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ROLE OF PLANT GROWTH REGULATORS IN PETAL SENESCENCE OF *CALENDULA OFFICINALIS* L. AND *COREOPSIS LANCEOLATA* L.

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Freshly cut flowers of *Calendula* as a detached system and flowers of *Coreopsis* in an intact system were taken to carry out a comparative study of petal senescence. Changes in proteins, amount of sugars and MDA (Malondialdehyde) content were estimated with the advancement of flower senescence. Activities of peroxidase and protease registered a steady increase during 0 to 8-day and 0 to 16-day in *C. officinalis* and *C. lanceolata* respectively. Higher concentration of each plant growth substance was effective to slow down the enzymatic activity and reduced the degradation of various metabolites. The distribution pattern of sugars and degree of effectiveness of growth regulators were not identical in these two flowers. The overall order of effectiveness among the applied plant growth regulators was KN> SA> MOR in both the flowers.

Key words -Enzymatic activities, senescence, kinetin, morphactin and salicylic acid.

Flower senescence represents the last stage of flower development leading to death of the petals. It is generally rapid and continuous similar to whole plant or leaf senescence (Voleti *et al*. 2000) and the later stages of senescence share many characteristics of programmed cell death (Rubinstein 2000).

The biochemical changes associated with petal senescence include an increase in hydrolytic enzymes, degradation of macromolecules and an increase in respiratory activity(Reid and Wu 1992).Although, low concentrations of various chemical solutions such as sugars, biocides, plant growth regulators and mineral ions ,besides water have been used as holding solutions to prolong vase life of cut flowers (Skutnik *et al*.2006),detailed physiological and biochemical studies are not many in the area of flower senescence. Further, a comparative assessment of senescence changes in flowers with respect to intact system and detached system have not been made earlier. Attempt was made, therefore, to study some of the selected metabolic alterations in petals of *Coreopsis*(intact system) and that of *Calendula* (detached system) in the natural control system and also after the flowers were treated with any one of the selected plant growth regulators like kinetin, salicylic acid and morphactin, which are widely used to delay senescence.

MATERIALS AND METHODS

Flowers of *Calendula officinalis* L. and *Coreopsis lanceolata,* L. belonging to family Asteraceae have been selected for the present study. Fresh flowers of *Calendula* were harvested from the plants growing in the experimental plots in university botanical garden, Kurukshetra. After harvesting the scapes in the morning hours they were cut under water using a sharp scalpel to prevent cavitation and were immediately brought to the laboratory. The basal few centimeters of scapes were recut under water to obtain an uniform length. Only the healthy scapes were transferred to different test solutions for experimentation. Conical flasks (Borosil-make)of 100 ml capacity were used to have different concentrations of kinetin (KN), salicylic acid (SA) and morphactin (MOR, Chlorflurenol methyl ester 74050).Selected concentrations of KN and SA were 3.75 and 37.5MM whereas that of MOR were 3.64 and

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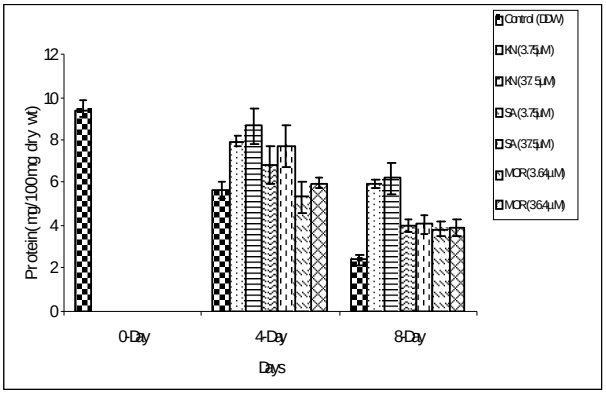

Figure-1 Protein content of *Calendula officinalis*

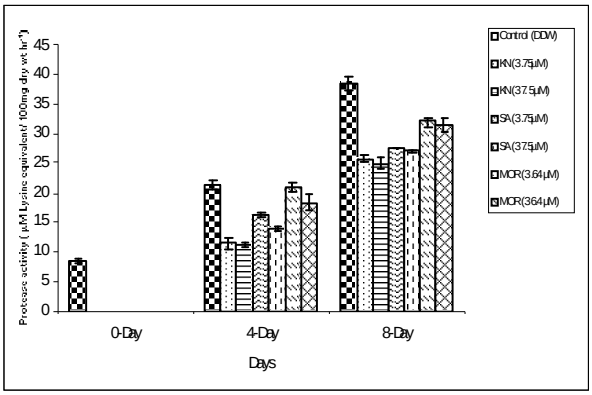

Figure-5 Protease activity in *Calendula officinalis*

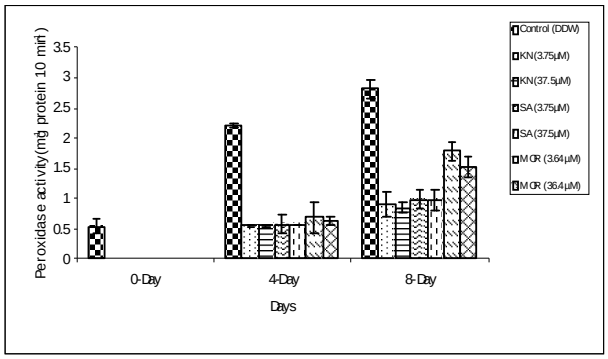

Figure-2 Activity Peroxidase in *Calendula officinalis*

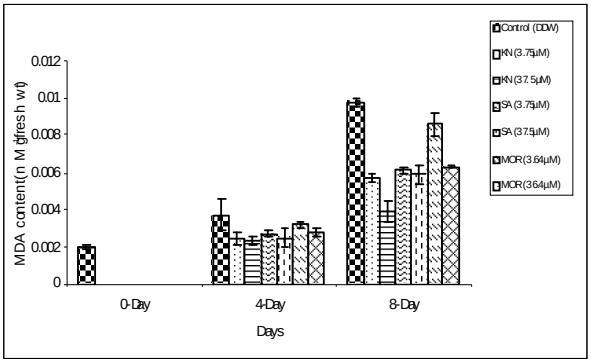

Figure-6 MDA content in *Calendula officinalis*

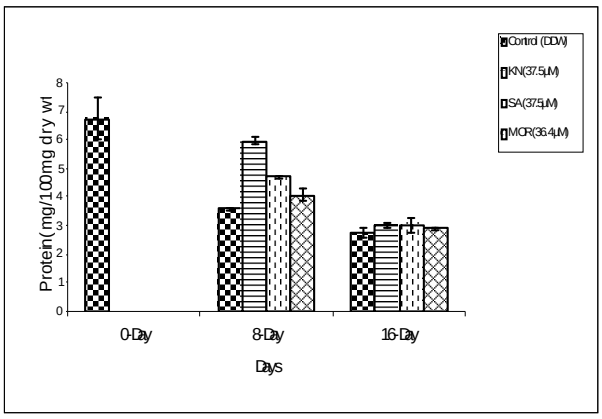

Figure-3 Protein content of *Coreopsis lanceolata*

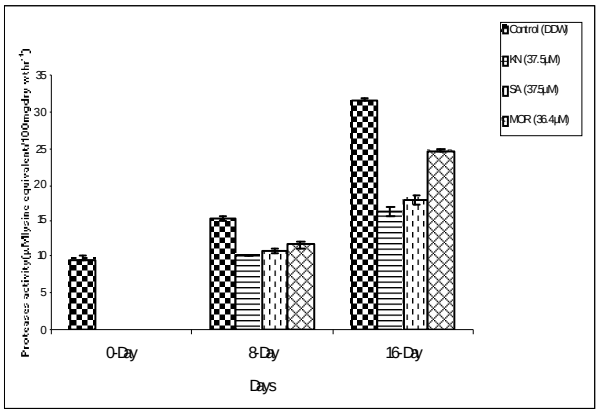

Figure-7 Protease activity in *Coreopsis lanceolata*

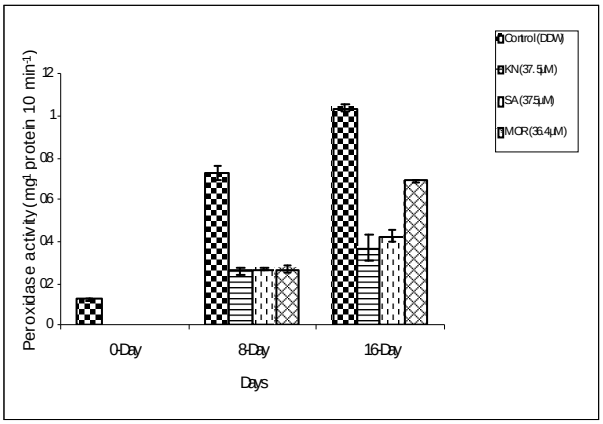

Figure-4 Activity Peroxidase in *Coreopsis lanceolata*

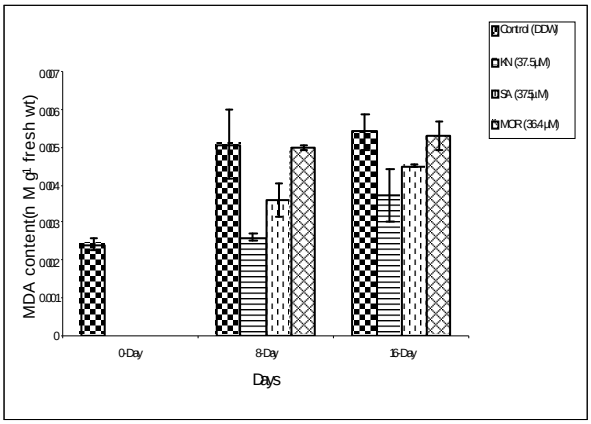

Figure-8 MDA content in *Coreopsis lanceolata*

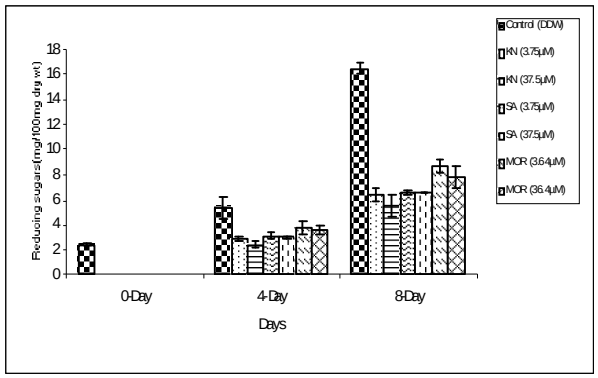

Figure-9 Reducing sugars in *Calendula officinalis*

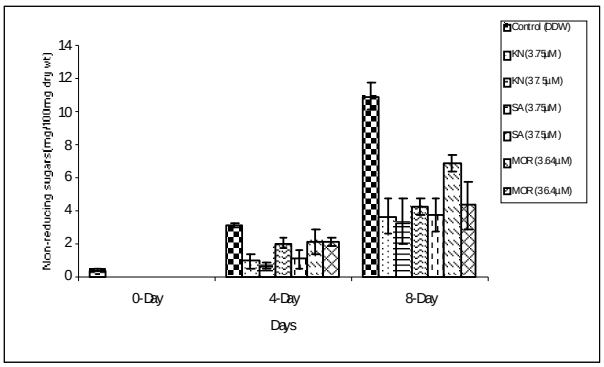

Figure-10 Non reducing sugars in *Calendula officinalis*

36.4 M . For each concentration, 10 conical flasks were used and 30 ml test solution was kept in each flask. Conical flasks containing 30 ml double distilled water (DDW) in each served as controls. Three scapes were kept in each flask. The experiment was set at 25±1°C with light intensity of 2.24µmol m^{-2} s^{-1} during day and 1.13µmol m^{-2} s^{-1} in night.

Flowers of *Coreopsis* were used to study the petal senescence in intact system. Saplings of *Coreopsis* were transferred in earthenware pots which were kept in protected cage in university botanical garden, Kurukshetra. After 4-day, higher concentration of each plant growth regulator in equal volume (5 ml) was sprayed on flower bud in each pot. DDW sprays were carried out on the potted plants kept as controls. Samples were collected at different time intervals. Flower petals were plucked up 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 3 replicates were available for each biochemical extraction and analysis. After recording the fresh weight, the samples were transferred to the deep freezer before carrying out various biochemical analyses. Further, visible effect, longevity, moisture content and flower diameter were recorded during this investigation and all data were statistically analyzed.

Protein estimation and measurement of protease activity-

The total soluble protein was estimated by the method of Bradford (1976) using Coomassie brilliant blue G-250 (a protein binding dye). The procedure of protease extraction was a slight modification of that described by Yomo and Varner (1973).The ninhydrin method was followed for the estimation of protease activity; originally described by Yemm and Cocking (1955) and modified by Reimerdes and Klostermeyer (1976).

Measurement of peroxidase activity-

The total peroxidase activity was measured by the method of Maehly (1954) and was expressed as the activity mg^{-1}protein min^{-10}.

Measurement of Lipid peroxidation (MDA content)-

The level of lipid peroxidation was measured in terms of MDA content (Heath and Packer 1968).

Estimation of total soluble sugars-

Total sugars were estimated by Anthrone method of Hart and Fisher (1971).

RESULTS AND DISCUSSION

Overall results of flower diameter and moisture content indicated a decreasing trend in *C. officinalis* and *C. lanceolata* undergoing

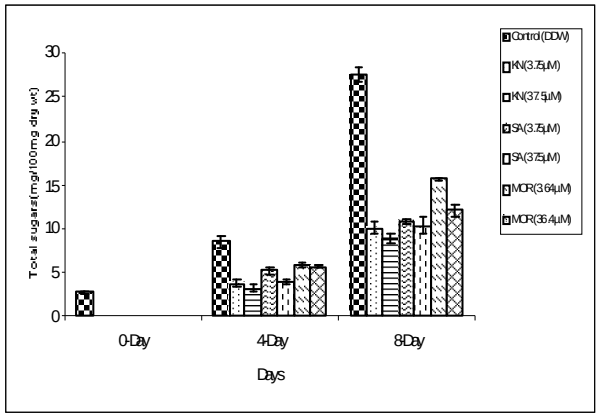

Fig-11 Total sugars in *Calendula officinalis*

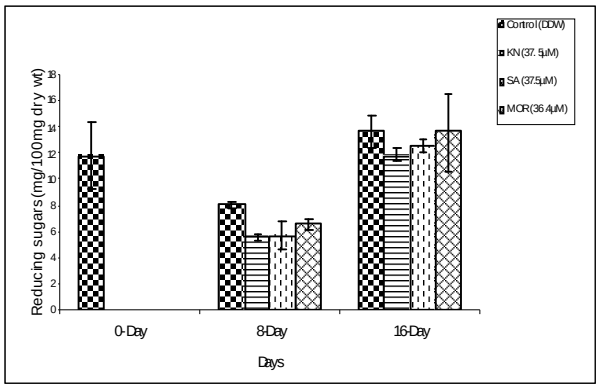

Figure-12 Reducing sugars in *Coreopsis lanceolata*

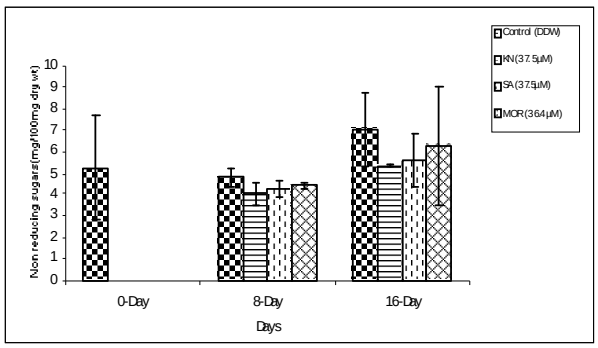

Figure-13 Non reducing sugars in *Coreopsis lanceolata*

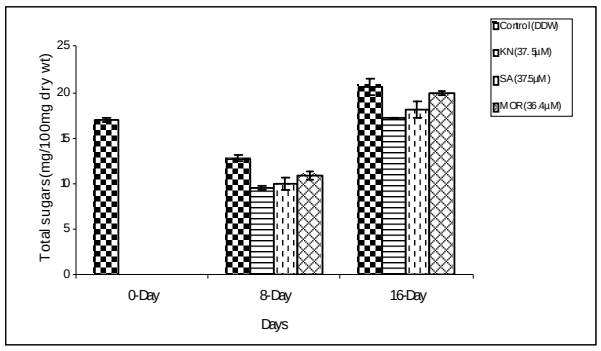

Figure-14 Total sugars in *Coreopsis lanceolata*

maturity and senescence. Applied concentrations of plant growth regulators (PGR'S) were, however, unique in checking shrinkage in flower diameters in both the plants, the best result was obtained with kinetin(KN) followed by salicylic acid(SA) and morphactin(MOR).

Protein and Peroxidase activity:

Figures 1 and 2 showed a significant decline in protein contents and significant increment in peroxidase activity of flower petals of *Calendula* at 8-day.At 4-day, protein breakdown was reduced by both the concentrations of KN and SA whereas after 8-day the effectiveness of MOR was also noticed. Likewise, Figures 3 and 4 showed changes in protein contents and peroxidase activity of *Coreopsis.* The difference in the specific activity of peroxidase between untreated samples and treated ones was highly significant in attached as well as in detached system and KN was observed most effective than other two growth substances. The loss of protein during senescence has been reported by a number of workers including Celikel and Van Doorn (1995), Stephenson and Rubinstein(1998) and Wagstaff *et al.*(2002).

In the present study, there is a gradual increment in specific activity of peroxidase (POX) with the progress of senescence.Similar trend was observed in petals of carnation(Bartoli *et al.* 1995) and daylily(Panavas and Rubinstein1998)during senescence process. These findings indicate that peroxidase is involved in the senescence of flower petals because it catalyzes the decomposition of H_2O_2. The common feature in both the flowers was a sharp decline in POX activity after KN, SA, and MOR treatments in comparison to controls.

Protease activity and MDA content:

Figure 5 and 6 showed a sharp increase in protease activity and MDA content in flower petals of *Calendula* from 0 to 8-day. These

flowers having treatments with plant growth regulators, exhibited smaller increments especially at 4-day stage. Wagstaff *et al.* (2002) also observed increment in proteolytic activity during senescence of *Alstroemeria* petals. Specific activity of protease and MDA content of *Coreopsis* as shown in figures7-8, revealed increments in the former at 8 and 16-day stages respectively.Significant increment in MDA content was also observed in carnation (Paulin and Droillard 1989) and daylily (Chakrabarty *et al*.2009).MDA,a decomposition product of polyunsaturated fatty acid hydroperoxides, has been utilized very often as a suitable biomarker for lipid peroxidation(Bailley *et al.* 1996),which occurs during advanced stages of senescence and is responsible for membrane leakage. Higher concentration of all the three growth regulators could appreciably bring down the enzymatic activity to enable the flowers in reducing protease activity and lipid peroxidation in this study and among all plant growth regulators, kinetin was highly effective.

Sugars:

Figures 9-11 clearly showed that at 0-day, the amount of reducing sugars was significantly higher than non-reducing sugars. Further, a sharp increase in reducing, non-reducing and total sugars was noticed in *Calendula.* Figures12-14 showed that values of reducing, non-reducing and total sugars in *Coreopsis* decreased a little at 8-day but at 16-day stage when senescence occurred in petals, these values had increasing trend. Initial values of reducing, non- reducing and total sugars at 0-day stage were about12, 5 and 17 percent respectively and at 8-day stage in control changed to about 8, 5 and 13 percent respectively. Applied plant growth regulators again minimized the rise in the quantity of sugars. Increment in the amount of sugars was due to degradation of starch and sucrose, which are present in petals.Van Doorn (2001)opined that in most flowers the levels of soluble sugars in the petals are still high at the time of flower wilting, which shows that cells still have adequate reserves at the time of wilting, which is contradictory to the effect of exogenous requirement of sugars. According to Halevy and Mayak (1979), for flower opening, large amounts of soluble carbohydrates are required for respiration and cell wall synthesis and as an osmolyte. As sugar reserves in cut rose flowers are gradually consumed, the vase life of cut roses may be thus shortened (Ichimura 2003).

Flowers of *Calendula* and *Coreopsis* showed more longevity with applied growth regulators (KN, SA and MOR) in comparison to control. Among the three, KN-treated flowers had longer life.Van Staden *et al.* (1988) suggested an inverse relationship between cytokinin content and senescence in some flowers. Van Doorn *et al.* (2002) also noticed the role of cytokinin in improving postharvest quality of rose cut flowers. Increase of endogenous cytokinins is delay is reported to flower senescence by inhibiting ethylene production (Guo and Sheng 1999).

Salicylic acid (SA), an endogenous growth regulator of phenolic nature, participates in the regulation of physiological process in plant and improves all growth characters (Hussein *et al*.2007).The vase solution having 5-SSA (5-sulfosalicylic acid) significantly increased not only cumulative uptake of solution, vase life, number of opened florets,etc.but also lowered respiration rates, lipid peroxidation and lipoxygenase(LOX)activity; and increased membrane stability, soluble protein concentration and activity of superoxide dismutase(SOD) and catalase in comparison to controls(Ezhilmathi *et al*.2007).Khurana and Cleland(1992) observed that continuous presence of SA or benzoic acid in the culture medium is essential to obtain maximal flowering and stated that SA is by far the most effective substance for induction of flowering in *L. paucicostata* LP6.

The application of morphactins may increase or decrease the number of flowers, depending on time and concentration of application. Earlier studies clearly demonstrated the ability of morphactins in delaying not only leaf senescence and chlorophyll breakdown but also in the regulation of flower senescence(Schneider 1970).Single morphactin treatment significantly checked the abscission and shedding of flowers there by increasing the number of fruits per plant(Mukherjee 1980).

CONCLUSION

From the overall discussion it can be concluded that all the three selected plant growth regulators were effective in controlling the petal senescence and extending the vase life/longevity of *C. officinalis* and *C. lanceolata*. These plant growth regulators were effective in both attached and detached systems.However, the most effective among the three was kinetin followed by salicylic acid and morphactin.

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