063	0.184±0.06	202.50	52.07	360.0
Morphactin	0.074±0.012	0.172±0.003	85.00	132.43	330.0
8-HQ	0.141±0.003	0.162±0.005	252.50	14.89	305.0
Sucrose +MOR	0.182±0.017	0.205±0.006	355.00	12.64	412.5
Sucrose +8-HQ	0.153±0.038	0.182±0.005	282.50	18.95	355.0
Sucrose +MOR+8-HQ	0.184±0.028	0.190±0.009	360.00	3.26	375.0

Table 7. *C. officinalis* showing changes in MDA content (in nM g^{-1} fresh weight ± S.E.) in flower petals of scapes maintained in sucrose (0.1M), morphactin (MOR, 20µM), 8-hydroxyquinoline (8-HQ, 1.5 mM), sucrose (0.1M) + MOR (20µM), sucrose (0.1M) +8-HQ (1.5mM) and sucrose (0.1M) + MOR (20 µM) + 8-HQ (1.5mM). [Initial value 7.36 ± 0.90 nM g^{-1}].

Holding solutions			% Difference Between		
	3-DAY	6-DAY	0 to 3-DAY	3 to 6-DAY	0 to 6-DAY
Control (DDW)	17.29±0.06	32.34±0.40	134.92	87.04	339.40
Sucrose	16.58±0.68	29.28±1.17	125.27	76.60	297.83
Morphactin	8.84±0.42	15.48±0.32	20.11	75.11	110.33
8-HQ	9.48±0.73	16.79±0.35	28.80	77.11	128.13
Sucrose +MOR	8.32±0.33	13.67±0.93	13.04	64.30	85.73
Sucrose +8-HQ	9.04±0.45	11.93±1.29	22.83	31.97	62.09
Sucrose +MOR+ 8-HQ	8.00±0.35	11.48±0.34	8.70	43.50	55.98

Table 7 clearly showed a sharp rise in MDA content in petals of cut flowers during 6-day period. Percent rise was much higher in petals of those scapes getting either DDW or sucrose (0.1M). Treatments of MOR and 8-HQ were very effective in lowering the lipid peroxidation process. Further, both MOR and 8-HQ when applied along with sucrose, MDA content declined further. The order of effectiveness in controlling or lowering lipid peroxidation was sucrose + MOR + 8- HQ > sucrose + 8-HQ > sucrose + MOR > MOR > 8-HQ > sucrose > DDW. Investigations carried out earlier indicated increment in MDA content during flower senescence as in tulips (Jones and Mc Conchie, 1995), roses (Fukuchi-Mizutani et al., 2000) and *Gladiolus* (Ezhilmathi et al., 2007). Several studies undertaken earlier by the corresponding author has been described elsewhere (Kaur and Mukherjee, 2016).

Conclusion

From the overall account, the effective role of sucrose + 8-HQ as well as sucrose+MOR+8-HQ was clearly evident which reduced the loss of fresh weight of scapes and moisture content. Besides these combinations, individual sucrose treatment and sucrose + MOR as holding solution need special mention as they reduced the loss in flower diameter. Sucrose, MOR and 8-HQ reduced lipid peroxidation and protein degradation.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

Authors are grateful to the Chairman, Department of Botany, Kurukshetra University, Kurukshetra and Principal, University College, Kurukshetra for providing laboratory facilities. One of us (D.M.) wishes to thank Dr. N. Bhushan Mandava, Repar Corp., Maryland, USA for providing a gift sample of MCF. The financial assistance to D.M. as Asutosh Mookerjee Fellowship from Indian Science Congress Association, Kolkata (India) is also gratefully acknowledged.

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How to cite this article:
Singh, S., Mukherjee, S., Jakhar, S., Mukherjee, D., 2018. Interaction of sucrose, a morphactin and 8-hydroxyquinoline in the regulation of petal senescence of *Calendula officinalis* L. cut flowers. Int. J. Curr. Res. Biosci. Plant Biol. 5(12), 28-38. **doi:** https://doi.org/10.20546/ijcrbp.2018.512.004