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# Tomato Fruits Regulating Petal Senescence and Affecting Levels of UV-B Absorbing Pigments and Proline in Cut Flowers of *Calendula* and Two Species of *Chrysanthemum*

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***Abstracts* – The study was aimed to find out how any exogenous source of ethylene like 'just ripe' tomato fruit could regulate petal senescence in some common ornamental cut flowers like *Calendula officinalis* and two species of *Chrysanthemum* (*C. dendranthema grandiflorum* and *C. coronarium*) where no significant ethylene production has been recorded during senescence. Flowers placed in distilled water as holding solution have 'just ripe' tomato fruit and covered with a bell jar showed a marked decline in flower diameter, fresh weight, cumulative uptake of vase solution and vase life in comparison to sets maintained in similar manner without fruit. Presence of fruit has induced greater magnitude of lipid peroxidation in all the flower petals. Between initial and final day, a sharp decrease was noticed in the amount of protein and UV-B absorbing pigments specially in those sets having fruit. It was interesting to note a small decrease in UV-B absorbing pigments in the petals where no tomato fruit was kept. The difference in the amount of UV-B absorbing flavonoids was smaller between two sets (flower with and without 'just ripe' tomato fruit) in *Calendula* petals whereas it was much greater in *Chrysanthemum.* Amount of proline increased in cut flowers on last day but the increment was of lesser magnitude in sets having fruit inside the bell jar than sets without it. Very high concentration of proline in cut flowers without fruit may point out protective device against wilting and membrane damage. Many fold increment in proline concentration in sets without fruit compared to initial day level in *Chrysanthemum* spp. may indicate effective measure to tackle the stress. The degree of increment was relatively smaller in *Calendula*. Inclusion of fruit has accelerated senescence resulting shorter vase life.**

***Keywords* – Cut Flower, Flavonoids, Fruit, Lipid Peroxidation, Membrane Stability, Senescence.**

## I. INTRODUCTION

Flower senescence is the final developmental phase between the maturity and death of a flower or flower part and it is an integral process that allows the removal of a metabolically costly tissue (i.e. petal), after it has attracted pollinators for sexual development, and signals the initiation of ovule development and seed production [1]. Respiration in living cells that mediates the release of energy through the oxidative breakdown of carbon compounds (starch, sugars and organic acids) and the formation of carbon skeletons, is necessary for maintenance and synthetic reactions after harvest [2]. The senescence process is divided into the pre-climacteric, climacteric- and post- climacteric phases [3]. Increment in respiration has been noticed from pre-climacteric to the climacteric phase followed by a decrease in post-climacteric phase until the imminent death of the plant organ. Ethylene synthesis in flowers is triphasic having a low steady level during the pre-climacteric phase followed by a climacteric-like surge and then a sharp decline during the post-climacteric phase.

Ripening and senescence are the ultimate phases in the developmental events of fruits that result in expression of the quality characteristics inherent to the fruit [4]. Ethylene, a phytohormone is not only associated with plant growth and development but also regulates ripening and senescence [5]. A dramatic increase in ethylene initiates wilting of a number of cut flowers [6]-[8]. It induces flower senescence and bud abscission in hibiscus plants [9]-[10]. Senescence is characterized by a number of catabolic processes such as degradation of proteins, lipids and nucleic acids; lipid peroxidation, disruption of cell membranes, etc., but at the same time, it is associated with controlled degradation, remobilization and re-utilization of cell components [11]-[13].

Selected flowers in the present investigation belong to family Asteraceae where no significant rise in ethylene production has been recorded during senescence process as the amount of this phytohormone released is very small [8]. They have also stated that flowers belonging to Compositae (Asteraceae) and Umbelliferae in the dicotyledons do not exhibit dramatic senescence effects unlike *Dianthus, Petunia,* etc. even by exogenous ethylene application. To test whether the indirect supply of ethylene can initiate senescence processes in flowers of Asteraceae viz. *Calendula* and two species of *Chrysanthemum* and also what would be the magnitude of physiological and biochemical difference between these genera, it was thought to place 'just ripe' tomato fruits inside the closed chamber where scapes were already kept in flasks containing double distilled water (DDW). Flowers without fruits in the enclosed bell jars were also maintained for a comparison. Specific changes such as uptake of vase solution, flower diameter, lipid peroxidation; amounts of proline, protein and UV-B absorbing flavonoid were observed in sets having tomato fruits or without them to assess the role of ripe tomato in regulating petal senescence in cut flowers.

## II. MATERIALS AND METHODS

### A. Plant materials and growth conditions

Plants of *Calendula officinalis* L. and *Chrysanthemum sp.* like *Chrysanthemum dendranthema grandiflorum* var. Chandrima and *C. coronarium* (*Glebianis coronarium*), known to be excellent indoor and outdoor ornamentals, were grown in uniformly maintained experimental plots of University Botanical Garden, Kurukshetra under natural condition of day and night. The length, breadth and height of the cage were 12×12×2.5 m respectively. Within the cage three experimental plots each were used for growing *C. officinalis* and two species of *Chrysanthemum* plants. The dimension of each plot was 3×2 $m^2$ and was provided with 40 Kg of dung manure. The pH of the soil and soil conductivity were 8.50 and 0.12 dc respectively. The seeds of deep orange-flowered variant of *C. officinalis* and white-flowered variant of *Chrysanthemum* were sown in the pulverized seed beds of the cage in rows during September-October and flowers were produced during January-March. The spacing between adjacent plants and between rows was 30 cm. Uniformity in length and flower head diameter was maintained while flowers were tagged in mid-January. Variations in day/night temperature were 12-15 ºC/4-7 ºC. Pesticides were not used at any stage of growth and development. Water was provided to these plants at 3-day intervals during vegetative and flowering periods. The tagged flowers were harvested at fully open stage (see Plate 1).

### B. Harvesting

The scapes harvested in the morning hours, cut under water to prevent cavitations and were immediately brought to laboratory. The basal few centimeters were recut under double distilled water (DDW) to obtain an uniform length of 14 cm. These healthy scapes were transferred to conical flasks (Borosil-make, India) of 100 ml capacity having DDW (50 ml in case of *C. dendranthema grandiflorum* and 30 ml in scapes of two other plants). Each flask with three cut flowers in holding solution was covered by a bell jar and it was made air tight. Flowers of *Chrysanthemum* were placed with two tomato fruits while one tomato was placed with *Calendula* flowers as the former exhibited longer vase life as compared to *Calendula*. Entire experimental set up was maintained at a temperature of 23 ± 2 ºC under aseptic condition in the laboratory. The light intensity of fluorescent tubes was 2.24 µmol $m^{-2}$ $s^{-1}$ photon flux density (PFD) during day and 1.13 µmol $m^{-2}s^{-1}$ PFD in night.

### C. Samplings

Samples were collected at initial representing 'fully open stage' and final day when petals of cut flowers start wilting. Flower petals were plucked with the help of forceps from different treatments, washed and dried in the folds of filter paper and samples were made in such a manner that three replicates were available for each biochemical extraction and analysis. After recording the fresh weight samples were put in the deep freezer for storage to arrest any biochemical change. Triplicate samples were also put in the oven for collecting dry weight data.

### D. Flower fresh weight and moisture content

Changes in moisture content as well as fresh weight were determined at each flower development stage for each species. Fresh weight of each flower (only flower head) was measured immediately after harvest.

Dry mass was determined by drying at least three replicates of 100 mg each of the petal sample in oven at 70 ºC for 24 h until reaching a constant weight. Moisture content was determined as the difference between fresh and dry mass.

### E. Vase life

External appearance and freshness of flowers during the course of post-harvest life were recorded regularly.

### F. pH of vase solution

pH of vase solutions was recorded at the initial and final day of experimentation.

### G. Determination of MDA and Protein content

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the method of Heath and Packer [14]. Two hundred milligram of petal 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 0.5ml aliquot of the supernatant, 2 ml of 5 g $L^{-1}$ thiobarbituric acid (TBA) in 200 g $L^{-1}$ trichloroacetic acid (TCA) was added. The mixture was heated at 90 ºC for 30 min in the water bath and then quickly cooled in an ice 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 $mM^{-1}cm^{-1}$.

Protein content was estimated by the method of Bradford [15] using Coomassie Brilliant blue G-250. Bovine Serum Albumin (BSA) was used for making a standard curve for protein estimation.

### H. Determination of proline content

Proline content was measured by the method of Bates et al. [16]. Two hundred mg of petal sample was homogenized in 2 ml of 3 % sulfosalicylic acid. The homogenate was centrifuged for 15 min at 10, 000 rpm in a Remi centrifuge (R-8C). Free proline in the supernatant was treated with 2 ml of 3 % GAA (glacial acetic acid) and 2 ml of acid ninhydrin. Reaction mixture in the test tubes was kept in boiling water bath for 1 hour at 95ºC. Reaction was terminated in the ice bath and colour complex was extracted in 4 ml of pure toluene. The absorbance was recorded at 520 nm in a uv-vis spectrophotometer (Specord 205, Analytic Jena, Germany). The amount was calculated using a standard curve for proline estimation.

### I. Flavonoids

UV-B absorbing pigments were extracted from 50 mg flower petals by keeping them in acidified methanol (methanol: water: HCl= 78: 20: 2; v/v/v) for 24 h at 4 ºC [17]. The filtered extract was then used for measuring the absorbance at 320 nm, which was indicative of relative concentration of UV-B absorbing flavonoid contents, expressed as absorbance $g^{-1}$ fresh mass of tissue at 320 nm.

## III. RESULTS AND DISCUSSION

Changes in fresh weight, moisture content, cumulative uptake of holding solutions and vase life of cut chrysanthemums (*C. dendranthema grandiflorum, C. coronarium*) and calendula (*C. officinalis*) have been presented in Table 1-2. Initial stage represented fresh cut flowers of fully open stage attaining maximum diameter. Flowers getting the supply of DDW and tomato fruits nearby within the enclosure (bell jar) started wilting on first, third and seventh day in *C. officinalis, C. coronarium* and *C. dendranthema grandiflorum* respectively. Similar setup of flowers without fruit have maximum vase life of four, six and ten days in cut flowers of *C. officinalis, C. coronarium* and *C. dendranthema grandiflorum* respectively. Reduction in flower diameter, fresh weight and moisture content was noticed in those cut flowers which had tomato fruits (Table 1). Such reduction in flower diameter and fresh weight is normal phenomenon and observed elsewhere and recently it has been found in *Helleborus orientalis Lam.* cv. Olympicus [18].

No microbial activity was found in these vase solutions regardless of having ripe tomatoes inside the bell jar, as flow of air has been completely eliminated. pH changes were recorded considerably higher in sets devoid of tomatoes whereas negligible change was seen in *C. officinalis* sets without fruits. *C. dendranthema grandiflorum* sets without fruits recorded higher pH in vase solution on final day while the other species of chrysanthemum (*C. coronarium*) exhibited lower value. Vascular blockage is one of the important reasons resulting slow uptake of holding solution and short vase life in cut flowers [19]. Presence of microorganisms is responsible for reduction in pH of vase solution [20]. Reduced pH generally has been considered to improve vase life of flower and most flower preservatives contain acid to reduce the pH of vase solution [21]. There was no vascular blockage and no microbial invasion has been witnessed in these flowers but increments of fungal attack at the centre of flower heads have been detected (see Plate 2).

Other visible symptoms of senescence like wilting, fading of colours in petals, browning of petals can be seen on last day in flowers placed with fruit. As fruit (Tomato) starts ripening with the progressive day, release of ethylene might have started. Lower whorls of petals were more affected by fruit ripening process as they are closer than upper whorls. Petals show colour change as shown in Plate 3. Although ethylene is the major promoter of flower senescence in ethylene sensitive flowers, coordinating senescence pathways and floral abscission [8], [22] flowers belonging to Asteraceae, as in the present study clearly demonstrated quick response to released ethylene by ripe tomato fruits. In a number of cut flowers, a dramatic increase in the amount of released ethylene has been well established in accordance with the wilting of a number of cut flowers [6]-[8].

### A. Lipid peroxidation

Changes in MDA content in petals of cut flowers have been depicted in Table 2. Lipid peroxidation increased markedly in these flowers from initial values; maximum rise was noticed in *C. officinalis* having tomato fruit followed by *C. coronarium* and *C. dendranthema grandiflorum*. Sets having ripe fruits inside bell jars had much larger values of MDA than those without fruits. Increments in lipid peroxidation were observed earlier during senescence in *Chrysanthemum* [23] and daylily [24]. MDA is known to be a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury [25]. Lipid peroxidation not only threatens the integrity and function of membranes and membranous proteins but also produce a variety of toxic aldehydes and ketones [26], [27].

### B. Protein and flavonoids content

Protein content shows a significant decline on final day in all three cases. These decreases were higher in flowers having ripe tomato as compared to sets without fruit (Table 2). Protein breakdown occurs in all cell organelles viz. proteasomes, vacuoles, mitochondria, nucleus and chloroplasts. However, greater degradation has been noticed in vacuoles [28]. Drastic reduction in protein levels has been noticed in *Ipomoea* [29], [30] and *Petunia* [31] petals prior to visible senescence. The decrease in protein levels during senescence may be due to decreased synthesis and/or increased degradation [31]. In *Hemerocallis*, a sharp decrease in protein levels has been shown to precede the visible symptoms of senescence [32]. No upsurge of in vitro protease activity was detected in *Ipomoea* during petal senescence [29], [30] but a marked increase in protease activity was recorded in many flowers including *Hemerocallis* [34] and *Petunia* [31].

Amount of flavonoids also decreased on last day but the magnitude of decline was smaller in sets without tomato in comparison to sets having tomato specially in both species of *Chrysanthemum*. In *Calendula* petals, the difference in the amount of flavonoids was smaller between two sets (flower with and without fruits) whereas it was much greater in *Chrysanthemums*. Anthocyanins and anthocynidins are coloured flavonoids while flavones anf flavonols appear colourless in visible light as they generally absorb light at shorter wavelengths than anthocyanins do [35]-[37]. The major function of flavones and flavonoids is to protect cell from excessive UV-B radiation (280-320 nm). They possess significant antioxidant and chelating properties [38]. During senescence, sugars as respiratory substrate get depleted and protein degraded to release free amino groups resulting more alkaline pH in the cell [39]. Rise in pH is responsible for 'blueing' where a shift from red to blue colouration has been noticed in petals with the advanced growth [2], [35]. Available data of flavonoids with respect to UV-B absorbing pigments indicate that presence of tomato is responsible for the decline in these pigments. Therefore, the release of ethylene (from ripe tomato) is responsible for the drastic decline in these pigments; which was much greater in two species of *Chrysanthemum* than *Calendula*. The vase life of *Chrysanthemum* flowers was much better than *Calendula* owing to higher concentration of UV-B absorbing pigments of flavonoids.

Table 1: The pH of vase solutions, flower diameter and fresh weight in cut flowers of *Chrysanthemum dendranthema grandiflorum* var. Chandrima, *Chrysanthemum coronarium* and *Calendula officinalis* on initial and final day and vase life when experiment was set with and without ripe tomato fruits.

| ***Chrysanthemum dendranthema grandiflorum* var. Chandrima** | | | | |
|---|---|---|---|---|
| | **With Fruit** | | **Without Fruit** | |
| | **Initial Day** | **Final Day** | **Initial Day** | **Final Day** |
| pH of vase solutions | 7.04 | 7.52 | 7.02 | 7.42 |
| Flower diameter (cm) | 6.96 ±0.42 | 5.41±0.39 | 6.98 ±0.264 | 6.08 ±0.50 |
| Fresh weight (fr. wt.) (gm) | 3.19 ±0.20 | 2.54 ±0.61 | 3.20 ±0.44 | 2.68 ±0.38 |
| Percent decline (in fr. wt.) | 48.25 | | 29.80 | |
| Vase life (day) | 3 | | 6 | |
| ***Chrysanthemum coronarium*** | | | | |
| pH of vase solutions | 7.02 | 7.83 | 7.01 | 6.79 |
| Flower diameter (cm) | 12.8 ±0.41 | 6.90 ±0.33 | 13.1 ±0.14 | 10.22 ±0.25 |
| Fresh weight (fr. wt.) (gm) | 14.9 ±0.55 | 7.72 ±0.68 | 15.1 ±0.35 | 10.60 ±0.61 |
| Percent decline (in fr. wt.) | 48.25 | | 29.80 | |
| Vase life (day) | 3 | | 6 | |
| ***Calendula officinalis* L.** | | | | |
| pH of vase solutions | 7.04 | 7.98 | 7.04 | 6.95 |
| Flower diameter (cm) | 6.80 ±0.39 | 4.20 ±0.57 | 7.00 ±0.38 | 5.90 ±0.42 |
| Fresh weight (fr. wt.) (gm) | 3.95 ±0.47 | 1.86 ±0.14 | 4.02 ±0.36 | 2.45 ±0.12 |
| Percent decline (in fr. wt.) | 52.91 | | 39.05 | |
| Vase life (day) | 1 | | 4 | |

Each value indicates ±SE.

Table 2: Effect of ripe tomato fruits on cumulative uptake of vase solution (ml), moisture content (%), lipid peroxidation (n mol mg$^{-1}$ protein min$^{-1}$), proline content (µg proline g$^{-1}$ fr. wt.), amount of protein (mg 100mg$^{-1}$ dry wt.) and flavonoid content (absorbance g$^{-1}$ fr. wt.) in cut flowers of *C. dendranthema grandiflorum*, *C. coronarium* and *C. officinalis*.

| **Flowers** | ***Chrysanthemum dendranthema grandiflorum*** | | | ***Chrysanthemum coronarium*** | | | ***Calendula officinalis* L.** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Initial Day** | **Final Day** | | **Initial Day** | **Final Day** | | **Initial Day** | **Final Day** | |
| | | **With Fruit** | **Without Fruit** | | **With Fruit** | **Without Fruit** | | **With Fruit** | **Without Fruit** |
| Vase solution uptake | * | 14 ±0.24 | 22±0.36 | ** | 9 ±0.07 | 16±0.41 | ** | 8±0.46 | 16±0.15 |
| Moisture content | 91.7 ±0.17 | 85.7 ±0.43 | 89.6 ±0.53 | 92.0 ±0.91 | 74.4 ±0.31 | 80.6 ±0.27 | 84 ±0.62 | 62.4 ±0.43 | 70.8 ±0.21 |
| Lipid peroxidation | 1.05 ±0.08 | 2.04 ±0.06 | 1.51 ±0.03 | 0.82 ±0.02 | 2.98 ±0.06 | 1.02 ±0.01 | 0.226±0.04 | 3.46 ±0.04 | 1.81 ±0.03 |
| Proline content | 1.5±0.02 | 6.8 ±0.041 | 27 ±0.56 | 2.3 ±0.58 | 7.4 ±0.46 | 15±0.33 | 0.08±0.001 | 0.195±0.01 | 0.261±0.003 |
| Protein content | 13.05±0.61 | 2.68 ±0.02 | 4.59 ±0.03 | 17.2 ±0.29 | 4.8 ±0.056 | 6.95 ±0.18 | 8.46 ±0.06 | 3.2 ±0.11 | 4.9±0.26 |
| Flavonoid content | 18.4 ±0.43 | 7.9 ±0.054 | 16.25±0.22 | 16.6 ±0.24 | 7.18 ±0.08 | 12.5 ±0.37 | 18.1 ±0.55 | 10.56±0.38 | 12.18 ±0.44 |

*= Volume of vase solution at initial day is 50 ml; **= Volume of vase solution at initial day is 30 ml.
Each value indicates ±SE.

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**Initial Day of Experimental set up** **Final Day of Experiment**

***Chrysanthemum dendranthema grandiflorum* var. Chandrima.**

***Chrysanthemum coronarium***

***Calendula officinalis* L.**

Plate 1**:** Photographs taken on initial and final day of experimental set up for morphological observations.

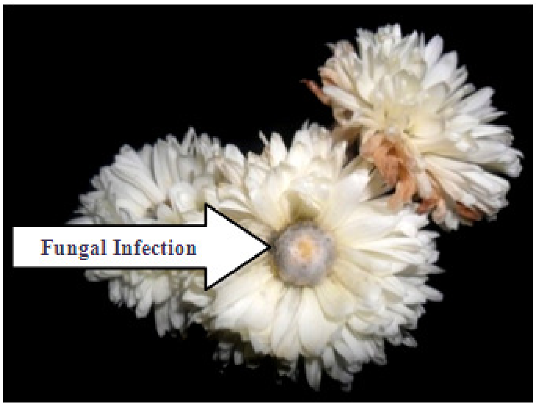

Plate 2: Picture showing fungal infection at the centre of its flower head

Plate 3: Picture showing petal discoloration.

### *C. Proline content*

The quantity of proline at initial day was very low; about 1.5, 2.3 and 0.08 µg/ml were recorded in cut flowers of *C. dendranthema grandiflorum, C. coronarium* and *C. officinalis* respectively (as shown in Table 2).. These values increased by 353, 221 and 143 percent in cut flowers of above mentioned plants respectively when placed with fruits. However, in these cut flowers without fruits, the percent increments were 1700, 552 and 226 respectively. It appears that sets without fruits developed more stress resulting much higher concentration of proline to ensure protection of flower petals from wilting and membrane damage. In sets having ripe tomato, release of ethylene could not be avoided so that the level of proline was much lower. Rapid accumulation of free proline is a common phenomenon of stress as has been noticed in several studies [40].

## IV. CONCLUSION

Finally, it can be concluded that inclusion of 'just ripe' tomato fruit in the experimental set up was responsible for a drastic change in flower diameter, vase solution uptake and various physiological changes resulting shorter vase life due to probable release of ethylene. Notable changes observed in petals as a result of fruit are membrane damage, significant decrease in protein and UV-B absorbing pigments. Amount of proline continued to increase in both sets but much higher level was recorded in sets without fruit.

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