# Regulation of lipoxygenase and superoxide dismutase activities and longevity of chrysanthemum cut flowers by ethanol and methanol

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## ABSTRACT

**Experiments were conducted with *Chrysanthemum sinense* cut flowers to find out post harvest behaviour and the effect of alcohols on the vase-life as well as senescence associated changes like lipid peroxidation, lipoxygenase (LOX) and total protease activities. Attempt was also made to assess how different concentrations of ethanol and methanol (2, 4 and 6%) used as holding solutions could minimize the degradation of protein and reduction in superoxide dismutase (SOD) activity. A gradual breakdown of proteins and increment in MDA content have been noticed during 6-day period. Activities of total protease and lipoxygenase registered a steady increasing trend while SOD activity exhibited a gradual decrease during the same period. Ethanol and methanol were not only effective to maintain higher values of flower diameter and moisture content but also bring about a reduction in total protease and lipoxygenase activities. Amount of proteins and SOD activity were higher in petals having alcohols as vase solutions than in controls. Our findings revealed that among all concentrations of alcohols, 2% ethanol was the best followed by 2% methanol to delay senescence and to increase the vase-life of *Chrysanthemum* cut flowers.**

**Key words:** *Chrysanthemum sinense*, lipoxygenase, protease, superoxide dismutase, vase-life.

## INTRODUCTION

Being one of the oldest ornamental plants chrysanthemums are used for large scale breeding and cultivation by big and small companies to be sold for consumers. In Germany and Netherlands, chrysanthemums occupy position one and three, respectively regarding the production and sale of potted plants and cut flowers. Therefore, extension of vase-life is an important area of investigation as it reveals structural, biochemical and molecular changes that can be regulated in various ways (Rubinstein, 17).

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 and Mc Conchie, 11). The biochemical changes associated with petal senescence include increase in hydrolytic enzymes, degradation of macromolecules and an increase in respiratory activity (Ezhilmathi *et al.*, 5). The longevity of cut flowers referred to as vase-life is influenced by harvesting conditions and post harvest handling and is an important quality criterion for cut flowers, often measured in days from the time flowers are harvested and placed in the holding solution to that of withering. Harvesting of flowers lead to petal senescence. The vase-life of carnation flowers has been generally determined by observing senescence profiles, *i.e.*, inrolling of petal margin and wilting of whole petals as well as ethylene production. Use of alcohols as holding solutions has revealed most effective role of ethanol to increase the vase-life of carnation flowers by inhibiting ethylene biosynthesis as well as its action (Wu *et al.*, 20). Exogenous application of ethanol has been shown to delay senescence of tomatoes (Kelly and Saltveit, 12) and improved vase-life of carnation and chrysanthemum flowers (Wu *et al.*, 20; Petridou *et al.*, 15).

This investigation has been undertaken, therefore, to find out how ethanol and methanol regulate the vase-life of chrysanthemum flowers taking some important biochemical changes like lipid peroxidation, protein content; and activities of protease, superoxide dismutase and lipoxygenase as little information is available on this aspect.

## MATERIALS AND METHODS

Flowers of *Chrysanthemum sinense* Sabine ex Sweet were harvested from the plants growing in the experimental plots at the University Botanical Garden, Kurukshetra. After harvesting the scapes in the morning hours they were cut under water using a sharp scalpel, brought to the laboratory and the basal few centimetres of scapes were recut under water to obtain an uniform length of 14 cm. Only the healthy scapes were transferred to different test solutions for experimentation. The day of transfer of scapes to the holding solutions was designated as zero day.

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Different concentrations of ethanol and methanol like 2,4 and 6% were used as holding solutions. For each concentration, 10 conical flasks were taken having 30 ml test solution. Double-distilled water (DDW) was served as control in each case. Three scapes were put in each flask. Entire experimental set up was maintained at a temperature of 25 ± 2°C under aseptic condition in the laboratory. The light intensity of fluorescent tubes was 2.24 µmol.$m^{-2}s^{-1}$during day and 1.13 µmol. $m^{-2}s^{-1}$ in night. Flowers were collected at 0, 2, 4 and 6 day and samples were made in such a manner that three replicates were available for each biochemical extraction and analysis. After recording the fresh weight, the samples were transferred to the deep freezer (-20°C) before carrying out various biochemical analyses. Triplicate samples were also put in the oven for collecting dry weight data. Throughout the course of the investigation various observations such as visible effect, longevity, volume of holding solutions absorbed, moisture content and flower diameter were also recorded.

For protein extraction, 100 mg of flower petals was homogenized in 80% ethanol. This was centrifuged at 5000 rpm for 10 min. The residue was re-extracted with 10 ml of 5% perchloric acid with same speed and time. The residue was re-extracted with 1N NaOH (5 ml) and kept in warm water (40-50°C) for 20 to 30 min. The clear supernatant containing total soluble protein was estimated by the method of Bradford (2) using Coomassie brilliant blue G-250. The ninhydrin method was followed for the estimation of protease activity as described elsewhere (Jakhar and Mukherjee, 9). The level of lipid peroxidation was measured in terms of MDA content (Heath and Packer, 8), while lipoxygenase (LOX) activity has been estimated according to the method of Doderer *et al.* (3). Superoxide dismutase (SOD) activity has been estimated by the method of Giannopolitis and Ries (7). The reaction was carried out in similar test tubes at 25°C for 15 min. in 100 µmol photon $m^{-2}s^{-1}$ 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, which under the experimental conditions caused a 50% inhibition of the reaction observed in the absence of enzyme.

## RESULTS AND DISCUSSION

Flowers were fresh at day-0, exhibited shrinkage after 2 days and the process was further intensified at day-4 and day-6 stages. Maximum shrinkage was noticed in those flowers which were maintained as control and less in those which were kept in 2% concentration of ethanol and methanol. The most effective concentration was 2% ethanol in delaying shrinkage, extending vase-life and maintaining freshness in flowers (Table 1). Reports are available that ethanol (4 and 6%) increased the vase-life of carnation flowers and cultivars showed variable responses to ethanol treatment with regard to vase-life increment. Two percent ethanol along with 2.5% sucrose can delay senescence in *Lisianthus* cut flowers. Ethanol also inhibits the conversion of ACC (1-amino cyclopropane-1 carboxylic acid) to ethylene as well as the action of ACC synthase, and even the formation of ACC (Wu *et al.*, 20).

Having higher moisture content of fresh cut flowers at 0-day, value decreased in treated and control sets with the advancement of senescence. Here also maximum moisture content up to 6 days was noticed in those flowers, treated with 2% ethanol and methanol (Table 1). Ethanol has the ability to extend the vase life of cut carnation flowers when applied as low concentration holding solution. As seen in Fig.1, a significant decline in protein contents in flower petals of *Chrysanthemum* was noted during 6-day. Protein breakdown was highly reduced at day-2 by the concentrations of ethanol and methanol in comparison to control. The percent decline in protein content at 2, 4 and 6 day in control were 19.457,49.538 and 80.598 respectively, whereas 2% ethanol appreciably brought down the degradation to just 3.261% at 2-day, 21.612% at 4 day and 56.431% at 6 day. One of the most important processes of petal senescence is protein degradation and remobilization (Wagstaff *et al.*, 19). Elanchezhian and Srivastava (4) have suggested that in *Chrysanthemum*, decrease in protein amount may be due to the inhibition of protein synthesis and/or enhanced protein degradation by proteases, which lead to loss of functional capability of membrane resulting in higher efflux of ions and finally senescence and death.

Like wise our investigation showed a sharp increase in protease activity and MDA content in flower petals from 0 to 6-day (Figs. 2 & 3).These flowers having treatments with different concentrations of ethanol and methanol, exhibited smaller increments especially at 2-day stage. Lower concentration of ethanol and methanol (2%) could appreciably bring down the protease activity and lipid peroxidation. Most of the studies on petal senescence have revealed a sharp rise in protease activity, e.g., *Hemerocallis* (Stephenson and Rubinstein, 18), *Petunia* (Jones *et al.,* 10) and *Iris* (Pak and van Doorn, 13). The percent increment values in MDA at 2, 4 and 6-day stages in control were 61.424, 117.804 and 193.175 respectively, whereas among all concentrations of alcohols, 2% ethanol was showing the best result. Lipid peroxidation (expressed as MDA level) is considered

**Table 1.** Flower diameter and moisture content of *Chrysanthemum sinense* Sabine ex Sweet., treated with different concentrations of ethanol and methanol (Mean ± SE, n = 10).

| Day | Treatment | Flower diameter (cm) | Moisture content (%) |
|---|---|---|---|
| 0-day | Initial value | 5.4 ± 0.076 | 86.90 ± 0.087 |
| 2-day | Contro l(DDW) | 4.5 ± 0.103 | 81.43 ± 0.074 |
| | Ethanol 2% | 5.4 ± 0.080 | 86.50 ± 0.086 |
| | Ethanol 4% | 5.3 ± 0.058 | 85.43 ± 0.076 |
| | Ethanol 6% | 5.1 ± 0.073 | 83.60 ± 0.074 |
| | Methanol 2% | 5.4 ± 0.079 | 86.20 ± 0.056 |
| | Methanol 4% | 5.2 ± 0.053 | 85.37 ± 0.068 |
| | Methanol 6% | 5.0 ± 0.083 | 82.24 ± 0.080 |
| 4-day | Control (DDW) | 3.6 ± 0.077 | 76.40 ± 0.064 |
| | Ethanol 2% | 5.1 ± 0.075 | 84.35 ± 0.057 |
| | Ethanol 4% | 4.8 ± 0.086 | 82.10 ± 0.083 |
| | Ethanol 6% | 4.1 ± 0.082 | 80.45 ± 0.090 |
| | Methanol 2% | 5.0 ± 0.083 | 84.13 ± 0.070 |
| | Methanol 4% | 4.7 ± 0.075 | 81.38 ± 0.050 |
| | Methanol 6% | 3.9 ± 0.065 | 80.17 ± 0.101 |
| 6-day | Control (DDW) | 1.9 ± 0.083 | 74.35 ± 0.065 |
| | Ethanol 2% | 4.4 ± 0.084 | 80.70 ± 0.084 |
| | Ethanol 4% | 4.0 ± 0.088 | 80.44 ± 0.100 |
| | Ethanol 6% | 2.0 ± 0.073 | 78.57 ± 0.080 |
| | Methanol 2% | 4.3 ± 0.068 | 80.63 ± 0.076 |
| | Methanol 4% | 3.8 ± 0.052 | 80.42 ± 0.081 |
| | Methanol 6% | 1.9 ± 0.052 | 78.20 ± 0.090 |

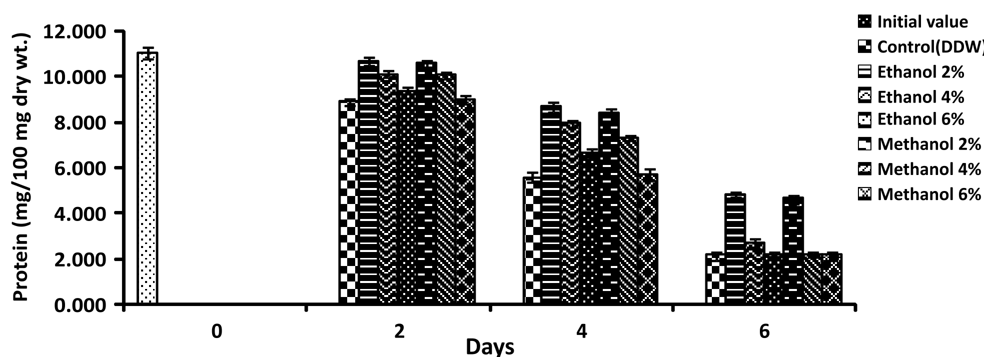


**Fig. 1.** Changes in protein content in *Chrysanthemum sinense* cut flowers after the application of alcohols.

to be an important mechanism of leaf senescence (Jakhar and Mukherjee, 9), which is induced by active oxygen species (AOS) and is considered to be associated with membrane deterioration. Various studies have indicated an increment in MDA content during senescence as in tulips (Jones and Mc Conchie, 11), roses (Fukuchi-Mizutani *et al.*, 6), and gladiolus (Ezhilmathi *et al.*, 5).

Changes in SOD activity has been shown in Fig.4 for cut scapes of *Chrysanthemum* kept in different concentrations of ethanol and methanol. Total SOD activity decreased regularly and gradually from initial

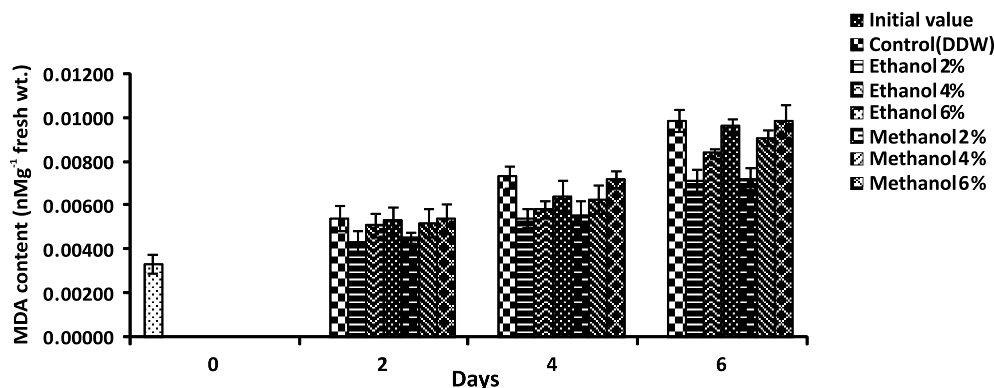


**Fig. 2.** Changes in MDA content in *C. sinense* cut flowers after the application of alcohols.

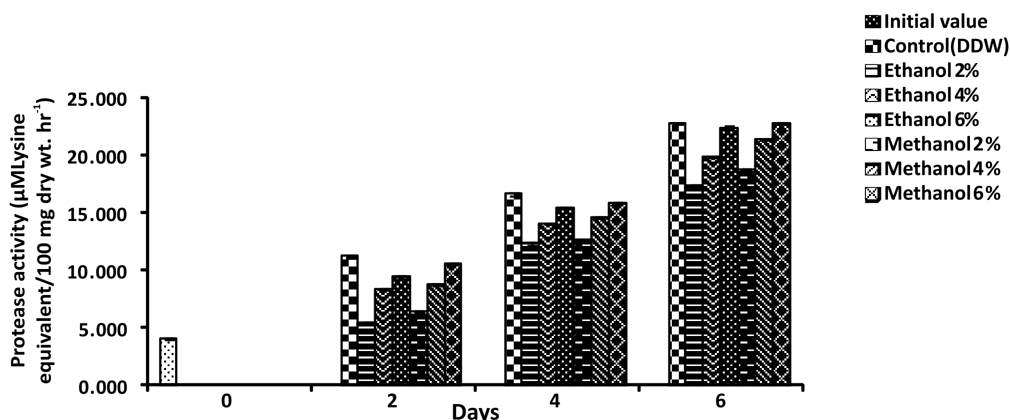


**Fig. 3.** Changes in activity of total protease in *C. sinense* cut flowers after the application of alcohols.

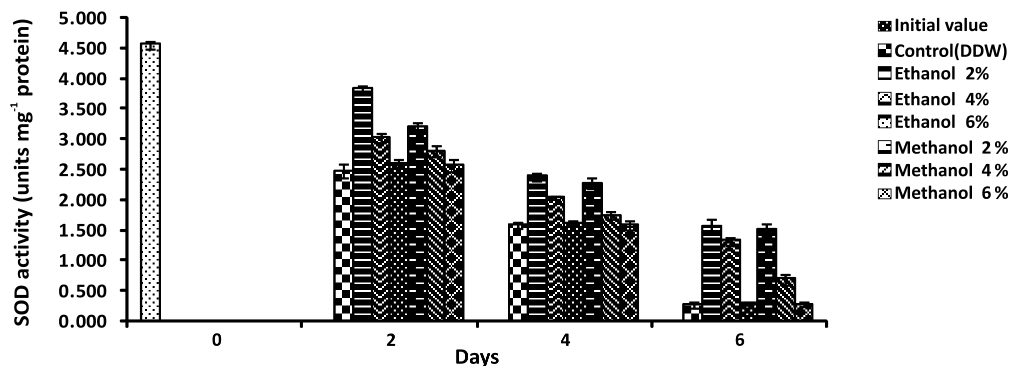


**Fig. 4.** Changes in SOD activity in *C. sinense* cut flowers after the application of alcohols.

to 6-day as cut scapes progressed to senescence. In untreated cut scapes total SOD activity declined by about 45, 65 and 94 percent during 2, 4 and 6-day, respectively. All concentrations of ethanol and methanol were found to be effective to curtail the reduction in SOD activity up to 4-day, but 2% concentration of ethanol and methanol was effective up to 6-day. Ethanol (2%) was able to curtail the reduction in SOD activity by about 29, 18 and 28 percent at 2, 4 and 6-day respectively, whereas 2% methanol increased

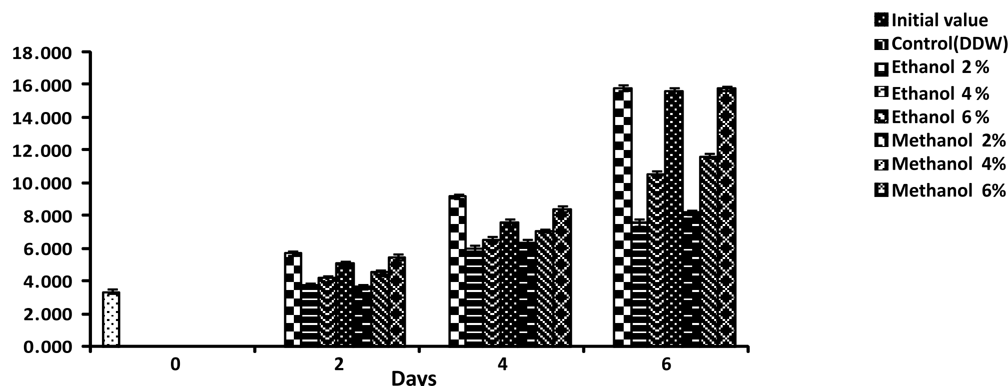


**Fig. 5.** Changes in LOX activity in *C. sinense* cut flowers after the application of alcohols.

the SOD by about 16, 15 and 27 percent at 2,4 and 6-day respectively when compared to untreated flower scapes. The increment in the SOD activity in ethanol and methanol (2%) treated flowers may be due to decrease in reactive oxygen species, which are the intrinsic part of plant senescence and inhibit the process of oxidative deterioration. Antioxidative enzymes such as SOD, catalase (CAT) and peroxidise (POD) are considered to be the main protective enzymes engaged in the removal of free radicals and AOS (Blokhina *et al.*,1). Advancement of senescence is characterized by the decline in SOD activity and the initiation of wilting process that emerged in the present study.

LOX activity increased by about 371 per cent during 0 to 6-day in untreated cut scapes. Ethanol and methanol concentrations of 2 and 4% were found highly effective in preventing it's increment up to 6-day, whereas 6% ethanol and 6% methanol also found effective up to 4-day only. Among all, 2% concentration of ethanol and methanol were found highly effective. Two percent ethanol prevented it's increment by about 58, 95 and 244 per cent during 2, 4 and 6-day, respectively when compared to control. The application of methanol (2%) too prevented rise in LOX activity over a period of 6-day in comparison to control. Elevated LOX activity has been cited as a common feature of senescent plant tissue by a number of studies such as in carnation flower petals (Rouet-Mayer *et al.*, 16), tulips (Jones and Mc Conchie, 11) and fully open *Gladiolus* flowers (Ezhilmathi *et al.*, 5). However, there are reports of decline in LOX activity during senescence in other plant systems such as in soybean cotyledons (Peterson and Siedow, 14). LOX is thought to play a primary role in generating peroxidative damage in membrane lipids, resulting in decreased level of lipid unsaturation and membrane fluidity.

From the overall discussion it can be concluded that all the selected concentrations of ethanol and methanol were effective in controlling the petal senescence by reducing degradation of SOD activity, protein breakdown, activities of protease, LOX and lipid peroxidation. However, the most effective among them was 2% ethanol in extending the vase-life of chrysanthemum cut flowers.

## ACKNOWLEDGEMENTS

University Research Scholarship received from Kurukshetra University, Kurukshetra, to the first author and Emeritus Fellowship of UGC, New Delhi to the corresponding author are gratefully acknowledged.

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Received : March, 2011; Revised : August, 2012; Accepted : October, 2012