DELAYING POST HARVEST SENESCENCE OF *Matricaria parthenium* L. FLOWERS USING ETHANOL, METHANOL AND SUCROSE

Kaur, P. and Mukherjee, D.[*]
Department of Botany, Kurukshetra University, Kurukshetra-136119, India
*****Chairman of Botany Department
Department of Botany, Kurukshetra University
Kurukshetra-136119, India
Tel : 09416038893
E-mail : dibumukherjee@gmail.com

ABSTRACT

Post harvest behavior and the effect of ethanol, methanol, and sucrose on the vase life of Matricaria parthenium cut flowers were studied. For this purpose, flower scapes were treated with different holding solutions. Scapes were immersed in the following preservative solutions: 4% sucrose, 2% ethanol, 2% ethanol + 4% sucrose, 2% methanol and 2% methanol + 4% sucrose. Data were recorded for moisture content, flower diameter and solution uptake at 2, 4 and 8-days after treatment. Among metabolites and enzymes changes in amount of total sugars, starch, specific activities of α-amylase and peroxidase, and lipid peroxidation were also determined and analyzed. Sucrose was found to be most effective in controlling senescence of cut flowers and this was further increased when 2% ethanol was added to the holding solution. Combined application of sucrose and 2% ethanol was able to reduce the increment in the amount of sugars, specific activities of peroxidase and α-amylase, malondialdehyde content and decline in starch. Thus the longevity was extended by 4 days more with this treatment.

Keywords Vase Life, Starch, α-Amylase, Sugar, Peroxidase, Malondialdehyde

INTRODUCTION

Flowers have a species-specific life span, and their senescence process is generally rapid and predictable compared with other plant organs. They are excellent model organ to study the mechanisms of plant senescence. Flower senescence is the terminal phase of developmental processes that lead to the death of flower, which include, flower wilting, shedding of flower parts and fading of blossoms (Voleti et al. 2000, Tripathi & Tuteja 2007, Seo et al. 2009). Senescence of flower petals is a complex process involving an increase of cell membrane permeability that results in wilting, pigment degradation and petal collapse (Jones & Mc Conchie 1995). Senescence is a highly controlled process and involves the progressive shut down of several biosynthetic pathways and the expression of different hydrolases that

hydrolyze polymers such as carbohydrates, proteins, lipids and nucleic acids. The hydrolyzed products are then transported to newly growing tissues where they are needed. Thus, senescence in flowers is connected with controlled degradation, remobilization and reutilization of cell components (Rubinstein 2000, O'Donoghue et al. 2002, Erdelska & Ovecka 2004). Senescence of cut flowers can be induced by several factors, e.g., water stress (Sankat & Mujaffar 1994), carbohydrate depletion (Ketsa 1989), micro-organisms (Witte & van Doorn 1991), and ethylene effects (Wu et al. 1992). Short post harvest vase life is one of the most important problems of the cut flowers. However, longevity of vase life is an important factor in consumer preference (Kader 2003, da Silva 2003). Ethanol has been found to be effective in increasing vase life of carnation flowers by inhibiting ethylene biosynthesis (Heins & Blakely 1980, Wu et al. 1992) as well as its action (Wu et al. 1991). Podd & Staden (2004) suggested that low concentration of either ethanol or acetaldehyde reduces the action of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, ACC oxidase and results in loss of protein which ultimately decreases the formation of ethylene. Hossain et al. (2007) reported that bougainvillea flowers longevity increased by applying 4, 8, and 10% ethanol. Metabolic sugars play an important role in maintaining flower quality during the postharvest period since they (sugars) are the main carbon-source utilized for energy in the cut flower metabolism (Ho & Nichols 1977; Kuiper et al. 1995; Monteiro et al. 2002). The translocated sugars accumulate in the petals, increasing their osmotic concentration, and improving their ability to absorb water and maintain turgidity (Rogers 1973, Ketsa et al. 1995, Kuiper et al. 1995, Ichimura & Hisamatsu. 1999, O'Donoghue et al. 2002). Applied sugars also act as respirable substrate and carbon source for the production of other compounds such as proteins, soluble carbohydrates and starch (Ho & Nichols 1977, Kuiper et al. 1995). Treatment with sucrose suppresses ethylene synthesis and reduces ethylene sensitivity (Mayak & Dilley 1976).

However, fewer studies have been carried out to show metabolic changes like starch, sugars and proteins as well as malondialdehyde (MDA) content and enzymatic activities (α-amylase and peroxidase) after the application of alcohols. Further, to know the alteration pattern of starch and sugars with the existing carbohydrate level, scapes of *Matricaria parthenium* were used for this investigation. Flowers of this plant are very attractive and grow in this part of India; and on which earlier work has been carried out in our laboratory (Rajni 2008- unpublished data).

MATERIALS AND METHODS

Plant material and treatments

Popular ornamental flowers of *Matricaria parthenium* L. (family- Asteraceae) were harvested from plants growing in the experimental plots in university botanical garden, Kurukshetra. For this experiment, 180 flowers *were*

collected for six treatments. Scapes (flower twigs without leaves) were cut under water to prevent cavitation and were immediately brought to the laboratory. The basal few centimeters of scapes were recut under distilled water to obtain an uniform length of 14 cm. The flowers were transferred to different test solutions in conical flasks, each containing 30 mL of the holding solution and three scapes. The treatments were 4% sucrose, 2% ethanol, 2% ethanol + 4% sucrose, 2% methanol and 2% methanol + 4% sucrose. Control sets with double distilled water (DDW) were also maintained. Petal samples were collected at 0, 2, 4 and 8 days after treatment. Observations on vase life, solution uptake, moisture content and flower diameter were recorded during the experimental set up.

Petal samples were collected for various biochemical determination of total sugars (reducing and non-reducing sugars); amount of starch; specific activities of α-amylase and peroxidase (POX) and malondialdehyde (MDA) content.

Determination of starch, α-amylase activity and total soluble sugars

The method of Hart & Fisher (1971) was also used to determine the amount of starch from the residue left after the separation of aqueous extract which was used for the determination of sugar. The standard curve of glucose at 630 nm was drawn. The amount of starch was calculated by multiplying the starch value in terms of glucose with 0.90.

The specific activity of α-amylase was measured by the method of Bernfeld (1951). The extract was prepared from two hundred mg of petals with 10 ml of DDW; centrifuged at 5000 rpm and the supernatant was used for determination of the enzyme activity. The reaction mixture contained 1% starch as substrate besides enzyme extract (1 mL each) and was incubated for 3 min. at 20 ºC. The enzyme reaction was interrupted by the addition of 2 ml of 3, 5-dinitrosalicylic acid over boiling water bath at 100 ºC for 5 min. Thereafter; it was cooled in running tap water. 10 ml DDW was added. Specific activity was expressed as α-amylase activity per mg protein. Protein was determined by the method of Bradford (1976). 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 by inversion. Protein was assayed spectrophotometrically at 595 nm using uv-vis spectrophotometer (Specord-205, Analytic Jena, Germany).

Determination of total soluble sugars was carried out by the anthrone method of Hart & Fisher (1971). Amount of reducing and non reducing sugars were calculated against a standard curve of glucose.

Determination of peroxidase (POX) activity

Total peroxidase (POX) activity was measured by the method of Maehly (1954). POX was assayed by measuring spectrophotometrically at 430 nm in a reaction medium consisting of 2 mL of crude enzyme, 2 mL of phosphate buffer (pH- 7.0), 2 mL of 20 mM guaiacol and 2 mL of 10 mM H_2O_2. It was expressed in terms of mg protein per 10 min. Protein was determined from the same extract following the procedure of Bradford (1976) as mentioned above.

Determination of malondialdehyde (MDA)

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content (Heath & Packer 1968). Two hundred mg of plant sample was homogenized in 2 mL of 50 mM phosphate buffer (pH 7.0).The homogenate was centrifuged at 8000 rpm for 20 min in a Remi centrifuge (R-8C). To 0.5 mL aliquot of the supernatant, 2 mL of 5 $g.L^{-1}$ thiobarbituric acid (TBA) in 200 $g.L^{-1}$ tri-chloro acetic acid (TCA) was added. The mixture was heated at 90 ºC for 30 min in the water bath and then quickly cooled in an iced water bath. After centrifugation at 8000 rpm for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption of each sample at 600 nm was also recorded and subtracted from the absorption recorded at 532 nm. The concentration of MDA, an end product of lipid peroxidation, was calculated according to its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Data were statistically analyzed using Prism version 3.0 and one-way ANOVA (Bonferroni Multiple Comparison Test) was used to find out the level of significance of various parameters.

RESULTS AND DISCUSSION

The longevity of the flowers was recorded as shown in Table 1. Applications of Suc, EtOH, EtOH + Suc, MetOH and MetOH + Suc as holding solutions extended the vase life of the flowers by 25%, 150%, 175%, 125%, and 50% respectively. Flower stems kept in flasks containing EtOH along with Suc had significantly increased vase life as well as cumulative uptake (mL flower-$scape^{-3}$) in comparison to the water control and all other holding solutions (Table 1). Vascular blockage has been cited as one of the reasons resulting water deficit followed by short vase life in cut flowers (van Doorn 1997). Farokhzad et al. (2005) have succeeded earlier to delay senescence in cut *Eustoma* flowers with 2 % EtOH along with 2.5 % Suc. Present findings further confirm the role of EtOH not only as a disinfectant but also, in improving conductance by reducing occlusions.

Moisture content of cut flowers having Suc and EtOH exhibited higher values than that of control. However, MetOH application showed lower values after 8days. Eight day old flowers showed a considerable reduction in diameter; the loss was minimized by Suc and both alcohols. A combination of 4 % Suc and 2 % EtOH brought about the best result (Table 2). Wilting was recorded in control flowers having only distilled water as holding solution.

Table 1: Effect of test solutions on cumulative uptake of vase solution and vase life in cut flowers of *Matricaria parthenium* L.

Treatments	Solution uptake*† (ml flower scape^{-3})	Vase life (days)
Control (DDW)	12.1±0.54	4
4%Suc	16.6±0.42	5
2%EtOH	17.1±0.57	9
2%EtOH + 4%Suc	17.3±0.59	10
2%MetOH	15.6±0.23	8
2%MetOH + 4%Suc	13.8±0.30	6

*Initial volume taken=30mL

†Each value indicates mean of 10 replicates and is significant at 5 % level.

Table 2: Flower diameter and moisture content of Matricaria *parthenium* L.

Days	Treatments	Moisture content (%) †	Flower diameter (cm)†
Initial stage	Control (DDW)	85.00±0.02	8.0±0.17
8-day	Control (DDW)	78.00±0.03	3.8±0.32
	4%Suc	82.00±0.05	5.0±0.18
	2%EtOH	86.40±0.01	6.6±0.13
	2%EtOH + 4%Suc	89.00±0.05	7.2±0.10
	2%MetOH	75.00±0.04	5.3±0.35
	2%MetOH + 4% Suc	74.00±0.01	5.0±0.31

†Each value indicates mean of 10 replicates and is significant at 5 % level.

Starch breakdown and increment in the amount of α-amylase activity were also noticed in this investigation during senescence of *M. parthenium* cut flowers which explained the rise in the level of sugars [Fig. 1(a)]. Initially starch content was 1.622 mg/100mg dry weight which gradually declined at 2nd, 4th and 8th day having an amount of 1.15, 0.41 and 0.26 mg/100mg dry weight respectively.

However, the amount of starch was higher for EtOH + Suc treated flowers than all other treatments. The decline in starch was in the order of EtOH + Suc ≈ MetOH ≈ MetOH + Suc ≈ EtOH < Suc < control.

α-Amylase activity of flower petals increased due to onset of senescence. At day 0, the specific activity of α-amylase was 0.01205 mg^{-1} protein and it was continuously increased with the passage of time. Among all the treatments, EtOH in combination with Suc was also most effective in retarding α-amylase activity; the percent increments were 56.06, 234 and 407 in comparison to control having 300.5, 491 and 721 % values respectively at 2^{nd}, 4^{th} and 8^{th} -day [Fig 1(b)]. The increment in α-amylase activity was in the order of EtOH < EtOH + Suc < MetOH < MetOH + Suc < Suc < control (DDW).

At initial stage, among the total sugars the amount of non-reducing sugars was significantly higher than reducing sugars. Further, a sharp increase in reducing, non-reducing and total sugars was noticed in *Matricaria* [Fig 1(c; d; e)]. The effectiveness of various holding solutions in lowering the quantity of reducing sugars was in the order of EtOH + Suc > MetOH + Suc > EtOH > MetOH > control > sucrose whereas for non-reducing sugars, sucrose alone could also bring down the concentration considerably besides EtOH and MetOH. Total sugar content in the petals varies throughout the evaluation period; showing a rise after 4 days possibly because of an increase in respiration rate and due to decline in starch content. On the whole, a combination of Suc + EtOH was found to be most effective in lowering the level of total sugars after 8days.

Petal senescence in *Iris* (van Doorn et al. 2003) and *Astroemeria* (Breeze et al. 2004) revealed the abundance of gene encoding a triose phosphate isomerase and sucrose synthase resulting considerable high concentration of sugars. Phloem exudate showed very high level of sucrose among different sugars which may be transported to various cells to fulfill requirements. As sugars are essential to improve osmotic solutes and to provide energy and carbon need of senescent petals (van Doorn & Woltering 2008), starch gets depleted constantly to release sugars as witnessed in the present investigation.

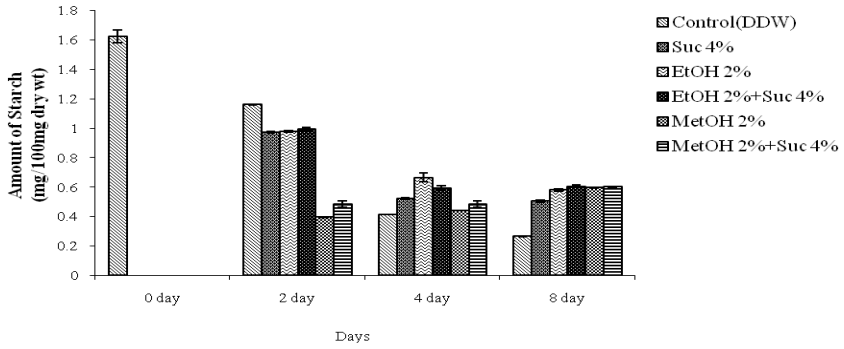

Figure1 (a) Effect of treatments on starch. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

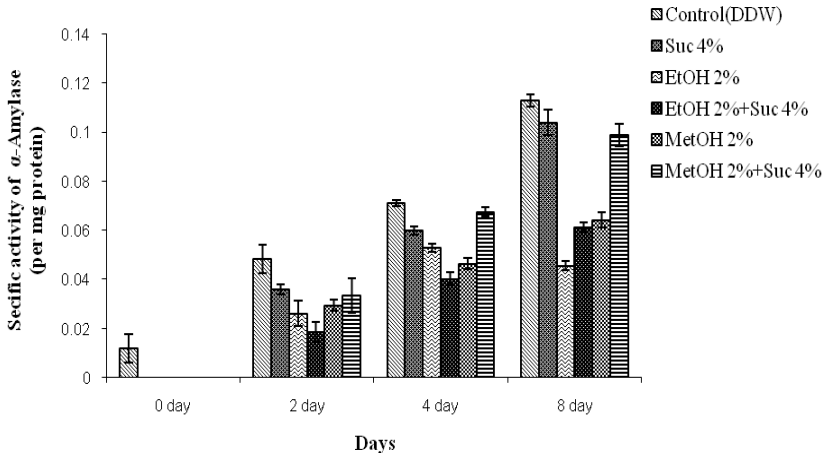

Figure1 (b) Effect of treatments on. α-amylase activity. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

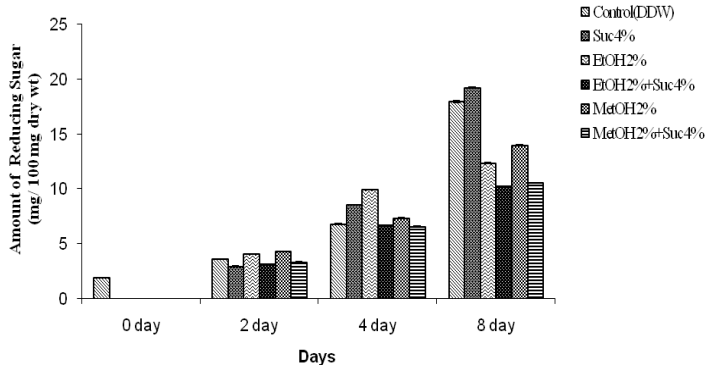

Figure1(c) Effect of treatments on reducing sugar. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

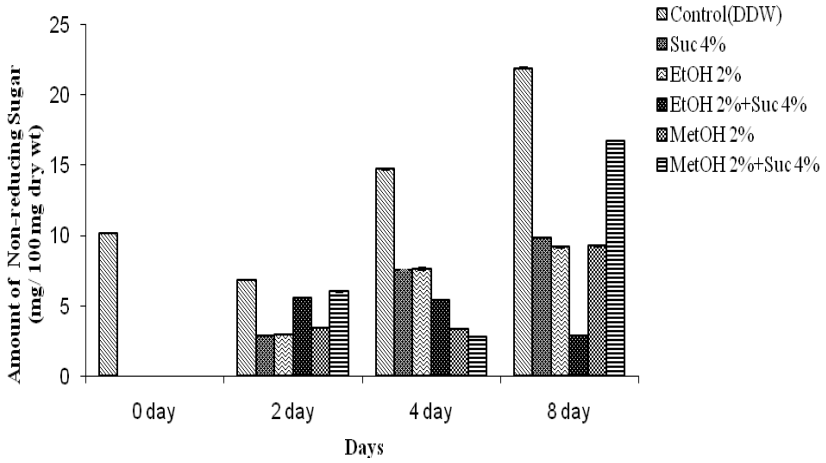

Figure1(d) Effect of treatments on non-reducing sugar. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

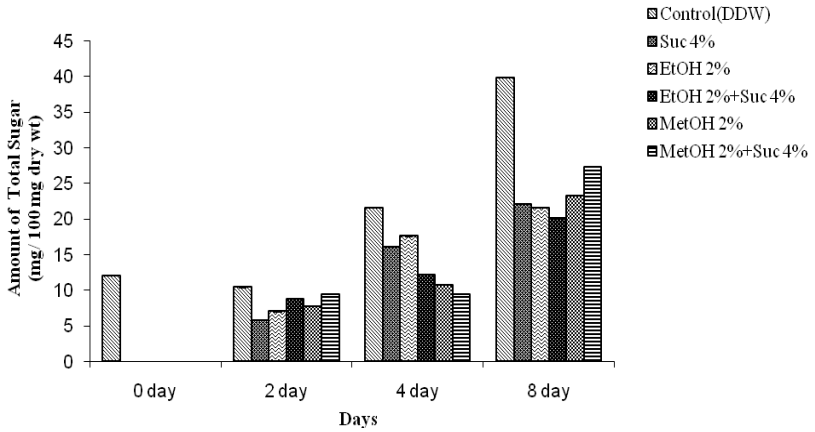

Figure1(e) Effect of treatments on total sugar. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

Regular and steady increment in peroxidase (POX) activity was noticed in *M. parthenium* from 0 to 8-day both in control and treated flowers [Fig 2(a)]. POX activity increased in association with senescence of petals of daylily (Panavas & Rubinstein 1998) and tulip (Carfantan & Daussant 1975). These findings and our results indicate that POX is involved in the senescence of *Matricaria* petals because it catalyzes the decomposition of H_2O_2. The application of EtOH + Suc showed least increment in POX activity as compared to other treatments on any specific day. At day-0 the activity was 0.05099 mg^{-1} protein 10 min^{-1}in control (untreated) flowers. At 2, 4 and 8-day amount of POX activity increased to 0.063627, 0.6275 and 1.19028 per mg^{-1} protein 10 min^{-1} respectively. These increments were successfully reduced by EtOH in combination with Suc having 0.048793, 0.1146 and 0.085565 per mg^{-1} protein 10 min^{-1} activity on 2, 4 and 8-day respectively. MetOH application could also lower the peroxidase activity but it was less effective than ethanol. These results indicate that EtOH + Suc caused significant decrease in POX activity compared to other treatments. Highest values of POX activity was found in cut flowers treated with Suc and control.

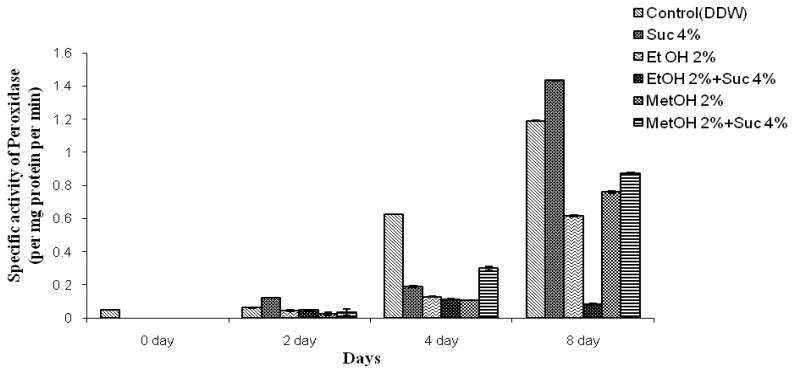

Figure 2 (a) Effect of treatments on peroxidase activity. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

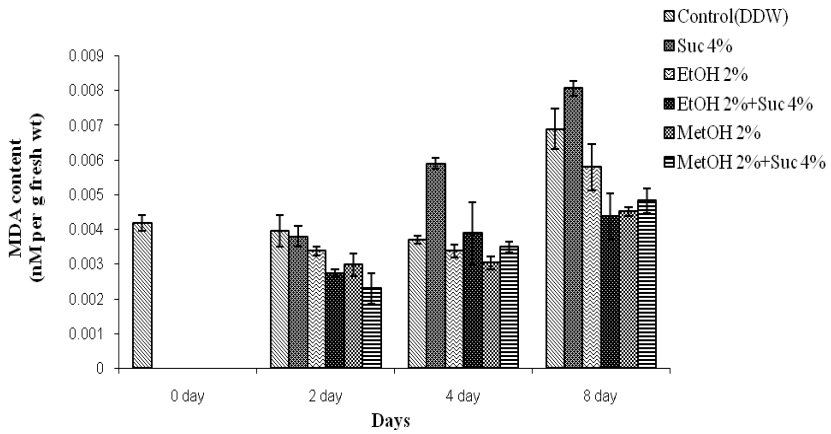

Figure 2(b) Effect of treatments on MDA content. Vertical bars represent standard errors (n=3). All values are significant at 1 % level according to Bonferroni Multiple Comparison Test (BMCT).

The actual role of peroxidase (POX) in senescence is very ambiguous because of their ability to produce lignin and to reduce growth by cross-linking wall materials (Lee & Lin 1995). Paliyath & Pinhero (2000) established that peroxidase activity is increased during flower development and especially senescence. Enhanced peroxidase activity has been found to be associated with an increase in the level of peroxides and free radicals, which reacted with cellular constituent (Fridovich 1975) and may be involved in the promotion of senescence (Brennan & Frenkel 1977). Increase in

specific activity of peroxidase in gladiolus (Yamane et al 1999; Hossain et al 2006) and daylily (Panavas & Rubinstein 1998) petals may be due to decrease in the total protein after flower opening (Lay-Yee et al. 1992).

A small reduction in MDA content was noticed between 2 and 4 day which again showed a sharp increase at day-8 during flower senescence in control scapes [Fig. 2(b)]. Individual sucrose treatment was not able to bring down MDA level as compared with controls but in combination with alcohols, the level was reduced appreciably. MDA content was reduced by EtOH + Suc and was significantly lower in preservative mixtures containing just MetOH compared to other treatments.

Loss of membrane integrity is the final and irreversible phase of senescence and is closely linked with membrane lipid peroxidation (Paulin et al. 1986). Increment in lipid peroxidation has been recorded earlier in studies with senescence of carnation flowers (Sylvestre et al. 1989, Smirnoff 1993, Bartoli et al. 1995) and petals in daylily (Panavas et al. 1998). Lipid peroxidation induced by AOS (active oxygen species) is considered as an important phenomenon of membrane deterioration (Irigoyen et al. 1992). Lipid peroxidation not only threatens the integrity and function of membranes and membranous proteins but also produces a variety of aldehydes and ketones (Valentine et al 1998, Wilhelmova et al. 2006). MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury (Janero 1990)

A low concentration of EtOH presumably decreased the formation of sugars due to control over starch degradation and α-amylase activity. Further, it also inhibits formation of MDA content and peroxidase activity which rise as senescence progresses.

CONCLUSION

From overall discussion, it can be concluded that 2% ethanol + 4% sucrose was effective in controlling the petal senescence and extending vase life/longevity of *Matricaria parthenium.*

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